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Title: Mouse Models of Epididymitis Induced by Pathogen-Associated Molecular Patterns

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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? **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. **Yes.**

Current Protocol Length
Number of Steps: 25
Number of Shots: 49

Introduction

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

INTRODUCTION:

1.1. **Alexandre Andrade:** We investigate the impact of inflammation on the physiology of the epididymis and male fertility.

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

1.2. **Alexandre Andrade:** Precise compartmentalized injection of inflammation inducers without damaging the epididymal duct in mouse models requires advanced microsurgical skills and training.

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

CONCLUSION:

1.3. **Alexandre Andrade:** Lack of reproducible animal models to study localized epididymal inflammation and its impact on male fertility.

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

1.4. **Alexandre Andrade:** Direct regional injection of inflammation-inducing chemicals enables precise analysis of region-specific immune responses and epididymitis outcomes.

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

1.5. **Alexandre Andrade:** Future research will evaluate mechanisms of immune regulation and novel therapeutic strategies to mitigate the effects of epididymitis on sperm parameters.

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.

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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

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

1.6. **Alexandre Andrade (Postdoctoral researcher at São Paulo 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. *Suggested B-roll: 4.3.1*

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.)

1.7. **Erick Silva (Associate Professor at São Paulo State University)**: (authors will present their testimonial statements live)

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

Authors: Could you please also deliver the above statements in Portuguese?
Videographer: Please film the testimonials in both English and Portuguese

Ethics Title Card

This research has been approved by the Ethics Committee for the Use of Experimental Animals at São Paulo State University (UNESP)

Protocol

2. Interstitial Injection in the Initial Segment of the Epididymis

Demonstrator: Alexandre Andrade

2.1. To begin, place the anesthetized animal in a supine position on the surgical field **[1-TXT]**. Using an electric trimmer pen, perform trichotomy of the mouse abdomen to remove the fur completely **[2]**.

2.1.1. WIDE: Talent positioning the anesthetized mouse in a supine position on the surgical field. **TXT: Anesthesia: Ketamine/Xylazine (60/20 mg/kg, i.p.)**

2.1.2. Talent shaving the abdominal area of the mouse using an electric trimmer pen.

2.2. Then, use sterile cotton soaked with 0.25% chlorhexidine solution to perform abdominal asepsis **[1]**. With a size-15 scalpel, make an approximately 4-millimeter vertical incision on the right side of the abdominal wall **[2]**.

2.2.1. Talent disinfecting the mouse abdomen with sterile cotton wetted in 0.25 percent chlorhexidine solution.

2.2.2. Talent making a vertical 4-millimeter incision on the right abdominal wall using scalpel size 15.

2.3. Now, gently separate the skin with angled blunt-ended surgical forceps **[1]**. Then, using the same scalpel, make another approximately 4-millimeter vertical incision on the peritoneum at the site of the first incision **[2]**.

2.3.1. Talent separating the skin gently using angled blunt-ended surgical forceps.

2.3.2. Talent making a vertical 4 millimeter incision on the peritoneum with scalpel size 15.

2.4. Carefully lift the epididymis and testis from the abdominal cavity by gently pushing the right side of the scrotum **[1]**.

2.4.1. Talent carefully elevating the epididymis and testis by pushing the right side of the scrotum.

2.5. Using curved surgical forceps, gently pull the epididymal white adipose tissue to localize

the initial segment of the epididymis **[1]**. With the help of the epididymal white adipose tissue, expose the initial segment at the incision site **[2]**.

2.5.1. Talent identifying and pulling the epididymal white adipose tissue with curved surgical forceps.

2.5.2. Talent exposing the initial segment of the epididymis at the incision site using the white adipose tissue.

2.6. Next, position the Hamilton syringe, preloaded with the injection solution, at an angle of approximately 90 degrees parallel to segment number 1 of the initial segment **[1]**. Carefully insert the needle into the interstitial space with the bevel oriented downward **[2]**.

2.6.1. Talent aligning the Hamilton syringe at a 90-degree angle relative to segment number 1.

2.6.2. Talent inserting the syringe needle into the interstitial compartment with the bevel facing downward. **NOTE**: Steps 2.6.2, 2.7.1, 2.7.2, and 2.8.1 are combined

2.7. Then, position the curved surgical forceps at the needle insertion site and gently pinch the epididymal capsule and the needle **[1]**. Slowly depress the syringe plunger until the entire stimulus solution has been injected **[2]**.

