validated against standard microbiome methods, other molecular methods or microscopy. We draw the attention towards the fact that the mentioned 'external validation' study and the referenced protocol publication (Koedooder *et al.*, 2018)—also retrospectively registered—are not able to compensate for the abovementioned shortcomings. Moreover, the external validation cohort is not sufficiently described—e.g. no description of baseline patient characteristics etc.

Secondly, according to the data presented, IS-pro cannot be used to stratify samples into community state types (CSTs) as previously described by Ravel *et al.* (2011). Certainly, the clustering in Fig. 1 does not define five CSTs that would match the ones probably obtained by Illumina sequencing as described in the protocol paper (Koedooder *et al.*, 2018). In fact, the gray bars in Fig. 1 show that many samples could not be correctly assigned. Moreover, microbiome methods—including IS-pro—do not sufficiently take into account the total abundance of bacteria, which could lead to a serious misclassification (Haahr *et al.*, 2019a). Hence, the reported stratification into subgroups as based on relative *Lactobacillus (L.) crispatus/L. iners* counts is not feasible. Furthermore, we find Fig. 3 misleading as Panel A is described in Panel C and vice versa.

Finally, authors entitled this trial as a prospective study—yet it was retrospectively registered as also stated by the authors which may lead to serious bias when evaluating results as a basis for predictive models. In fact, the authors state that their final analysis compared the group of pregnant patients to the group of non-pregnant patients to find the predictive cut-off levels and to *post hoc* determine an 'unfavorable microbiome'—this definitely suggests that the study by Koedooder et al. was not prospective.

Previously, we stated that it is of the utmost importance to use validated, transparent and thus repeatable diagnostics (Haahr *et al.*, 2019b) when stratifying vaginal dysbiosis and evaluating reproductive outcome. Unfortunately, this study does not meet such standards and we suggest that the results reported herein should be interpreted with caution.

Conflict of interest

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, SpeeDx 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'.

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doi:10.1093/humrep/dez167
Advance Access Publication on October 1, 2019

Reply: Non-transparent and insufficient descriptions of non-validated microbiome methods and related reproductive outcome results should be interpreted with caution

Sir,

We thank Haahr *et al.* for their critical notifications towards our recent publication. We would like to elaborate on our study and on the points raised:

Concerning their first comment that the IS-pro technique has not been externally validated, we would like to point out that a brief PubMed search reveals a series of papers validating all aspects of the IS-pro technique (Budding *et al.*, 2010; Budding *et al.*, 2014; Daniels *et al.*, 2014; Budding *et al.*, 2016; Eck *et al.*, 2017a; Eck *et al.*, 2017b) and highlighting its applicability as a highly reproducible assay that can be applied to clinical diagnostics. This is in contrast to current sequenc-

ing approaches, which do not have the inter-hospital reproducibility required for clinical diagnostics (Lozupone *et al.*, 2013).

Furthermore, as can be read in our protocol paper (Koedooder *et al.*, 2018), we performed the analyses with both 16s rRNA gene sequencing and the IS-pro technique. The manuscript that compares these analyses with each other is in preparation, but the scope will be that the results yielded very similar vaginal microbiome profiles, with a median Pearson's R2 of 0.97. This indicates a high level of similarity between 16s rRNA gene sequencing and IS-pro results.

We could have described the external validation group in more detail. However, restrictions in manuscript length forced us to make choices in what information to include. The baseline characteristics of the original study group showed no differences between women with a favourable and unfavourable profile. Moreover, the treatment in Germany is comparable to the treatment in the Netherlands.

Regarding the comments raised concerning the community state types (CSTs), it is important to note that there are no formal definitions of the CSTs, and true beta-diversity in vaginal samples is larger than what can be captured by the artificial classification in five CSTs. This is reflected in Fig. 1 of our paper. We decided to include the CST classification merely to provide easier insight into the data set, and CST classification is no part of our predictive algorithm.

Regarding the remark that 'microbiome methods do not sufficiently take into account the total abundance of bacteria', we wonder what 'microbiome methods' are referred to here. Moreover, we wonder what would be 'sufficient' for what purposes and what 'misclassification' is referred to. Some clarity in these important points would be essential to address them properly.

