

Journal of Visualized Experiments

Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model

--Manuscript Draft--

Manuscript Number:	JoVE51214R2
Full Title:	Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model
Article Type:	Invited Methods Article - JoVE Produced Video
Keywords:	blastocyst; chimera; lentivirus; uterine transfer; oviductal transfer; uterotubal transfer; anesthesia
Manuscript Classifications:	1.16.254.500: Blastocyst; 1.16.254.500.766: Trophoblasts; 1.16.615: Morula; 1.16.94: Blastomeres; 1.5.360.319.114.373: Fallopian Tubes; 1.5.360.319.114.630: Ovary; 1.5.360.319.679: Uterus; 95.51.24: life sciences; 95.51.3: animal biology; 95.51.4: animal models
Corresponding Author:	Pablo Bermejo-Alvarez University of Maryland Beltsville, MD UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	borrilobermejo@hotmail.com
Corresponding Author's Institution:	University of Maryland
Corresponding Author's Secondary Institution:	
First Author:	Pablo Bermejo-Alvarez
First Author Secondary Information:	
Other Authors:	Ki-Eun Park Bhanu P. Telugu
Order of Authors Secondary Information:	
Abstract:	<p>The transfer of preimplantation embryos to a surrogate female is a required step for the production of transgenic mice or to study the effects of epigenetic alterations originated during preimplantation development on subsequent fetal development and adult health. The use of an effective and consistent embryo transfer technique is crucial to enhance the generation of transgenic animals and to determine the effect of different treatments on implantation rates and survival to term. Embryos at the blastocyst stage are usually transferred by uterine transfer, performing a puncture in the uterine wall to introduce the embryo manipulation pipette. The orifice performed in the uterus does not close after the pipette has been withdrawn, and the embryos can outflow to the abdominal cavity due to the positive pressure of the uterus. The puncture can also produce a hemorrhage that impairs implantation, blocks the transfer pipette and may affect embryo development, especially when embryos without zona are transferred. Consequently, this technique often results in very variable and overall low embryo survival rates. Avoiding these negative effects, utero-tubal embryo transfer take advantage of the utero-tubal junction as a natural barrier that impedes embryo outflow and avoid the puncture of the uterine wall. Vasectomized males are required for obtaining pseudopregnant recipients. A technique to perform vasectomy is described as a complement to the utero-tubal embryo transfer.</p>
Author Comments:	The type of access preferred is standard access.
Additional Information:	
Question	Response



DEPARTMENT OF ANIMAL AND AVIAN SCIENCES

Animal Sciences Center
College Park, Maryland 20742-2311
301-504-5295 TEL
301-504-5753 FAX

April 25, 2013

Dear Editor,

I am submitting the manuscript "Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model" for consideration by Journal of Visual Experiments. I was invited to submit a protocol regarding to my previous work published in Molecular Reproduction and Development (ref. 2 in this manuscript) by the associate editor Jane Hannon.

In this manuscript we describe a protocol for transferring preimplantation mouse embryos to the uterus of a pseudopregnant recipient following an improved technique that minimizes uterine damage and embryo loss. The article also describes the anesthesia protocol used and a technique to perform vasectomy. We consider that the multimedia format of JoVE is ideal to describe these protocols, as surgical techniques are difficult to describe by the sole use of words. More importantly, despite being probably the most frequently performed surgery in the mouse model and being an essential step to generate transgenic mice, there is not any publicly available video to learn the technique.

I hope you will find our manuscript both interesting and acceptable for publication in JoVE. You can find a list of 6 possible peer reviewers on the following page.

Sincerely,

Pablo Bermejo-Alvarez, DVM, PhD
ANRI, ARS, USDA
Bldg 230, Room 102, BARC-East
10300 Baltimore Ave
Beltsville, MD 20705-2350
Tel: 01-573-825-7541
Contact email: borrilobermejo@hotmail.com

Peer reviewers:

Inchul Choi

Department of Animal Science, Michigan State University, USA.

icchoi@msu.edu

Celia de Frutos

Animal Sciences Department, University of California at Davis, USA.

cdefrutos@ucdavis.edu

Juan de Dios Hourcade

Transgenesis Unit, National Center for Cardiovascular Disease (CNIC), Madrid, Spain.

jhourcade@cnic.csic.es

Belen Pintado

Transgenesis Unit, National Biotechnology Center (CNB), Madrid, Spain.

bpintado@cnb.csic.es

Rocio M Rivera

Department of Animal Sciences, University of Missouri, USA.

riverarm@missouri.edu

Laura Schulz

Department of Obstetrics and Gynecology, University of Missouri, USA.

schulzl@health.missouri.edu

Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model

Authors:

Pablo Bermejo-Alvarez, Ki-Eun Park, Bhanu P. Telugu

Authors: institution(s)/affiliation(s) for each author:

Pablo Bermejo-Alvarez
Animal Bioscience and Biotechnology Laboratory.
United States Department of Agriculture.
Department of Animal and Avian Sciences.
University of Maryland.
borrillobermejo@hotmail.com

Ki-Eun Park
Animal Bioscience and Biotechnology Laboratory.
United States Department of Agriculture.
Department of Animal and Avian Sciences.
University of Maryland.
kepark@umd.edu

Bhanu Prakash Telugu
Animal Bioscience and Biotechnology Laboratory.
United States Department of Agriculture.
Department of Animal and Avian Sciences.
University of Maryland.
btelugu@umd.edu

Corresponding author: Pablo Bermejo-Alvarez, D.V.M. Ph.D.

Keywords: Blastocyst, chimera, lentivirus, uterine transfer, oviductal transfer, uterotubal transfer, anesthesia.

Short Abstract:

Utero-tubal embryo transfer uses the utero-tubal junction as a barrier to prevent the embryo outflow that may occur when performing uterine transfer. Vasectomized males are required to obtain pseudopregnant recipients for embryo transfer. Both techniques are discussed.

Long Abstract:

The transfer of preimplantation embryos to a surrogate female is a required step for the production of genetically modified mice or to study the effects of epigenetic alterations originated during preimplantation development on subsequent fetal development and adult

health. The use of an effective and consistent embryo transfer technique is crucial to enhance the generation of genetically modified animals and to determine the effect of different treatments on implantation rates and survival to term. Embryos at the blastocyst stage are usually transferred by uterine transfer, performing a puncture in the uterine wall to introduce the embryo manipulation pipette. The orifice performed in the uterus does not close after the pipette has been withdrawn, and the embryos can outflow to the abdominal cavity due to the positive pressure of the uterus. The puncture can also produce a hemorrhage that impairs implantation, blocks the transfer pipette and may affect embryo development, especially when embryos without *zona* are transferred. Consequently, this technique often results in very variable and overall low embryo survival rates. Avoiding these negative effects, utero-tubal embryo transfer take advantage of the utero-tubal junction as a natural barrier that impedes embryo outflow and avoid the puncture of the uterine wall. Vasectomized males are required for obtaining pseudopregnant recipients. A technique to perform vasectomy is described as a complement to the utero-tubal embryo transfer.

