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Title: Spatula Montevideo Device for the Vitrification of Mammalian Embryos

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, done** **NOTE:** They have filmed the microscope-system's screen for the shots performed under microscope, hence they are labeled as SCREEN. Footage was reviewed for scope/screen capture shots only and added

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **06/02/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 19
Number of Shots: 37 (6 SC)

Introduction

1.1. **Geraldine Schlapp:** We generate genetically modified mouse models and then cryopreserve the lines as a backup. For this, we need an easy, low-cost, efficient and reproducible method of vitrifying embryos.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1* 68176_ screenshot_Interview 1.1

What are the most recent developments in your field of research?

1.2. **Geraldine Schlapp:** Microinjection or electroporation of CRISPR-Cas9 could be done in vitrified-warmed mouse and livestock zygotes, resulting in a more flexible schedule and reducing the animals in use.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1* 68176_ screenshot_Interview 1.2

What technologies are currently used to advance research in your field?

1.3. **María Noel Meikle:** Embryo vitrification uses ultra-rapid cooling to prevent ice crystal formation, preserving cellular integrity. This technique advances cryobiology research, enabling studies on embryo viability, genetic stability, and long-term storage outcomes

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1* 68176_ screenshot_Interview 1.3

What advantage does your protocol offer compared to other techniques?

1.4. **María Noel Meikle:** Both the Spatula MVD and the solutions are home-made. The Spatula is sealed with a 0.5 mL straw, taking up minimal space in the nitrogen dewar.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1* 68176_ screenshot_Interview 1.4

Ethics Title Card

This research has been approved by the Institut Pasteur de Montevideo Animal Care and Use Committee (permit number #007-18) and the Institutional Animal Care Committee Fundación IRAUy (protocol #001–2017).

Protocol

NOTE: The APF filenames and timestamps are added as provided by the authors. The writer has not reviewed the footage for regular talent shots. The authors labeled protocol file names from 1.1 onwards by mistake, instead of 2.1 (Example: filename 1.1.1 is actually shot 2.1.1 and so on.). **Please refer to the correct filename added against each shot below in red font**.

2. Spatula Montevideo (MVD) Device Preparation

Demonstrator: María Noel Meikle

2.1. To begin, cut off the first 10 millimeters and the tip cone of a gel-loader tip **[1]**.

2.1.1. WIDE: Talent using scissors or blade to cut off 10 millimeters and the tip cone of the gel-loader tip. 68176_ screenshot_1.1.1.mp4 00:00-00:22

2.2. Using watchmaker's number 5 forceps, hold the end of the gel-loader tip approximately 1 centimeter from the tip **[1]** and gently place it into the Bunsen burner flame for 8 to 10 seconds to form a petal-like platform **[2]**.

2.2.1. Talent holding the gel-loader tip with forceps 1 centimeter from the end. 68176_ screenshot_1.2.1 and 1.2.2 and 1.3.1. 00:00-00:07

2.2.2. Talent placing the tip into a Bunsen burner flame and maintaining it to form a flat platform. . 68176_ screenshot_1.2.1 and 1.2.2 and 1.3.1. 00:08-00-19

2.3. Now, remove the tip from the flame and continue holding it tightly with the forceps for 5 seconds **[1]**.

2.3.1. Talent removing the tip from the flame and maintaining grip with forceps for 5 seconds. 68176_ screenshot_1.2.1 and 1.2.2 and 1.3.1. 00:20-00:26

2.4. Using a stereomicroscope, check that the petal-like platform of approximately 1 square millimeter has formed **[1]** and that the distal edge is sealed **[2]**.

2.4.1. SCREEN: 68176_ screenshot_1.4.1

2.4.2. SCREEN: 68176_ screenshot_1.4.2.

~~2.5. Repeat the spatula fabrication steps to prepare the required number of spatulas, with each spatula capable of handling up to 40 mouse embryos **[1]**.~~

~~2.5.1. Shot of multiple spatulas arranged on the work surface.~~ **NOTE**: Delete the shot and VO

2.6. Sterilize the fabricated spatulas using ultraviolet rays and store them in a sterile tube or box **[1]**.

2.6.1. Talent placing the finished spatulas under a UV chamber. **TXT: Alternatively, use ethylene oxide for sterilization** 68176_ screenshot_1.6.1 00:00-00:13

~~2.6.2. Talent transferring the sterilized spatulas to a sterile box.~~ **NOTE**: Not filmed, VO merged with the previous shot

2.7. On the day of vitrification, label as many rods as spatulas to vitrify with a permanent marker or a liquid nitrogen-resistant sticker label **[1]**. One by one, retrieve the spatulas, handling them from the opposite end of the platform **[2]**, insert an identification rod into each spatula **[3]** and place them on the stereomicroscope base **[4]**.

