FINAL SCRIPT: APPROVED FOR FILMING

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Title: A Simple Microaspiration Technique for Isolating Somatic Cells from Cryopreserved Equine Semen as Nuclear Donors for Cloning

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**

   Authors: Please create scope videos of the shots labeled as SCOPE and upload the files to your project page as soon as possible: **https://review.jove.com/account/file-uploader?src=21168268**

   SCOPE: 3.2.1, 3.2.2, 3.2.3, 3.3.1, 3.3.2,3.3.3, 3.3.4, 3.4.1, 3.4.2, 3.6.1, 3.6.2, 3.6.3, 3.7.1

   NOTE: SCOPE shots were shot by the videographer

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length
Number of Steps: 11
Number of Shots: 28

Introduction

Videographer: Obtain headshots for all authors available at the filming location.
Videographer's Note: Headshot "EEC_4360" is Dr. José Ernesto Hernández Pichardo

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Alejandro Ávalos Rodríguez:** We aim to show that somatic cells from cryopreserved equine semen can be useful to recover individuals of high genetic value.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll:4.1.1*~~
Videographer's Note: Is unslated in clip "EEC_4335"

~~What are the current experimental challenges?~~

1.2. **Boris Ramos Serrano:** Our current challenge is to be able to select somatic cells with intact, diploid DNA to be used as nuclear donors.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll:3.6*~~

CONCLUSION:

~~What significant findings have you established in your field?~~

1.3. **Alejandro Ávalos Rodríguez:** We identified a population of somatic cells that differs in morphology and viability in cryopreserved equine semen, which may be useful as nuclear donors.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What advantage does your protocol offer compared to other techniques?~~

1.4. **Boris Ramos Serrano:** The cells can be selected based on their morphology, and the procedure does not require the establishment of primary cultures.

~~1.4.1.~~ INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll:3.2.3*~~

~~What questions will future research focus on?~~

1.5. **Boris Ramos Serrano:** We will focus on improving the viability of these cells after thawing equine semen.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the the *Norma Oficial Mexicana NOM-027-ZOO-1995: Proceso Zoosanitario del Semen de Animales Domésticos*, issued by the Secretaría de Agricultura, Ganadería y Desarrollo Rural

Protocol

Videographer's Note: The SCOPE shots were recorded in 4K, so there is more room for close up. All the SCOPE shots are numbered by voice.

2. Preparation of Cryopreserved Equine Semen for Somatic Cell Isolation

Demonstrator: Boris Ramos Serrano

2.1. To begin, remove the equine semen straws from the liquid nitrogen tank **[1]**. Expose them to air for 10 seconds **[2]** and thaw them in a water bath at 37 degrees Celsius for 30 seconds **[3]**.

2.1.1. WIDE: Talent opening the liquid nitrogen tank and removing semen straws using protective gloves.

2.1.2. Talent holding the straws in ambient air for 10 seconds.

2.1.3. Talent placing the straws into a 37 degrees Celsius water bath.

2.2. Take 250 microliters sample of the thawed semen **[1].** Dilute it with 1 milliliter of supplemented DMEM/F12 *(D-M-E-M-F-Twelve)* medium **[2]**. Incubate the semen mixture at 37 degrees Celsius for 30 minutes **[3]**.

2.2.1. Talent pipetting 250 microliters of thawed semen into a tube.

2.2.2. Talent pipetting 1 milliliter of supplemented DMEM/F12 medium.
AND
TEXT ON PLAIN BACKGROUND:
DMEM-F12 supplementation:
10% FBS
0.1 mg/mL BSA
12.5 µM β-mercaptoethanol
1% antibiotic-antimycotic
Video Editor: Please play both shots side by side in a split screen

2.2.3. Talent placing the tube containing the semen dilution into a 37 degrees Celsius incubator.

2.3. Pipette out 25 microliters from the incubated dilution into a tube **[1]** and dilute it in 975 microliters of DPBS supplemented with 0.1 milligram per milliliter polyvinyl alcohol or polyvinylpyrrolidone **[2]**. ~~Deposit this mixture into a 1.5-milliliter microcentrifuge tube **[3]** and~~ Gently mix the suspension **[4]**.