Introduction:

Embryo transfer is probably the most frequent surgical procedure performed in the mouse model. This technique is essential to obtain offspring from embryos subjected to *in vitro* manipulation techniques and, therefore, constitutes a necessary step for the development of genetically modified models by pronuclear injection, lentiviral transduction, or chimera formation. Besides, the technique allows the study of the developmental effects of diverse insults occurring during preimplantation development. The use of artificial reproduction techniques¹ or the exposure to abnormal concentrations of different substances or metabolites² may affect embryo development resulting in implantation or placentation failures and long term effects in the offspring. A reliable and reproducible embryo transfer technique is crucial to test the possible negative effects of the experimental treatment on implantation and fetal development in a consistent manner.

Murine preimplantation embryos can be transferred to a recipient female either into the oviduct via the ampullae of 0.5 days post coitum (dpc) pseudopregnant recipients (oviduct transfer)^{3,4} or into the uterus of 2.5 dpc pseudopregnant recipient (uterine transfer)^{5,6} depending on their developmental stage. Embryos at the blastocyst stage, such as those used to generate chimeric mice by injection of embryonic or induced pluripotent stem cells, are usually transferred by uterine transfer. Blastocysts can also be transferred to the oviduct of a 0.5 dpc recipient, but it constitutes a less physiological test for developmental disruptors, because the embryo undergoes diapause and has 2 days to recover from the insult before implantation takes place. Uterine transfer involves puncturing the uterine wall with a narrow needle in order to generate an aperture that allows the access of an embryo manipulation pipette into the uterine lumen. Although this technique can yield good results, the survival to term (i.e. the percentage of embryos transferred that develop to a pup) is often low and unpredictable^{7,8}.

The puncture of the uterine wall entails some detrimental side effects. First, myometrium is a highly vascularized tissue and its puncture often results in a small hemorrhage. Blood may block the embryo transfer pipette or invade the uterine lumen causing embryonic death and/or implantation failure. This is particularly relevant when embryos without *zona* are transferred, as the blood cells and debris can attach to the blastomeres. Second, the opening performed does not seal after the embryos have been transferred, so they can flow back through the orifice and be expelled to the abdominal cavity when a too large volume has been introduced into the uterus. The utero-tubal embryo transfer described herein takes advantage of the utero-tubal junction to deliver the embryos into the uterus without the need of puncturing the uterine wall and thereby avoiding its adverse consequences⁹.

The pseudopregnant recipient females used for embryo transfer are obtained by natural mating with vasectomized males⁸. The seminal secretions produced by a sterile male are required for the uterus to become receptive to the transferred embryos. To obtain a recipient, a maximum of 2 females of 8 weeks to 6 months of age are placed with a vasectomized male in the afternoon. The following morning, females are checked for the presence of a vaginal copulation plug, a clump of coagulated proteins from the male seminal fluid. As mating usually occurs during midnight, the day of vaginal plug detection is considered to be 0.5 dpc. Although vasectomized males can be purchased from some vendors, the surgical procedure described herein is relatively easy and does not require any additional instruments than required for embryo transfer.

Protocol Text

All animal experiments were approved by the Beltsville Area Animal Care and Use Committees (BAACUC 11-015) in accordance with USDA Animal Care and Use Guidelines.

1. Anesthesia and analgesia (common for both surgical procedures).

1.1) **Weigh** the mouse and load the following **anesthetics** and analgesic in two 1 ml syringes with 27G needles:

1.1.1) Ketamine (0.1 mg/g: 0.01 ml/g of a 10 mg/ml solution) and xylazine (0.01 mg/g: 0.005 ml/g of a 2 mg/ml solution).

1.1.2) Buprenorphine (0.1 µg/g: 0.01 ml of a 0.01 mg/ml solution).

1.2) Immobilize the mouse by picking up the scruff of its neck as close to the ears as possible with the thumb and index fingers and holding the tail between the little and ring fingers.

1.3) Inject **Ketamine-Xylazine** mixture **intraperitoneally**. In order to avoid puncturing internal organs, hold the mouse with its head slightly below the level of its hips (**Fig. 1A**)

1.4) Inject **Buprenorphine subcutaneously** in the scruff of neck hold between the thumb and index fingers (**Fig. 1B**).

1.5) Leave the mouse in the cage (clean and without any other animal) on a warm stage.

1.6) Once unconscious, check for the absence of **rear foot reflex** (checked by toe pinch). Apply **eye ointment** to avoid dryness of the eye and to check for the absence of **palpebral reflex** (**Fig. 1C**).

1.7) This protocol provides a surgical anesthesia plane for a minimum of 30 minutes, enough to perform the procedures described below (2 and 3). If longer times are required, an additional injection of ketamine + xylazine with half of the dosage described in 1.1.1 can be applied after 30 min. A change in the breathing pattern to a faster and irregular one indicates the loss of the proper anesthesia plane.

2. Vasectomy

2.1) Use a male with a **proven mating performance**.

2.2) **Sterilize** surgical instruments, clean the surfaces where surgery will be performed and wipe them with 70 % ethanol.

2.3) Perform **anesthesia** as previously detailed (1), checking for loss of reflexes.

2.4) Place the mouse on a warm stage, **remove fur** with electric clippers from the ventral area between two imaginary transversal lines placed 0.5 cm and 2.5 cm above the penis (**Fig. 2A**).

2.5) **Sanitize** the shaved area by sequential wiping with 10 % povidone iodine and 70 % ethanol.

2.6) Place the mouse in supine position with its tail towards the surgeon and cover with a sterile towel with a hole exposing the shaved area. Illuminate the surgical area.

2.7) Perform a **10-15 mm longitudinal skin incision** in the medial line of the abdomen, about 1 cm above the penis. Hold the skin with dressing serrated forceps and then cut with scissors (**Fig. 2A, B**).

2.8) Perform a **5-10 mm longitudinal incision in the *linea alba***. Hold the muscle with micro dissecting serrated forceps and cut with scissors (**Fig. 2C**).

2.9) Grab the testicular adipose pad of one side with micro dissecting serrated forceps and pull it to **expose testis, vas deferens and epididymis**. *Vas deferens* is located medial to the testis and it is a clearly distinguishable free tube (not attached to the testis wall like the epididymis) with a blood vessel running along one side (**Fig. 2D**).

