from patients with RVVC/VVC remain sensitive to conventional antifungal agents, the ineffectiveness of these drugs in curing RVVC is reported frequently. This suggests that microbial strategies other than intrinsic resistance might be involved. Such possible strategies include biofilm formation and production of persister cells (a small population of "transiently resistant" cells). Supporting this suggestion is the observation that the recurrence pattern of RVVC coincides with the model of relapsing biofilm infections proposed by Lewis[2] (2010), in which persister cell are major culprits.

Harriot et al[3] (2010) successfully demonstrated vaginal epithelium-based *Candida* biofilms using a mouse VVC model and raised the hypothesis that formation of such biofilms may be an initiating event in VVC. Using the same model, Zhang et al[4] (2013) found a dynamic change of *Candida* growth on vaginal epithelia along with VVC progression. *Candida* pseudohyphae and yeast cells were found on mouse vaginal epithelia 3 days after the infection; these adherent biofilms were replaced gradually by the endocytosed hyphae, which is a unique growth mode also reported by Swidsinski et al.[1] The possibility of the presence of such transient biofilms in patients whose infections have progressed to a later stage should not be excluded. Furthermore, although the endocytosed yeast/hyphal cells in vaginal epithelia do not present any structural characteristics of typical multilayer biofilms, such as the presence of self-producing polymeric matrix or 3-dimensional mushroom structures, they might resemble monolayer biofilms and share some important traits that drive the recurrence of infections, which includes harboring antifungal-tolerant persister cells. We have found that dense growth of monolayer biofilms in a confined space stimulated persister cell production and led to antimicrobial tolerance in vitro.[5] The further clarification of the possible dynamic change of *Candida* growth in the human vagina VVC and whether the endocytosed hyphal cell growth mode promotes persister cell production needs collaborative efforts from experimental microbiologists and clinical investigators.

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REPLY

Phantom limb sensations are obviously very strong in the *Candida*-biofilm research community. It is striking that this and previous letters to the editor with regard to our publication,[1] cite Harriott et al[2] as work that definitively demonstrated the vaginal candida biofilm in rodents. That is not true. We likewise cite the same author in our article and state that "The only publication claiming *Candida albicans* forms biofilms on the vaginal mucosa exclusively includes pictures of smears from vagina without vaginal epithelium."

Neither Harriott nor anybody else have ever presented microscopic proof of candida biofilm on the vaginal wall. In addition, there are major differences between the experimental model of rodent *Candida* vaginal infection and human vaginal candidiasis, for example, microbiota, pH, and the enormous inoculum of yeast organisms that is used in the animal model, which alone may simulate biofilm-similar discharge. Until the visual proof of biofilm on the surface of intact human vaginal epithelium is available, all speculations about the existence of vaginal candida biofilms will remain scientifically unsound.

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In women with transplantable euploid blastocysts, age does not affect transplant potential

Check for updates

TO THE EDITORS: The published study by Irani et al[1] entitled "Does maternal age at retrieval influence the implantation potential of euploid blastocysts?" was well designed. However, the grouping of morphological ratings may have some of the following issues: 2BC, 2CB, and 2CC were inconsistent in the *Methods* and abstract and lack the grouping of morphological ratings, such as AC, CA, and 1-2AA. More importantly, placing AB and BA in the same group may be controversial.

According to Nazem et al,[2] blastocysts with an inner cell mass grade of A had a greater odds of ongoing pregnancy/live birth (odds ratio, 1.5; 99% confidence interval, 1.1−2.0) and clinical pregnancy (odds ratio, 1.4; 99% confidence interval, 1.1−1.9) compared with B.

We further analyzed the trophectoderm grade of A and B in this paper of Nazem et al[2] and found no statistically significant difference in any of the results between the 2 grades ($P > .05$; SPSS version 20.0). The abovementioned points indicate that it may be unreasonable to include AB and BA in the same group.

This may explain why the implantation rates of the excellent- and good-quality groups were not significantly different according to the grouping method of Irani et al[1], possibly because of the relatively small number of cycles in which excellent-quality blastocysts were transferred, as suggested by Irani et al.

With this in mind, a preliminary analysis including 94 frozen-thawed cycles from our own center was performed for verification. When grouping blastocyst morphological ratings according to the method of Irani et al,[1] we reached the same conclusion reported in their paper. However, after changing the grouping strategy, AB and AA were grouped together (n = 37), while BA and BB were grouped together (n = 57). A statistically significant difference in implantation rates was found between the 2 groups (75.7% vs 50.9%; $P = .016$).

This suggests that changes in morphological grouping strategies can lead to different results, and this may further affect the distribution of embryo quality across age groups. More importantly, AB may be a better choice for transplantation than BA, especially if no AA is available for transplantation. Although this remains to be verified, it may have a positive effect on improving the quality of the assisted reproductive cycle.

We hope that Irani and colleagues, as well as researchers at other centers with large data, could consider the previously mentioned issues and further verify the effect of expansion grade on pregnancy outcomes proposed by Nazem et al.[2]

In addition, Irani et al[1] concluded that differences in spontaneous abortion at different ages were not statistically significant, but older women (>40 years) still showed an increasing trend that should not be ignored. In addition, euploid embryo transfer can help older women achieve live birth rates that are similar to those of younger women; however, this does not mean that their pregnancy qualities are comparable. Other pregnancy outcomes for older women, such as preterm birth and low birthweight, should also be assessed.

Finally, the study included only those cycles from women with surviving euploid embryos for transplantation after biopsy and freeze-thawing,[1] which limits the study population. Therefore, we believe that the conclusion should be that age does not affect the implantation potential of the embryo among women with a transplantable euploid blastocyst. ■

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REPLY

Check for updates

We appreciate Drs Y. Guo, S. Guo, and Zhang for their interest in our work. After studying the role of blastocyst morphology in selecting among euploid embryos, we have demonstrated