# Evaluation of the Relationship Between Vaginal, Placental and Neonatal Mycobiota and Microbiome with Preterm Birth in Women with Short Cervical Length

EBRU CELIK
KOC UNIVERSITY SCHOOL OF MEDICINE

**STUDY PROTOCOL**

**Introduction**

The term microbiota is used to describe "ecological commensal, symbiotic and pathological communities" living on and / or within all multicellular microorganisms, from plants to animals. Microbiota contributes to the immunological, hormonal and metabolic homeostasis of the host. As in all natural orifices in the body, there is also a microbiota and mycobiota specific to the vagina.

On the other hand, the sonographic short cervix in the second 3 months of pregnancy is associated with preterm delivery, which may be an important cause of mortality and morbidity in the neonatal period. American Society of Obstetricians and Gynecologists (ACOG), British Royal Society of Obstetricians and Gynecologists (RCOG) and the American Society of Maternal Fetal Medicine (SMFM) suggests the measurement of transvaginal sonographic cervical length at 20-24 gestational weeks for screening of preterm birth.  Aforementioned associations also recommend the use of progesterone in the treatment of women who diagnosed with short cervix by transvaginal ultrasonography due to the fact that progesterone is effective in prevention of preterm birth (Grade B) (1-2).

Previous vaginal microbiota studies have shown that some bacterial species, such as Lactobacillus insers, cause a predisposition to premature labor in patients with short cervix (3). However, the prominent deficiency in these studies is that the eukaryotic fungi in abundant vaginal flora have not been evaluated.

On the other hand, it is already known that progesterone treatment is not able to prevent all preterm birth in women with short cervical length. As a research group, our hypothesis is that the variety of microbiological and / or mycobiotal in pregnancies resulting in preterm birth are different than those who give birth at term although patients with sonographic short cervical length receive progesterone regularly from the second trimester. In this study, the aim of this study is to evaluate the patterns of microbiota and mycobiota from vaginal swabs of women who had preterm birth due to sonographic short cervical length and postpartum swabs of placenta and fetal oral cavity.

In this study, it has already known that some species in vaginal flora present a greater risk for preterm delivery. Women with short cervical length tend to give preterm birth, especially in the second trimester of pregnancy (between 20-28 weeks of gestation). Babies born in this early period are exposed to high risk of morbidity, such as severe neurodevelopmental sequelae, even if they survive. Early diagnosis and appropriate treatment of cervical / vaginal mycobiota and microbiota changes in women with short cervical length might reduce the rate of preterm birth and the associated neonatal mortality and morbidity.

Yet, measurements of cervical length are routinely performed in all pregnant women at 11-14th and 18-22 th  gestational weeks by transvaginal ultrasonography. Of these patients, cervical swabs will be obtained just before the scans from women who agree to participate in the study will be kept in the -80 closet in our hospital until the samples are studied. Study group will include women who had cervical length 25 mm or less than 25 mm at 20-24 th gestational week and underwent birth (preterm delivery) before 37 + 0 weeks of gestation. The control group had pregnancies with a cervical length above 25 mm and delivered at term (37 + 0 gestational week and above). During the follow-up of pregnancies, care will be taken not to use antibiotics. Plaques of placental tissue in sterile

**KOÇ UNIVERSITY**

conditions and oral swab samples from newborns will be taken from women whose follow-up and delivery are performed in our hospital and by our team.

**The inclusion criteria are:**

- Singleton pregnancies
- The pregnant women who are willing to participitate sign the informed consent form

**The exclusion criteria are:**

- Multiple pregnancies
- The presence of fetal anomaly
- Finding the intrauterine mort de fetus
- Antibiotic and / or antifungal use at any time of pregnancy
- Pregnant women under 18 years of age
- Women with previous cervical surgery
- Women who do not accept to participate to be in the study
- The presence of uterine anomaly
- Women with vaginal bleeding at the time of cervical swabs taken

**Study Procedure**

The measurements of cervical length will be performed by the perinatology team in two different periods of pregnancy (11-14 and 18-22 weeks of gestation) in the singleton pregnancies. Firstly, the vaginal swabs of women who accept to participate in the study will be collected at 11-14th weeks of gestation just before the measurement of cervical length. Also, the vaginal swabs through the aferomentioned procedure will be collected at

