Dear Dr.　Bing Wu,

Thank you very much for the second reviews and the comments to our manuscript entitled “Ovarian Tissue Culture to Visualize Phenomena in mouse ovary” (JoVE57794). In the attached document, we described the changes made in response to the reviewer’s comments.

**Editorial comments:**  
  
The manuscript has been modified and the updated manuscript, **57794\_R1.docx**, is attached and located in your Editorial Manager account. **Please use the updated version to make your revisions.**  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We revised the manuscript after we got English proofreading again.

2. Please do not highlight notes for filming.

We deleted “Note” from highlighting for filming.

3. Please highlight more protocol steps for filming (less than 2.75 pages).  
 We added protocol steps, step 4-7, to highlight for filming.

4. Step 2.2: How to excise the ovaries? Using what? How to trim the tissues?

We revised this part.

“Excise the ovaries from 4-week-old female ICR mice and trim the tissues surrounding the ovary **using a scissors and a tweezers** under a stereoscopic microscope.” (line 113-114)

5. 2.2.1: What’s the size of the blade?

We added the information of the blade’s size in Table of Materials.

6. 3.2: How many ovary samples are placed in the 1 mL medium?

We revised the sentence to clear the number of samples in a drop.

“3.2 Place **single sliced specimens** into **one drop** of culture medium on cell culture inserts using tweezers (see step 3.1)

In addition to this point, we revised the volume of a drop, from “1 mL” to “0.5 L” (line 130)

7. 5.1: Please specify the imaging conditions.

We showed the conditions of time-lapse imaging in Table 1, so we added the word, “Table 1” (line 159). Furthermore, we modified Table 1 to clear the condition under which each figure and movie was captured.

8. 5.1.1: What laser intensities and exposure time are used?

We showed the conditions of time-lapse imaging in Table 1, so we added the word, “Table 1” (line 163). Furthermore, we modified Table 1 to clear the condition under which each figure and movie was captured.

9. 5.1.2: How to compare?

We revised this part.

“5.1.2. Compare follicle growth under each differing culture conditions **by measuring follicle areas in images of control samples at 24-h intervals and in time-lapse imaging samples. (See step 6)**.”

10. 5.2: How to capture the images?

We revised this part

“5.2. Capture images at 30-min intervals using the **time-lapse** imaging system **under the determined condition (Table 1)**.”

11. 7.3: Please specify the settings.  
 We added the information of setting condition of each figure and movie in Table 1.

Sincerely,

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