Lactobacillus crispatus dominance in the vaginal microbiome reduces the occurrence of spontaneous preterm birth in women with a short cervical length

Dominância de Lactobacillus crispatus *no microbioma vaginal reduz a ocorrência de parto prematuro espontâneo em mulheres com encurtamento do colo uterino*

Antonio Fernandes Moron[1,2], Steven Sol Witkin[3,4], Iara Moreno Linhares[5], Alan Roberto Hatanaka[1], Stéphanno Gomes Pereira Sarmento[1], Marcelo Santucci França[1], Francisco Herlânio Costa Carvalho[6], Rosiane Mattar[1], Larry Jay Forney[7]

ABSTRACT

Introduction: The majority of pregnant women with a short cervix will deliver at term and, thus, may unnecessarily receive advanced monitoring and treatment. It is still necessary to define more accurately which sub-population of women with a short cervix is at elevated risk for early delivery. **Objective**: To determine if vaginal microbiome composition influenced the rate of spontaneous preterm birth in women with a short cervical length. **Methods**: In an exploratory, observational prospective study, vaginal secretions were obtained from 591 women at 21–24 week gestation. Vaginal microbiome composition was determined by analyzing the V1–V3 region of the bacterial 16S ribosomal RNA gene. **Results***: Lactobacillus crispatus* was numerically dominant in the vagina in 41.7% of subjects, followed by *L. iners* in 32% and *Gardnerella vaginalis* in 12%. In women whose cervix was ≤25mm, the sensitivity to predict an spontaneous preterm birth was 11.8%. However, when *L. crispatus* was not the dominant vaginal bacterium, this sensitivity increased to 81.8%. Similarly, in women with a cervical length ≤30mm, the sensitivity to predict an spontaneous preterm birth increased from 21.7 to 78.3% when *L. crispatus* was not the dominant vaginal bacterium. In women with a prior spontaneous preterm birth and a cervix ≤25 or ≤30mm, *L. crispatus* dominance was also associated with a reduced rate of spontaneous preterm birth in the current pregnancy (p<0.001). **Conclusion**: In pregnant women with a cervix ≤25mm or ≤30mm, the risk for an spontaneous preterm birth is increased if *L. crispatus* is not dominant in the vagina.

Keywords: Cervical length measurement. *Lactobacillus crispatus*. Mycobiome. Pregnancy. Obstetric labor, premature.

RESUMO

Introdução: A maioria das mulheres grávidas com colo do útero curto dará à luz a termo e, portanto, pode receber desnecessariamente monitoramento e tratamento avançados. Permanece a necessidade de definir com mais precisão qual subpopulação de mulheres com colo do útero curto está em risco elevado de parto prematuro. **Objetivo**: Determinar se a composição do microbioma vaginal influenciou a taxa de parto prematuro espontâneo em mulheres com colo curto. **Métodos**: Em um estudo prospectivo exploratório observacional, os conteúdos vaginais foram obtidos de 591 mulheres com 21–24 semanas de gestação. A composição do microbioma vaginal foi determinada pela análise da região V1–V3 do gene de RNA ribossômico bacteriano 16S. **Resultados**: *Lactobacillus crispatus* foi numericamente dominante na vagina em 41,7% dos indivíduos, seguido por *L. iners* em 32% e *Gardnerella vaginalis* em 12%. Em mulheres cujo colo do útero era <25 mm, a sensibilidade para prever uma taxa de parto prematuro espontâneo foi de 11,8%. No entanto, quando *L. crispatus* não era a bactéria vaginal dominante, essa sensibilidade aumentou para 81,8%. Da mesma forma, em mulheres com comprimento cervical <30 mm, a sensibilidade para prever uma taxa de parto prematuro espontâneo aumentou de 21,7 para 78,3% quando *L. crispatus* não era a bactéria vaginal dominante. Em mulheres com taxa de parto prematuro espontâneo anterior e colo do útero <25 ou <30 mm, a dominância de *L. crispatus* também foi associada a uma taxa reduzida de taxa de parto prematuro espontâneo na gravidez atual (p<0,001). **Conclusão**: Em mulheres grávidas com colo do útero <25 ou <30 mm, o risco de parto prematuro espontâneo é aumentado se *L. crispatus* não for dominante na vagina.