2.7.1. Talent labeling rods using permanent markers or stickers 68176_ screenshot_1.7.1 to 1.7.4. 00:00-00:10

2.7.2. Talent picking up sterilized spatulas by the opposite end of the platform. 68176_ screenshot_1.7.1 to 1.7.4. 00:11-00:14

2.7.3. Talent inserting identification rod into a spatula. 68176_ screenshot_1.7.1 to 1.7.4. 00:15-00:17

2.7.4. Talent placing spatulas on the microscope base. 68176_ screenshot_1.7.1 to 1.7.4 . 00:18-00:24

3. Embryo Dehydration and Vitrification Process

Demonstrator: Geraldine Schlapp

3.1. Pour liquid nitrogen into an insulated polystyrene container measuring 10 by 30 by 20 centimeters **[1]**. Place one 0.5 milliliter straw for each spatula into the container **[2]**.

3.1.1. Talent pouring liquid nitrogen into the container. 68176_ screenshot_2.1.1. 00:00-00:22

3.1.2. Talent placing straws into the liquid nitrogen. 68176_ screenshot_2.1.2. 00:00-00:05

3.2. For each spatula, prepare drops of 100 microliters of M2 solution, 20 microliters of PV solution, and 20 microliters of V *(We)* solution on a 100-millimeter plastic Petri dish **[1-**

TXT].

3.2.1. Talent pipetting and placing the respective volumes of M2, PV, and V solutions onto the Petri dish. **TXT: PV: Pre-vitrification; V: Vitrification** 68176_ screenshot_2.2.1. 00:00-00:59

3.3. Under the stereomicroscope, place 30 to 40 embryos into the M2 drop **[1]**. Using a pulled glass capillary attached to an aspirator tube assembly, pre-load the capillary with M2 medium and at least 1 microliter of PV solution **[2]**. Aspirate the embryos and incubate them in the PV solution drop for 30 seconds **[3]**. Load the capillary with at least 1 microliter of V solution **[4]** and transfer the embryos to the V solution drop, incubate for another 30 seconds at room temperature **[5]**.

3.3.1. Talent transferring embryos into the M2 drop. 68176_ screenshot_2.3.1 00:00-00:05

3.3.2. Talent preparing the glass capillary, attached to an aspirator assembly, by drawing M2 and at least 1 microliter of PV solution 68176_ screenshot_2.3.2 to 2.3.5 .00:00-00:06

3.3.3. Talentmoving embryos into PV solution. 68176_ screenshot_2.3.2 to 2.3.5 .00:07-00:29

3.3.4. Talent loading the glass capillary with 1 microliter of V solution. 68176_ screenshot_2.3.2 to 2.3.5 .00:30-00:37

3.3.5. Talent transferring embryos into V solution. 68176_ screenshot_2.3.2 to 2.3.5 .00:38-01:19

3.4. Aspirate the embryos using minimal V solution, approximately 0.1 microliter **[1]**, and quickly place them onto the spatula platform **[2]**.

3.4.1. Talent aspirating embryos with the pre-loaded capillary using minimal V solution. 68176_ screenshot_2.4.1 to 2.5.3 00:00-00:18

3.4.2. Talent transferring embryos quickly onto the spatula platform. 68176_ screenshot_2.4.1 to 2.5.3. 00:19-00:39

3.5. Immediately immerse the spatula platform into liquid nitrogen for 5 seconds while holding the spatula by its rod **[1]**. Retrieve a 0.5 milliliter straw in the container using long stainless-steel tweezers and remove the LN-2 from it **[2].** Lift up the spatula a little bit, maintaining it in the LN_2 vapor and seal it with the straw **[3]**.

3.5.1. Talent immersing the spatula platform into the liquid nitrogen container. 68176_ screenshot_2.4.1 to 2.5.3. 00:40-00:49

3.5.2. Talent removing LN_2 from a straw before sealing to prevent expansion during warming. 68176_ screenshot_2.4.1 to 2.5.3. 00:50-00:52

3.5.3. Talent sealing the spatula in a straw using tweezers while maintaining it in nitrogen vapor. 68176_ screenshot_2.4.1 to 2.5.3. 00:53-01:04

4. Embryo Warming Process

Demonstrator: María Noel Meikle

4.1. On a 100-millimeter plastic Petri dish, place the required sucrose drops for each spatula to be warmed **[1-TXT]** and add three 100 microliter drops of M2 medium for washing **[2]**.

