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Title: An Animal Model of Pulpitis Based on an Outbred Mice Strain and Its Histological Analysis

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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. **Interview statements:** Which interview statement filming option is the most appropriate for your group?

☒ Interviewees self-record interview statements. JoVE can provide support for this option.

3. **Proposed interview filming date:** Please indicate the proposed date that your group will self-film interviews: **MM/DD/YYYY**

Author's NOTE: We will fill out a date of our convenience.

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

Current Protocol Length

Number of Steps: 17

Number of Shots: 29

Introduction

INTRODUCTION:

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

- 1.1 **Arshad Hasan:** This study establishes NMRI mouse as a model to investigate the development and progression of pulpitis and its underlying mechanisms.

- 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 **Arshad Hasan:** Existing histological methods lack sufficient detail, leading to trial-and-error and unnecessary animal loss. This protocol standardizes pulpitis induction and analysis.

- 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. **Arshad Hasan:** This protocol enables researchers to evaluate pulp inflammation for up to seventy-two hours at defined time intervals.

- 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. **Arshad Hasan:** In developing this protocol, the greatest challenge was optimizing histological processing, which often lacks sufficient methodological detail in published reports.

- 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. **Arshad Hasan:** Future studies can use this model to investigate regenerative strategies and targeted modulation of pulpal inflammation.

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 Review Board (IRB) for Animal Research and Ethics of Dow University of Health Sciences

Protocol

2. Jaw Retraction Clip Fabrication

Demonstrator: Arshad Hasan

2.1 To begin, take a 100-millimeter stainless steel orthodontic wire and form a 5-millimeter loop at the center [1-TXT].

2.1.1. Talent taking the 100 mm wire and making a 5 mm loop at the center.
TXT: Wire diameter - 0.7mm File name: Mouse Loop.mp4

2.2 Bend both parts of the wire 5 millimeters from the loop at right angles, avoiding sharp bends [1].

2.2.1. Talent bending both wire ends 5 mm from the loop at right angles, avoiding sharp bends.

2.3 Measure 25 millimeters from each right-angle bend, make a notch [1] and turn the free ends outward [2].

2.3.1. Talent measuring 25 mm from each right-angle bend and creating a notch.

2.3.2. Talent turning the free ends outward on both sides. *Videographer: Please film the outcome to show the loop and ends turned outside for 2-3 seconds.*

And/or LAB MEDIA: Figure 1A. *Video editor: Please show Figure 1A as an inset if the footage does not show the outcome properly as shown in the images.*

NOTE: Insert a chapter title "Pulpitis Induction"

2.4 For pulpitis induction, first divide the desired number of mice into groups A and B [1].

2.4.1. Talent placing mice in two cages labeled as Group A and Group B.

~~2.5 Administer an intraperitoneal injection of Ketamine-Xylazine cocktail to anesthetize the mouse [1-TXT].~~ **NOTE: Do not show this**

~~2.5.1. Talent holding the mouse and injecting the cocktail intraperitoneally. TXT: Ketamine (87.5 mg/kg) + Xylazine (12.5 mg/kg), Dosage 0.1 mL/20 g body weight~~

- 2.6 After anesthetizing the mouse, adjust the 10-time long-arm stereomicroscope to obtain a direct view of the retracted maxillary teeth [1-TXT].
- 2.6.1. Talent positioning the stereomicroscope to obtain a direct view of the retracted maxillary teeth. **TXT: Anesthesia: 0.1 mL/20 g Ketamine-Xylazine Cocktail**
- 2.7 Then, squeeze the clip and position the notched segment between the maxillary and mandibular incisors on either side [1]. Release the clip and secure the free ends with adhesive tape [2].
- 2.7.1. Talent squeezing the clip and positioning the notched segment between the maxillary and mandibular incisors on either side.
- 2.7.2. Talent releasing the clip and securing the free ends with adhesive tape.
- 2.8 Once the clip is in place, use a dental tweezer to expand the cheeks of the animal [1].
- 2.8.1. Talent using a dental tweezer to expand the cheeks of the animal.
- 2.9 Then, under 10X (*Ten-X*) magnification, use a bur on a slow-speed handpiece to prepare a half-depth Class I (*one*) cavity in the center of the right maxillary first molar [1-TXT]. Operate the bur gently at low speed using a sweeping motion [2].
- 2.9.1. Talent using a bur to prepare the cavity in the center of the right maxillary first molar. **TXT: Use #1/4 round carbide bur** File name: Pulpitis Model1.1.mp4, Pulpitis Model1.mp4, Pulpitis Model2.1.mp4, Pulpitis Model2.mp4: 2.9. 2.10
NOTE: Duplicate files. Choose the best one
- 2.9.2. Talent running the bur gently at low speed in a sweeping motion.
- 2.10 Next, use a number 15 K-type endodontic file to expose the pulp [1-TXT]. For Group A, leave the pulp exposed to induce pulpitis [2].
- 2.10.1. Talent using a #15 K-file in the cavity to expose the pulp.
- 2.10.2. Shot of the exposed pulp.
- 2.11 For Group B, apply 0.2 microliters of Zymosan solution onto the exposed pulp and leave it exposed to induce pulpitis [1-TXT].
- 2.11.1. Talent applying Zymosan solution onto the exposed pulp. *Videographer: Please film 2-3 seconds of exposed pulp.* File name: Zymosan Pulpitis.mp4

3. Sample Preparation for Histological Studies

Demonstrator: Muhammad Adeel

NOTE: Muhammad Adeel's affiliation is Dow University of Health Sciences.

