

Submission ID #: 71397

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21776133>

Title: Laparoscopic Ovum Pick-Up for In Vivo Oocyte Retrieval in Alpacas

Authors and Affiliations:

Julio Landinez-Aponte, Myeongdon Joo, Rusty Stott, Nathan Merrill, James Cisneros, Yanan Liu, Zhongde Wang

Department of Animal, Dairy and Veterinary Sciences, S.J. and Jessie E. Quinney
College of Agriculture and Natural Resources

Corresponding Authors:

Zhongde Wang zonda.wang@usu.edu

Email Addresses for All Authors:

Julio Landinez-Aponte	julio.landinezaponte@usu.edu
Myeongdon Joo	myeong-don.joo@usu.edu
Rusty Stott	rusty.stott@usu.edu
Nathan Merrill	nathan.merrill@usu.edu
James Cisneros	james.cisneros@usu.edu
Yanan Liu	yanan.liu@usu.edu
Zhongde Wang	zonda.wang@usu.edu

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? **Yes**

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Laparoscope: 2.9.2; 2.11.1, 2.11.2; 2.12.1, 2.12.2; 2.13.2, 2.13.3; 2.16.1; 2.17.1, 2.17.2, 2.17.3.

Stereomicroscope: 4.1.2, 4.1.3; 4.2.1, 4.2.2, 4.2.3

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

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: **06/09/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 25

Number of Shots: 53 (16 Scope)

Interviews

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Julio Landinez-Aponte**: My research focuses on improving assisted reproductive technologies in alpacas, specifically optimizing oocyte collection through laparoscopic ovum pick-up.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Nathan Merrill**: Transvaginal approaches are limited in alpacas due to anatomical constraints; this laparoscopic protocol provides a more effective and reliable alternative.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3., **Nathan Merrill**: This protocol enables oocyte yield assessment and provides material for downstream procedures, including IVF and genetic engineering in alpacas.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Julio Landinez-Aponte**: Correctly synchronizing the follicular wave and carefully managing the factors that directly affect aspiration efficiency are the most critical steps.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Julio Landinez-Aponte:** Future studies can optimize in vitro maturation, fertilization, and gene editing protocols to advance reproductive technologies in Alpacas.

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at the Utah State University

Protocol

2. Laparoscopic Aspiration of Ovarian Follicles in Huacaya Female Alpacas

Demonstrator: Julio Landinez-Aponte

2.1. To begin, position the anesthetized and intubated Huacaya female alpaca [1] on a gurney bed [2].

2.1.1. WIDE: Talent positioning the anesthetized intubated animal on a gurney.

2.1.2. TEXT ON 2.1.1's BACKGROUND:

Anesthesia: BKX cocktail and isoflurane (12 h after the final FSH dose)

BKX cocktail:

Ketamine 1,000 mg/10 mL + Xylazine 100 mg/mL + Butorphanol 10 mg/mL

Administer 1 mL/18 kg IM

Isoflurane:

Induction: 5% with 1- 2 L/min O₂

Maintenance: 1 - 3% with 1 - 2 L/min O₂

2.2. Review the animal's treatment record to confirm that the alpaca has undergone [1] follicular wave synchronization with GnRH (*G-N-R-H*) and ovarian super-stimulation with FSH (*F-S-H*) before oocyte collection [2-TXT].

2.2.1. Talent looking at the animal's treatment chart.

2.2.2. LAB MEDIA: Figure 2 *Video editor: Show only the bottom table with its labels*

TXT: GnRH: Gonadotropin-Releasing Hormone; FSH: Follicle-Stimulating Hormone

2.3. Shave the abdominal region [1]. Thoroughly cleanse the area with surgical soap [2], disinfect with ethanol [3], and apply an iodine-based antiseptic solution [4]. **NOTE: The VO is edited for the deleted shot**

