

Submission ID #: 71258

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21733738>

Title: Unilateral Gonadectomy in Adult Male *Xenopus laevis*

Authors and Affiliations:

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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? **No**

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? **Yes**

500 yds

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. **Yes**

Current Protocol Length

Number of Steps: 17

Number of Shots: 26

Introduction

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

INTRODUCTION:

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

- 1.1. **Rhoda Adjarkey**: This technique focuses on a procedure in *Xenopus laevis* males, specifically the unilateral removal of one testis to enable its use while preserving the second for future experiments.
 - 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. **Christa Merzdorf**: IVF methods in *Xenopus* typically require sacrificing male frogs. This protocol enables using each male frog twice, reducing numbers and supporting 3Rs compliance in IACUC-approved research.
 - 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. **Christa Merzdorf**: This protocol contributes to more sustainable colony management.
 - 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. **Rhoda Adjarkey**: The main challenge is avoiding intestinal damage, as the intestines are partially exteriorized during surgery and must be carefully returned to the body cavity after harvesting the testis.

- 1.4.1. 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.5. **Christa Merzdorf, Senior Researcher, Montana State University**: (authors will present their testimonial statements live)
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

How does the video format of your article help you to share your work with the broader scientific community?

- 1.6. **Christa Merzdorf, Senior Researcher, Montana State University**: (authors will present their testimonial statements live)
 - 1.6.1. 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 (IACUC) at Montana State University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Pre-surgical Preparation of Frogs

Demonstrator: Rhoda Adjartey

2.1. Begin by autoclaving all instruments, including forceps, spay hook, scissors, needle driver, scalpel handle, retractor, and paper towel wrapped in aluminum foil [1].

2.1.1. WIDE: Talent removing the autoclaved instruments and aluminium foil/paper towel from the autoclave.

2.2. Prepare 200-250 milliliters of 0.15% tricaine methane sulfonate in distilled water, adjusting the pH to 7.0 with sodium bicarbonate for frog anesthesia [1]. Place the frog in the Tricaine solution and keep it moist [2]. **NOTE: The VO is edited for the deleted shot**

2.2.1. Talent at the working bench with the bottle of Tricaine and of sodium bicarbonate placed in front.

Added shot: 2.2b.1. Talent places frog into Tricaine solution and keeps the frog moist.

2.3. After anesthetizing the frog, verify a surgical level of anesthesia by gently pinching the fleshy area of both hind feet with forceps or a hemostat and ensuring no response [1].

2.3.1. Talent pinching the rear feet of the frog with forceps.

2.4. Place the frog on its back on sterile paper towels wetted with the Tricaine methane sulfonate solution on a large piece of sterile aluminum foil [1]. Then, inject Meloxicam at a dose of 0.1 milligram per kilogram intramuscularly into a calf muscle [2]. Apply strips of paper towel wetted in the Tricaine solution to the periphery to keep the frog moist and maintain anesthesia [3]. Then, place the surgical drape over the frog [4]. **NOTE: The VO is edited for the additional shot and moved shot**

2.4.1. Talent positioning the frog on wetted sterile paper towels over aluminum foil.

2.7.1. Talent injecting Meloxicam into the calf muscle.

NOTE: Authors requested to move 2.7.1 between 2.4.1. and 2.4.2

2.4.2 Talent placing moist paper towel strips on the frog's legs and torso.

Added shot: Talent placing the drape over the frog

2.5. Rinse the incision site with 0.9 percent sterile saline for at least 5 seconds using a bulb pipette [1].

2.5.1. Talent rinsing the incision site with a bulb pipette.

2.6. Next, using a sterile cotton swab, dab Bupivacaine diluted to 0.25 percent with sterile water onto the skin in the incision area [1]. Allow 5 minutes for absorption before making the incision [2].

