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Title: An Immature Murine Model of Reversible Unilateral Ureteral Obstruction

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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**

MÖLLER-WEDEL INTERNATIONAL, MICROFLEX REF-655 190, SN-0605 (Made in Germany).

Videographer: Please use the SCOPE kit to capture the following SCOPE shots:

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, In the first floor and fourth floor of the same building.**

Current Protocol Length
Number of Steps: 15
Number of Shots: 34

Introduction

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

REQUIRED:

1.1. **Ping Li:** This study establishes a reversible UUO model in immature rats to investigate kidney injury recovery and assess its reliability and application in studying post-obstruction renal repair.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9*

What advantage does your protocol offer compared to other techniques?

1.2. **Ping Li:** A key consideration in this approach is minimizing ureteral damage during surgery, as ureteral injury can induce inflammation, potentially leading to adhesion, closure, and recanalization failure.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

What research questions will your laboratory focus on in the future?

1.3. **Ping Li:** We will focus on the dynamic recovery of renal function and kidney injury following obstruction release, particularly in the context of the immature RUUO kidney.

1.3.1. 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:

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.4. **Ping Li:** Jove's video format makes our model more accessible, enhancing reproducibility, visibility, and impact across the scientific community.

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.5. **Ping Li:** Publishing with JoVE enhances method reproducibility, reducing training time for new researchers and improving lab efficiency. We expect broader visibility, leading to increased citations and collaborations.

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

AUTHORS: Please deliver the above statements in Chinese and in English

Videographer: Please film the testimonials in Chinese and in English

Ethics Title Card

This research has been approved by the Research Ethics Committee at the Children's Hospital of Chongqing Medical University

Protocol

2. Surgical Procedure and Postoperative Assessments for Reversible Unilateral Ureteral Obstruction

Demonstrator: Ping Li

2.1. To begin, use sterilized scissors to cut a silicone tube into approximately 1-centimeter segments **[1]**. Make a longitudinal incision along one side of the tube wall for subsequent use **[2]**.
- 2.1.1. WIDE: Talent cutting the silicone tube into 1-centimeter segments.
- 2.1.2. Close-up of the tube with a longitudinal incision along its side.

2.2. Next, confirm adequate anesthesia of the mice by checking for the absence of reflex responses, such as the pedal withdrawal reflex, upon toe pinch **[1-TXT]**. Apply veterinary ophthalmic ointment to the eyes to prevent corneal drying **[2]**.
- 2.2.1. Talent performing a toe pinch test to confirm anesthesia. **TXT: Anesthesia: Pentobarbital injection (40 mg/kg) (i.p)**
- 2.2.2. Talent applying ophthalmic ointment to the rat's eyes.

2.3. Depilate the rat's abdomen from the xiphoid process to the pubic symphysis, extending bilaterally to the midline **[1]**. Then position the rat in a supine position on a heated surgical pad **[2]** and secure its limbs with rubber ropes **[3]**.
- 2.3.1. Talent using a depilatory tool to remove abdominal hair from the rat.
- 2.3.2. Talent placing the rat on a heated surgical pad
- 2.3.3. Shot of its limbs being secured with rubber ropes.

2.4. Wipe the skin with povidone-iodine solution **[1]**. Then, drape a sterile fenestrated sheet to maintain a sterile field **[2]**. NOTE: The VO has been edited.
2.4.2. Talent applying povidone-iodine solution to the rat's abdominal skin. NOTE: This shot is moved here.
2.4.1. Talent draping a sterile fenestrated sheet over the surgical site. NOTE: This shot is moved here.

2.5. Now make a midline skin incision along the abdomen, extending from the subxiphoid region to just below the umbilicus, to expose the kidneys and upper ureters **[1]**. With a pair of surgical scissors, incise the subcutaneous tissues and fascia along the midline **[2]**.
- 2.5.1. Shot of the midline skin incision being made, extending from the subxiphoid region to just below the umbilicus.
- 2.5.2. Shot of surgical scissors being used to the subcutaneous tissue and fascia.

