



INCREASED VAGINAL GRAM-NEGATIVE BACTERIAL DIVERSITY IN THIRD TRIMESTER OF PREGNANCY IN NHP (NON-HUMAN PRIMATES)



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ABSTRACT

Introduction: The composition of vaginal microbial community is important for pregnancy maintenance. Decreased diversity of vaginal microbiome in third trimester of human pregnancy is associated with the preterm birth. NHP have been used as a model of pregnancy-related research for decades, being especially crucial for development of therapies, counteracting effects of human teratogens. The understanding of bacterial changes in pregnancy in NHP is critical for data interpretation and analyses. We and others reported decreased presence of Lactobacilli in baboons (*Papio* spp), compared to human vaginal milieu, however, the reports regarding pregnancy-related changes in these species are sparse.

Methods: Vaginal swabs were taken from 5 pregnant and 5 non-pregnant baboons (*Papio* spp) at the end of gestation. The specimens were evaluated for the presence of colony forming units [CFU].

Results: The gram-negative bacterial CFU were increased in pregnant animals (n= 26) compared to non-pregnant (n=9) and included spp., which are probably *Achromobacter*, *Staphylococcus* spp., *Diphtheroids*, etc., including novel subspecies.

Conclusions: Increase vaginal bacterial diversity in pregnancy might be universal evolutionary phenomenon, which is independent on presence of *Lactobacilli* spp.

MATERIAL AND METHODS



Figure 1. External genitalia in non-pregnant baboon (*Papio* spp.)

Five non-pregnant healthy female baboons (*Papio* spp.) and five pregnant baboons near term (165-175 days of gestation) were housed in the outdoor coral facilities. Vaginal swabs were taken according to the approved IACUC protocol, using previously described technique (SciRep 2016, [PMC4880931](https://doi.org/10.1038/s41598-016-04880-9)). Swabs were placed in 10 ml of physiological solution and transported over night for further evaluation. The standard microbiological techniques were used to grow bacterial colonies.

