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Lactobacillus iners: a Marker of Changes in the Vaginal Flora?[▽]

Lactobacillus iners seems to be a species of lactobacilli occurring in the human vagina that deserves close scrutiny, as it was not found in earlier studies due to its peculiar culture requirements but is now discussed as one of the normal vaginal bacteria (1, 4). Following up on the interesting paper by Ferris et al. on changes in vaginal flora after the treatment of bacterial vaginosis with metronidazole, and especially their finding of *L. iners* DNA clones posttreatment, we would like to add to the picture our findings from investigations on vaginal flora changes during estrogen treatment for the purpose of in vitro fertilization (IVF) (2).

Observations in previous studies of healthy women of childbearing age using the Nugent score and Amsel criteria indicate that *L. crispatus*, *L. iners*, *L. gasseri*, and *L. jensenii* are the *Lactobacillus* species most likely to be part of the normal flora in a healthy vagina (4). These studies, however, were not set up to illuminate any possible changes in the flora over time, e.g., throughout a normal menstrual cycle. Earlier studies, using mostly phenotypic characteristics to type cultured vaginal lactobacilli, were not powerful enough to guide in designing experiments investigating the influence of treatment, time course, and physiological parameters such as estrogen levels on the natural flora. The interplay between physiological parameters and floral changes may be subtle, and thus, using *Lactobacillus* genotyping methods described in an earlier study from our group, we resorted to the study of possible *Lactobacillus* flora changes associated with the artificially high estrogen levels that occur during IVF in women seeking help to conceive (3).

Thirty-four Swedish women, ages 23 to 39, from the normal IVF program of Linköping University Hospital, Linköping, Sweden, were enrolled in the study. According to the IVF protocol used at the time of the study, the women were treated with doxycycline and metronidazole. At intervals after the antimicrobial treatment, vaginal samples were taken for culture, vaginal fluid for Gram staining on slides and Nugent scoring was obtained from the upper third of the vagina, and samples were evaluated according to the Amsel criteria (4). Plasma estradiol levels were determined at each visit. A normal vaginal status (as defined by Nugent scores and Amsel criteria) throughout the study period was found for 22 women from whom sufficient culture data were collected. 16S rRNA DNA sequence analysis identified one or two dominant *Lactobacillus* species in each woman, most frequently *L. iners*, *L. gasseri*, and *L. jensenii*, that did not change among these women over the study period (3).

Three women had abnormal vaginal floras, evident from high Nugent scores, high vaginal pHs, and the occurrence of *Mobiluncus* species or high numbers of gram-positive cocci, before the antibiotic treatment. Samples from two of these women yielded *L. iners* in cultures after metronidazole treatment, and the flora in the third woman changed to include *L. iners* in conjunction with higher estradiol levels in plasma. Two patients with normal floras had *L. iners* even before antibiotic treatment. This group of five patients retained *L. iners* throughout the remainder of the study period (up to 6 weeks).

Our findings not only support those of Ferris et al. but also suggest that *L. iners* is a dominant part of the vaginal flora when the flora is in a transitional stage between abnormal and normal, either because of treatment or because of physiological changes such as varying estrogen levels. Additional studies using molecular tools, such as the one used by Ferris et al., are thus warranted to elucidate the finer details of vaginal *Lactobacillus* colonization, especially that by *L. iners*, in relation to the long-overdue description of the interplay between normal physiology and vaginal bacteria.

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Tell Jakobsson
Urban Forsum*
Division of Clinical Microbiology
Linköping University
Linköping, Sweden

*Phone: 4613222090
Fax: 4613224596
E-mail: urban.forsum@imk.liu.se

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