2.10) Holding the *vas deferens* with a micro dissecting serrated forceps, **flame** dressing forceps until they turn red (**Fig. 2E**), and then use them to cut and **cauterize the *vas deferens*** in two points at once (**Fig. 2F**). The cut should remove a portion of about 5 mm and leave two clearly separated cauterized ends (**Fig. 2G**).

2.11) Move the testicle, epididymis and *vas deferens* back to the abdominal cavity.

2.12) **Proceed from step 9 in the other testicle.**

2.13) **Suture the muscle** with one or two horizontal mattress stitches made with 5/0 absorbable suture (**Fig. 2H**).

2.14) **Suture the skin** with one or two wound clippers (**Fig. 2I**).

2.15) Identify the vasectomized male (ear ring, finger tattoo...), move it the cage placed on a warm stage and **observe until it recovers from anesthesia** (conscious and maintain sternal recumbancy). A 0.5-1 ml subcutaneous injection of warm saline solution improves recovery. Record the possible incidences occurring during vasectomy transfer, add antibiotic to the drinking water.

2.16) Wound **clips** can be **removed** 10 days after vasectomy with a wound clipper remover or a pair of teeth forceps. The vasectomized male will be ready to mate 2 weeks after surgery.

2.17) **Test the infertility** of the vasectomized male by mating with fertile females before using it to obtain recipients.

3. Utero-tubal embryo transfer

3.1) Mouse morulae or blastocysts can be transferred by this technique to a **pseudopregnant recipient** female at **2.5 dpc**.

3.2) Prepare **embryo manipulation glass pipette**:

3.2.1) Polish the tips of the glass capillaries in order to avoid damaging the pipette holder.

3.2.2) **Soften** a middle portion of the glass capillary by heating with a fine flame while slightly rotating the capillary with both hands synchronously. Once the capillary section becomes soft and malleable (light red color), withdraw it quickly from the flame and **pull** both ends to narrow its external diameter to 130-150 μm .

3.2.3) Wait for the glass to cool down and then **cut** it by lightly scoring the narrow part with a diamond-point pencil, abrasive stone or nail file and pulling from both sides. The break should be clean and perpendicular

3.3.3) **Polish** the tip by very quickly flaming, leaving a 100-130 μm aperture. Pipettes can be stored for later use.

3.4) Warm embryo manipulation **media** (CZBH or M2, see discussion).

3.5) **Sterilize** surgical instruments, clean the surfaces where surgery will be performed and wipe them with 70 % ethanol.

3.6) Perform **anesthesia** as previously detailed (1), checking for loss of reflexes.

3.7) Keeping the mouse on a warm stage, **remove fur** with electric clippers from the dorsal area between the knees and the distal ribs (**Fig 3A, B**).

3.8) **Sanitize** the shaved area by sequential wiping with 10 % povidone iodine and 70 % ethanol.

3.9) **Move the embryos** from the incubator to the prewarmed embryo manipulation media.

3.10) Move the recipient to a warm stage under the stereomicroscope and place it in prone position laterally to the surgeon (with its head looking to the right or left side of the surgeon).

3.11) Cover the area with a sterile towel with a hole exposing the shaved area and illuminate the surgical area.

3.12) Perform a 1 cm transversal (vertical) **incision in the skin** in a spot located on the cranial 1/3 of the line between the last rib and the hips and the dorsal 1/3 of the line between the back and the abdomen (**Fig. 3A, B**). Hold the skin with dressing serrated forceps and cut with scissors (**Fig. 3C**).

3.13) Once the skin has been cut, ovary (red/orange) or the adipose pad surrounding the ovary (white) can be visualized through the body wall. Perform a 0.3-0.5 cm transversal (vertical) **incision in the body wall** over the ovary or adipose pad in a spot where the incision does not cut any large blood vessel. Hold the muscle with micro dissecting serrated forceps and cut with scissors (**Fig. 3D**).

3.14) Move the mouse to have its head facing towards the surgeon.

3.15) **Load** the embryo manipulation **pipette** (**Fig. 3E**):

3.15.1) Allow CZBH media to ascend by capillarity through the narrow portion of the manipulation pipette until about 5 mm of the wider part.

3.15.2) Take up a small air bubble (0.2-0.5 mm).

3.15.3) Introduce the embryos (5-10) in a minimal amount of media (2-4 mm).

3.15.4) Take up another small air bubble (0.2-0.5 mm) and a small amount of media (0.5-1 mm).

3.15.5) Leave the glass pipette attached to the mouth aspirator holder or to the hand-operated device, ready for step 3.17.

3.16) Grab the adipose pad surrounding the ovary with micro dissecting serrated forceps and pull it towards the mouse head to **expose the ovary, oviduct and** a small portion of the upper **uterus** out of the abdominal cavity (**Fig. 3F, G**).

3.17) Holding the aspirator mouth piece in the mouth, ready to be used, grab the adipose pad with micro dissecting serrated forceps to move the oviduct and expose the utero-tubal junction (i.e. where the oviduct meets the uterus).

3.18) Keeping the utero-tubal junction accessible, take slight curved micro dissection forceps with your left hand (if right handed) and place them just below the utero-tubal junction **grabbing the oviduct** about 2 mm above that portion.

3.19) Holding the utero-tubal junction with the slight curved micro dissection forceps, **puncture the oviduct** section close to the forceps with a 27G needle (**Fig. 3H**).

3.20) **Insert** the embryo manipulation **pipette** into the orifice performed with the needle and **advance** to the uterus through the utero-tubal junction (**Fig. 3I, J**). Once the pipette has passed the utero-tubal junction (**Fig. 3K**) it slides easily. Do not progress very far into the uterus to prevent endometrial damage (no more than 3 mm) and pipette blocking by the debris.

3.21) **Release the embryos** into the uterus by gently blowing (**Fig. 3L**). Both air bubbles must pass through the uterus. Some of the media above the first bubble may also be released into the uterus, but avoid introducing more air, as it may impede implantation.

3.22) **Remove the pipette** just after embryos have been released into the uterus.

3.23) Move the oviduct and ovary back to the abdominal cavity by grabbing the adipose pad.

3.24) **Suture the muscle** with a horizontal mattress stitch with 5/0 absorbable suture (**Fig. 3M**).

3.25) **Suture the skin** with a wound clipper (**Fig. 3N**).

3.26) Proceed from step 10 on the **other side if required**.

3.27) Identify the recipient (ear ring, finger tattoo...), move it to its cage (placed on a warm stage) and **observe until it recovers from anesthesia** (conscious and maintain sternal recumbancy).

3.28) Annotate the possible incidences occurring during embryo transfer and add antibiotic to the drinking water. A 0.5-1 ml subcutaneous injection of warm saline solution improves recovery.