2.3.1. Talent shaving the abdominal region of the animal.

2.3.2. Talent removing previously placed staples from the abdominal region, if present.
NOTE: Not filmed

2.3.3. Talent cleansing the abdominal region with surgical soap.

- 2.3.4. Talent disinfecting the abdominal region with ethanol.
- 2.3.5. Talent applying an iodine-based antiseptic solution to the abdominal region.
- 2.4. Transfer the animal to the designated surgical area [1] and secure it in place while maintaining proper alignment and aseptic conditions [2].
 - 2.4.1. Talent transferring the animal to the designated surgical area.
 - 2.4.2. Talent securing the animal in place while maintaining proper alignment and aseptic conditions.
- 2.5. Cover the abdominal region with a sterile surgical drape [1] and create a fenestration to expose only the operative field [2].
 - 2.5.1. Talent covering the abdominal region with a sterile surgical drape.
 - 2.5.2. Talent creating a fenestration in the sterile surgical drape to expose only the operative field.
- 2.6. ~~Place the female alpaca in the Trendelenburg position [1].~~ NOTE: This step is placed after step 2.10
 - 2.6.1. ~~Talent positioning the female alpaca in the Trendelenburg position.~~
- 2.7. Using a scalpel, make a midline skin incision approximately 10 to 15 centimeters cranial to the udder [1].
 - 2.7.1. Talent performing a midline skin incision approximately 10 to 15 centimeters cranial to the udder with a scalpel.
- 2.8. Introduce a blunt insufflation needle into the abdominal cavity [1]. Establish a capnoperitoneum using carbon dioxide and continue insufflation until adequate abdominal distension is achieved [2].
 - 2.8.1. Talent introducing a blunt insufflation needle into the abdominal cavity.
 - 2.8.2. Talent insufflating the abdominal cavity with carbon dioxide until adequate

abdominal distension is observed.

2.9. Insert a 5-millimeter valved trocar through the initial incision to access the abdominal cavity [1] and introduce the laparoscopic video camera [2].

2.9.1. Talent inserting a 5-millimeter valved trocar through the initial incision to access the abdominal cavity.

2.9.2. SCOPE: SCOPE_2.9.2.mp4: 00:02-00:10

2.10. Place two additional 5-millimeter valved trocars, one on each side of the first port, 10 centimeters lateral to the midline [1] and 5 centimeters cranial to the udder [2].

2.10.1. Talent placing an additional 5-millimeter valved trocars at 10 centimeters lateral to the midline.

2.10.2. Talent placing another trocar at 5 centimeters cranial to the udder.

2.6. Place the female alpaca in the Trendelenburg position [1]. **NOTE: This step is moved here as per author's request**

2.6.1. Talent positioning the female alpaca in the Trendelenburg position.

~~2.11. Use one port to introduce atraumatic Babcock forceps for ovarian manipulation and stabilization on the mesovarium while avoiding tissue injury [1]. Insert the aspiration needle into the second port [2].~~

~~2.11.1. **SCOPE:** introducing atraumatic Babcock forceps through one port to manipulate and stabilize the ovary on the mesovarium.~~

~~2.11.2. **SCOPE:** inserting the aspiration needle through the second port. **NOTE: Step is placed just before 2.14.2**~~

2.12. With atraumatic forceps, gently manipulate and mobilize the ovary to optimize follicle exposure [1] and release it from the infundibulum [2].

2.12.1. SCOPE: SCOPE_2.12.1.mp4: 00:05-00:13

2.12.2. SCOPE: SCOPE_2.12.2.mp4: 00:05-00:10.

2.13. Gather a single-lumen aspiration system consisting of a 20-gauge short-bevel needle connected to a 50-centimeter cannula [1], a silicone stopper, and a 50-milliliter collection tube prefilled with collection medium [2].

2.13.1. Talent showing and placing the single-lumen aspiration system consisting of a 20-gauge short-bevel needle connected to a 50-centimeter cannula on the table.

2.13.2. Talent placing a silicone stopper, and a 50-milliliter collection tube prefilled with collection medium on the table.

