Dear reviewers and editor,

In this document we respond appropriately to your recommendations.

**Changes recommended by the JoVE Scientific Review Editor:**  
  
• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.  
  
• **Abstracts:** Please re-word the Short Abstract to more clearly state the goal of the protocol. For example, “This protocol/manuscript describes…”

***The abstract has been revised and rewritten.***  
  
• **Introduction:**  
1) The following lines need literature references: 80-81, 81-82.

***The text has been revised and literature references have been added.***  
  
• **Protocol Language:** Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

1. Some examples NOT in imperative tense: Line 167, 174. 191-93 etc.

***The protocol text has been revised and the imperative voice implemented. In this sense, supplementary information is added as a “note”, following the editorial recommendations.***

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.) your protocol steps.**There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

***These recommendations have been taken into account and the appropriate corrections made.***

1) Line 172: Is the dosage fixed regardless of body weight? Dosage should ideally be provided as ug/kg.

***We have clarified this issue in the manuscript. Normally, 0.8 µg is a suitable dose for ovulation induction in medium size rabbits (4-5 Kg), so 1 µg generally guarantees the ovulation.***

2) Line 186: Unclear what is meant by “insert and fix”; is the needle left inserted throughout the remaining steps?

***One more step is added to clarify this issue.***

3) Lines 200-201: This should be moved up to before the induction of anesthesia or to the start of 1.4.

***The duration of the step has been clarified.***

4) Line 222: Do you mean 70%?

***The symbol error has been corrected.***

5) Line 236: Please describe all the surgery steps including tools used. E.g, it is unclear how and where the initial incisions are made. How large and deep should they be?

***No initial incision is present before the trocar introduction; the CO2 was insufflated through the inserted trocar. The process has been clarified.***

6) Line 239: Which port are you referring to? The one from 1.4.8.? Please describe what is to do be done here clearly: “It is important to look at the reproductive tract arrangement, determining the status and position of the infundibulum and ampulla before embryo transfer to facilitate the next steps”

***The sentence has been clarified. The part highlighted in “” quotes is added as a note in the manuscript, as it merely advises the reader that identifying the reproductive tract could facilitate the following steps.***

7) Line 244: how is it identified?

***Graphical information is available in figure 2. This has been clarified in the manuscript.***

8) Line 248: Can you quantify how deep to insert? Perhaps in millimeters?

***Data have been added.***

9) Line 286: What age?

***The age of the embryos depends on the experimental process in which the manuscript could be useful. Only the embryo receptor females should be prepared accordingly and this is stated in the paper.***

10) 288: How are the embryos handled?

***This issue has been added as a note in the previous step.***

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

***The text protocol numbering has been corrected.***

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.  
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.  
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.  
4) Notes cannot be filmed and should be excluded from highlighting.

***The protocol steps to the video are accordingly highlighted in yellow.***

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

***The recommendations are taken into account. However, this article has some particularities, such as the implementation of two different procedures (embryo vitrification and embryo transfer) with one common goal. We want to focus the work mainly on the embryo transfer process, which is the main new feature that will allow the researchers to use the rabbit as a model for reproductive studies. For this reason, discussion is mainly focused on this technique. In this context, a comprehensive narration was performed, including in two paragraphs the troubleshooting, limitations and comparison to the existing methods in each technique. Then, limitations are discussed after each of the above mentioned paragraphs and the future applications are discussed in the last part of the discussion. This structure is constructed to provide one cohesive description of the protocol, making references to the results presented in order to contextualize this protocol with the laboratory work.***

• **Table of Materials:** Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as rabbit strain, all drugs.

***Rabbit strain is added to the table, and the drugs have been revised.***

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

***The re-print permission from the editorial is included in the supplementary files. The figure is accordingly cited in the manuscript.***

**Comments from Peer-Reviewers:**   
  
**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
I have completed my review of the article entitled 'Minimally invasive embryo transfer and embryo vitrification at the optimal embryo stage in rabbit model' by Marco-Jimenez, Garcia-Dominguez, Viudes-de-Castro and Vicente. I find the article/protocol to be of importance and a valuable contribution. The protocol provide a minimally invasive technique compared to open laparotomy and the recovery of animals is an improvement. The protocol is detailed and provide fine instructions.  
Even though there are numerous problems, and I recommend that it be accepted only after major revisions. These problems, which I detail below, may be summarized as (1) poor grammar and style throughout, especially in the introduction and discussion (2) The results are misleading in terms of the description of non-compact vs. compact in table 1 and 2. (3) The discussion summarize interesting studies but due to the unclear writing the statements vanish.  
In the following I outline the above-mentioned points in more detail, it is crucial that a qualified English editor review this article for grammar, as there are numerous grammatical errors, some of which I have not altered, as it is not within my purview as peer reviewer  
  
Major Concerns:  
-The need of proofreading by a qualified English editor considering typos and grammar.