3.29) Wound **clips** can be **removed** 10 days after embryo transfer with a wound clipper remover or two pairs of forceps (**Fig. 3O**). The recipient can be weighed on that day to assess pregnancy and estimate the number of pups. Provide nestling material to the recipient 15 days after embryo transfer.

Representative Results

Utero-tubal embryo transfer provides a mean to transfer embryos to the uterus avoiding some of the complications associated to the uterine embryo transfer^{2,9,10}. In **table 1** we show some representative result we have obtained transferring CD1 blastocysts subjected to different kinds of manipulations to CD1 recipients following the protocol described. The survival to term (% of embryos resulting in a pup) or survival to E15 (in the case of lentivirus exposed) is similar between embryos simply cultured *in vitro* from the zygote stage (IVC), embryos subjected to iPSC injection to generate chimeric pups and embryos that had their *zona* removed and were exposed to lentivirus for 7 hours before embryo transfer. Therefore, utero-tubal embryo transfer is a reliable technique to transfer embryos particularly complicated such as those lacking *zona pellucida* and incubated with lentivirus.

Tables and Figures:

Figure 1: Injection of anesthetics and analgesics. A) Intraperitoneal injection of Ketamine-Xylazine. B) Subcutaneous injection of Buprenorphine. C) Application of eye ointment.

Figure 2: Vasectomy protocol. A) The incision point (depicted with a red “X”) is placed about 1 cm above the penis; remove fur from 0.5 to 2.5 cm above penis (black lines). B) Skin incision. C) Muscle incision. D) *Vas deferens* (black arrow) and testis (*). E) Flaming of the forceps for cauterization. F) Cauterization of the *Vas deferens* in two points at once. G) *Vas deferens* clearly separated into two cauterized ends. H) Muscle suture. I) Skin suture.

Figure 3: Utero-tubal embryo transfer protocol. A, B) Dorsal and lateral view of the incision point (depicted with a red “X”); black lines between the last rib and hips (A, B) and between back and abdomen (B) serve as a guide. C) Skin incision. D) Muscle incision. E) Embryo manipulation glass pipette loaded with 5 blastocysts ready to be transferred. F, G) Representative ovaries with (F) or without (G) corpora lutea, indicated with black arrows. H-K) For representation purposes, a simulation of utero-tubal embryo transfer was performed loading a glass manipulation pipette with 0.4 % trypan blue solution. H) Puncture of the oviduct (black arrow) close to the uterus (*). I) Introduction of the embryo manipulation pipette into the orifice performed previously. J) The embryo manipulation pipette advances through the utero-tubal junction. K) A volume of trypan blue solution equivalent to that released in an embryo transfer has been expelled in the uterine lumen (black arrow). M) To test the efficient

closure produced by the utero-tubal junction, all the content of the manipulation pipette was released into the uterus; utero-tubal junction (arrow) impedes the back flow of the trypan blue solution released into the uterus (*). M) Muscle suture. N) Skin suture. O) Clip removal. P) 15 days after embryo transfer provide nestling material to the recipient. Q) Chimeric litter obtained following this technique.

Table 1: Representative results obtained after transferring three different groups of manipulated embryos following utero-tubal embryo transfer: 1) Embryos cultured *in vitro* (IVC) from the zygote stage to the blastocyst stage, 2) *In vivo* produced blastocysts injected with 10 mouse iPSC to generate chimeric mice, 3) *In vivo* produced blastocysts that had their *zona* removed and were incubated for 7 h with a lentivirus expressing GFP. Data represent the number of pups born out of the number of embryos transferred except for the lentivirus exposed group, where they represent the number of viable fetuses 10 days after embryo transfer, as gestation was not allowed to progress further.

Discussion

Vasectomy is a relatively straight forward surgical technique that does not involve major difficulties. When sanitizing with povidone iodine and ethanol make sure that the last wash (with ethanol) removes povidone iodine, as it may irritate the peritoneum. The access to *vas deferens* can also be achieved by the scrotum or performing a transversal incision in the abdomen⁸. Scrotal incision has been recommended to transversal abdominal incision due to the comparatively smaller incision needed and slightly better postoperative behavior¹¹. However, we prefer the abdominal incision over the scrotal because it provides an easier and clearer access to the *vasa deferentia* from both testicles, preventing novice surgeons from cauterizing twice the same *vas deferens* and leaving a functional *vas deferens*. Between both abdominal techniques, we favor the longitudinal incision in the *linea alba* over the transversal because it does not cut any abdominal muscular fiber, avoiding the development of abdominal hernias. However, both vasectomy and utero-tubal embryo transfer protocols can be adapted to the equipment available or to the personal likes of the researcher. For instance, a glass bead sterilizer can be used to heat the forceps used to cauterize the *vas deferens*. In any case, it is essential to follow an aseptic technique, to provide proper anesthesia and analgesia in order to maximize animal welfare, and to comply with local regulations.

Another aspect susceptible for modifications is the anesthesia protocol. If inhalational anesthesia (isoflurane) is available, we encourage its use instead of parenteral (injectable) anesthesia, as it provides a very stable anesthesia plane and rapid recovery. However, it is important to state that inhalational anesthesia still requires the use of an analgesic for intra- and post-operative pain, which should be administered before the surgery as premedication. Buprenorphine is an opioid that provides long term analgesia in mouse¹². Parenteral anesthesia also provides a good anesthetic plane for short procedures such as vasectomy and embryo transfer. Ketamine-Xylazine is a very reliable combination for mouse surgery¹³ and we have never observed any anesthetic complications using this combination in conjunction with premedication with buprenorphine. Other parenteral combinations may be used¹², but we

discourage the use of the narcotic mixture avertin (tribromoethanol), as only a single dose can be administered and multiple articles have reported diverse complications associated with its use such as local irritation, poor analgesia, ileus, fibrous adhesions in the abdominal cavity, necrosis of subperitoneal muscle fibers and abdominal organs surface, and even mortality¹⁴⁻¹⁸.

Embryo transfer requires good management of both embryos and recipient. As a general rule for mammalian embryo transfer, the developmental stage of the embryos can be more advanced than the pseudopregnancy stage of the recipient, but not the opposite. In other words, the embryos can wait for the mother, but the mother cannot wait for the embryos, so this technique can be used to transfer morulae or blastocysts, but not earlier stages. Utero-tubal embryo transfer can be performed two days after the detection of the vaginal plug from noon (2.5 dpc) to evening-night (3 dpc), when the utero-tubal junction is open to allow the natural transit of embryos from the oviduct to uterine horns. Considering that the embryos transferred may have already suffered from some kind of manipulation, embryo handling should minimize any further damage. An excellent guide for mouse embryo manipulation can be found in⁸. The two most commonly used mouse embryo manipulation media, which hold a physiological pH in regular atmosphere, are CZBH¹⁹ and M2²⁰. Although *in vivo* produced mouse embryos can overcome the exposure to cold temperature or abnormal pH for long periods²¹, manipulation media should be pre-warmed. If media is pre-warmed in a culture dish, avoid warming for long periods (more than 40 min), as the osmolarity of the media will increase due to water evaporation and that can be more harmful than cold manipulation media. Similarly, the time the embryos spent inside the manipulation pipette should be minimized due to the small volume present in the pipette.

