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Reply: More details on microbiome profile and IVF, would allow readers to judge for themselves

Sir,

We thank Gruteke *et al.* (2021) for their suggestions and requests regarding our recent publication (Koedooder *et al.*, 2019a). We would like to elaborate on our study and on the points raised.

Concerning their first suggestion, to provide a supplementary table listing presence of the four risk elements and outcome in all the 34 individuals classified as 'unfavourable microbiome profile', we would like to respond that due to patent developments this additional information cannot yet be made public. This has already been made known

to Dr Gruteke in an earlier conversation before we received this letter to the editor.

Regarding their second request to clarify the role of the different *Gardnerella* subtypes, we can answer that the *Gardnerella* with IS-pro (or IS) type I seemed to play an important role and was found in 17 of the 34 women with an unfavourable profile.

Their final issue concerning the presence of *Atopobium vaginae*, which is part of the bacterial vaginosis qPCR, but not part of the unfavourable profile of the ReceptIVFity test. In our study, we found that the presence of *A. vaginae* was not an independent predictor of pregnancy outcome. Instead, it was co-correlated with the presence of other species. To maximize generalizability of the predictive algorithm, we included only the most predictive species and left out co-correlating species that did not add to predictive accuracy.

Finally, we would like to conclude with the opinion that the results of different analysis methods (e.g. qPCR and IS-pro) are difficult to compare head on. More information about this can be read in our review (Koedooder *et al.*, 2019b) and our latest paper concerning this issue (to be published *BMC Microbiology* April 2021).

Conflict of interest

The authors would like to state a number of competing interests. The author A.E.B. is co-founder of InBiome B.V. and co-inventor of the IS-pro technology. The authors R.K. and J.D.d.J. report that they are an employee at ARTPred B.V. J.S.E.L. reports consultancy fees from Titus Health Care and is co-inventor of the patent 'Method and kit for prediction success of *in vitro* fertilization' (US-9896733-B2) which is assigned to ARTPred B.V. The author S.S. declares that there is no conflict of interest.

J.D.d.J. and A.E.B. have obtained patents 'Microbial population analysis' (9506109) and 'Microbial population analysis' (20170159108), both licenced to ARTPred B.V. J.D.d.J. and A.E.B. report co-inventorship on patent applications 'Method and kit for predicting the outcome of an assisted reproductive technology procedure' (392EPP0) and patent 'Method and kit for altering the outcome of an assisted reproductive technology procedure' by ARTPred.

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Is a randomized controlled design sufficient for a trial to be valuable?

Sir,

We read with great interest the manuscript 'Transfer of fresh or frozen embryos: a randomised controlled trial' recently published by Wong *et al.* (2021). The authors summarized the data of their registered randomized controlled trial (RCT) conducted between 2013 and 2015 on 205 unselected couples clustered in two groups: first transfer performed fresh in day 5 versus freeze-all of all day 6 embryos. Based on these data, already in the abstract, the authors claim: 'the freeze-all strategy was inferior to the conventional strategy in terms of cumulative ongoing pregnancy rate per woman' and 'there might be no benefit of a freeze-all strategy in terms of cumulative ongoing pregnancy rates'. However, we want to question the quality of the evidence produced. The term RCT does not confer absolute value to a study per se, but in some cases can result even misleading for the readers. In our view, Wong's paper might fall in this category.

Firstly, the sample size analysis is odd. In particular, Wong *et al.* state: 'we expected a cumulative ongoing pregnancy rate after one cycle of 40% in the freeze-all strategy and of 20% in the conventional group'. A 20%-absolute difference in the cumulative pregnancy rate (i.e. twice as much as the control group) is excessive. To the best of our knowledge, no technology of recent implementation in ART can improve the rate of success to such extent. Thus, it is reasonable to argue that such a peculiar premise has been made to allow them performing an RCT with a small sample size (about 200 patients and 80% statistical power). For instance, to exclude a more reasonable 5%-absolute difference in the two groups, Wong *et al.* would have needed more than 2000 patients, a sample size 10 times higher than theirs but

definitely more appropriate to answer their study question. This limitation is even more worrisome, as the authors themselves recognized, because the population of patients was unselected (e.g. maternal age: 18–43 years) and encompassed both good and poor prognosis patients.

Secondly, the results after vitrification raise some doubts on Wong *et al.* performance when conducting vitrification and warming procedures. Before running an RCT, the results of an IVF clinic must equate (if not exceed) the key performance indicators (KPIs) set for the technique under investigation. In the case of this study, the 'The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting' represents a valuable reference (Alpha Scientists In Reproductive Medicine, 2012). In this consensus, a panel of experts clearly stated that, when it comes to the implantation of cryopreserved blastocysts, the competence value should be '≤10% (relative) lower than that for the comparable population of fresh embryos at the centre' and the benchmark value should be 'the same as for the comparable population of fresh embryos at the centre'. Hence, the implantation rates reported in Wong's study (34% after fresh transfer in day 5 versus 7–12% after vitrified-warmed transfer in day 6 in both study groups) raise some doubts on their expertise with a technique which is essential in a MODEM IVF clinic to cryopreserve (surplus) oocytes/embryos, independently of the strategy commonly adopted for the first transfer (fresh or frozen). Moreover, the protocol used in the cryo-cycles is surprisingly different from the gold standard approach. Specifically, all embryos were cryopreserved on Day 6 regardless of their stage, quality, and likely prognosis, as well as progesterone was administered for 7.5 days before transfer. This scheme is in counter-tendency with the concept of embryonic-endometrial synchronization (Mackens *et al.*, 2017; Franasiak *et al.*, 2018) and might have contributed to impairing the outcomes, further limiting the value of the study and the generalizability of its conclusions.

A last concern is the lack throughout the manuscript of pivotal information about putative confounders (e.g. patients' distribution across different maternal age group, blastocyst quality, etcetera) which are essential for adjusting the outcomes. This is probably imputable again to the low sample size, in turn preventing relevant sub-analyses and/or multivariate regressions.

In conclusion, our question remains: is a randomized controlled design sufficient for a trial to be valuable? In our opinion, the premises to calculate the sample size must be reasonable, the center entitled of conducting the study must at minimum equate the KPIs set by national/international scientific societies, the protocols adopted in the study must be representative of the real life of an IVF clinic, and all information must be comprehensively disclosed across the manuscript. Unfortunately, Wong's study does not satisfy these prerequisites. Therefore, in our view, it does not provide first class data helpful in the dispute over the efficacy and efficiency of freeze-all in an unselected population of patients.

Conflict of interest

The authors declare no conflict of interest related with this study.