2.14. After connecting the aspiration pump, set it to a vacuum pressure of 25 to 30 millimeters of mercury and a flow rate of 13 to 15 milliliters per minute [1]. Use one port to introduce atraumatic Babcock forceps for ovarian manipulation and stabilization on the mesovarium while avoiding tissue injury [2]. Insert the aspiration needle into the second port [3]. Then, puncture each follicle to aspirate the follicular fluid [4]. Rotate the needle gently within the follicle to ensure complete aspiration while minimizing mechanical disruption of the cumulus-oocyte complexes [5]. NOTE: The VO for the moved shot is added

2.14.1. Talent adjusting the vacuum pressure to 25 to 30 millimeters of mercury and the flow rate to 13 to 15 milliliters per minute.

2.11.1 SCOPE: SCOPE_2.11.1.mp4: 00:04-00:10 NOTE: 2.11.1. and 2.11.2 are moved here as per authors request

2.11.2 SCOPE: SCOPE_2.11.2.mp4: 00:03-00:10

2.14.2 SCOPE: SCOPE_2.14.2.mp4: 00:05-00:10

2.14.3 SCOPE: SCOPE_2.14.3.mp4: 00:03-00:10.

2.15 Next, rinse the ovarian surface with warm sterile saline solution containing heparin to remove residual blood [1-TXT].

2.15.1 SCOPE: SCOPE_2.15.1.mp4: 00:03-end. TXT: Heparin: 0.1 mg/mL

2.16 Using a 10-milliliter syringe introduced through the cannula, gently maneuver the ovary toward the cannula [1] while simultaneously moving the cannula over the ovarian surface [2]. Then, flush the ovary and lightly agitate it to ensure thorough removal of blood and minimize adhesion formation [3].

2.16.1 SCOPE: SCOPE_2.16.1.mp4

2.16.2 SCOPE: SCOPE_2.16.2.mp4

2.16.3 SCOPE: SCOPE_2.16.3.mp4

3 Wound Closure and Postoperative Recovery

3.1 Close the skin incision with surgical staples [1].

3.1.1 Talent closing skin incisions with surgical staples.

3.2 Clean the surgical wounds with povidone-iodine [1] and apply a topical repellent and healing ointment [2].

3.2.1 Talent cleaning the surgical wounds with povidone-iodine.

3.2.2 Talent applying a topical repellent and healing ointment to the surgical wounds.

3.3 Administer 2 milliliters of cloprostenol intramuscularly if a corpus luteum is identified during the laparoscopic ovum pick-up procedure [1]. Next, inject ampicillin intramuscularly at a dose of 8.8 milligrams per kilogram of body weight [2].

3.3.1 Talent administering cloprostenol intramuscularly.

3.3.2 Talent administering ampicillin intramuscularly.

3.4 At the end of the procedure, reduce the vaporizer setting to 0 percent [1] and maintain oxygen flow to facilitate anesthetic washout until the animal exhibits signs of recovery [2].

3.4.1 Talent reducing the vaporizer setting to 0 percent.

3.4.2 Talent showing oxygen flow settings maintained and the animal exhibiting signs of recovery.

3.5 Monitor the animal during recovery by assessing pulse oximetry [1], body temperature, respiratory effort, and overall behavior [2].

3.5.1 Talent assessing pulse oximetry values.

3.5.2 Talent assessing body temperature with a temperature probe.

3.6 Using a 20-milliliter syringe, deflate the endotracheal tube cuff [1] and carefully remove the endotracheal tube once protective reflexes return [2]. Discontinue the oxygen supply following completion of anesthetic recovery [3].

3.6.1 Talent deflating the endotracheal tube cuff with a 20-milliliter syringe.

3.6.2 Talent carefully removing the endotracheal tube once protective reflexes are seen.

3.6.3 Talent discontinuing the oxygen supply after recovery.

4 Oocyte Recovery and Morphological Classification

Demonstrator: Nathan Merrill

4.1 Transfer the contents of the 50-milliliter conical collection tube containing follicular fluid and collection medium into a sterile Petri dish using a trimmed 1-milliliter pipette tip [1]. Examine the Petri dish under a stereomicroscope to [2] identify cumulus-oocyte complexes [3].