Concerning IS-pro, we would like to point out that this technique is semi-quantitative and certainly reflects the total abundance of bacteria. However, as Haahr *et al.* point out themselves, as we are comparing 'relative' abundances, total load is not important for this goal.

Concerning Fig. 3, we were aware of the error in Fig. 3, provided the correct version of Fig. 3, and in the meantime the journal has decided to publish a correction in the form of a corrigendum.

While this study was indeed set up as a prospective cohort study, we did use the pregnancy outcomes to optimize the cut-off levels of the predictive algorithm.

Finally, we fully agree with Haahr *et al.* that it is of the utmost importance to use validated and repeatable diagnostics. Therefore, we used the only tool that has—to our knowledge—met these standards (Budding *et al.*, 2010; Budding *et al.*, 2014; Daniels *et al.*, 2014; Budding *et al.*, 2016; Eck *et al.*, 2017a; Eck *et al.*, 2017b). Outside the published validations, the IS-pro assay has been Conformité Européenne/in-vitrodiagnostics (CE/IVD) marked to meet the highest international demands for standardization in clinical diagnostics.

Conflict of interest

None.

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doi:10.1093/humrep/dez168
Advance Access Publication on October 1, 2019

Methodological concerns on 'Assessment of ovarian reserve after cystectomy versus "one-step" laser vaporization in the treatment of ovarian endometrioma: a small randomized clinical trial'

Sir,

We read the recently published randomized controlled trial (RCT) comparing the effect on ovarian reserve of laparoscopic stripping of endometriomas versus CO_2 laser vaporization. This study demonstrated that laser treatment was associated with a significant increase in antral follicle count (AFC) in the operated ovary without reduction of anti-Müllerian hormone (AMH) levels while laparoscopic stripping caused only a significant reduction of AMH levels without significantly improving AFC (Candiani et al., 2018).

It would be interesting to have more details about the study. Firstly, since the trial investigates the impact of surgery of endometriomas on ovarian reserve, we deem that a deeper explanation about surgical techniques should be provided. In particular, the authors state that 'patients in Group 1 underwent a standardized laparoscopic stripping technique to treat endometriomas' without providing information on the hemostatic method used to manage the bleeding. A little more information is available on Clinicaltrial.gov website where the original study design was registered (NCT03227640) where it is indicated that hemostasis was achieved by selective bipolar coagulation. However, neither the type of hemostatic device used by the surgeons nor the power settings for bipolar coagulation are reported. In addition, it would be relevant to know which kind of hemostatic technique was used in event of bleeding in the group of patients treated by CO_2 laser vaporization. Several studies have investigated the best hemostatic technique to achieve hemostasis after stripping of endometriomas (Ferrero et al., 2012; Kang et al., 2015; Deckers et al., 2018). Available evidence suggests limiting/avoiding the use of bipolar coagulation in favor of less invasive techniques, such as the use of hemostatic sealants or intracorporeal suturing that are associated with less damage on ovarian reserve especially for patients with reproductive goals (Deckers et al., 2018, Kang et al., 2015). Thus, we consider that trials comparing CO_2 laser vaporization with stripping combined with hemostatic sealants or intracorporeal suturing should be planned to verify the magnitude of the incremental benefit, if any, of the laser technique over the currently used standard treatments.

Secondly, the authors chose the comparison of AFC changes before and after treatment as primary endpoint of the study. It may be advocated that AFC could be a better tool than AMH levels to estimate ovarian reserve after surgery for ovarian endometrioma, because in patients with unilateral endometrioma the laterality of the ovary is considered, thus evaluating the impact of the surgery only in the operated ovaries. The results of the RCT seem to confirm this theory but the authors should try to explain why a reduction in AMH levels was observed in both study groups despite an increase in AFC. Furthermore, this finding is surprising since the magnitude of AFC increase is very large (3.6 ± 1.9 before surgery versus 8.6 ± 4.2 in the laser group) and almost all previous studies showed a decrease of AFC after surgery