The use of a proper embryo manipulation pipette is crucial for the success of the protocol. Manipulation pipettes can be made from glass capillaries with a thin glass wall. Pasteur pipettes, often used to handle large animal embryos, can also be employed, but due to its shorter distance from the handle to the tip, manipulation pipettes made from glass capillaries are more comfortable to maneuver. The exposure to the flame and speed of pulling determine the wall thickness and inner diameter of the pipette. Although manipulation pipettes can be easily done by hand after some practice, puller and micro-forge may also be used. It is important to invest time in producing several optimal manipulation pipettes. The manipulation pipette aperture should be wider than an embryo to allow a smooth flow, but small enough to easily penetrate and progress through the utero-tubal junction. If *zona pellucida* has been removed before the transfer, blastocysts usually expand to a larger diameter. In this case, it is advisable to make wider pipettes with a wider aperture (130-180 μm) to avoid any damage to the trophectoderm cells. Tip polish is important to avoid damaging the oviduct, uterine wall and the embryo –especially if *zona* has been removed–, and to avoid the pipette tip from being blocked by debris. However, after polishing, the aperture should not be too small compared with the inner diameter of the pipette, as a sharp decrease in diameter will cause abrupt changes in flow speed. Aspirator mouthpiece provides a more precise flow control as well as a more comfortable hand position compared to hand-controlled devices. Nevertheless, hand-controlled device may be used if required (for instance when manipulating lentivirus-treated embryos). For an optimal flow and to avoid the transfer of mineral oil, it is also advisable to use a new pipette for embryo transfer instead of that used to move the embryos from the culture

media to the manipulation media. Finally, while introducing the pipette through the oviduct, the capillary should be handled directly, i.e. grabbing the glass and not the plastic handle of the aspirator, to obtain a firm grip. The magnification used for embryo transfer is a personal matter that depends on the visual acuity of the surgeon. We prefer to use low magnification to have a wide visual field, so we use a 10X final magnification (10X ocular and 1X objective).

The recipients should be at least 8 weeks old and weigh between 27 and 40 g. Outbred mice such as CD1 or Swiss Webster display a good maternal behavior and are excellent recipients. It is advisable to set up breeding to obtain extra recipients, as those not used will recover its normal cycling activity in two weeks. Despite being mated, some females may not have corpora lutea (**Fig. 3G**) and therefore will not be receptive to the transferred embryos. For this reason, ovaries should be checked for the presence of corpora lutea, which at 2.5 dpc can be clearly identified as bright red structures in the ovary (**Fig. 3F**). During the surgery it is important to minimize the contact with the reproductive tract by grabbing ovarian adipose pad instead; excessive manipulation of the ovary and uterus may result in luteolysis. The introduction of the manipulation pipette into the oviduct is the most complicated step of the protocol. In some recipients, the oviduct is very twisted and there is not a 2 mm straight stretch from the junction with the uterus. In that case arrange the oviduct and perform the puncture in the first bend. As detailed above, a nice manipulation pipette really makes a difference. Once the pipette has passed through the utero-tubal junction, it slides easily. If it does not, the pipette may have deviated from the oviduct and not reached the uterine lumen. Once inside the uterus, if the media does not flow, move the pipette slightly out or in and try again. If it still does not flow, the pipette is clogged, take it out from the uterus, release the content in the dish with manipulation media and reload the same or other pipette. Delivery usually takes place 17 days after embryo transfer. To prevent cannibalism, provide nestling material 2 days in advance and do not change the cage during the first days after delivery.

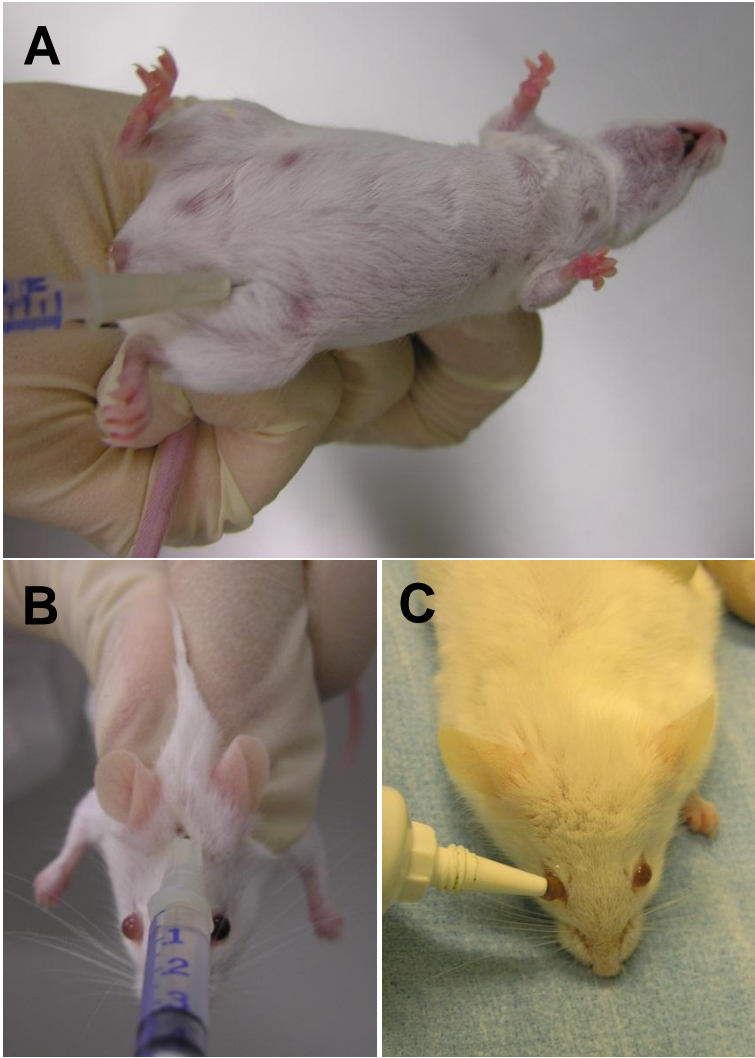
Acknowledgements: This work was supported by funds from the Department of Animal and Avian Sciences to BT.