2.6. Dissect the skin and underlying tissues meticulously layer by layer **[1]**. Fully expose the retroperitoneal space using tissue forceps **[2]**.
2.6.1. Talent dissecting the underlying tissues layer by layer.
2.6.2. Shot of the retroperitoneal space being fully exposed.

2.7. For reversible unilateral ureteral obstruction, first retract the bowel to the right side of the abdominal cavity using a sterile swab **[1]**. Cover the ureter with saline-soaked gauze to prevent desiccation **[2]**.
2.7.1. Talent using a sterile swab to retract the bowel to the right side.
Videographer's NOTE: Shots 2.7.1 and 2.7.2 were captured consecutively, with the video file named c8949.mp4.
2.7.2. Shot of the bowel being covered with saline-soaked gauze.

2.8. With microscopic forceps, dissect and mobilize the left ureter, freeing approximately 1.5 centimeters from the surrounding tissues **[1]**. Then place a 1-centimeter-long silicone tube beneath the freed ureter **[2]**. Use forceps to ensure complete encasement within the tube **[3]**.
Videographer: Please capture the shots labelled SCOPE with a SCOPE kit
2.8.1. SCOPE: The left ureter is being dissected and mobilized. Author's NOTE: 2.8.1 and 2.8.2 are combined.
2.8.2. SCOPE: The silicone tube is being placed beneath the freed ureter.
Videographer's NOTE: Shots 2.8.2 and 2.8.3 were filmed sequentially, and all files in the SCOPE folder have been renamed with corresponding numbering.
2.8.3. SCOPE: The ureter is being secured within the tube.

2.9. Use 3-0 *(Three-zero)* silk thread to ligate the silicone tube and the middle portion of the ureter and induce ureteral obstruction **[1-TXT]**. Gradually pull the silicone tube along the ureter's longitudinal axis to ensure secure but non-slipping ligation **[2]**.
2.9.1. SCOPE: The ureter is being ligated with 3-0 silk thread. **TXT: Avoid excessive ligation force**
2.9.2. SCOPE: The silicone tube is being gently pulled along the ureter's axis.

2.10. Reposition the bowel within the peritoneal cavity carefully, ensuring proper alignment without tension or obstruction **[1]**. Suture the abdominal muscle and fascial layers using a 2-0 *(Two-zero)* non-absorbable suture with a curved cutting needle in a continuous manner **[2]**. Then, disinfect the muscle layer by applying povidone-iodine to the incision of the muscle **[3]** and close the skin with a 4-0 *(Four-zero)* non-absorbable suture, ensuring anatomical alignment and even tension **[4]**. NOTE: The VO has been edited.
2.10.1. Talent repositioning the bowel within the peritoneal cavity.
2.10.2. Shot of continuous suturing of the abdominal muscle and fascia.
Added shot: 2.10.2-1: Shot of applying povidone-iodine to the incision of the muscle.

2.10.3 Shot of skin closure with 4-0 non-absorbable sutures.

2.11. Disinfect the incision site with povidone-iodine solution **[1]**. Allow the rats to recover under controlled conditions at a constant temperature for 7 days **[2]**.
2.11.1. Talent applying povidone-iodine to the incision site.
2.11.2. Shot of the rat being placed in a controlled-temperature recovery chamber.

2.12. For the relief surgery, first dissect the knot of the silicone tube carefully using a scalpel blade **[1]**. Remove the silicone tube and the silk sutures **[2]**, and irrigate the abdominal cavity with normal saline to minimize adhesion and infection risk **[3]**. NOTE: The VO has been edited.
2.12.1. SCOPE: The scalpel blade is cutting the ligation knot.
Added shot: 2.12.1-1: SCOPE: Move out the silicone tube and silk suture.
2.12.2. SCOPE: The abdominal cavity is being irrigated with normal saline.