2.7.1. Talent positioning the curved surgical forceps to stabilize the capsule and the needle.

2.7.2. Talent slowly lowering the syringe plunger to deliver the full injection volume.

2.8. Keeping the curved surgical forceps in place, carefully remove the needle **[1]** and release the curved surgical forceps after approximately 30 seconds **[2]**.

2.8.1. Talent gently withdrawing the needle while holding the forceps steady.

2.8.2. Talent removing the curved surgical forceps after a brief pause of about 30 seconds.

2.9. Next, using the epididymal white adipose tissue, gently return the testicular tissue to the abdominal cavity **[1]**.

2.9.1. Talent repositioning the testis and epididymis back into the abdominal cavity using the white adipose tissue.

2.10. Identify the peritoneum and close it by making two suture stitches using a needle holder

and size 5-0 *(5-oh)* surgical suture **[1]** and close the skin incision with two suture stitches **[2-TXT]**.

2.10.1. Talent suturing the peritoneum with two stitches using a needle holder and 5-0 surgical suture.

2.10.2. Talent closing the skin incision with two stitches using the needle holder and 5-0 suture. **TXT: Repeat the interstitial injection procedure on the left side**

2.11. After surgery, observe the mouse to ensure appropriate postoperative recovery **[1-TXT]**.

2.11.1. Talent observing the mouse in its cage after surgery. **TXT: The mouse may move slowly for up to 24 h before resuming normal behavior**

3. Intravasal Injection in the Cauda Epididymidis

3.1. After administering anesthesia, place the mouse in a supine position on the surgical field **[1-TXT]**.

3.1.1. Talent positioning the anesthetized mouse in a supine position on the surgical field. **TXT: Anesthesia: Ketamine/Xylazine (60/20 mg/kg, i.p.)**

3.2. Place the index finger and thumb below the scrotum to palpate and confirm the position of the testes and epididymides **[1]**. If necessary, gently massage the sides of the abdomen toward the scrotum to guide the testicles and epididymides into position **[2]**.

3.2.1. Talent placing the index finger and thumb under the scrotum to locate the testes and epididymides.

3.2.2. Talent gently massaging the abdominal sides to reposition the testicles and epididymides into the scrotum.

3.3. Using an electric trimmer pen, perform a trichotomy of the scrotum to remove the hair completely **[1]** and disinfect the area with sterile cotton soaked in 0.25 percent chlorhexidine solution **[2]**.

3.3.1. Talent shaving the scrotal area with an electric trimmer pen to ensure a clean surgical field.

3.3.2. Talent disinfecting the scrotal area using sterile cotton wetted in 0.25 percent chlorhexidine solution.

3.4. With the index finger and thumb below the scrotum, gently stretch the scrotal skin **[1]**. Using a size-15 scalpel, make an approximately 2-millimeter vertical incision on the medial portion of the scrotum **[2-TXT]**.

3.4.1. Talent positioning fingers below the scrotum to stretch the skin slightly.

3.4.2. Talent making a 2-millimeter vertical incision in the center of the scrotum with scalpel size 15. **TXT: Cut through the skin, tunica dartos and external spermatic fascia**

3.5. Now, gently move the left testis and epididymis back into the abdominal cavity while keeping the right testis and epididymis in the scrotum **[1-TXT]**.

3.5.1. Talent carefully pushing the left testis and epididymis back toward the abdomen while maintaining the right side in the scrotum.

3.6. With the index finger and thumb positioned below the right testis and epididymis, keep the scrotal skin stretched gently **[1]**. Then, using angled blunt-ended surgical forceps, carefully open and close the tips over the internal spermatic fascia to rupture the internal spermatic fascia and vaginal tunic **[2]**.

3.6.1. Talent holding the scrotum gently stretched with fingers beneath the testis and epididymis.

3.6.2. Talent repeatedly opening and closing the angled blunt-ended surgical forceps to break the internal spermatic fascia and vaginal tunic.

3.7. Next, relieve the pressure from the index finger and thumb **[1]** and insert the angled surgical forceps through the opening in the vaginal tunic toward the base of the scrotum **[2]**. Using the forceps, carefully pull the distal epididymal fat to localize the cauda epididymidis **[3]**.

3.7.1. Talent relaxing the grip of the index finger and thumb on the scrotum. **NOTE**: Steps 3.7.1 and 3.7.2 are combined

3.7.2. Talent inserting angled surgical forceps into the vaginal tunic opening and directing them toward the scrotal base.

3.7.3. Talent pulling the distal epididymal fat gently to reveal the cauda epididymidis.

3.8. Now, localize the vas deferens and position the straight surgical forceps beneath it **[1]**. Place the bulldog tweezer clamp on the vas deferens, ensuring enough space between

the cauda epididymidis and the clamp **[2-TXT]**.