2.3.1. Talent pipetting 25 microliters from the first dilution into a fresh tube.

2.3.2. Talent pipetting 975 microliters of DPBS with PVA or PVP into the sample aliquot.

~~2.3.3. Talent transferring the mixture into a 1.5 mL microcentrifuge tube.~~
Videographer's Note: Shot deleted

2.3.4. Talent gently mixing the contents in the microcentrifuge tube.

2.4. Maintain the diluted semen mixture at 37 degrees Celsius throughout the entire cell collection process **[1]**.

2.4.1. Talent placing the tube containing the final dilution into a 37 degrees Celsius warming block.

3. Micromanipulation and Washing of Somatic Cells Isolated from Cryopreserved Equine Semen

3.1. Place a Petri dish lid containing diluted equine semen on the microscope stage to prepare for somatic cell collection **[1]**. Select a micropipette of suitable diameter for somatic cell collection and connect it to the mechanical aspiration system **[2]**.

3.1.1. Talent positioning the ~~labeled~~ lid onto the microscope stage.

3.1.2. Talent selecting a micropipette and attaching it to the aspiration setup.

3.2. Carefully insert the micropipette into the microdrop and align it with the dish lid base **[1]**. Once the pipette aspirates culture medium **[2]**, begin individual capture of somatic cells **[3]**.
NOTE: SCOPE shots were shot by the videographer

3.2.1. SCOPE: Talent inserting the micropipette into the microdrop and adjusting its position near the bottom of the lid.

3.2.2. SCOPE: Shot of the pipette aspirating the culture medium.

3.2.3. SCOPE: Shot of the somatic cells

3.3. Focus on the bottom of the microdrop where various cell morphologies are visible **[1]**. Position the micropipette tip next to each somatic cell **[2]** and aspirate under negative pressure **[3]**. Collect all round and elongated cells regardless of size **[4]**.

3.3.1. SCOPE: Shot showing different cell morphologies within the microdrop.
Videographer's Note:
- 3.3.1 Take 1: Elongated Cell
- 3.3.1 Take 2: Squamous Cell
- 3.3.1 Take 3: Large Round Cell
- 3.3.1 Take 4: (Clip EEC_4331) Small Round Cell

3.3.2. SCOPE: Shot of the micropipette tip being positioned next to an individual cell.

3.3.3. SCOPE: Shot of the cell being aspirated.

3.3.4. SCOPE: Shot of aspiration of both round and elongated cells into the micropipette.
Videographer's Note: Shot split into two. 3.3.4 : Shot of of aspiration of round cell into de micropipette and 3.3.5: Shot of of aspiration of elongated cell into de micropipette

3.4. After collecting 10 cells, deposit them into a 10 microliter microdrop located on the upper surface of the dish lid **[1-TXT]**. Retain all collected somatic cells within this microdrop during the entire collection process **[2]**.

3.4.1. SCOPE: Talent releasing 10 collected cells into the designated microdrop.**TXT: Repeat until required number of cells for cloning is obtained**

3.4.2. SCOPE:: Shot of the microdrop
Videographer's Note: Takes 2-4: Take 2: View of the 2 microdrops on the microscope stage

3.5. Once the desired number of somatic cells is collected, perform a simple washing procedure using microdrops to remove sperm, debris, and microbial contaminants **[1]**. Using a second dish lid, perform a sequential three-step washing of somatic cells **[2]**.

3.5.1. Talent preparing for washing by arranging microdrops for sequential cleaning.

3.5.2. Talent placing a new lid onto the microscope stage with three microdrops arranged.

3.6. Use a micropipette to transfer the somatic cells into the first drop and allow them to settle for 3 to 5 minutes **[1]**. Transfer the cells from the first drop to the second drop using the same procedure **[2]**. Repeat the transfer once more and place the somatic cells into the third drop **[3]**.

3.6.1. SCOPE: Talent transferring somatic cells into the first drop and observing settling under the microscope.

3.6.2. SCOPE: Talent aspirating cells from the first drop and releasing them into the second drop.

3.6.3. SCOPE: Talent moving somatic cells from the second drop into the third drop.
Added shot: 3.6.4: View of the 3 microdrops on the microscope stage

3.7. Finally, deposit the isolated somatic cells into a prepared microdrop designated for somatic cell nuclear transfer **[1]**.

