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Letter to the Editor

In Reply: Revisiting Claims of the Continued Absence of Functional Germline Stem Cells in Adult Ovaries

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In their Letter to the Editor, Woods and Tilly[1] repeat their concerns[2] about our research on DDX4 antibody positive cells (DDX4 Ab+) isolated from adult human ovarian cortex.[3,4] We show that these cells are perivascular cells instead of so-called oogonial stem cells (OSC), and hence recommend researchers working on the topic to be cautious if following the protocol developed by Tilly and co-workers.[5] As we have responded to these concerns previously,[6] we will keep our response short. For example, we already extensively addressed the issues related to cell numbers and viability, evolution of bioinformatic tools, and detection of ovarian cell types by scRNA-seq.[6]

As Woods and Tilly wrote[1], there have been 3 clinical trials where women have been subjected to the AUGMENT treatment.[7-9] A piece of their ovaries was surgically removed and dissociated into single cells for isolation of DDX4 Ab+ cells. Then, mitochondria were isolated and stored for simultaneous injection with sperm into oocytes collected from the same women during a regular ovarian hyperstimulation cycle.[10] As we have previously highlighted, the only blinded randomized controlled trial was terminated prematurely due to a significantly lower rate of blastocyst formation in the AUGMENT therapy group.[8] The shortcomings of the other two trials have been raised and discussed elsewhere.[8,11] Our studies add concerns about the nature of the DDX4 Ab+ cells that were used for mitochondrial isolation.

Our first single-cell transcriptomic analysis of the DDX4 Ab+ cells was published in 2015.[4] Among the top genes expressed in the DDX4 Ab+ cells, two well-known perivascular cell markers, *ACTA2* and *TAGLN*, were found.[4] Further, we have already shown that these cells keep their perivascular gene expression profile even when cultured for extended time under OSC conditions.[3] Hence, in contrast to what Woods and Tilly suggest, the data sets we presented in 2015 and 2020 are not in disagreement with each other but instead unequivocally support our conclusions.

Woods and Tilly point to 9 rodent studies as evidence showing that DDX4 Ab+ cell-derived oocytes can give rise to viable offspring. However, the majority of these studies did not use DDX4 Ab for cell isolation from ovaries but rather other markers (*Ifitm3)* or GFP expression in the germline. When DDX4 Ab was used, the DDX4 Ab+ cells were not been systematically compared to DDX4 Ab– cells, which is troublesome, knowing the unspecific nature of this antibody,[3,6] and as such the studies do not help to answer the claims of DDX4 Ab+ cells being germline stem cells.

We notice that the hypothesis that Tilly and his co-workers presented earlier regarding accidental sorting of perivascular cells based on autofluorescence in our laboratory[2] was not repeated in the current letter,[1] suggesting that the new data we presented cleared that concern.[6]

In closing, the data presented by Woods and Tilly to support the hypothesis that DDX4 Ab+ cells are germline stem cells continue to have significant gaps. Our results demonstrate that the use of the recommended Abcam rabbit polyclonal Ab toward DDX4 for the isolation human of germline stem cells[5] instead leads to the isolation of perivascular cells.[3,6] As such, we continue to encourage researchers working with the protocol to thoroughly characterize the DDX4 Ab+ cells and their derivatives, with DDX Ab– cells

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as controls, using single–cell technologies in combination with functional studies.

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Conflict of Interest

The authors declared no potential conflicts of interest.

Data Availability

No new data were generated or analyzed in support of this research.

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