Palavras-chave: Medida do comprimento cervical. *Lactobacillus crispatus*. Microbioma. Gravidez. Trabalho de parto prematuro.

[1]Universidade Federal de São Paulo, Department of Obstetrics – São Paulo (SP), Brazil.
[2]Universidade de São Paulo, Institute of Tropical Medicine – São Paulo (SP), Brazil.
[3]Weill Cornell Medicine, Department of Obstetrics and Gynecology – New York, United States of America.
[4]Universidade de São Paulo, Institute of Tropical Medicine, Virology Laboratory – São Paulo (SP), Brazil.
[5]Universidade de São Paulo, Medical School, Department of Obstetrics and Gynecology – São Paulo (SP), Brazil.
[6]Universidade Federal do Ceará, Department of Gynecology and Obstetrics – Ceará (CE), Brazil.
[7]University of Idaho, Institute for Bioinformatics and Evolutionary Studies, Department of Biological Sciences – Idaho, United States of America.

INTRODUCTION

A major risk factor for a spontaneous preterm birth (SPTB), defined as spontaneous delivery at <37 weeks of gestation, is the presence of a short cervical length, typically defined as ≤ 25mm on a mid-trimester vaginal ultrasound[1,2]. However, the predictive value of a short cervix for an SPTB is low[3]. The majority of pregnant women with a short cervix will deliver at term and, thus, may unnecessarily receive advanced monitoring and treatment. It is still necessary to define more accurately which sub-population of women with a short cervix is at elevated risk for early delivery. Also, the

optimal cutoff value that best defines a short cervical length remains incompletely resolved[1,4,5].

It has been consistently observed that *Lactobacillus iners* or *Gardnerella vaginalis* dominance in the vaginal microbiome in pregnant women is associated with the increased detection of a short cervical length that predisposes to SPTB[6-13]. However, whether the consequence of a shortened cervix is influenced by differential bacterial dominance in the vagina has scarcely been evaluated. An exception is a study by Gerson, who reported that, in women with a short cervix, an SPTB was more frequent if their vaginal microbiome was dominated by bacteria other than lactobacilli[13].

OBJECTIVE

The present study investigated whether the risk for an SPTB in women with different measurements of cervical length was modified by the relative abundance of individual vaginal bacteria.

METHODS

The subjects in this observational, exploratory prospective study were 591 mid-trimester pregnant women with singleton gestations seen at three outpatient hospital-based obstetric clinics in Brazil: Universidade Federal de São Paulo, Universidade Federal do Ceara and Jundiai Medical School. Women were enrolled regardless of their pregnancy history, and so both high risk and low risk women were included. Exclusion criteria were multiple gestation, signs or symptoms consistent with vaginal dysbiosis, use of antibiotics in the previous two weeks, vaginal intercourse in the preceding 7 days, a history of an immune or endocrine disorder, or the inability to give informed consent. The study was approved by the Institutional Review Board at Universidade Federal de São Paulo, and all subjects gave their written informed consent.

Cervical length was routinely evaluated in each subject by transvaginal sonography using a 5–9MHz probe (Accuvix XQ and V10, Medison, S.Korea; Voluson Expert 730, USA) as previously reported.[6] All ultrasound examinations were performed by physicians with extensive training in fetal medicine. As per the routine at all participating institutions, women with a cervical length ≤25mm received a 200 mg daily dose of vaginal progesterone (Utrogestan®) up to a 36-week gestation or until the time of delivery.

Just prior to the ultrasound cervical length measurement, the Copan ESwab collection system (Fisher Scientific, Pittsburgh, PA) was used to obtain a sample from the posterior vaginal wall of each subject to determine the vaginal microbiome composition. The samples were sent on dry ice to the Forney lab at the University of Idaho.