18-22 weeks of gestation. Micronized progesterone will be started in women with a cervical length equal or less than 25 mm (smaller than 3rd percentile) at 18-22 weeks of gestation. The medication (progesterone 200mg) will be administered intravaginally every night before bedtime and will continue until 36th gestational week unless the patient gives birth. Measurement of cervical length will be repeated transvaginally at 28th and 32nd gestational weeks, and samples will be taken with vaginal swab before these measurements. In order to evaluate the presence of placental mycobiota and microbiota after birth, 1 cm x 1 cm x 1 cm of tissue sample from placenta that is 3 cm lateral of the cord insertion will be taken under sterile conditions. In order to evaluate the amniotic compartment, buccal mucosal swabs will be taken from the neonates immediately after birth. All samples taken during pregnancy follow-up and after delivery will be delivered to our Research Laboratory immediately and will be kept and stored in -80 degrees cabinet until the evaluation.

**Study population**

The study will plan to take 2 years for recruitment of patients. The enrollment of 60 participants with singleton pregnancies who have cervical length of 25 mm or less would give the study a power of 85% to show a treatment effect at a two-sided alpha level of 5%. The risk of preterm birth in a pregnant with a cervical length of 25 mm or less is 30%. The rate of preterm birth is 12% in the whole population. Thirty-two women who delivered at ter with cervical length of longer than 25 mm will be in the control group. For the recruitment of study group, 1429 women with  singleton pregnancies will need to be screened.

The patients will be included in the study group;

1) Pregnant women with cervical length of 25mm or less (n=60)

A) Of 60 patients, 30% will be expected to give a birth before 37 gestational weeks (n=18)

B) Of 60 patients, almost 70% will be expected to give a birt at term (n=42)

2) As a control group, women with a cervical length of more than 25 mm who give a birth at term will be included (n=32)

A total of 1429 women will need to be screened to be able to recruit the above numbers of patients.

**Analysis of Microbiota**

**Isolation of DNA**

Genomic DNA will be stored at 80 0 C until the analysis microbiota is performed by using the BIO PowerSoil DNA isolation kit.

**Bacterial microbiota**

The V3-V4 regions by the 16s ribosomal RNA sequencing method will be sequenced with the Illumina MiSeq device and the data will be analyzed according to the protocols standardized in the Human Microbiome Project.

**Fungal Microbiota**

DNA samples which will be ranked from the data to be sequenced in accordance with the ITS1 region metabiota protocol will be evaluated within the Human Microbiome Project. Pseudomonas, Escherichia, Neisseria, Streptococcus, Lactobacillus, Candida, Actinomyces will be used as positive controls and, V3-V4 and ITS regions will be matched for bacterial microbiota and Fungal microbiota.

**KOÇ UNIVERSITY**

**Evaluation of Results**

After the "Operational taxonomic units" of the amplicons are configured with the VSEARCH program, the analyzes will be carried out with GENBANK microbiota and micobiota data.

Recruitment and care of patients, collection of materials and management plan will be carried out by Perinatology team at Koç University Hospital Gynecology and Obstetrics Clinic during the study. The evaluation of materials will be carried out by the Department of Microbiology of Koç University, School of Medicine.

Only the research team will have access to the identity information of the participants. Only physicians in the perinatology department of Koç University Hospital, Department of Obstetrics and Gynecology, have access to the GE Voluson E8 Ultrasonography device and the hospital-approved Viewpoint archiving system, which is already in place at the Perinatology Department. After the research is completed, the forms will be destroyed by paper milling machine after 5 years.

