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Reply: Exposure of human fallopian tube epithelium to elevated testosterone results in alteration of cilia gene expression and beating

Sir,

We thank Drs Saridogan, Ertan Kervancioglu and Djahanbakhch (Saridogan *et al.*, 2021) for their comments and critiques on our paper (Jackson-Bey *et al.*, 2020).

Regarding the argument that we required a thin layer of tissue, this is indeed critical to obtain high-resolution microscopy since the cilia need to be close to the objective to obtain a high-resolution video. We agree that the epithelium is not completely free of stroma. By microscopy, you can focus on cells bearing cilia, and reveal the columnar structure while recording cilia moving. Stroma could represent an issue in the quantitative PCR (qPCR), but since we are only considering cilia genes, in this specific paper, any contamination of stromal cell mRNA should not alter those transcripts. For future qPCR experiments, we are indeed including a stroma control. We also included immunohistochemistry (IHC) images to confirm key changes in the ciliated cells, such as FOXJ1.

The 'superficial secretion' is not reliably reported by a single, representative IHC picture. It is important to keep in mind that this is from one patient and there is significant variability. In each assay, we include multiple patient samples to account for the variability, but due to publication limitations, we show one image.

The tissues were fixed in 4%, there is indeed a typo in the IHC method session. For Fig. 6, we calculated folds, but we had four different patients and for each patient we calculate fold and then averaged them. Therefore, the control, being 1 for each patient will give a mean = 1 and SEM = 0; for the high testosterone condition folds are also calculated for each patient and the mean is 0.75, so <1, also, in this case, the four measurements do not give the same value and therefore SEM is not equal to zero, which is reported in the paper.

We will keep all these critiques in consideration for future studies.

Note: All schematics found in the paper were created using BioRender.com.

Conflict of interest

None.

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ICSI does not improve reproductive outcomes in autologous ovarian response cycles with non-male factor subfertility: a need for clarification

Sir,

We read with great interest the article written by Supramaniam and colleagues and just published in Human Reproduction (Supramaniam *et al.*, 2020). This paper based on the retrospective analysis of the whole HFEA database from 1991 to 2016 clearly shows that ICSI does not improve reproductive outcomes in autologous ovarian response cycles with non-male factor subfertility. We would like to congratulate the authors for such a relevant piece of work, raising once again the issue of ICSI overuse in non-male factor infertility.

Although we do not question the relevance of the statistical approach used by the authors, we would like to point out some inconsistencies that we found in the results presented and which might deserve clarification. More specifically, the numbers presented for the IVF group in the abstract, results section and table III are not consistent. While the number of IVF cycles included for all autologous ovarian response categories is 272 433 in the abstract and in the results section, the number of live births reported in this group is 84 725,