The methods used to determine the bacterial species composition of the vaginal microbiome have been previously reported in detail[6,14]. Briefly, bacterial cells were lysed with an enzyme cocktail and bead beating, and genomic DNA was isolated using a QIAamp DNA Mini kit. DNA yield was determined by fluorometry and DNA size and integrity were verified with an Agilent Bioanalyzer. The V1 to V3 regions of bacterial 16S rRNA genes were amplified using a polymerase chain reaction protocol that attached sample barcodes and sequencing adapters. DADA2 software (v1.8) was used to identify distinct sequence variants (DSVs) and remove sequence chimeras. The DSVs were classified to the genus level using the RDP naive Bayesian classifier (v11.5) in combination with the Silva reference database, then assigned to the species using SPINGO software. Data were cleaned to include only samples with more than 3,000 reads.

The vaginal microbiome analysis in the subjects from São Paulo have been previously reported[6]. The present study is, therefore, an extension and expansion of that initial investigation. The unique aspects of the present study, employing a larger study group, are determination of associations between the vaginal microbiome composition and SPTB in women with a short cervical length, as well as examining the inclusion of subjects with a cervical length between 26 and 30mm in the definition of a short cervix.

Differences in continuous clinical and laboratory variables between women with a median cervical length of ≤25mm, 26–30mm and >30mm were determined by the Kruskal-Wallis test or the Spearman rank correlation test, as appropriate. Differences between any two cervical lengths were assessed by the Student *t* test. Variations in discrete variables were analyzed by Fisher's exact test. Sensitivity, specificity and positive and negative predictive values were determined to predict SPTB according to cervical length and *Lactobacillus crispatus* dominance. A two-sided $p<0.05$ was considered significant.

RESULTS

A cervical length ≤25mm was identified in 82 (13.9%) women, 76 (12.9%) had a cervical length between 26–30mm and, in 433 women (73.2%), their cervix was >30mm. Associations between women in the three cervical length classes, clinical variables and pregnancy outcome are shown in **Table 1**. The mean maternal age increased, while the occurrence of nulliparity decreased, in proportion to cervical length ($p<0.001$). There were no significant differences in race, body mass index, years of education and smoking status between the three groups. An SPTB was documented in 12.2% of women whose cervix was ≤25mm, 14% in those with a cervix between 26–30mm, and 6.5% in women with a cervix >30mm. A medically induced preterm birth (IPTB) was not present in any of the women whose cervix was

Table 1 – Association between cervical length and clinical variables.

	Cervical length (mm)		
	<25	26–30	>30
	n=82	n=76	n=433
Mean maternal age (SD)	25.7 (7.0)	28.0 (7.2)	29.5 (7.0)[a]
Nulliparity (%)	58	51.3	41.4[b]
Mean body mass index (SD)	26.1 (5.3)	26.8 (5.3)	27.2 (5.5)
Prior miscarriages >12 weeks (%)	6.1	6.7	4.4
Race (%)			
White (N = 284)	48.8	49.3	50.4
Mixed race (N = 236)	41.5	38.7	42.1
Black (N = 48)	9.8	12.3	7.5
Mean education (years)	10.9 (2.6)	10.6 (3.0)	11.4 (2.8)
Smoker (%)	9.8	2.7	6.8
Spontaneous preterm birth (%)	10 (12.2)	12 (14)[c]	28 (6.5)
Induced preterm birth	0[d]	2 (2.6)	27 (6.2)

[a]p<0.001 vs.<25 and 26-30mm; [b]p<0.01 vs.<25 and 26–30mm; cp<0.01 vs.>30mm; [d]p=0.01 vs.>30mm

≤25mm, in 2 women (2.6%) with a cervix between 26–30mm and in 27 women (6.2%) whose cervix was >30mm.

In 5 women, 2 with a cervix between 26–30mm and 3 whose cervix was >30mm, their SPTB was preceded by premature rupture of membranes. In the IPTB group, 12 had preeclampsia, 5 had gestational diabetes mellitus, 3 each had preexisting diabetes mellitus, hypertension or intrauterine growth restriction, and 1 each had placenta previa, chorioamnionitis or thrombophilia.

In the total patient population, *L. crispatus* was the numerically dominant bacterium (≥50% of the total detected) in 237 women (41.7%), followed by *L. iners* in 182 women (32%) and *Gardnerella vaginalis* in 68 women (12%). The relative abundance of *L. crispatus* increased in relation to cervical length (Spearman rank correlation, p=0.0078); the relative abundance of *G. vaginalis* decreased as cervical length increased (p=0.0010), while there was no relationship between the *L. iners* abundance and cervical length (p>0.05).