4.1.1. Shot of adding the drops onto a plate. **TXT: 500 µL drop of 0.5 M sucrose; 50 µL drop of 0.5 M sucrose; 50 µL drop of 0.25 M sucrose** 68176_ screenshot_3.1.1 00:00-00:47

4.1.2. Shot of adding three 100 microliter M2 medium drops on the same Petri dish containing sucrose drops. 68176_ screenshot_3.1.2. 00:00-00:18

4.2. Retrieve the sealed spatulas from the dewar **[1]** and place them in an appropriate container filled with liquid nitrogen **[2]**.

4.2.1. Talent opening the dewar, removing a visotube containing spatulas. 68176_ screenshot_3.2.1 to 3.2.2. 00:00-00:17

4.2.2. Talent transferring the visotube into a container with liquid nitrogen. 68176_ screenshot_3.2.1 to 3.2.2. 00:18-00:33

4.3. Now, place the warming plate on the stereomicroscope **[1]**. One at a time, hold the base of the spatula with stainless-steel tweezers **[2]**. With a gloved hand, pull out the identification rod and then the straw **[3]**. Quickly dip the platform containing the embryos into the 500-microliter drop of 0.5 molar sucrose **[4-TXT]**.

4.3.1. SCREEN: 68176_ screenshot_3.3.1. 00:00-00:19

4.3.2. Talent holding the base of spatula with tweezers. 68176_ screenshot_3.3.2 to 3.3.4 . 00:00-00:04

4.3.3. Talent separating the straw using gloved hand. 68176_ screenshot_3.3.2 to 3.3.4 . 00:04-00:14

4.3.4. SCREEN: 68176_ screenshot_3.3.2 to 3.3.4 00:15-00:18 and 01:02-01:06 **TXT:**

Ensure all embryos fall into the sucrose drop

4.4. ~~. If necessary, gently move the spatula to aid their release **[1].**~~ Then, load the embryos into a pulled glass capillary **[1]** and place them into the 50-microliter drop of 0.5 molar sucrose solution **[2]** and incubate for 2 minutes **[3]**. **NOTE**: VO adjusted

~~4.4.1. Talent adjusting the spatula to release embryos into the solution.~~

4.4.2. Talent collecting embryos with a glass capillary. 68176_ screenshot_3.4.2 and 3.4.3. 00:00-00:12

4.4.3. Talent transferring embryos to the smaller 0.5 molar sucrose. 68176_ screenshot_3.4.2 and 3.4.3. 00:13-00:21

Added shot: SCREEN: 68176_ screenshot_3.4.4 00:00-00:12

4.5. Next, place the embryos into the 50-microliter drop of 0.25 molar sucrose solution and incubate for another 2 minutes **[1]**.

4.5.1. SCREEN: 68176_ screenshot_3.5.1 00:10-00:18

4.6. Finally, wash the embryos three times in separate drops of M2 medium to remove sucrose **[1]** and incubate them under conditions appropriate **[2]** for the species for at least 1 hour before use **[3]**.

4.6.1. Talent transferring embryos through three sequential M2 medium drops. 68176_ screenshot_3.6.1 00:00-00:50

Added shot: SCREEN: 68176_ screenshot_3.6.2 00:00-00:06

4.6.2. Talent placing embryos in an incubator. 68176_ screenshot_3.6.3. 00:00-00:12

Results

5. Results

5.1. In both in vivo derived 8-cell and in vitro produced 2-cell mouse embryos, spatula MVD resulted in a significantly higher survival rate compared to the slow freezing method **[1]**, and a significantly higher embryo development rate at 3.5 days post coitum **[2]**.

5.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled "Spatula MVD" under the column "Survival rate at 2.5 dpc".*

5.1.2. LAB MEDIA: Table 1. *Video editor: Highlight the "Embryo development rate at 3.5 dpc" column value for "Spatula MVD row"*.

5.2. Despite both cryopreservation methods yielding hatching rates, these were significantly lower **[1]** than in the control fresh embryo group **[2]**.

5.2.1. LAB MEDIA: Table 1. *Video editor: Highlight the columns "Hatching rate at 5.5 dpc" for rows Spatula MVD and Slow Freezing rows*.

5.2.2. LAB MEDIA: Table 1. *Video editor: Highlight the column value "Hatching rate at 5.5 dpc" for "Fresh embryos" row*.

5.3. No significant differences were observed in pregnancy and birth rates between vitrified, warmed and fresh 2-cell embryos transferred to recipient mice **[1]**.