2.6.1. Talent applying Bupivacaine with a cotton swab to the incision area.

2.6.2. Shot of the treated skin area during absorption period.

~~2.7. Then, inject Meloxicam at a dose of 0.1 milligram per kilogram intramuscularly into a calf muscle [1].~~ NOTE: 2.7 is moved after 2.4.1

~~Talent injecting Meloxicam into the calf muscle.~~

3. Surgical Procedure and Postoperative Monitoring

~~3.1. Using Adson dressing forceps or rat tooth forceps, grasp the skin in the lower left or right ventral abdominal region slightly lateral to the midline [1].~~ NOTE: Not filmed

~~3.1.1. Talent grasping the abdominal skin with forceps.~~

~~3.2. Then, elevate the skin [1].~~ Use a number 15 scalpel to make a 1-centimeter skin incision [1]. Then cut through the abdominal muscle layer [2]. NOTE: The VO is edited as per the shots

~~3.2.1. Talent creating a skin fold.~~ NOTE: Not filmed

3.2.2. Talent making 1 cm incision in the skin.

3.2.3. Added shot: Talent cutting through the abdominal muscle layer

3.3. Next, use a skin retractor to visualize the intestine and fat body [1]. Use a spay hook to gently exteriorize a portion of the small intestine and abdominal fat body [2]. Follow the abdominal fat body medially to locate and remove the testis [2]. NOTE: The VO is edited for the additional shots

Added shot: Talent using the skin retractor to visualize the intestine and the fat body.

3.3.1. Talent using a spay hook to exteriorize the fat body and intestine. NOTE: 3.3.1

and 3.3.2 are filmed in a single shot

3.3.2 Talent locating and removing the testis.

3.4. Place the testis in Modified Barth's Saline with 10% calf or lamb serum [1]. Store initially on ice, then at 4 degrees Celsius [2].

3.4.1. Talent placing the testis into a tube with Modified Barth's Saline.

3.4.2. Talent placing the tube into an ice bucket.

3.5. Using a blunt instrument, guide the intestines and the abdominal fat body back into the body cavity [1].

3.5.1. Talent repositioning the intestines and fat body into the body cavity using a spay hook.

3.6. Close the muscle layer using a 4-0 (*four-oh*) absorbable monofilament suture with a tapered point needle in two or three simple interrupted sutures [1]. Then close the skin layer separately using the same suture technique [2].

3.6.1. Talent suturing the muscle layer with simple interrupted sutures.

3.6.2. Talent suturing the skin layer with simple interrupted sutures.

~~4. Postsurgical Monitoring and Housing of the Operated Animal~~ NOTE: Please merge this section with the previous one due to insufficient shots

4.1. Rinse the frog in clean, chlorine-free water to remove residual Tricaine solution [1].

4.1.1. Talent rinsing the frog with clean water.

4.2. Place the frog into a container with fresh chlorine-free water while supporting the head above water and keeping the skin moist [1]. Observe continuously until the frog is moving normally, which may take 20 to 30 minutes [2].

4.2.1. Talent placing the frog into a container with its head supported above water.

4.2.2. Talent observing the frog during recovery.

4.3. Transfer the frog into a stand-alone recovery tank with just enough water to fully submerge the frog for 24 hours, once normal movement resumes [1].

4.3.1. Talent transferring the frog into a recovery tank.

~~4.4. Finally, return the frog to a standard housing tank separate from other frogs [1]. Monitor daily for normal eating and swimming, signs of skin lesions or loss of skin, swollen or red legs, and incisional healing [2].~~ **NOTE: Not filmed**

~~4.4.1. Talent placing the frog into a housing tank separate from others.~~

~~4.4.2. Talent observing the frog for recovery indicators.~~

Results

5. Results

- 5.1. Embryos from surgically removed [1] and contralateral testes developed similarly across cleavage, gastrulation, neurulation, and tailbud stages [2], showing normal morphology with no differences in development or viability [3].

5.1.1. LAB MEDIA: Figure 4. *Video editor: Please highlight panel A*

5.1.2. LAB MEDIA: Figure 4. *Video editor: Please highlight panel B*

5.1.3. LAB MEDIA: Figure 4A and B

- 5.2. Frog usage averaged 6.2 frogs per two-month interval from January 2023 to October 2024 [1], decreasing to 3.4 frogs per interval after November 2024 [2].