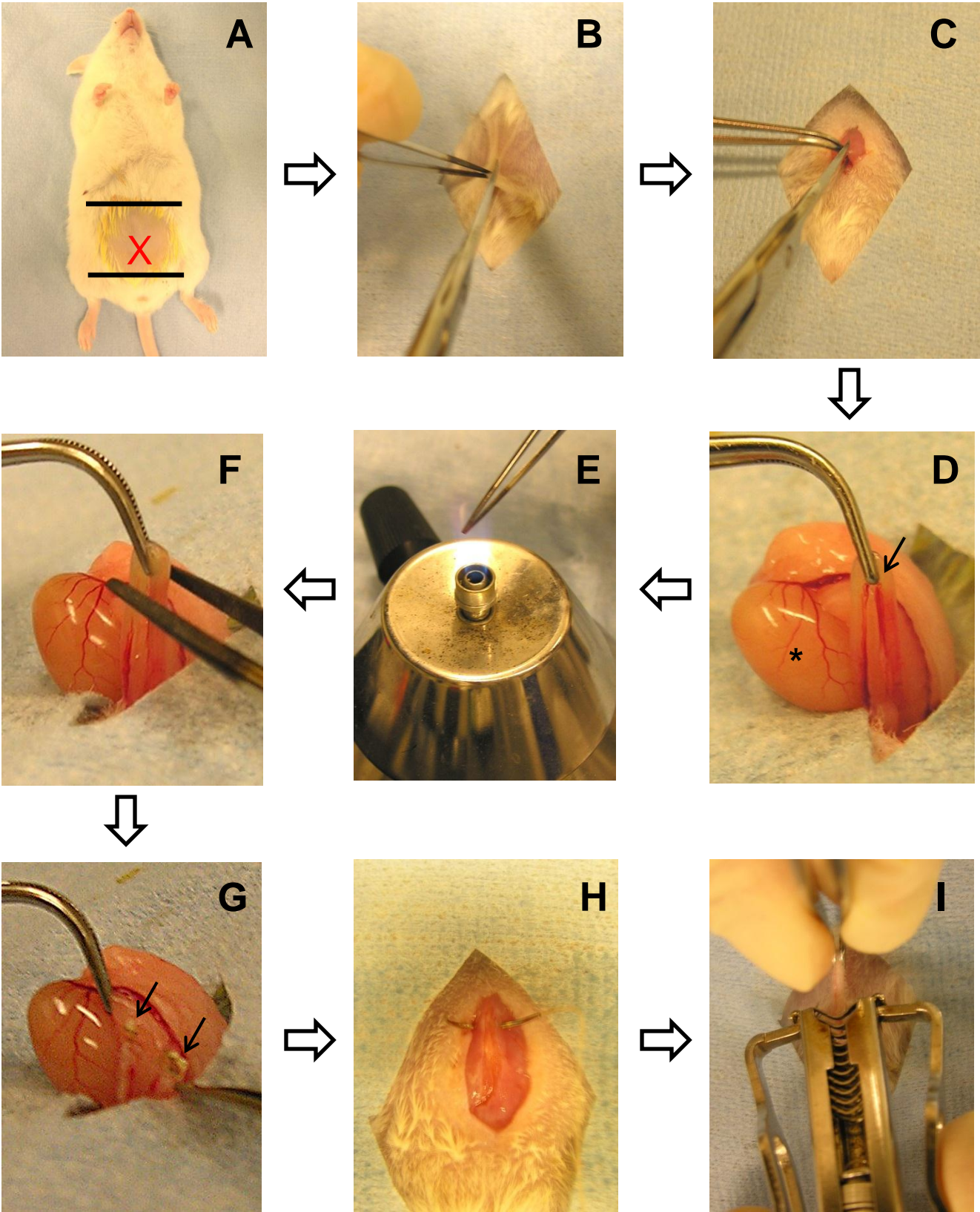
Disclosures: The authors declare no competing financial interests.

References

- 1 Fernandez-Gonzalez, R. *et al.* Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *Proc. Natl. Acad. Sci. USA* **101**, 5880-5885 (2004).
- 2 Bermejo-Alvarez, P., Roberts, R. M. & Rosenfeld, C. S. Effect of glucose concentration during in vitro culture of mouse embryos on development to blastocyst, success of embryo transfer, and litter sex ratio. *Mol. Reprod. Dev.* **79**, 329-336 (2012).
- 3 Tarkowski, A. K. Experiments on the development of isolated blastomers of mouse eggs. *Nature* **184**, 1286-1287 (1959).
- 4 Whittingham, D. G. Fertilization of mouse eggs in vitro. *Nature* **220**, 592-593 (1968).

- 5 McLaren, A. & Biggers, J. D. Successful development and birth of mice cultivated in vitro as early as early embryos. *Nature* **182**, 877-878 (1958).
- 6 McLaren, A. & Michie, D. Studies on the transfer of fertilized mouse eggs to uterine foster-mothers. I. Factors affecting the implantation and survival of native and transferred eggs. *J. Exp. Biol.* **33**, 394-416 (1956).
- 7 Goto, Y. *et al.* The fate of embryos transferred into the uterus. *J. Assist. Reprod. Gen.* **10**, 197-201 (1993).
- 8 Nagy, A., Gertsenstein, M., Vintersten, K. & Behringer, R. *Manipulating the Mouse Embryo: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (2003).
- 9 Chin, H. J. & Wang, C. K. Utero-tubal transfer of mouse embryos. *Genesis* **30**, 77-81 (2001).
- 10 Ramirez, M. A., Fernandez-Gonzalez, R., Perez-Crespo, M., Pericuesta, E. & Gutierrez-Adan, A. Effect of stem cell activation, culture media of manipulated embryos, and site of embryo transfer in the production of F0 embryonic stem cell mice. *Biol. Reprod.* **80**, 1216-1222 (2009).
- 11 Miller, A. M., Wright-Williams, S. L., Flecknell, P. A. & Roughan, J. V. A comparison of abdominal and scrotal approach methods of vasectomy and the influence of analgesic treatment in laboratory mice. *Lab. Anim.* **46**, 304-310 (2012).
- 12 Flecknell, P. A. *Laboratory Animal Anaesthesia*. Academic Press, Oxford, UK, (2009).
- 13 Erhardt, W., Hebestedt, A., Aschenbrenner, G., Pichotka, B. & Blumel, G. A comparative study with various anesthetics in mice (pentobarbitone, ketamine-xylazine, carfentanyl-etomidate). *Res. Exp. Med.* **184**, 159-169 (1984).
- 14 Tarin, D. & Sturdee, A. Surgical anaesthesia of mice: evaluation of tribromo-ethanol, ether, halothane and methoxyflurane and development of a reliable technique. *Lab. Anim.* **6**, 79-84 (1972).
- 15 Zeller, W., Meier, G., Burki, K. & Panoussis, B. Adverse effects of tribromoethanol as used in the production of transgenic mice. *Lab. Anim.* **32**, 407-413 (1998).
- 16 Lieggi, C. C. *et al.* Efficacy and safety of stored and newly prepared tribromoethanol in ICR mice. *Contemp. Top. Lab. Anim. Sci.* **44**, 17-22 (2005).
- 17 Lieggi, C. C. *et al.* An evaluation of preparation methods and storage conditions of tribromoethanol. *Contemp. Top. Lab. Anim. Sci.* **44**, 11-16 (2005).
- 18 Meyer, R. E. & Fish, R. E. A review of tribromoethanol anesthesia for production of genetically engineered mice and rats. *Lab. Anim.* **34**, 47-52, (2005).
- 19 Chatot, C. L., Lewis, J. L., Torres, I. & Ziomek, C. A. Development of 1-cell embryos from different strains of mice in CZB medium. *Biol. Reprod.* **42**, 432-440 (1990).
- 20 Quinn, P., Barros, C. & Whittingham, D. G. Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa. *J. Reprod. Fertil.* **66**, 161-168 (1982).
- 21 de Dios Hourcade, J., Perez-Crespo, M., Serrano, A., Gutierrez-Adan, A. & Pintado, B. In vitro and in vivo development of mice morulae after storage in non-frozen conditions. *Reprod. Biol. Endocrinol.* **10**, 62, (2012).