5.3.1. LAB MEDIA: Table 2. *Video editor: Highlight the pregnancy rate and birth rate (last 2) columns*.

5.4. In sheep embryos, survival and hatching rates at both 3 hours and 24 hours post-warming were similar between Spatula MVD and a commercial device **[1]**, but both were significantly lower than those of the fresh embryo control group in terms of blastocyst development by day 8 **[2]**.

5.4.1. LAB MEDIA: Table 3. *Video editor: Highlight the "survival rates" and "hatching rates" columns for both Spatula MVD and Commercial device rows*.

5.4.2. LAB MEDIA: Table 3. *Video editor: Highlight the "Development rate" and "Hatching rate" for the Control group "row"*.

5.5. Across various genetically modified mouse lines, Spatula MVD vitrification resulted in an average recovery rate of 90.4%, survival rate of 96.8%, pregnancy rate of 80%, and birth rate of 31.5%, with variability likely due to genetic background differences **[1]**.

5.5.1. LAB MEDIA: Table 5. *Video editor: Highlight the average row at the bottom*.

1. vitrification

Pronunciation link:
https://www.merriam-webster.com/dictionary/vitrification YouTubeOxford Learner's Dictionaries+12Merriam-Webster+12Collins Dictionary+12
IPA: /ˌvɪt.rəˌfiˈkeɪ.ʃən/
Phonetic spelling: vit-ruh-fi-KAY-shuhn

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2. polystyrene

Pronunciation link:
https://www.merriam-webster.com/dictionary/polystyrene Wikipedia+12Merriam-Webster+12Merriam-Webster+12
IPA: /ˌpɑː.lɪˈstaɪ.riːn/
Phonetic spelling: pah-li-STY-reen

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3. aspirator

Pronunciation link:
https://www.merriam-webster.com/dictionary/aspirator Merriam-Webster+14Merriam-Webster+14Merriam-Webster+14
IPA: /ˈæs.pəˌreɪ.ɾɚ/
Phonetic spelling: AS-puh-RAY-tuhr

1. distal

Pronunciation link: https://www.merriam-webster.com/dictionary/distal Collins Dictionary+8Definitions+8Cambridge Dictionary+8
IPA: /ˈdɪs.təl/
Phonetic Spelling: DIS-tuhl

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2. Dewar

Pronunciation link: https://www.merriam-webster.com/dictionary/dewar Merriam-Webster
IPA: /ˈduː.ɑr/
Phonetic Spelling: DOO-ar

3. ethylene

Pronunciation link: https://www.merriam-webster.com/dictionary/ethylene Merriam-Webster
IPA: /ˈɛθ.əˌliːn/
Phonetic Spelling: ETH-uh-leen

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4. microliter

Pronunciation link: https://www.merriam-webster.com/dictionary/microliter Merriam-Webster
IPA: /ˈmaɪ.kroʊˌliː.tər/
Phonetic Spelling: MY-kroh-LEE-tuhr

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5. molar

Pronunciation link: https://www.merriam-webster.com/dictionary/molar Merriam-Webster
IPA: /ˈmoʊ.lɚ/
Phonetic Spelling: MOH-luhr

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6. capillary

Pronunciation link: https://www.merriam-webster.com/dictionary/capillary Merriam-Webster
IPA: /ˈkæp.əˌlɛr.i/
Phonetic Spelling: KAP-uh-L AIR-ee

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7. aspirator

Pronunciation link: https://www.merriam-webster.com/dictionary/aspirator Merriam-Webster
IPA: /ˈæs.pəˌreɪ.tɚ/
Phonetic Spelling: AS-puh-RAY-tuhr

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8. polystyrene

Pronunciation link: https://www.merriam-webster.com/dictionary/polystyrene Merriam-Webster
IPA: /ˌpɑ.lɪˈstaɪ.riːn/
Phonetic Spelling: pah-lee-STYLE-reen

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9. vitrification

Pronunciation link: https://www.merriam-webster.com/dictionary/vitrification Merriam-Webster
IPA: /ˌvɪt.rəˌfɪˈkeɪ.ʃən/
Phonetic Spelling: vit-ruh-fi-KAY-shuhn

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10. semivitrification

Pronunciation link: https://www.merriam-webster.com/dictionary/semivitrification Merriam-Webster
IPA: /ˌsɛ.miˌvɪt.rəˌfɪˈkeɪ.ʃən/
Phonetic Spelling: SEM-ee-vit-ruh-fi-KAY-shuhn