The sensitivity, specificity and positive and negative predictive values for the occurrence of SPTB in women with a shortened cervix is presented in **Table 2**. In the total study population, a cervical length ≤25mm had an 11.8% sensitivity to predict SPTB. This value markedly increased to 81.8% when *L. crispatus* was not dominant in the vaginal microbiome. Similarly, in women with a cervical length ≤30mm, the sensitivity to predict SPTB increased from 21.7 to 78.3% when *L. crispatus* was not the dominant vaginal bacterium.

Focusing on the 30 women with a short cervix who had a previous SPTB, the influence of *L. crispatus* dominance on the occurrence of SPTB is shown in **Figure 1**. In those with a cervical length ≤25mm, the rate of SPTB in the current pregnancy decreased from 71.4 to 36.7% in the presence of *L. crispatus* dominance (p<0.001). Similarly, in women whose cervix was between 26–30mm, the rate of SPTB decreased from 83.7 to 57.1% when *L. crispatus* was dominant (p<0.001).

DISCUSSION

It has previously been reported that the rate of SPTB in women with a short cervix is greatest when bacteria other than lactobacilli predominate in the vagina[13]. The present study is consistent with this observation and extends this finding by highlighting the association between *L. crispatus* dominance and a reduced rate of SPTB in these women. In our patient population, the occurrence of SPTB in women with a cervical length ≤25mm or ≤30mm was markedly lower if *L. crispatus* was dominant in their vaginal microbiota.

The reasons for this observation remain speculative. Previous studies have suggested potential unique properties of *L. crispatus,* especially in contrast to *L. iners* and G. vaginalis, which may at last partially explain this occurrence. *L.* crispatus, along with the less prevalent *L. jensenii* and L. *gasseri,* produce both the D- and L-isomers of lactic acid. *L. iners* produces only L-lactic acid, while

Table 2 – Predictive value of cervical length plus the absence of *L. crispatus* dominance for spontaneous preterm birth.

Cervical length	*L. crispatus dominance*	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
<25mm	Yes	7.4	88.6	11.8	88.6
	No	16.7	92.6	81.8	35.7
<30mm	Yes	8.6	81.8	21.7	60.4
	No	18.1	91.4	78.3	39.6

PPV: positive predictive value; NPV: negative predictive value Informações adicionais sobre autoria: Kawanami Hamamoto