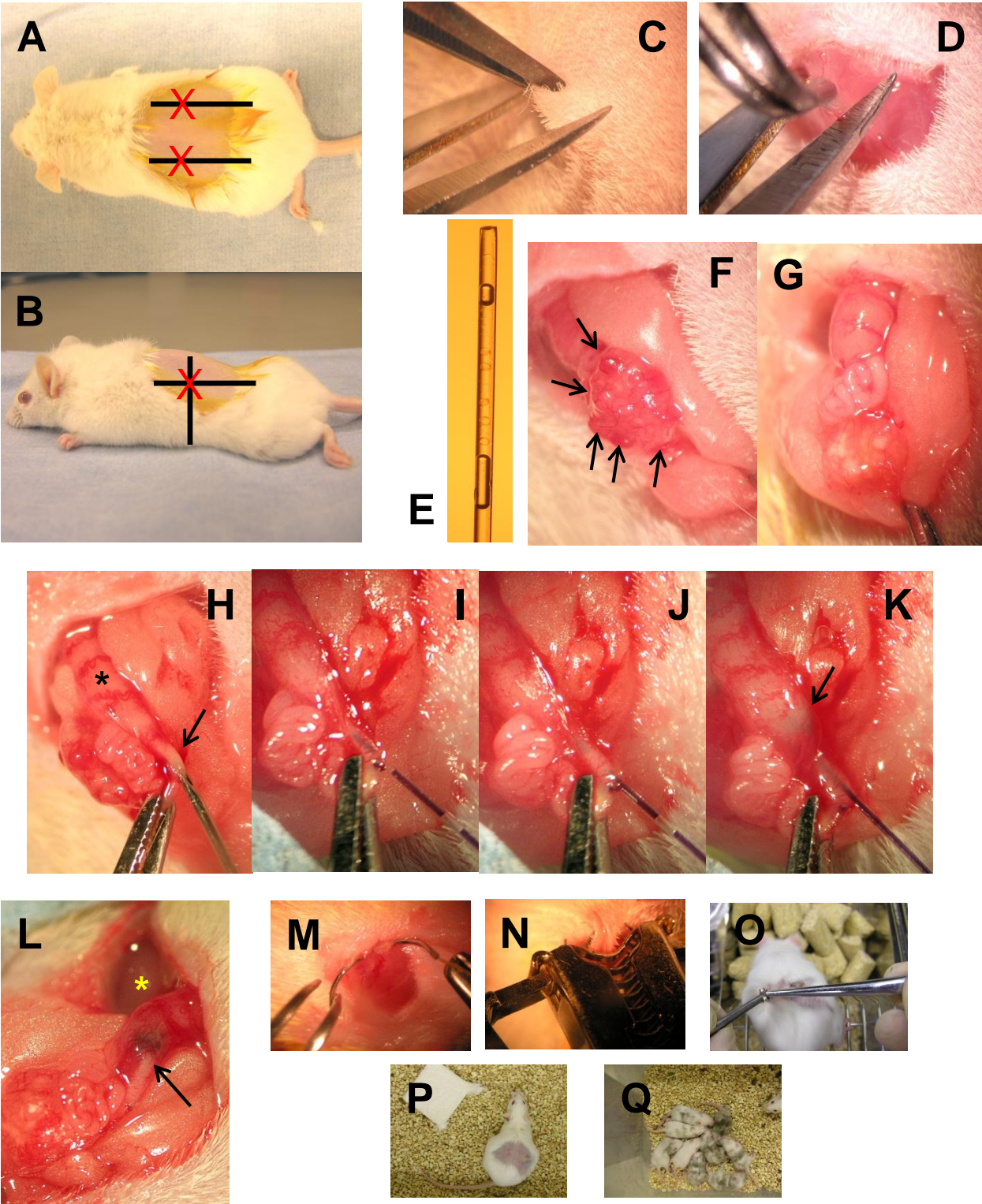


Table
[Click here to download Table: Bermejo-AlvarezetalJoVETable1.xlsx](#)

Treatment	Number of transfers	Embryos transferred	Pups delivered	Survival to term (%)
IVC	11	110	82	74.5
iPSC injection	3	50	35	70.0
Lentivirus	6	60	44	73.3

Name of the material/equipment	Company	Catalog Number
Ketamine	VEDCO	Ketaved ANADA 200-257
Xylazine	Lloyd laboratories	Anased NADA #139-236
Buprenorphine	Generic	NDC 400-42-010-01
Eye ointment	Novartis	Genteal
Antibiotic	Pfizer	Clavamox NADA #55-101.
Dressing serrated forceps	ROBOZ	RS-8120
Micro dissecting serrated forceps	ROBOZ	RS-5137
Slight curved micro dissection forceps	ROBOZ	RS-5136
Scissors	ROBOZ	RS-5880
27G needles	Beckton-Dickinson	305136
Clip applier	MiKRon	42763
9 mm Clips	MiKRon	427631
Clip remover	MiKRon	7637
Suture needle holder	ROBOZ	RS-7820
Suture	Dowist Gell	5-0 Dexon S 7204-21
Glass capillaries	VWR	100 ul calibrated pipettes 53432-921
Burner	KISAG AG	Typ 2002
Stereomicroscope	Leica	MZFLIII
Fiber optics illumination	Dolan Jenner	Fiber lite
Warm stages	American scope	http://store.amscope.com/tcs-100.html
Culture dishes for embryo manipulation	Falcon	353001

Comments/Description (optional)

To be ordered by a licensed veterinarian.