RESULTS

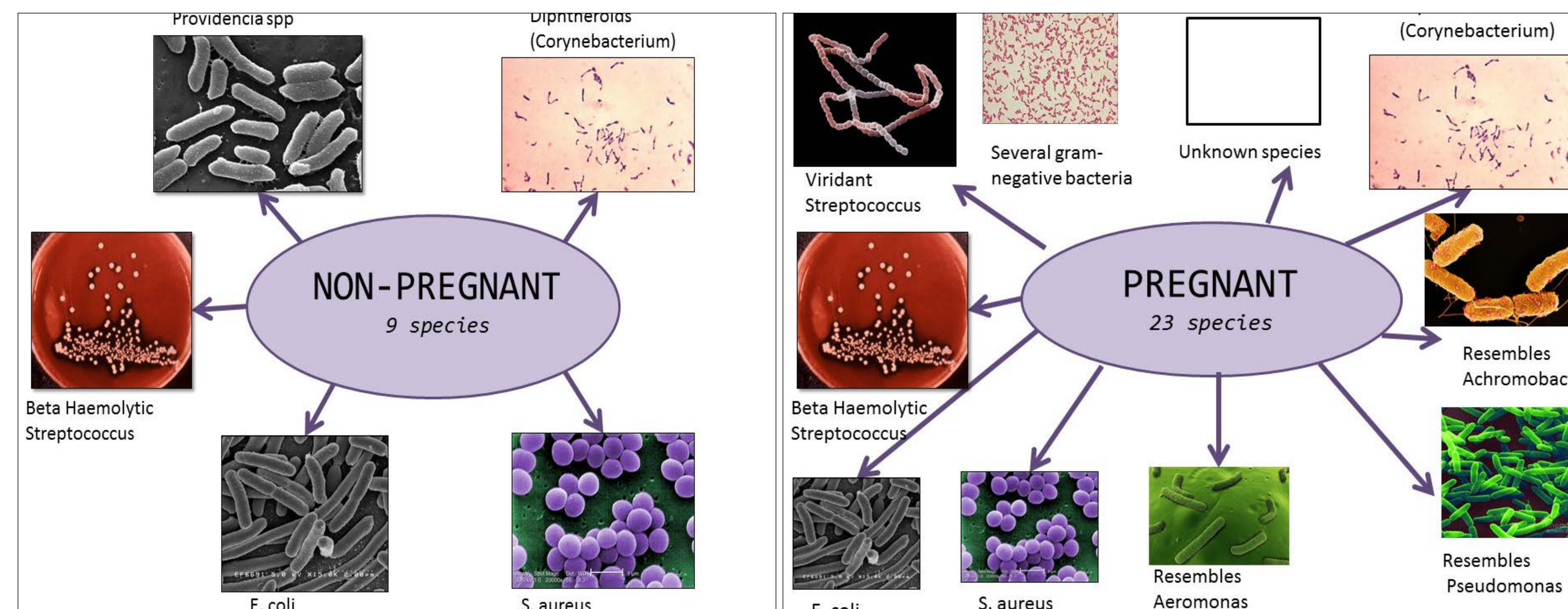


Figure 2. Graphical representation of the vaginal colony-forming units present in non-pregnant and pregnant baboons.

NON-PREGNANT BABOONS (<i>Papio</i> spp., n=5)	PREGNANT BABOONS (<i>Papio</i> spp., n=5) NEAR TERM
1) Presumptive <i>E. coli</i> . Gram Negative Enteric species, probably <i>Enterobacter</i> . <i>Staph. aureus</i> .	6) Two types of <i>Staphylococcus aureus</i> <i>Staphylococcus</i> spp. Yellow mucoid oxidase positive (resembles aeromonas). White mucoid NH oxidase positive (resembles <i>Pseudomonas aeruginosa</i>). Gray waxy spread oxidase positive (resembles <i>Achromobacter</i>).
2) Culture overgrown with <i>Proteus mirabilis</i> . Able to isolate <i>Staphylococcus</i> spp.	7) Probable * <i>Staphylococcus</i> spp #1 (NH, mannitol positive, LAT=, CONS) * <i>Diphtheroids</i> (Eg. <i>Corynebacterium</i> spp or other) * Gram-negative bacillus (OX+) most likely <i>Pseudomonas aeruginosa</i> <i>Staphylococcus</i> spp #2 slight H, LAT=, mucoids, CONS <i>Staphylococcus</i> spp #3 <i>S. aureus</i> 4+H, M+1, LAT+ <i>Staphylococcus</i> spp #4 yellow NH but LAT+, M+1, chromogenic NOT <i>S. aureus</i> <i>Diphtheroids</i> #2
3) <i>Staphylococcus aureus</i> . <i>Diphtheroids</i> (Eg. <i>Corynebacterium</i> or other). Beta Haemolytic <i>Streptococcus</i> .	8) Probable <i>E. coli</i> <i>Pseudomonas</i> Viridans streptococci <i>Streptococcus</i> spp <i>Staphylococcus aureus</i> <i>Diphtheroids</i> X2 or 3 species Beta haemolytic Strep
4) Probable <i>Providencia</i> spp <i>Staphylococcus aureus</i> Beta haemolytic <i>Streptococci</i>	9) Probable <i>Pseudomonas</i> spp x2 (or more) Mucoid coliform (<i>Enterobacter</i> spp or <i>E. coli</i>) Red gram-neg bacillus <i>Staphylococcus</i> spp Other gram-negative bacilli
5) <i>Staphylococcus</i> spp (not <i>Staph. aureus</i>) Coliform- probably not <i>.coli</i> Beta- haemolytic <i>Streptococcus</i> sp <i>Diphtheroids</i> (eg. <i>Corynebacterium</i> spp)	10) Probable <i>Staphylococcus aureus</i> Coliform NOT <i>E. coli</i> <i>Staphylococcus</i> spp "CONS" white NH Other organisms

Table 1. List of different bacterial species found in the vagina of non-pregnant and pregnant baboons.