2.13. Reposition the intestine **[1]** and suture the abdominal wall incision in layers using 4-0 non-absorbable sutures **[2]**. Then suture the skin **[3]** and sterilize the incision site with povidone-iodine solution before transferring the rat into the recovery chamber for 7 days **[4]**. NOTE: The VO has been edited.
2.13.1. Talent repositioning the intestine within the peritoneal cavity.
2.13.2. Talent suturing the abdominal wall layers.
Added shot: 2.13.2-1: Talent suturing the skin.
2.13.3. Shot of the incision site being wiped with povidone-iodine solution.

2.14. On postoperative day 14, section the kidneys into 2 halves to collect kidney samples **[1-TXT]**. Store one half in half in 4% paraformaldehyde for histopathological examination **[2]** and rapidly freeze the other half in liquid nitrogen for storage at minus 80 degrees Celsius for subsequent molecular analysis **[3]**.
2.14.1. Shot of halved kidneys. **TXT: Also, collect blood samples**
2.14.2. Shot of ½ kidney being placed in 4% PFA.
2.14.3. Shot of ½ kidney being rapidly frozen in liquid nitrogen.

2.15. Perform the on-screen assessment for surgical follow-up **[1]**.
2.15.1. TEXT ON PLAIN BACKGROUND:
Body weight (Post-RUUO): Compare weights of control vs. UUO groups
Kidney weight
Kidney volume
Serum creatinine: Indicator of renal function improvement
Urethral peristalsis: Inject methylene blue into renal pelvis and observe coloration post recovery
H& E Staining: Assessment of Tubular integrity

Masson's Trichome Staining: Renal Interstitial Fibrosis Regression analysis
Renal Damage scores

Results

3. Results

3.1. The morphology of the kidneys changed significantly following unilateral ureteral obstruction, with the UUO *(U-U-O)* kidney appearing enlarged and swollen compared to the native kidney **[1-TXT]**. After release of the obstruction, the kidney size reduced but remained larger than the native kidney **[2]**.

3.1.1. LAB MEDIA: Figure 1. **TXT: UUO: Unilateral Ureteral Obstruction** *Video Editor: Please sequentially emphasize the images from left to right*

3.1.2. LAB MEDIA: Figure 1 *Please highlight the Native and RUUO kidney images*

3.2. The ureteral obstruction was confirmed using methylene blue injection, which failed to pass through the blocked ureter **[1]**. After the obstruction was released, the ureter at the ligation site exhibited a ruddy color and showed peristalsis **[2]**.

3.2.1. LAB MEDIA: Figure 2A

3.2.2. LAB MEDIA: Figure 2B

3.3. Histological analysis showed intact renal structures in the native kidney **[1]**, whereas the UUO kidney exhibited tubular atrophy, vacuolation, and cell debris within the lumens **[2]**. The RUUO kidney demonstrated partial recovery, with fewer damaged tubules than the UUO kidney **[3]**.

3.3.1. LAB MEDIA: Figure 3 *Video Editor: Please highlight the images corresponding to Native Kidney (Both top and bottom panel)*

3.3.2. LAB MEDIA: Figure 3 *Video Editor: Please highlight the images corresponding to UUO Kidney (Both top and bottom panel)*

3.3.3. LAB MEDIA: Figure 3 *Video Editor: Please highlight the images corresponding to RUUO Kidney (Both top and bottom panel)*

3.4. Masson's trichrome staining revealed severe interstitial fibrosis in the UUO kidney, indicated by increased, blue-stained collagen fibers **[1]**. The RUUO kidney displayed reduced fibrosis compared to the UUO kidney **[2]**.

3.4.1. LAB MEDIA: Figure 4. *Video Editor: Please highlight the images corresponding to UUO*

3.4.2. LAB MEDIA: Figure 4. *Video Editor: Please highlight the images corresponding to UUO and RUUO*

3.5. The renal damage score was significantly increased in the UUO group compared to the native group **[1]**. The RUUO group had a lower renal injury score than the UUO group but remained significantly higher than the native kidney **[2]**.