5.2.1 LAB MEDIA: Figure 4C. *Video editor: Highlight the left portion of the graph from January 2023 to October 2024 where the data points are higher.*

5.2.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the right portion of the graph after November 2024 where the data points are lower, near the arrow labeled "Begin Gonadectomies".*

1. Autoclaving
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔː.tuː.kleɪ.vɪŋ/
Phonetic Spelling: aw·toh·klay·ving
2. Tricaine
Pronunciation link: <https://www.merriam-webster.com/medical/tricaine>
IPA: /'traɪ.keɪn/
Phonetic Spelling: try·kayn
3. Methane
Pronunciation link: <https://www.merriam-webster.com/dictionary/methane>
IPA: /'meθ.əm/
Phonetic Spelling: meth·ayn
4. Sulfonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/sulfonate>
IPA: /'sʌl.fəˌneɪt/
Phonetic Spelling: sul·fuh·nayt
5. Anesthesia
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>
IPA: /ˌæn.əs'θiː.zə/
Phonetic Spelling: an·uhs·thee·zhuh
6. Bicarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/bicarbonate>
IPA: /ˌbaɪˈkɑːr.bəˌneɪt/
Phonetic Spelling: bye·kar·buh·nuht **NOTE: Author suggested to use "'bye-kar-buh-nate" - as in 'he "ate" the apple'**
7. Hemostat
Pronunciation link: <https://www.merriam-webster.com/medical/hemostat>
IPA: /'hiː.məˌstæt/
Phonetic Spelling: hee·muh·stat
8. Intramuscularly
Pronunciation link: <https://www.merriam-webster.com/dictionary/intramuscular>
IPA: /ˌɪn.trə'mʌs.kjə.lə.li/
Phonetic Spelling: in·truh·mus·kyuh·ler·lee
9. Meloxicam
Pronunciation link: <https://www.merriam-webster.com/medical/meloxicam>
IPA: /mə'ləˌk.sɪ.kæm/
Phonetic Spelling: muh·lok·sih·kam
10. Saline
Pronunciation link: <https://www.merriam-webster.com/dictionary/saline>
IPA: /'seɪ.laɪn/
Phonetic Spelling: say·line **NOTE: Author suggested to use "'say-leen"**
11. "

12. Bupivacaine
 Pronunciation link: <https://www.merriam-webster.com/medical/bupivacaine>
 IPA: /bjuːˈpɪv.əˌkeɪn/
 Phonetic Spelling: byoo·piv·uh·kayn
13. Absorption
 Pronunciation link: <https://www.merriam-webster.com/dictionary/absorption>
 IPA: /əbˈzɔːr.pʃən/
 Phonetic Spelling: ub·zorp·shuhn
14. Ventral
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ventral>
 IPA: /ˈven.trəl/
 Phonetic Spelling: ven·truhl
15. Abdominal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/abdominal>
 IPA: /æbˈdɑː.mə.nəl/
 Phonetic Spelling: ab·dom·uh·nuhl
16. Exteriorize
 Pronunciation link: <https://www.merriam-webster.com/dictionary/exteriorize>
 IPA: /ɪkˈstri.i.əˌraɪz/
 Phonetic Spelling: ik·steer·ee·uh·ryze
17. Testis
 Pronunciation link: <https://www.merriam-webster.com/dictionary/testis>
 IPA: /ˈtes.tɪs/
 Phonetic Spelling: tes·tis
18. Gonadectomies
 Pronunciation link: <https://www.merriam-webster.com/medical/gonadectomy>
 IPA: /ˌɡoʊ.nəˈdek.tə.miː/
 Phonetic Spelling: goh·nuh·dek·tuh·mee
19. Neurulation
 Pronunciation link: <https://www.merriam-webster.com/medical/neurulation>
 IPA: /ˌnoʊr.jəˈleɪ.ʃən/
 Phonetic Spelling: noor·yuh·lay·shuhn
20. Contralateral
 Pronunciation link: <https://www.merriam-webster.com/dictionary/contralateral>
 IPA: /ˌkɑːn.trəˈlæt̬.ə.əl/
 Phonetic Spelling: kon·tru·lat·uh·ruhl
21. Morphology
 Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
 IPA: /mɔːrˈfɑː.lə.dʒi/
 Phonetic Spelling: mor·fol·uh·jee