4.1.1 Talent transferring the contents of a 50-milliliter conical collection tube containing follicular fluid and collection medium into a sterile Petri dish.

4.1.2 Talent placing the Petri dish under a stereomicroscope.

4.1.3 Talent pointing to the cumulus-oocyte complexes.

4.2 Using a sterile pipette, recover oocytes from the medium [1]. Transfer the oocytes into maturation medium [2] and grade them according to morphological characteristics [3].

4.2.1 SCOPE: Aspirating oocytes from the medium with a sterile pipette.

4.2.2 SCOPE: transferring the recovered oocytes into maturation medium. T

4.2.3 TEXT ON PLAIN BACKGROUND:

Oocyte classification criteria:

Grade I: ≥ 2 compact cumulus layers, homogeneous cytoplasm

Grade II: ≥ 1 intact cumulus layer, mostly homogeneous cytoplasm

Grade III: partial cumulus layers, cytoplasmic irregularity

Grade IV: absent cumulus cells, fragmented or degenerated cytoplasm

Results

5 Results

5.1 Animals synchronized and super-stimulated with FSH developed an average of about 14.88 ovarian follicles per session [1], of which around 10.94 were successfully aspirated [2], resulting in a follicle aspiration rate of about 74.18 percent [3].

5.1.1 LAB MEDIA: Table 1. *Video editor: Highlight the cell "14.88 ± 3.85" under "Total Ovarian Follicles"*

5.1.2 LAB MEDIA: Table 1. *Video editor: Highlight the cell "10.94 ± 3.62" under "Ovarian Follicles Aspirated"*

5.1.3 LAB MEDIA: Table 1. *Video editor: Highlight the cell "74.18 ± 8.91" under "Follicle Aspiration Rate"*

5.2 A mean of approximately 6.68 oocytes was recovered per procedure [1], corresponding to an oocyte recovery rate of around 61.30 [2].

5.2.1 LAB MEDIA: Table 1. *Video editor: Highlight the cell "6.68 ± 2.21" under "Oocytes Collected"*

5.2.2 LAB MEDIA: Table 1. *Video editor: Highlight the cell "61.30 ± 8.21" under "Oocyte Recovery Rate"*

5.3 Of the total oocytes collected, 59.05 percent were classified as Grade I to II [1].

5.3.1 LAB MEDIA: Table 1. *Video editor: Highlight the "G1" and "G2" columns under "Oocyte Morphological Classification"*

5.4 Follicles measuring 5 to 6 millimeters in diameter constituted a greater proportion of the ovarian follicular population [1] compared with follicles measuring 3 to 4 millimeters [2].

5.4.1 LAB MEDIA: Figure 4. *Video editor: Highlight the box labelled "5–6"*

5.4.2 LAB MEDIA: Figure 4. *Video editor: Highlight the box labelled "3–4"*

5.5 The micrographs illustrated well-preserved cumulus investment and homogeneous cytoplasm in the grade 1 and 2 oocytes [1]. Reduced cumulus coverage and cytoplasmic heterogeneity were observed in the grade 3 and 4 oocytes [2].