To be ordered by a licensed veterinarian.

To be ordered by a licensed veterinarian.

Added to drinking water (0.3 mg/ml of amoxicillin trihydrate and 0.075 mg/ml of clavulanate potassium). Add 1.5 ml of the reconstituted 15 ml bottle to 250 ml of water.

Any medium size surgical-grade steel straight forceps will work.

These ones are curved at a 90° angle. Straight forceps can be used if preferred.

This model is particularly useful to hold the oviduct.

Any regular surgical grade steel small straight scissors will work.

Smaller needles (30G) can be also used. 25G may be a bit too big.

Two pairs of teeth forceps (ROBOZ RS-8160) can be used instead.

Can be substituted for any 4-0 to 6-0 absorbable suture with a narrow curved needle.

It includes a mouth aspirator system that only requires to attach a 0.22 µm filter in the tubing to be ready to use.

More information can be obtained in ref. 8.

Gas operated burner, can be charged with Kigas (CH-4512, from the same vendor). Alcohol burners may be also used, but gas provides a higher temperature and this burner provides a small and precise flame.

This is an expensive stereomicroscope with fluorescence, that can be also used for other purposes. There are cheaper options such as Leica MZ8 or Nikon SMZ-10 or SMZ-2B, to name a few. It is better to use two stereomicroscopes, one for handling the embryos (which does not need to be a very nice one) and another one for the recipient. The one used for the recipient should display a long distance from the stage plate to the objective lense, in order to be able to focus 3-4 cm above the stage plate (where the oviduct will be placed) and still leave some room for the surgeon; most of the stereomicroscopes can do this, but some cannot.

To illuminate the surgical area. There are different systems available.

These can be placed over the stage plate of the stereomicroscope. Some modifications (inserting a stick to level the stage) may be needed if it is too short for the stereomicroscope. A big warm stage can be used for warming the cage if it is available. If not, a regular heating pad can be used, but temperature must be checked.

351008 may be also used, they made narrower drops.

Reagent	Sigma catalogue	mM (MW)	g/500 ml
NaCl	S5886	82 (58.44)	2.385
KCl	P5405	4.83 (74.55)	0.1815
KH ₂ PO ₄	P5655	1.18 (136.09)	0.0795
MgSO ₄	M2643	1.18 (120.4)	0.071
NaHCO ₃	S5761	3.48 (84)	0.146
Na pyruvate	P4562	0.26 (110)	0.0143
HEPES (acid free)	H6147	20 (238.3)	2.383
L-glutamine	G8540	1 (146.15)	0.073
Na ₂ EDTA·2H ₂ O	E6635	0.1 (372.2)	0.0186
CaCl ₂ ·2H ₂ O*	C7902	1.7 (147.2)	0.1255
Na lactate (60% syrup)	L7900	52.16 (112.1)	2.923 ml
Penicillin- streptomycin	Gibco 15140- 122	500 ug/ml	2.5 ml
Phenol red	P0290		1 ml

*Dissolve it in water before adding.
pH is adjusted with 1N NaOH to 7.4.
Osmolarity should be 280-290.
Add 2.5 g of BSA (A3311), dissolve and filter.



17 Sellers Street
Cambridge, MA 02139
tel: +1.617.945.9051
www.JoVE.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

UTERO-TUBAL EMBRYO TRANSFER AND VASECTOMY IN THE MOUSE MODEL

Author(s):

BERMEJO-ALVAREZ P., PARK K.E., TELUGU B.P.

Item 1 (check one box): The Author elects to have the Materials be made available (as described at <http://www.jove.com/publish>) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):

- ☐ The Author is NOT a United States government employee.
- ☒ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.
- ☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "Agreement" means this Article and Video License Agreement; "Article" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "Author" means the author who is a signatory to this Agreement; "Collective Work" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "CRC License" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; "Derivative Work" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "Institution" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "JoVE" means MyJoVE Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; "Materials" means the Article and / or the Video; "Parties" means the Author and JoVE; "Video" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to Sections 4 and 7 below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in Item 1 above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in Section 3 above, the

ARTICLE AND VIDEO LICENSE AGREEMENT

Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict

shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including,

ARTICLE AND VIDEO LICENSE AGREEMENT

without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or

damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

AUTHOR:

Name:

Pablo Bermejo-Alvarez

Department:

Animal and Avian Sciences

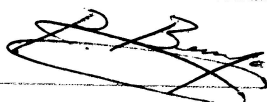
Institution:

University of Maryland

Article Title:

Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model

Signature:



Date:

04/25/2013

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy as a PDF to the JoVE submission site upon manuscript submission (preferred);
- 2) Fax the document to +1.866.381.2236; or
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 17 Sellers St / Cambridge, MA 02139

For questions, please email editorial@jove.com or call +1.617.945.9051.

MS # (internal use):

ARTICLE AND VIDEO LICENSE AGREEMENT

without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or

damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

AUTHOR:

Name:

Ki-Eun Park

Department:

Animal and Avian Sciences

Institution:

University of Maryland

Article Title:

Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model

Signature:



Date:

04/25/2013

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy as a PDF to the JoVE submission site upon manuscript submission (preferred);
- 2) Fax the document to +1.866.381.2236; or
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 17 Sellers St / Cambridge, MA 02139

For questions, please email editorial@jove.com or call +1.617.945.9051.

MS # (internal use):

ARTICLE AND VIDEO LICENSE AGREEMENT

without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or

damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

AUTHOR:

Name:

Bhanu Prakash Telugu

Department:

Animal and Avian Sciences

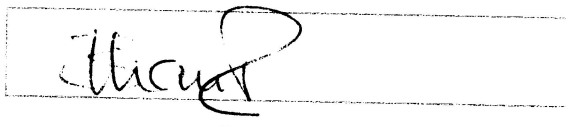
Institution:

University of Maryland

Article Title:

Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model

Signature:



Date:

04/25/2013

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy as a PDF to the JoVE submission site upon manuscript submission (preferred);
- 2) Fax the document to +1.866.381.2236; or
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 17 Sellers St / Cambridge, MA 02139

For questions, please email editorial@jove.com or call +1.617.945.9051.

MS # (internal use):

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 paragraph (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 incorrect. 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 incision 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 access. 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 Welfare Officers. Scrotal vasectomy is easily performed with a minimum 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 priori even though it does not demonstrate this technique is better than the traditional approach 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 is worth 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:

Authors 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 right 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.

Authors should change "transgenic" by "genetically modified", lines 38 and 55. For several years now, Transgenic refers exclusively to additive 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 reference by Ann McLaren (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.