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LETTER

Vaginal microbiota and IVF outcomes: poor diagnosis results in flawed conclusions

To the Editor

We read with interest a recent publication in this journal investigating the vaginal microbiota of oocyte recipients at the time of single blastocyst transfer (*Vergaro et al., 2018*). This study might be considered ideal to investigate the potential impact of the vaginal microbiota on reproductive outcomes. Unfortunately, the study suffers from many important methodological shortcomings, as follows. (i) The use of non-validated and arbitrary cut-off levels to stratify normal and abnormal vaginal microbiota based on a pilot study (n=32) investigating low biomass endometrial specimens by next generation sequencing rather than by quantitative polymerase chain reaction (qPCR) as was used in Vergaro et al. (*Moreno et al., 2016*). (ii) This arbitrary cut-off does not sufficiently take into account the alpha diversity within *Lactobacillus (L.)*-dominated samples (*Haahr et al., 2018*). For example, *L. iners* has a 15-fold higher concentration than *L. crispatus* (*Haahr et al., 2018*), rendering especially the *L. iners* group reported by Vergaro et al. subject to misclassification due to 'contamination' with large numbers of bacterial vaginosis-associated bacteria (BVAB). (iii) The authors failed to compare using the gold standard for vaginal dysbiosis, the Nugent score, as was done by others (*Haahr et al., 2016*). (iv) Raw outcome data was not reported. (v) DNA normalization was performed in several (some redundant) steps. For example, in the case of normalization according to human DNA, BVAB DNA would be heavily underestimated in patients with vaginal leukocytosis, who have high amounts of human DNA.

Despite these shortcomings, and in contrast to the conclusions of the authors (*Vergaro et al., 2018*), *L. crispatus*-dominated vaginal microbiota was actually associated with a significantly higher live birth rate in the study. In conclusion, we urge researchers in genital tract microbiota to thoroughly validate diagnostics and to share the underlying raw data when reporting reproductive outcome results.

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Received 1 April 2019; accepted 8 April 2019.

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Declaration: TH has received honoraria for lectures from Ferring and Merck. PH received unrestricted research grants from MSD, Merck, and Ferring as well as honoraria for lectures from MSD, Merck, Gedeon-Richter, Theramex, and IBSA. JSJ has received speaker's fee from Hologic, BD, and Cepheid and serves scientific advisory board of Roche Molecular Systems, Abbott Molecular, and Cepheid. PH, TH and JSJ received a research grant from Osel inc. which produces LACTIN-V, a live biotherapeutic product with *Lactobacillus crispatus*. PH and TH are listed as inventors in an international patent application (PCT/UK2018/040882) involving 'Use of vaginal lactobacilli for improving the success rate of in vitro fertilization'.