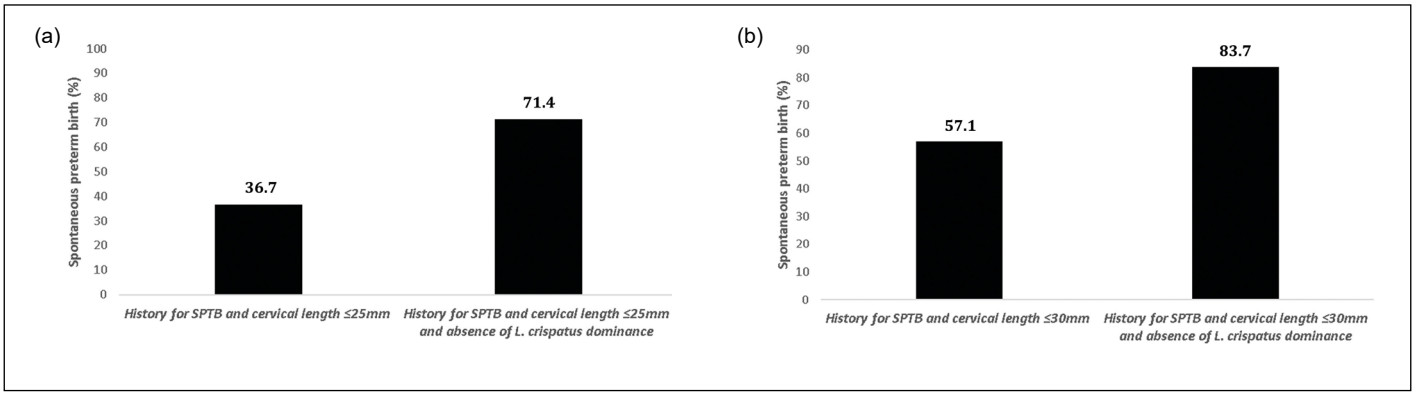

Figure 1 – Association between cervical length, vaginal microbiome and spontaneous preterm birth in women with a prior spontaneous preterm birth. The cervical length was measured by vaginal ultrasound, and the vaginal microbiome composition was assessed by amplification and analysis of the gene coding for bacterial 16S ribosomal RNA. (A) The occurrence of spontaneous preterm birth in women with a cervical length <25mm was 71.4% if *L. Crispatus* was not dominant versus 36.7% when bacterial dominance was not assessed (p<0.001). (B) The occurrence of spontaneous preterm birth in women with a cervical length <30mm was 83.7% if *L. Crispatus* was not dominant versus 57.1% when bacterial dominance was not assessed (p<0.001).

G. vaginalis also does not produces D-lactic acid, and no, or at best negligible, amounts of L-lactic acid[15]. Initial investigations have suggested that D-lactic acid possesses unique properties that may likely contribute to vaginal quiescence. There is a negative association between vaginal levels of D-lactic acid and matrix metalloproteinase-8 (MMP-8)[15]. MMP-8 involvement in degradation of the extracellular matrix may facilitate migration of bacteria from the lower to the upper genital tract during gestation and, therefore, induce preterm labor and delivery[16]. A reduced MMP-8 level as a result of *L. crispatus*-mediated release of D-lactic acid would minimize this consequence. A recent study has also demonstrated that the vaginal concentration of MMP-8 in pregnant women is positively associated with the level of histone deacetylase (HDAC)[17]. HDAC is an enzyme that regulates gene transcription by modulating the acetylation of histones in distinct chromosome regions[18]. There is a negative association between D-lactic acid and HDAC levels in the vagina during pregnancy[17]. Thus, by modulating the transcription of specific genes, D-lactic acid may enhance the ability of cells in the lower genital tract to successfully respond to exogenous or endogenous insults and maintain normal function.

It has also been shown that the presence of *L. crispatus* is tolerated better by the vaginal epithelium than other vaginal bacteria are. Production of the 70kDa heat shock protein (HSPA1A), an early indicator of non-physiological conditions[19], by vaginal epithelial cells is lowest, and vaginal cell autophagy, an intracellular process essential for promoting cell homeostasis and killing intracellular microorganisms, is higher when *L. crispatus* dominates the vaginal microbiota[20]. The differential production of short chain fatty acids by individual vaginal bacteria[21] is another potential contributor to variations in pregnancy outcome. Thus, the optimal maintenance of a quiescent local environment when *L. crispatus* is dominant in the vagina would be expected to best promote successful pregnancy progression.

Our study has several limitations. Due to ethical considerations, women with a cervical length ≤25mm were treated with vaginal progesterone. Thus, the reported SPTB rate in this population is probably lower than it would have been without this intervention. This most likely accounts for the observation that the preterm birth rate was higher in women with a cervical length between 26 and 30mm, a group not treated with progesterone, than in those whose cervix was ≤25mm. In addition, although our total study population was high, the number of women with an SPTB was relatively small. Subsequent investigations on larger numbers of women with an SPTB are needed to confirm our observations. The findings also have to be reproduced in other populations to determine their overall validity. Lastly, while a cut-off value of ≤30mm may be optimal for defining women with a short cervical length in Brazilian populations, it remains to be determined whether this will also be true for pregnant women in other countries. Women with symptomatic bacterial vaginosis were not included in the analysis since a symptomatic vaginal infection was an exclusion criterion. Women were not screened for vaginal bacterial composition by Gram stain, and so the possible involvement of asymptomatic BV in an SPTB related to cervical length was not assessed. Lastly, since our proposed prediction factors were not evaluated in an independent cohort, the present study must correctly be defined as exploratory.

Realistically, most hospital facilities are unable to characterize the vaginal microbiome in pregnant women, due to the cost and expertise necessary to perform gene amplification analyses. However, it has been demonstrated that the measurement of D- and L-lactic acid and tissue inhibitor of metalloproteinase-1 levels in vaginal secretions by a simple ELISA performed on vaginal fluid can accurately determine whether or not *L. crispatus* is dominant[6,22]. This should make identification of the dominant bacterium in the vaginal microbiome available in most clinical settings.