***English grammar and vocabulary has been revised.***  
-Results: Table 2: 70-72h is considered non-compacted whereas they are considered compacted in table 1? The text doesn't state anywhere why 70-72h Compact morulae are suddenly being referred to as non-compact in Table 2. This makes one question the experiment concerning " Viability of 70-72h non-compacted vs compact vitrified morulae".

***Further explanation of this issue is included in the table description to clarify the doubt.***

-Discussion: very interesting point in the discussion disappear in the unclear writing. I would recommend several of the paragraphs within the discussion to be rewritten

***Some paragraphs in the discussion have been rewritten in order to clarify the sense of the sentences. English grammar and vocabulary has been revised.***

Minor Concerns:

Line 53. Rewrite

***This recommendation has been implemented.***

58: rabbits, delete animal in animal model organism  
***This recommendation has been implemented.***

84: transferred to a surrogate

***This point has been revised.***

96: techniques

***This point has been revised.***

102: bovine, pigs and mice

***This point has been revised.***

102: rewrite sentence

***The sentence has been rewritten.***

105: endoscopy was used

***This point has been revised.***

114: rewrite

***This point has been rewritten.***

117: an ideal model (76 % model organisms are rodents)

***This point has been revised.***

136: ET procedure using rabbits as a model organism

***This point has been revised.***

136: rewrite the last sentence (the technique provide a model for further research…)

***The sentence has been rewritten.***

144: outcome

***This point has been revised.***

144-145: rewrite not clear

***This point has been revised.***

152: rabbits

***This point has been revised.***

152: model organism

***This point has been revised.***

192: time is

***This point has been revised.***

242: 17-G

***This point has been revised.***

301 Step 2.2.2 must take place…

***This point has been revised.***

303: nitrogen to achieve

***This point has been revised.***

304: Store the ministraw in

***This point has been revised.***

305: Thawing is completed in a..

***This point has been revised.***

312: °C

***This point has been revised.***

General: inconsistency using ℃ or ° C throughout the paper

***This point has been revised.***  
  
Table 1 : text: 2 survival rate … rewrite sentence

***The sentence has been rewritten.***

Table 2: text: 1 survival… rewrite sentence

***The sentence has been rewritten.***  
  
Discussion:  
Paragraph 1, 3 and 5: rewrite, the statements are unclear.

***These points have been revised.***  
  
I wish the authors success implementing my recommended changes, and I look forward to seeing this article published.  
  
  
**Reviewer #2:**  
Manuscript Summary:  
The manuscript describes a method of laparoscopic embryo transfer in rabbits.  
  
Major Concerns:  
None  
  
Minor Concerns:  
Many minor grammatical and stylistic suggestions for improvement on the attached.

***All the grammatical and stylistic suggestions have been implemented.***  
Recommend title change to: ...in rabbits.

***The title has been reconsidered, but taking into account that in this paper we present some techniques that allow the use of the rabbit as an ideal animal model for reproductive studies in large mammalian species such as humans, we think that the detailed title is consistent with the purpose of the paper.***  
  
  
**Reviewer #3:**  
Review of manuscript JoVE58055

Line 38: change "transference" to "embryo transfer"

***This point has been revised.***

Line 43: delete "today"

***This point has been revised.***

Line 44: delete "the" and hyphen

***This point has been revised.***

Line 45: rabbitS

***This point has been revised.***

Line 53: delete "Taking into account that"

***This point has been revised.***

Line 55: replace "final" with "and"

***This point has been revised.***

Line 58: replace "To date," with "The"; delete "as an animal model organism"

***This point has been revised.***

Line 60: delete "it has reproductive characteristics such as induced ovulation, a"

***This point has been revised.***

Line 61: delete "chronology of"

***This point has been revised.***

Lines 62-63: place "Such as intracytoplasmic sperm injection, embryo culture or cryopreservation" in parentheses.

***This point has been revised.***

Line 70: change "transference" to "transfer"

***This point has been revised.***

Line 71: Delete "So," and add "thus" after comma

***This point has been revised.***

Line 72: Delete "Interestingly,"

***This point has been revised.***

Line 73: change "laparoscopy-" to "laparoscopically"

***This point has been revised.***

Line 78: aimS

***This point has been revised.***

Line 81: add comma before "were"; delete "oocytes are produced by following a"

***This point has been revised.***

Line 82: change "treatment" to "treatments are given"; replace "and" with "to"; replace "these gametes" with "antral ovarian follicles"

***This point has been revised.***

Line 83: Replace "Thus," with "oocytes"; replace "eggs" with "from these follicles"; move "in vitro" before "until"

***This point has been revised.***

Line 91 and throughout manuscript: British English or American English? (characterized, behavior, hemochorial, anesthesia, etc.)