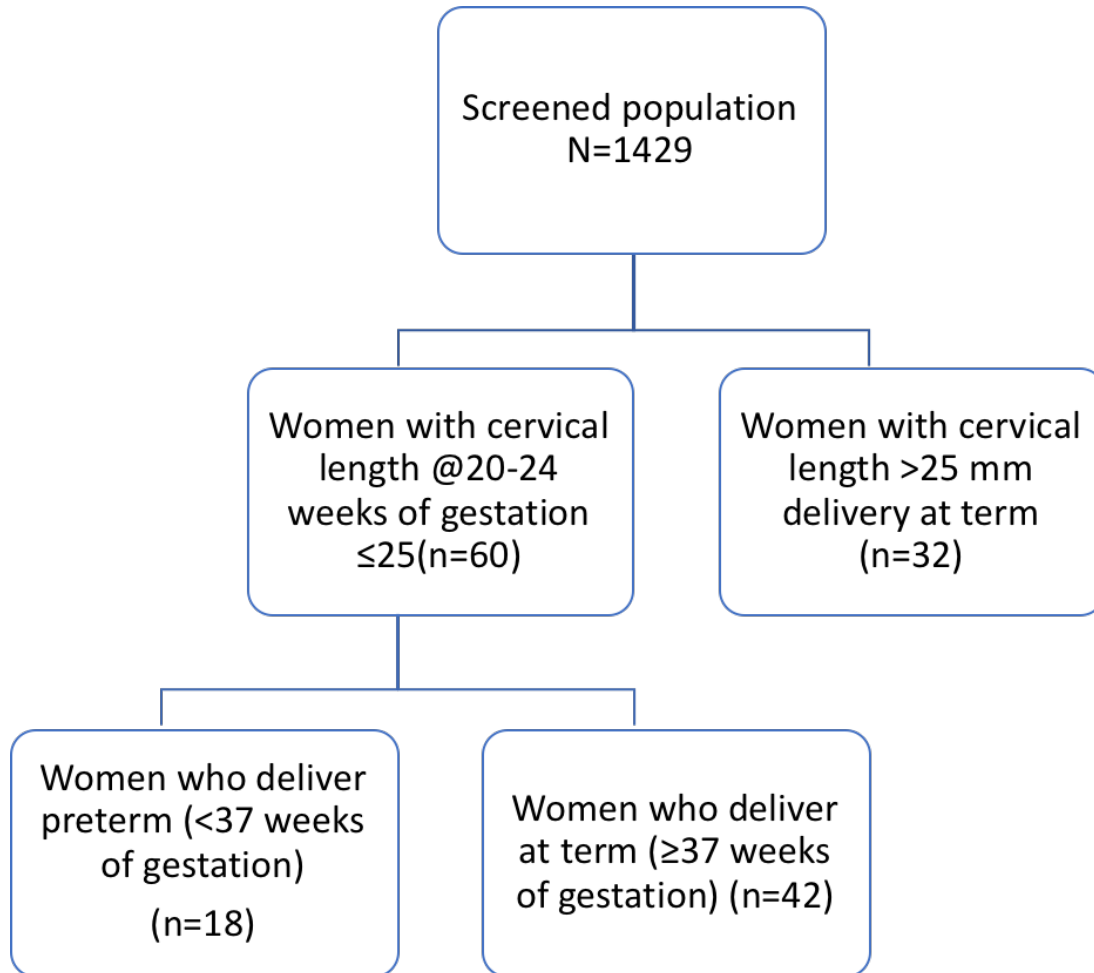Ethic No: 2019.093.IRB2.030                                              01.03.2019

## References:

[1]  Aagaard, K., Ma, J., Antony, K.M., Ganu, R., Petrosino, J., ve Versalovic, J. 2014. "The placenta harbors a unique microbiome", Sci Transl Med, 21(6),237.

[2]  DiGiulio, D.B., Romero, R., Amogan, H.P., Kusanovic, J.P., Bik, E.M., Gotsch, F., Kim, C.J., Erez, O., Edwin, S., ve Relman, D.A. 2008. "Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: A molecular and culture-based investigation", PLoS One, 3(8),30-56.

[3]  DiGiulio, D.B., Romero, R., Kusanovic, J.P., Gómez, R., Kim, C.J., Seok, K.S., Gotsch, F., Mazaki-Tovi, S., Vaisbuch, E., Sanders, K., Bik, E.M., Chaiworapongsa, T., Oyarzún, E., ve Relman, D.A. 2010. "Prevelance 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, 64(1),38–57.

[4]  Gardella, C., Riley, D.E., Hitti, J., Agnew, K., Krieger, J.N., ve Eschenbach, D. 2004. "Identification and sequencing of bacterial rDNAs in culture-negative amniotic fluid from women in premature labor", Am J Perinatol, 21(6),319-23.

[5]  Han, Y.W., Shen, T., Chung, P., Buhimschi, I.A., ve , C.S. 2009. "Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth," J Clin Microbiol, 47(1),38–47.

