Dear Dr. Rasahkam,

We want to submit the revised version of the manuscript JoVE51214R1 “Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model”. We believe we have addressed all the reviewers comments, which have result in a substantially improved manuscript. The type of access preferred is standard access.

We hope this resubmitted version is considered suitable for publication.

**Reviewer #3:**   
*Manuscript Summary:*   
This manuscript describes two techniques that are extensively used in many laboratories. The embryo transfer procedure described represents a novelty that refines the traditional approach.  
  
*Major Concerns:*  
Second parragraph (line 63) needs to be corrected. Authors state that mouse embryos can be transferred either to oviducts of 0.5 pseudopregnant recipients or to uterine horns of 2.5 recipients. This is uncorrect. Mouse blastocysts can be transferred as the authors describe, but if earlier stages are used, for instance 2-4 cell embryos, those will never implant if transferred to uterine horns of 2.5 recipients. Embryos can be transferred into recipients that are in earlier stages of pregnancy, but never will be able to implant in more advanced recipients.

We totally agree with the reviewer, the sentence in the second paragraph of the introduction may be misleading and we have modified it accordingly. As stated in the third paragraph of the discussion, this technique cannot be used to transfer early-cleavage state embryos.

The vasectomy technique described represents a refinement on the technique described in several handbooks of mouse embryology where laparotomy is performed through a transversal incission instead of a longitudinal one, as Bermejo et al describe. I agree that this approach is less traumatic and less prone to post operatory problems than the transversal accession. However, from the animal welfare point of view, scrotal vasectomy is the method of choice and should be the technique employed for a real refinement of the technology and the one that should be enforced IACUC or Animal Wefare Officers. Scrotal vasectomy is easily performed with a minimun training. It is consistently less traumatic than the abdominal access, and according to the 3Rs principle it should be the technique of choice. The authors state they prefer the abdominal access because it is easier for them (lines 281-282), in my opinion this is not reason enough and for sure it is not an argument to appear in a discussion.

We have modified the discussion and included a reference to acknowledge that scrotal incision as an alternative to the technique proposed herein. We agree that scrotal incision is a less invasive technique, however, *vasa deferentia* are more difficult to access from the scrotum and, thus, novice surgeons may have problems to distinguish from which testicle they come from. This situation may lead to cauterize twice the same *vas deferens*, leaving the other intact and to excessive tissue manipulation. Regarding to the new reference inserted, it would be interesting to analyze the possible differences in postoperative behavior between the longitudinal abdominal and scrotal incision performed under a strong postoperative analgesic (such as buprenorphine).

The table provided shows that the technique is reliable a priory even though it does not demonstrate this technique is better than the traditional aproach through the uterine wall, since they do not compare both approaches using the same experimental conditions. Real efficiency could be achieved if embryos treated in exactly the same way are transferred using the two systems. However, their results suggest it worths to try this approach

Our main concern with the uterine approach is that it leads to pretty inconsistent results: one particular transfer can result in about 70 % delivery rates, but others may completely fail. After switching to the utero-tubal embryo transfer, we are obtaining optimal results consistently, which improves the quality of science and reduces greatly the number of animals used.

*Minor Concerns:*  
Authos should mention magnification used to perform embryo transfer

A sentence has been added to the fourth paragraph of the discussion.

Authors should mention that the uterotubal junction can be easily passed only at the rigth time, this is, on the day when the natural transit from oviduct to uterine horns takes place during pregnancy.

A sentence has been added to the third paragraph of the discussion.

Authrs should change "transgenic" by "genetically modified", lines 38 and 55. For several years now, Transgenic refers exclusively to aditive modifications of the genome. Targeted mutations (KO, KI) are not transgenics,the term that includes both is "genetically modified"

Changes have been made.

*Additional Comments to Authors:*  
The authors should correct the freference by Ann Mc Laren (5) her name is missing as first author and since she was the real pioneer in rodent embryo transfer technology she deserves a correct reference.

Change has been made. As noticed by the reviewer, there is a mistake in the author abbreviation of that reference in the PubMed reference list for EndNotes.