3.7.1. SCOPE: Talent releasing the fully washed somatic cells into the final microdrop for nuclear transfer use.

Results

4. Results

4.1. The microaspiration technique successfully recovered somatic cells from cryopreserved equine semen, with capture rates ranging from 317.7 to 424.7 cells per hour across three stallions **[1]**. Stallion number 3 showing the highest yield **[2]**.

4.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the entire "Average of somatic cells capture/hour" column for stallions #01, #02, and #03.*

4.1.2. LAB MEDIA: Table 1. *Video editor: Highlight the row for stallion #03*

4.2. Five distinct somatic cell types were identified based on size and morphology **[1]**.

4.2.1. LAB MEDIA: Figure 3 *Video Editor: Please sequentially show A to D panels*

Pronunciation Guide:

 Microaspiration
Pronunciation link: https://www.merriam-webster.com/dictionary/microaspiration
IPA: /ˌmaɪ.kroʊˌæs.pəˈreɪ.ʃən/
Phonetic Spelling: my·kroh·as·puh·ray·shuhn

 Cryopreserved
Pronunciation link: https://www.merriam-webster.com/dictionary/cryopreserved
IPA: /ˌkraɪ.oʊ.prɪˈzɝːvd/
Phonetic Spelling: kry·oh·prih·zervd

 Equine
Pronunciation link: https://www.merriam-webster.com/dictionary/equine
IPA: /ˈiː.kwaɪn/
Phonetic Spelling: ee·kwyne

 Somatic
Pronunciation link: https://www.merriam-webster.com/dictionary/somatic
IPA: /soʊˈmæt̬.ɪk/
Phonetic Spelling: soh·mat·ik

 Micromanipulation
Pronunciation link: https://www.merriam-webster.com/dictionary/micromanipulation
IPA: /ˌmaɪ.kroʊ.məˌnɪp.jəˈleɪ.ʃən/
Phonetic Spelling: my·kroh·muh·nip·yuh·lay·shuhn

 Microcentrifuge
Pronunciation link: https://www.merriam-webster.com/dictionary/microcentrifuge
IPA: /ˌmaɪ.kroʊˈsɛn.trəˌfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj

 Microliters
Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪ.kroʊˌliː.ţɚ/
Phonetic Spelling: my·kroh·lee·ter

 Diploid
Pronunciation link: https://www.merriam-webster.com/dictionary/diploid
IPA: /ˈdɪp.lɔɪd/
Phonetic Spelling: dip·loyd

 Micropipette
Pronunciation link: https://www.merriam-webster.com/dictionary/micropipette
IPA: /ˌmaɪ.kroʊ.pɪˈpɛt/
Phonetic Spelling: my·kroh·pih·pet

 Polyvinylpyrrolidone
Pronunciation link: https://www.howtopronounce.com/polyvinylpyrrolidone
IPA: /ˌpɑː.liˌvaɪ.nəl.pɪˈrɑː.lɪˌdoʊn/
Phonetic Spelling: pah·lee·vy·nuhl·pih·rah·luh·dohn

 Polyvinyl alcohol
Pronunciation link: https://www.merriam-webster.com/dictionary/polyvinyl%20alcohol

IPA: /ˌpɑː.liˌvaɪ.nəlˈæl.kəˌhɔːl/
Phonetic Spelling: pah·lee·vy·nuhl·al·kuh·hawl
 β-mercaptoethanol
Pronunciation link: https://www.howtopronounce.com/beta-mercaptoethanol
IPA: /ˌbeɪ.ɾ̞ə.mɚˌkæp.toʊˈɛθ.əˌnɔːl/
Phonetic Spelling: bay·tuh·mur·kap·toh·eth·uh·nawl
 Microscopy
Pronunciation link: https://www.merriam-webster.com/dictionary/microscopy
IPA: /maɪˈkrɑː.skə.pi/
Phonetic Spelling: my·krah·skuh·pee
 Stereomicroscope
Pronunciation link: https://www.merriam-webster.com/dictionary/stereomicroscope
IPA: /ˌstɛr.i.oʊˈmaɪ.krəˌskoʊp/
Phonetic Spelling: stair·ee·oh·my·kruh·skohp
 Nuclear transfer
Pronunciation link: https://www.merriam-webster.com/dictionary/nuclear%20transfer
IPA: /ˈnuː.kli.ɚ ˈtræns.fɝː/
Phonetic Spelling: noo·klee·er trans·fer