CONCLUSION

We conclude that the absence of *L. crispatus* dominance in the vaginal microbiota may improve the positive predictive value of a short cervix as a risk factor for spontaneous preterm birth. These findings may be useful to design improvements in screening and medical interventions for preterm birth prevention. Future studies should repeat the present analyses on first trimester pregnant women to determine whether the risk for a preterm birth can be identified earlier in gestation. This would further enhance the opportunity for differential monitoring and treatment.

Approval by the Human Research Ethics Committee

The study was approved by Universidade Federal de São Paulo – UNIFESP/EPM n° 2.004.116.

Participation of each author

AFM: Conceptualization, Formal Analysis, Writing – original draft. SSW: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. IML: Formal Analysis, Writing – original draft. ARH: Data curation. SGPS: Data curation. MSF: Data curation. FHCC: Data curation. RM: Data curation. LJF: Formal Analysis, Supervision, Writing – review & editing.

Funding

This study was supported by a grant from Bill and Melinda Gates Foundation, the Brazilian Ministry of Health (DECIT) and the Brazilian National Research Council (CNPq – grant 401626/2013-0). Sequence data collection and analyses were performed by the IBEST Genomics Resources Core at the University of Idaho, which is supported in part by NIH COBRE grant P30GM103324.

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, et al. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996;334(9):567-72. https://doi.org/10.1056/NEJM199602293340904