DISCUSSION

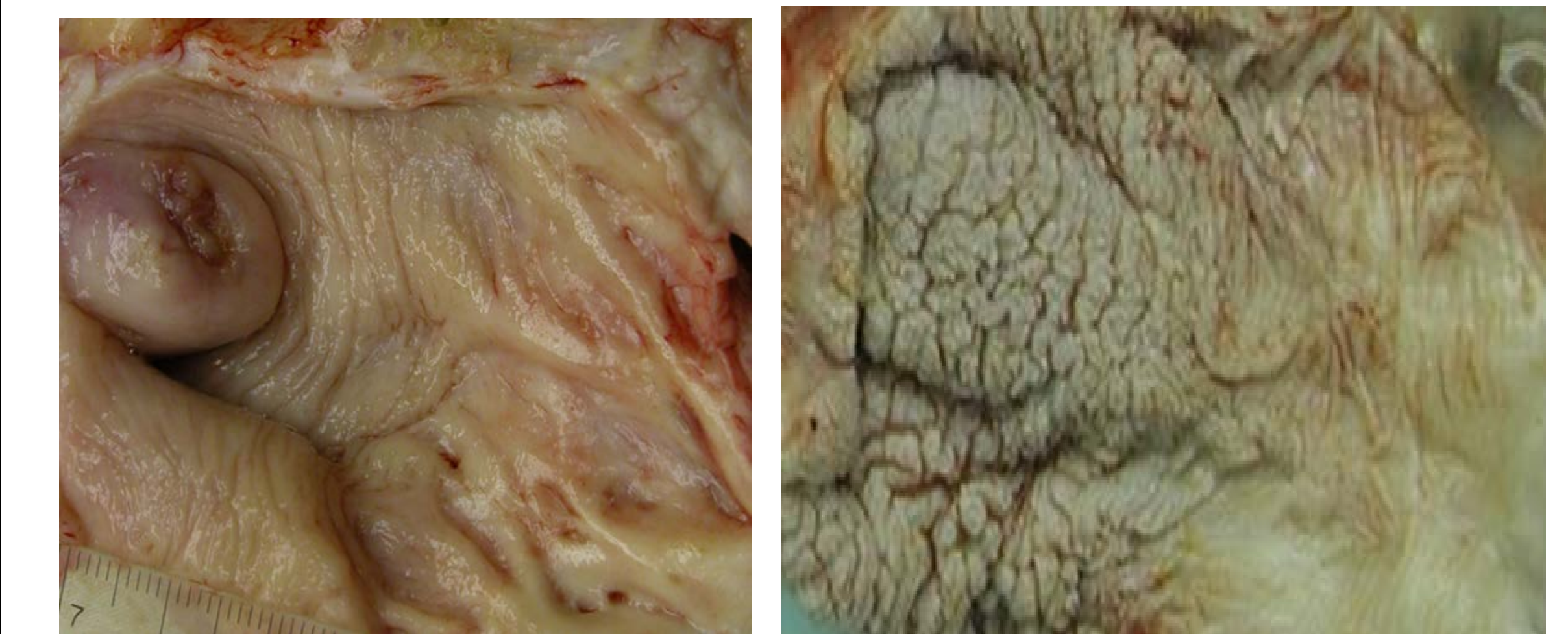
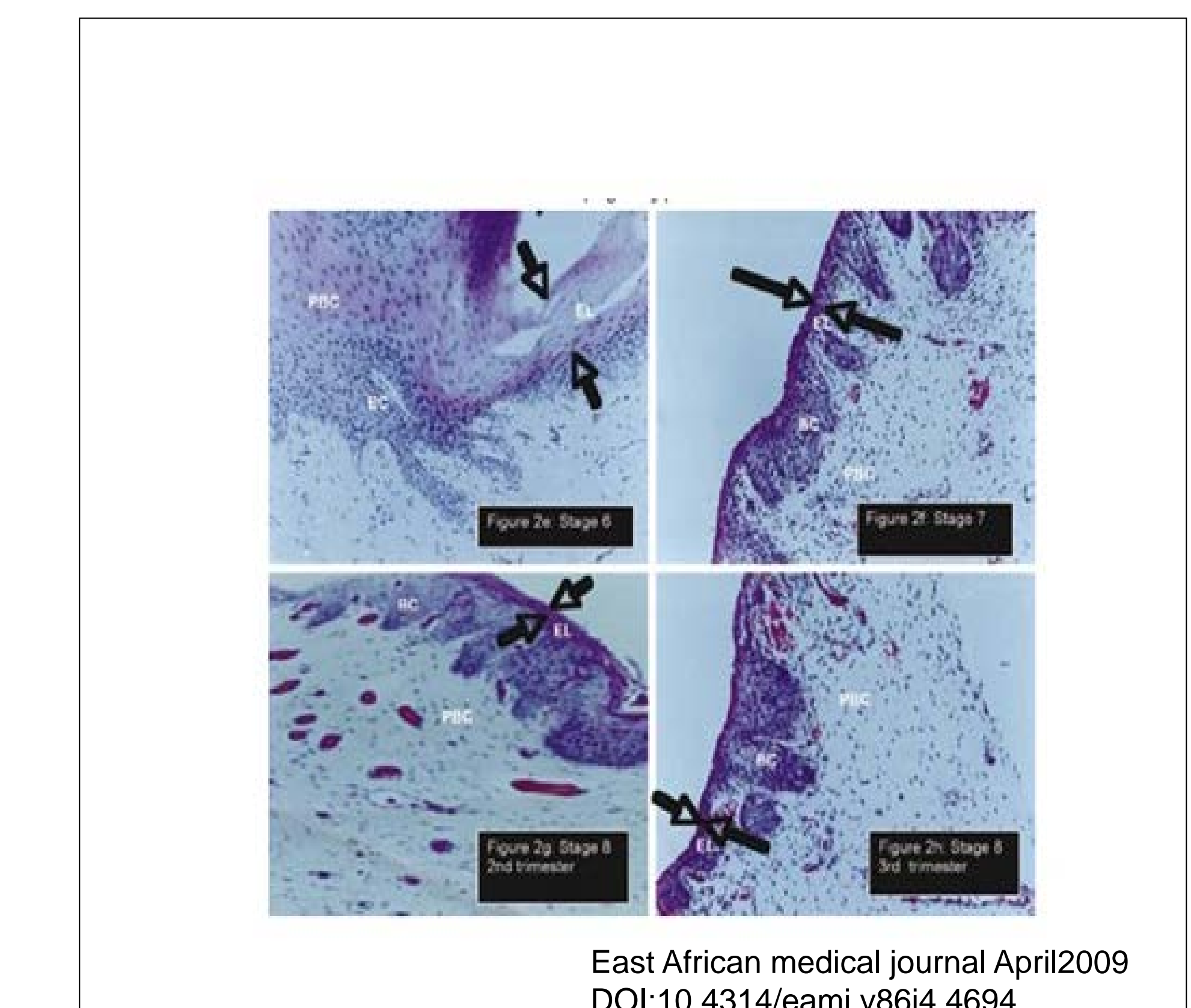
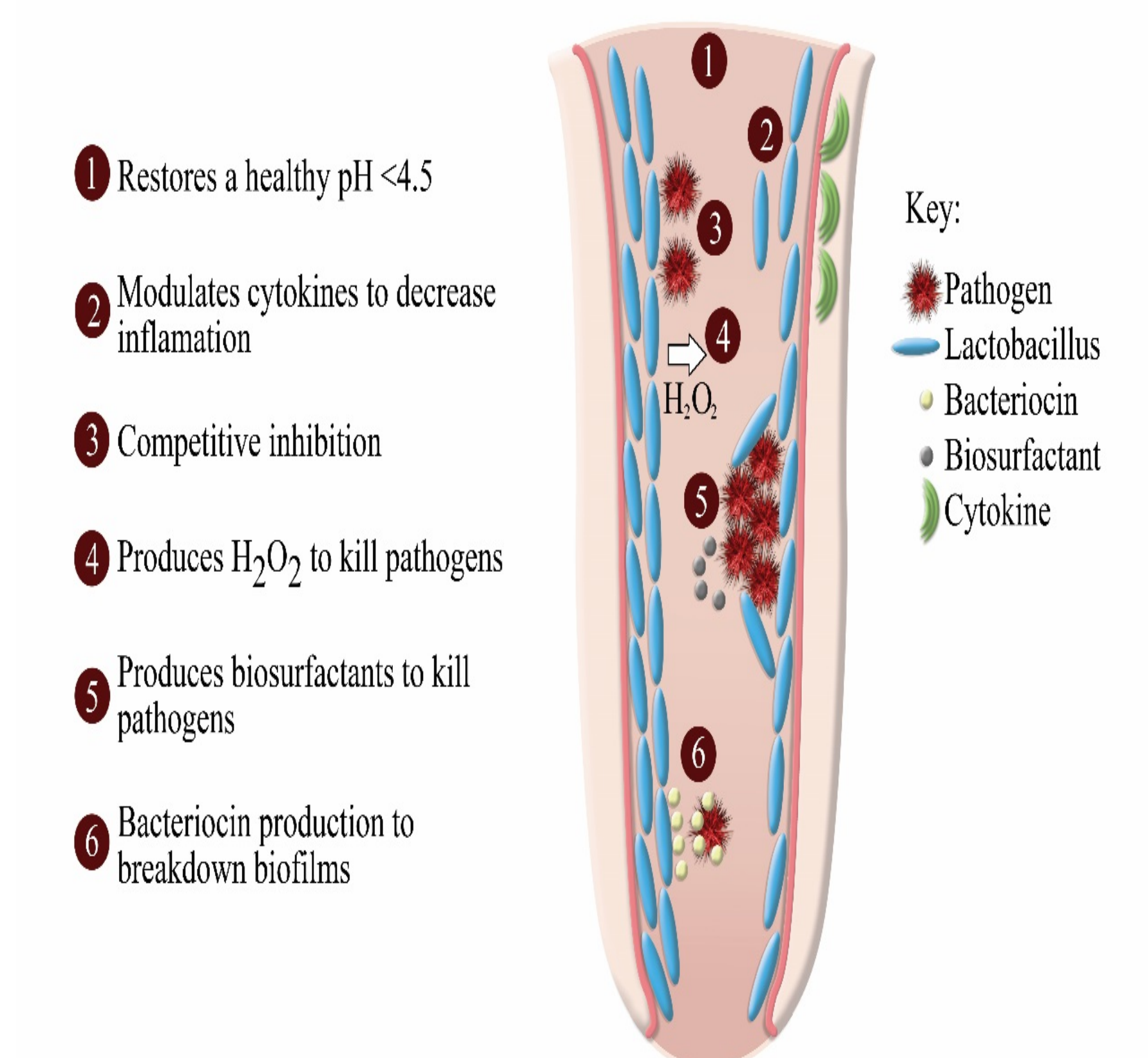


Figure 3. The vaginal epithelial folding in non-pregnant and pregnant baboon



The vaginal squamous epithelium layer becomes thin during the luteal phase (stage 6, Figure 2e) menstrual phase (stage 7, Figure 2f) and during pregnancy (stage 8, Figures 2g and 2h).

The decreased vaginal epithelial thickness, reported in the baboons and humans, (DOI:10.4314/eamj.v8i6.46946), might change properties of the vaginal bacterial biofilm, described by us in humans (Fig. below) and transform bacterial life stages.



(Letbetter et al., 2017; Ventolini et al., 2017)

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