***The manuscript is in UK English and has been revised for consistency.***

Line 94: delete hyphen

***This point has been revised.***

Line 95: delete "stages"

***This point has been revised.***

Line 96: Replace "Embryo transfer" with "The ET"

***This point has been revised.***

Line 98: delete hyphen

***This point has been revised.***

Line 99: replace "currently used" with "frequently"

***This point has been revised.***

Line 104: cervices

***This point has been revised.***

Line 111: takeS

***This point has been revised.***

Line 117: replace "where" with "and"

***This point has been revised.***

Line 124: use semi-colon after "months"

***This point has been revised.***

Line 126: replace "double" with "duplex"

***This point has been revised.***

Line 130: start new paragraph with "Today, non-surgical…"; rabbitS

***This point has been revised.***

Line 132: delivery

***This point has been revised.***

Line 135: in THE oviduct

***This point has been revised.***

Line 136: rabbitS

***This point has been revised.***

Line 137: Replace "embryo transfer" with "ET"

***This point has been revised.***

Line 139: replace "However, as" with "Because"

***This point has been revised.***

Line 141: replace "transfer time" with "ET"

***This point has been revised.***

Line 142: replace "retards" with "slows"; delete "the"; place "such as in vitro culture" in parentheses

***This point has been revised.***

Line 143: place "such as superovulation treatments" in parentheses

***This point has been revised.***

Line 147: giveS

***This point has been revised.***

Line 151: add "of animal research: before "with"

***This point has been revised.***

Line 152: replace "rabbit constitutes" with "rabbits constitute"

***This point has been revised.***

Line 153: add "in vivo" before "reproductive"

***This point has been revised.***

Line 168: replace "accommodate" with "adapt"

***This point has been revised.***

Line 200: for how many hours is water withheld?

***Animals have free access to water. It has been clarified in the paper.***

Line 220: add semi-colon after "gloves"; replace "wearing" with "wear"

***This point has been revised.***

Line 221: replace "cleaning" with "clean"  
***This point has been revised.***

Line 222: %  
***This point has been revised.***

Lines 226-229: uses a, b, c, d (instead of 1, 2, 3 ,4)  
***We follow the authors instruction for the numbering.***

Line 227: What size Foley catheter? 10 French?  
**The model is available in the materials Table.**

Line 233: add "properly" after "them"  
***This point has been revised.***

Line 234: add "(fenestration)" after "hole"  
***This point has been revised.***

Line 236 and throughout manuscript: replace "trocar endoscope" with "endoscopic trocar"  
***This point has been revised.***

Line 237: add a comma after "process"; add a hyphen after "pressure"  
***This point has been revised.***

Line 242: gauge  
***This point has been revised.***

Lines 247-248: Which is correct: "deep" or "do not progress very far"?  
***This confusing sentence has been corrected.***

Line 257: reaplce "is accumulated" with "remains"  
***This point has been revised.***

Line 258: pain and discomfort  
***This point has been revised.***

Line 259: from (not form)  
***This point has been revised.***

Line 280: VERY IMPORTANT CHANGE NEEDED: replace "thawing" with "warming"  
***This point has been revised.***

Line 282: degree symbol  
***This point has been revised.***

Line 283: warmER  
***This point has been revised.***

Line 289: sulFoxide  
***The manuscript is in British English (sulphoxide)* https://en.oxforddictionaries.com/definition/dimethyl\_sulphoxide**

Line 291: 125 microliters  
***This point has been revised.***

Line 294: 125 microliters  
***This point has been revised.***

Line 296: PLEASE EXPLAIN THE LARGE VOLUME (40 microliters) - is it because of the large diameter of the rabbit morula? Ruminant livestock species are typically vitrified in very small (< 5 microliters) volumes.

***The straw allows us to vitrify large numbers of embryos, unlike minimum volume devices. Furthermore, we choose the straw due to the easier handling and the similar results obtained compared with minimum volume devices (Cryotop). These details are implemented in the manuscript with the appropriate reference.***

Line 298: reachES  
***This point has been revised.***

Line 301: replace "be taken" with "take"  
***This point has been revised.***

Line 303: replace "for" with "to"  
***This point has been revised.***

Line 304: StorE ; delete "cryobank of"; add "dewar for" after "nitrogen"  
***This point has been revised.***

Line 305: Warming  
***This point has been revised.***

Line 308: where should crystallization be observed? Outside the mini-straw?

***Inside the mini-straw. It has been clarified in the paper.***

Line 311: volume of solution?

***This point has been revised.***

Line 314: volume of solution?  
***This point has been revised.***

Line 349: replace "checked" with "confirmed"  
***This point has been revised.***

Line 351: correctLY  
***This point has been revised.***

Line 371: delete "as" start new sentence with "The capacity"  
***This point has been revised.***

Table 1: specify fresh in vivo derived; pronuclear; 8-cell; morula (not morulae)

***This point has been revised.***

Line 641: change "was" to "were"; pronuclear; 8-cell, morula  
***This point has been revised.***

Table 2: need to add number of recipients. Line numbers cover superscripts of means; Was there a CONTROL GROUP?

***The number of recipients has been added. In this table we compare the viability after vitrification at two different stages of the embryo development; no control groups are required for this purpose.***