2. To MS, Skentou CA, Royston P, Yu CK, Nicolaides KH. Prediction of patient-specific risk of early preterm delivery using maternal history and sonographic measurement of cervical length: a population-based prospective study. Ultrasound Obstet Gynecol. 2006;27(4):362-7. https://doi.org/10.1002/uog.2773
3. Rosenbloom JI, Raghuraman N, Temming LA, Stout MJ, Tuuli MG, Dicke JM, et al. Predictive value of midtrimester universal cervical length screening based on parity. J Ultrasound Med. 2020;39(1):147-54. https://doi.org/10.1002/jum.15091
4. van der Ven AJ, van Os MA, Kleinrouweler CE, de Groot CJ, Haak MC, Mol BW, et al. Is cervical length associated with maternal characteristics? Eur J Obstet Gynecol Reprod Biol. 2015;188:12-6. https://doi.org/10.1016/j.ejogrb.2015.02.032
5. van Os MA, Kleinrouweler CE, Schuit E, van der Ven AJ, Pajkrt E, de Groot CJ, et al. Influence of cut-off value on prevalence of short cervical length. Ultrasound Obstet Gynecol. 2017;49(3):330-6. https://doi.org/10.1002/uog.15967
6. Witkin SS, Moron AF, Ridenhour BJ, Minis E, Hatanaka A, Sarmento SGP, et al. Vaginal Biomarkers That Predict Cervical Length and Dominant Bacteria in the Vaginal Microbiomes of Pregnant Women. mBio. 2019;10(5):e02242-19. https://doi.org/10.1128/mBio.02242-19
7. Kindinger LM, MacIntyre DA, Lee YS, Marchesi JR, Smith A, McDonald JA, et al. Relationship between vaginal microbial dysbiosis, inflammation, and pregnancy outcomes in cervical cerclage. Sci Transl Med. 2016;8(350):350ra102. https://doi.org/10.1126/scitranslmed.aag1026
8. Kindinger LM, Bennett PR, Lee YS, Marchesi JR, Smith A, Cacciatore S, et al. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. Microbiome. 2017;5(1):6. https://doi.org/10.1186/s40168-016-0223-9
9. Yoo HN, Park KH, Jung EY, Kim YM, Kook SY, Jeon SJ. Non-invasive prediction of preterm birth in women with cervical insufficiency or an asymptomatic short cervix (≤25 mm) by measurement of biomarkers in the cervicovaginal fluid. PLoS One. 2017;12(7):e0180878. https://doi.org/10.1371/journal.pone.0180878
10. Callahan BJ, DiGiulio DB, Goltsman DSA, Sun CL, Costello EK, Jeganathan P, et al. Replication and refinement of a vaginal microbial signature of preterm birth in two racially distinct cohorts of US women. Proc Natl Acad Sci U S A. 2017;114(37):9966-71. https://doi.org/10.1073/pnas.1705899114
11. Elovitz MA, Gajer P, Riis V, Brown AG, Humphrys MS, Holm JB, et al. Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. Nat Commun. 2019;10(1):1305. https://doi.org/10.1038/s41467-019-09285-9
12. Freitas AC, Bocking A, Hill JE, Money DM, VOGUE Research Group (2018). Increased richness and diversity of the vaginal microbiota and spontaneous preterm birth. Microbiome. 2018;6(1):117. https://doi.org/10.1186/s40168-018-0502-8
13. Gerson KD, McCarthy C, Elovitz MA, Ravel J, Sammel MD, Burris HH. Cervicovaginal microbial communities deficient in Lactobacillus species are associated with second trimester short cervix. Am J Obstet Gynecol. 2020;222(5):491.e1-491.e8. https://doi.org/10.1016/j.ajog.2019.11.1283
14. Nasioudis D, Forney LJ, Schneider GM, Gliniewicz K, France M, Boester A, et al. Influence of pregnancy history on the vaginal microbiome of pregnant women in their first trimester. Sci Rep. 2017;7(1):10201. https://doi.org/10.1038/s41598-017-09857-z
15. Witkin SS, Mendes-Soares H, Linhares IM, Jayaram A, Ledger WJ, Forney LJ. Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. mBio. 2013;4(4):e00460-13. https://doi.org/10.1128/mBio.00460-13
16. Chaemasaithong P, Romero R, Docheva N, Chaiyasit N, Bhatti G, Pacora P, et al. Comparison of rapid MMP-8 and interleukin-6 point-of-care tests to identify intra-amniotic inflammation/infection and impending preterm delivery in patients with preterm labor and intact membranes. J Matern Fetal Med. 2018;31:228-244. https://doi.org/10.1080/14767058.2017.1281904
17. Witkin SS, Nasioudis D, Leizer J, Minis E, Boester A, Forney LJ. Epigenetics and the vaginal microbiome: influence of the microbiota on the histone deacetylase level in vaginal epithelial cells from pregnant women. Minerva Ginecol. 2019;71(2):171-5. https://doi.org/10.23736/S0026-4784.18.04322-8
18. Latham T, Mackay L, Sproul D, Karim M, Culley J, Harrison DJ, et al. Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. Nucleic Acids Res. 2012;40(11):4794-803. https://doi.org/10.1093/nar/gks066
19. Craig EA. The stress response: changes in eukaryotic gene expression in response to environmental stress. Science. 1985;230(4727):800-1. https://doi.org/10.1126/science.230.4727.800-a
20. Leizer J, Nasioudis D, Forney LJ, Schneider GM, Gliniewicz K, Boester A, et al. Properties of epithelial cells and vaginal secretions in pregnant women when *Lactobacillus crispatus* or *Lactobacillus iners* dominate the vaginal microbiome. Reprod Sci. 2018;25(6):854-60. https://doi.org/10.1177/1933719117698583
21. Ceccarani C, Foschi C, Parolin C, D'Antuono A, Gaspari V, Consolandi C, et al. Diversity of vaginal microbiome and metabolome during genital infections. Sci Rep. 2019;9(1):14095. https://doi.org/10.1038/s41598-019-50410-x
22. Sarmento SGP, Moron AF, Forney LJ, Hatanaka AR, Carvalho FHC, França MS, et al. An exploratory study of associations with spontaneous preterm birth in primigravid pregnant women with a normal cervical length. J Matern Fetal Neonatal Med. 2021:1-6. https://doi.org/10.1080/14767058.2021.1879786

Address for correspondence
STEVEN S. WITKIN
Instituto de Medicina Tropical da Faculdade de Medicina da Universidade de São Paulo
Av. Doutor Arnaldo, 455, 2º andar, sala 2319 – Cerqueira César
São Paulo (SP), Brazil
CEP: 01246-903
E mail: switkin@med.cornell.edu

Received on: 03.05.2022
Approved on: 03.18.2022