3.8.1. Talent identifying the vas deferens and positioning straight surgical forceps below it.

3.8.2. Talent applying the bulldog tweezer clamp on the vas deferens while maintaining proper spacing. **TXT: If necessary, limit the opening of the straight surgical forceps**

3.9. Then, position the Hamilton syringe, preloaded with the injection solution, at an angle of approximately 10 to 15 degrees parallel to the vas deferens **[1]** and insert the needle into the vas deferens with the bevel facing upward **[2]**.

3.9.1. Talent aligning the Hamilton syringe at a shallow 10 to 15 degree angle relative to the vas deferens.

3.9.2. Talent inserting the syringe needle bevel-up into the vas deferens.

3.10. Gently squeeze the straight surgical forceps to release the vas deferens **[1]** and slowly depress the syringe plunger to inject the entire stimulus solution **[2]**.

3.10.1. Talent loosening the straight surgical forceps to release the vas deferens. **NOTE**: Steps 3.10.1 and 3.10.2 are combined.

3.10.2. Talent pressing the syringe plunger slowly to deliver the full volume of solution.

3.11. Now, remove the straight surgical forceps **[1]**. Using the curved surgical forceps, gently pinch the region around the needle insertion site **[2]**, then carefully withdraw the syringe needle **[3]**.

3.11.1. Talent removing the straight surgical forceps from beneath the vas deferens. **NOTE**: Steps 3.11.1, 3.11.2, and 3.11.3 are combined

3.11.2. Talent positioning the curved surgical forceps to gently stabilize the tissue near the injection site.

3.11.3. Talent carefully withdrawing the syringe needle from the vas deferens.

3.12. After approximately 30 seconds, remove the curved surgical forceps and the bulldog tweezer clamp **[1]** and gently return the vas deferens to the scrotum **[2]**.

3.12.1. Talent removing the curved surgical forceps and bulldog tweezer clamp after a short delay.

3.12.2. Talent repositioning the vas deferens gently back into the scrotum.

3.13. Next, identify the vaginal tunic and close it with one suture stitch using a needle holder and size 5-0 surgical suture **[1]**. Using the index finger, gently push the right testis and epididymis back into the abdomen **[2]**, and move the left testis and epididymis into the scrotum **[3-TXT]**.

3.13.1. Talent suturing the vaginal tunic with one stitch using a needle holder and 5-0 surgical suture.

3.13.2. Talent pushing the right testis and epididymis carefully into the abdominal cavity.

3.13.3. Talent repositioning the left testis and epididymis inside the scrotum. **TXT: Repeat the same intravasal injection procedure on the left side**

3.14. Finally, close the scrotal skin incision with two suture stitches using a needle holder and size 5-0 *(5-oh)* surgical sutures **[1]** and observe the mouse to ensure proper postoperative recovery **[2]**.

3.14.1. Talent closing the scrotal skin incision with two stitches using the needle holder and 5-0 surgical suture.

3.14.2. Talent looking at the mouse in its cage during postoperative recovery.

Results

4. Results

4.1. The interstitial injection in the initial segment resulted in the injected Blue Evans dye being restricted mainly to segment 1 for up to 30 minutes post-injection **[1]**, and later diffusing into neighboring segments of the initial segment and caput epididymidis **[2]**.

4.1.1. LAB MEDIA: Figure 2B. *Video editor: Highlight image 1*.

4.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight image 3*.

4.2. The intravasal injection into the vas deferens confined the Blue Evans dye within the lumen of segment 9 for up to 30 minutes post-injection **[1]**.

4.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the blue-coloured tube in 1 and 2*.

4.3. Histological photomicrographs confirmed that saline control injections did not alter epididymal morphology 72 hours post-treatment **[1]**.

4.3.1. LAB MEDIA: Figure 4. *Video editor: Focus on the panels labeled as 'saline' in A and B.*

4.4. Interstitial LPS injection in the initial segment induced interstitial edema and intense interstitial, intraepithelial, and intraluminal immune cell infiltrates 72 hours post-treatment **[1]**.

4.4.1. LAB MEDIA: Figure 4A. *Video editor: Zoom in on the 4 inset pictures under "LPS"*.

4.5. Intravasal injection of LPS or LTA in the cauda epididymidis produced interstitial edema and immune cell infiltrates in interstitial and intraluminal compartments 72 hours post-treatment **[1]**.

4.5.1. LAB MEDIA: Figure 4B. *Video editor: Zoom in on the 4 inset pictures under "LPS" and "LTA"*.