3.5.1. LAB MEDIA: Figure 5. *Video Editor: Please highlight the blue and red columns*

3.5.2. LAB MEDIA: Figure 5. *Video Editor: Please highlight the green and red columns*

3.6. The UUO group exhibited the largest kidney weight and volume compared to all other groups **[1]**. The RUUO group showed a reduction in kidney weight and volume but did not fully return to control levels **[2]**.

3.6.1. LAB MEDIA: Table 1 *Video Editor: Please highlight the row "UUO" and column "Kidney weight" and "kidney volume"*

3.6.2. MEDIA: Table 1. *Video Editor: Please highlight the row "RUUO" and column "Kidney weight" and "kidney volume"*

Pronunciation Guides:

1. Silicone

Pronunciation link: https://www.merriam-webster.com/dictionary/silicone
IPA: /ˈsɪlɪˌkoʊn/
Phonetic Spelling: sih-lih-kohn

2. Ophthalmic

Pronunciation link: https://www.merriam-webster.com/dictionary/ophthalmic
IPA: /ɑpˈθælmɪk/
Phonetic Spelling: ahf-thal-mik

3. Corneal

Pronunciation link: https://www.merriam-webster.com/dictionary/corneal
IPA: /ˈkɔːrniəl/
Phonetic Spelling: kor-nee-uhl

4. Xiphoid

Pronunciation link: https://www.merriam-webster.com/dictionary/xiphoid
IPA: /ˈzaɪˌfɔɪd/
Phonetic Spelling: zai-foyd

5. Povidone-iodine

Pronunciation link: https://www.howtopronounce.com/povidone-iodine
IPA: /ˈpoʊvɪˌdoʊn ˈaɪəˌdaɪn/
Phonetic Spelling: poh-vih-dohn eye-uh-dine

6. Fascia

Pronunciation link: https://www.merriam-webster.com/dictionary/fascia
IPA: /ˈfæʃ(i)ə/
Phonetic Spelling: fa-shuh

7. Retroperitoneal

Pronunciation link: https://www.howtopronounce.com/retroperitoneal
IPA: /ˌrɛtroʊˌpɛrɪtəˈniəl/
Phonetic Spelling: reh-troh-peh-rih-tuh-nee-uhl

8. Ureter

Pronunciation link: https://www.merriam-webster.com/dictionary/ureter
IPA: /ˈjʊrəˌtɚ/
Phonetic Spelling: yur-uh-ter

9. Peristalsis

Pronunciation link: https://www.merriam-webster.com/dictionary/peristalsis
IPA: /ˌperəˈstɔːlsɪs/
Phonetic Spelling: peh-ruh-stawl-sis

10. Paraformaldehyde

Pronunciation link: https://www.howtopronounce.com/paraformaldehyde
IPA: /ˌpærəfɔːrˈmældɪhaɪd/
Phonetic Spelling: pah-ruh-for-mal-duh-hide

11. Histopathological

Pronunciation link: https://www.howtopronounce.com/histopathological
IPA: /ˌhɪstoʊˌpæθəˈlɑːdʒɪkəl/
Phonetic Spelling: his-toh-path-uh-lah-juh-kuhl

12. Creatinine

Pronunciation link: https://www.merriam-webster.com/dictionary/creatinine
IPA: /kriˈætəˌniːn/
Phonetic Spelling: kree-a-tuh-neen

13. Methylene blue

Pronunciation link: https://www.merriam-webster.com/medical/methylene%20blue
IPA: /ˈmɛθəˌliːn blu/
Phonetic Spelling: meth-uh-leen bloo

14. Trichrome

Pronunciation link: https://www.merriam-webster.com/medical/trichrome
IPA: /ˈtraɪˌkroʊm/
Phonetic Spelling: try-krohm

15. Interstitial

Pronunciation link: https://www.merriam-webster.com/dictionary/interstitial
IPA: /ˌɪntəˈstɪʃəl/
Phonetic Spelling: in-ter-sti-shuhl