5.5.1 LAB MEDIA: Figure 5. *Video editor: Highlight the upper row showing Grade I and II oocytes*

5.5.2 LAB MEDIA: Figure 5. *Video editor: Highlight the lower row showing Grade III and IV oocytes*

1. Huacaya
Pronunciation link: <https://www.howtopronounce.com/huacaya>
IPA: /wə'kaɪ.ə/
Phonetic Spelling: wuh·ky·uh
2. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>
IPA: /,aɪ.sou'flʊr.ən/
Phonetic Spelling: eye·soh·floor·ayn
3. Gonadotropin-Releasing Hormone
Pronunciation link: <https://www.merriam-webster.com/medical/gonadotropin-releasing%20hormone>
IPA: /,ɡoʊ.nə.doo'troʊ.pɪn rɪ'liː.sɪŋ 'hɔr.moon/
Phonetic Spelling: goh·nuh·doh·troh·pin ree·lee·sing hor·mohn
4. Follicular
Pronunciation link: <https://www.merriam-webster.com/dictionary/follicular>
IPA: /fə'lik.jə.lə/
Phonetic Spelling: fuh·lik·yuh·ler
5. Trendelenburg
Pronunciation link: <https://www.merriam-webster.com/medical/Trendelenburg%20position>
IPA: /tren'del.ən.bɜːg/
Phonetic Spelling: tren·del·uhn·berg
6. Insufflation
Pronunciation link: <https://www.merriam-webster.com/dictionary/insufflation>
IPA: /,ɪn.sə'fleɪ.ʃən/
Phonetic Spelling: in·suh·flay·shuhn
7. Capnoperitoneum
Pronunciation link: <https://www.howtopronounce.com/capnoperitoneum>
IPA: /,kæp.noʊ.per.ɪ.tə'niː.əm/
Phonetic Spelling: kap·noh·pehr·ih·tuh·nee·uhm
8. Laparoscopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/laparoscopic>
IPA: /,ləp.ə.rə'skɑː.pɪk/
Phonetic Spelling: lap·uh·ruh·skop·ik

9. Trocar
Pronunciation link: <https://www.merriam-webster.com/medical/trocar>
IPA: /trou'kɑ:r/
Phonetic Spelling: troh·kar
10. Atraumatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/atraumatic>
IPA: /,ei.trə'mæt.ɪk/
Phonetic Spelling: ay·truh·mat·ik
11. Babcock
Pronunciation link: <https://www.howtopronounce.com/babcock>
IPA: /'bæb.kɑ:k/
Phonetic Spelling: bab·kok
12. Mesovarium
Pronunciation link: <https://www.howtopronounce.com/mesovarium>
IPA: /,mez.uu'ver.i.əm/
Phonetic Spelling: mez·oh·vair·ee·uhm
13. Infundibulum
Pronunciation link: <https://www.merriam-webster.com/medical/infundibulum>
IPA: /,ɪn.fən'dɪb.jə.ləm/
Phonetic Spelling: in·fun·dib·yuh·luhm
14. Cumulus-oocyte
Pronunciation link: <https://www.howtopronounce.com/cumulus-oocyte>
IPA: /'kju:.mjə.ləs 'ou.ə.sɪt/
Phonetic Spelling: kyoo·myuh·luhs oh·uh·syte
15. Cloprostenol
Pronunciation link: <https://www.howtopronounce.com/cloprostenol>
IPA: /,kloʊ.proʊ'sten.ɑ:l/
Phonetic Spelling: kloh·proh·sten·awl
16. Pulse oximetry
Pronunciation link: <https://www.merriam-webster.com/medical/pulse%20oximetry>
IPA: /pʌls ɑ:k'sɪm.ə.tri/
Phonetic Spelling: puls ok·sim·uh·tree
17. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/medical/stereomicroscope>
IPA: /,ster.i.uʊ'maɪ.krə'skoʊp/
Phonetic Spelling: stair·ee·oh·my·kruh·skohp
18. Morphological
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphological>
IPA: /,mɔr.fə'lɑ:.dʒɪ.kəl/
Phonetic Spelling: mor·fuh·loj·i·kuhl
19. Cytoplasmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoplasmic>
IPA: /,saɪ.t̬ə'plæz.mɪk/
Phonetic Spelling: sy·tuh·plaz·mik

20. Heterogeneity

Pronunciation link: <https://www.merriam-webster.com/dictionary/heterogeneity>

IPA: /ˌhɛt.ə.oo.dʒəˈniː.ə.ti/

Phonetic Spelling: het·uh·roh·juh·nee·uh·tee