[6]  Iams, J.D., Goldenberg, R.L., Meis, P.J., Mercer, B.M., Moawad, A., Das, A., Thom, E., McNellis, D., Copper, R.L., Johnson, F., ve Roberts, J.M. 1996. "The length of the cervix and the risk of spontaneous premature delivery", N Eng J Med, 334(9),567-72.

[7]  Kindinger, L.M., Bennett, P,B., Lee, Y.S., Marchesi, J.R., Smith, A., Cacciatore, S., Holmes, E., Nicholson, J.K., Teoh, T.G., ve MacIntyre, D.A. 2017. "The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk" Microbiome, 5,6.

[8]  McIntosh, J., Feltovich, H., Berghella, V., ve Manuck, T. 2016. "The role of routine cervical length screening in selected high-        and low-risk women for preterm birth prevention", Am J Obstet Gynecol, 215(3):2-7.

[9]  "Practice Bulletin No. 171: Management of Preterm Labor", 2016. Obstet Gynecol, 128(4):155-64

[10] "Preterm labour and birth", 2015. National Institute for Health and Care Excellence (NICE) guideline. National Collaborating Centre for Women's and Children's Health. London, United Kingdom. https://www.ncbi.nlm.nih.gov/books/NBK327571/  Son erişim tarihi: 28 Mart 2019

[11] Prince, A. 2012. "The bitter taste of infection", J. Clin. Invest, 122:3847-3849.

[12] Rautava, S., Collado, M.C., Salminen, S., ve Isolauri, E. 2012. "Probiotics modulate host–microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial", 102(3),178-84.

[13] Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., ve Forney, L.J. 2011. "Vaginal microbome of reproductive-age women", Proc Natl Acad Sci U S A, 15(3),108.

[14] Romero, R., Nicolaides, K.H., Conde-Agudelo, A., O'Brien, J.M., Cetingoz, E., Da Fonseca, E., Creasy, G.W., ve Hassan, S.S. 2016. "Vaginal progesterone decreases preterm birth ≤ 34 weeks of gestation in women with a singleton pregnancy and a short cervix: an updated meta-analysis including data from the OPPTIMUM study", Ultrasound Obstet Gynecol, 48(3),308-17.

[15] van de Wijgert, J.H., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., ve Jespers, V. 2014. "The vaginal microbiota: what have we learned after a decade of molecular characterization?", PLoS One, 22(8),105998.

[16] Younes, J.A., Lievens, E., Hummelen, R., van der Westen, R., Reid, G., ve Petrova, M.I. 2018. "Women and Their Microbes: The Unexpected Friendship", Trends Microbiol, 26(1),16-32.

**KOÇ
UNIVERSITY**

```
┌─────────────────────────┐
│   Screened population    │
│        N=1429            │
└─────────────────────────┘
             │
      ┌──────┴──────────────────┐
┌─────────────────┐    ┌─────────────────┐
│ Women with      │    │ Women with      │
│ cervical        │    │ cervical        │
│ length @20-24   │    │ length >25 mm   │
│ weeks of        │    │ delivery at term│
│ gestation       │    │ (n=32)          │
│ ≤25(n=60)       │    │                 │
└─────────────────┘    └─────────────────┘
         │
   ┌─────┴──────────┐
┌────────────────┐  ┌────────────────┐
│ Women who      │  │ Women who      │
│ deliver preterm│  │ deliver at term│
│ (<37 weeks of  │  │ (≥37 weeks of  │
│ gestation)     │  │ gestation)     │
│ (n=18)         │  │ (n=42)         │
└────────────────┘  └────────────────┘
```

Ethic No: 2019.093.IRB2.030                                      01.03.2019

KOÇ
UNIVERSITY

**Recruitment of patients**

The first trimester scan (11-13 weeks of gestation
- -Demographic data
- -Vaginal swabs
- - Measurement of cervical length

The second trimester scan (18-24 weeks of gestation
- -Demographic data
- -Vaginal swabs
- - Measurement of cervical

Administration of progesterone

CL≤ 25 mm

CL>25 mm

The second trimester scan (28-32 weeks of gestation
- -Vaginal swabs
- - Measurement of cervical length

- Birth;
- Collection of placental specimen
- Neonatal buccal swabs

Isolation fo 16s rRNA and 18s rRNA

Analysis of bioinformatic data