Dear Dr. Wu,

Thank you for the additional comments on my manuscript JoVE58352R1. I have addressed all the comments and updated the materials sheet.

Best regards

Hanne Hoffmann,  
  
  
  
**Editorial comments:**  
  
**Q1.** Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**R1.** We have thoroughly revised the manuscript and corrected spelling mistakes etc.

**Q2.** For in-text referencing, please put the reference number before a comma or period.

**R2.** Punctuation has been placed after references.

**Q3.** Please use h, min, s for time units.  
**R3.** International units have been used throughout .

**Q4.** Step 1.7.5: Please write this step in imperative tense.  
**R4.** The step has been reworded as follows.

Observe slides from step 1.7.4 on a bright field microscope at x10 or x20. Establish the stage of the estrous cycle (Figure 2B)20 by observing cell morphology which allows to distinguish the four distinct stages of the estrous cycle (Figure 2B); metestrus is characterized by a mixture of cornified squamous epithelial cells and leukocytes, diestrus by leukocytes, proestrus by a mixture of leukocytes and nucleated epithelial cells, and estrus by cornified epithelial cells (Figure 2B)20.

**Q5.** 3.7.1-3.7.3: Please write these steps in imperative tense.  
**R5.** This section has been revised as follows:

3.6 Place the breeding cage on the housing rack. Leave the breeding cage as undisturbed as possible for the full duration of the fertility study. Conduct the fertility study for 30 days. For each breeding pair keep detailed records and note 1) the date the mating is set up, 2) the date litters are born and 3) the number of pups born, include dead and live pups. Litter checks are done daily throughout the study.

3.7 Prolong the fertility study for an additional 30-60 days if no or a weak impact on fertility is established in step 3.6.

**Q6.** Line 239, 267: Please remove all headers in Representative Results.

**R6.** All headers have been removed.

**Q7.** Line 345, 366, 382, 412, 422: Please remove all headers from Discussion.

**R7.** All headers have been removed.

**Q8**. Line 348-364: Please do not use a list.  
**R8.** The list has been removed and the section has been written as a paragraph.

The overall wellbeing of the mice is critical for a successful fertility assay21. When performing a fertility assay it is important to not physically check on the mice every day as this can cause stress. Further avoid frequent cage changes, as these are also stressful. Ideally cage changes will be done no more than 1-2 times per week. Light exposure during the dark phase negatively impacts breeding in nocturnal rodents. Do not turn on lights in the breeding room during the dark hours. If entry to the room is required during the dark hours use dim red light illumination. Another stressor is the presence of excessive odors, vibrations or noise in the vivarium for all or parts of the study. To help alleviate stress and improve mouse wellbeing and breeding, give the mice nesting material or other types of enrichment. Nest building is an important behavior in mice and will increase breeding success. The nests also allow the mice a place to hide and play.

Healthy and well-nourished animals breed well, it is therefore important to provide *ad libitum* access to high quality food and water. If breeding pairs start to exhibit symptoms of disease, such as dermatitis (frequent in C57BL/6J), dental malocclusion or other, exclude these mice from the study. In addition, if the study is being done in immunodeficient mice it is key the mouse room is kept clean to preserve fertility.

**Q9.** Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

**R9.** Table of Equipment and Materials has been revised.

**Q10.**  Please revise the table of materials to include all essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

**R10.** Table of Equipment and Materials has been revised and equipment has been included.