- 3.1 After acquiring the fixed maxillary jaws following animal euthanasia, decalcify the jaws in 10 percent formic acid for three days [1].
 - 3.1.1. Talent immersing the fixed jaws in 10% formic acid.
- 3.2 Use a number 22 blade to make an incision parallel to the occlusal plane, aligned with the sagittal plane [1]. Then, position the blade palatally to the maxillary molars at the level of the palatal cusps and perform the cut [2].
 - 3.2.1. Talent using a No. 22 blade to make an incision parallel to the occlusal plane and aligned with the sagittal plane.
 - 3.2.2. Talent positioning the blade palatal to the palatal cusps of the maxillary molars and performing the cut.
- 3.3 Mark the occlusal surfaces with India ink to maintain orientation [1].
 - 3.3.1. Talent marking the occlusal surfaces with India ink to maintain orientation.
- 3.4 Pour molten paraffin into a stainless-steel base mold [1]. Embed the cut surface of the sectioned jaw facing the mold base and allow the block to solidify [2]. Then remove the hardened block from the mold [3] and mount the block on the microtome [4].
 - 3.4.1. Talent pouring molten paraffin into a stainless-steel base mold.
 - 3.4.2. Talent embedding the sectioned jaw with the cut surface facing the mold base.
 - 3.4.3. Talent removing the hardened paraffin block from the mold.
 - 3.4.4. Mounting the paraffin block onto the microtome.
- 3.5 Adjust the microtome thickness to 3 micrometers [1], cut the sagittal sections [2] and transfer sections onto a clean, charged microscope slide [3].
 - 3.5.1. Talent adjusting the microtome thickness setting to 3 μm .
 - 3.5.2. Show the microtome cutting thin sagittal sections.
 - 3.5.3. Talent transferring the sections onto a clean, charged microscope slide.

3.6 Examine the sections sequentially under a compound microscope [1] until the occlusal cavity becomes visible [2]. Identify the full pulp chamber and one or two roots to confirm the correct sagittal orientation [3].

3.6.1. Talent examining the sections sequentially under a compound microscope until the occlusal cavity becomes visible.

3.6.2. SCOPE: Show the occlusal cavity under the microscope and LAB MEDIA: Figure 3D. *Video Editor: Please show Figure 3D as an inset.*

3.6.3. SCOPE: Show the full pulp chamber and one or two roots to confirm the correct sagittal orientation and LAB MEDIA: Figure 3E. *Video Editor: Please show Figure 3E as an inset.*

Results

4. Results

4.1 Histological images of both groups demonstrated a progressive increase in inflammatory response from 0 to 72 hours compared to control tissue [1]. Images at higher magnification confirm red blood cell extravasation [2] and areas of necrosis [3].

4.1.1. LAB MEDIA: Figure 4A-H. *Video Editor. Please ensure that the images are placed in a sequential manner for easy comparison. Please label the images as follows: 4B - 0 hr, 4C - 6hrs, 4D - 9 hrs, 4E - 12 hrs, 4F - 24 hrs, 4G - 48 hrs, 4H - 72 hrs.*

4.1.2. LAB MEDIA: Figure 4 F1 *Video Editor: Please emphasize the area around the arrow.*

4.1.3. LAB MEDIA: Figure 4 G1 *Video Editor: Please emphasize the area around the triangle.*

4.2 Histomorphological analysis showed progressive inflammation in both groups over time, with significant differences between intervals indicating a time-dependent increase [1].

4.2.1. LAB MEDIA: Table 3 *Video Editor: In the parameter column, within "Location of inflammatory cells" combined row, emphasize the last row named "Pervaded in the entire coronal pulp (score 4)" from Control to 72 hours.*

Similarly, within "Intensity of the inflammatory infiltrate" combined row, emphasize the last row named "Over 80 inflammatory cells (score 4)" from Control to 72 hours;

Within "Inflammatory edema" combined row, emphasize the last row named "Present (score 2)" from Control to 72 hours;

Within "Vascular leakage" combined row, emphasize the last row named "Present (score 2)" from Control to 72 hours;

Within "Extension of pulp necrosis (coronal pulp)" combined row, emphasize the last row named "Over 20% necrotic area (score 4)" from Control to 72 hours.

.... In case of any confusion, please contact the scriptwriter.

5. Orthodontic
Pronunciation link: <https://www.merriam-webster.com/dictionary/orthodontic>
IPA: /ˌɔrθəˈdɒntɪk/
Phonetic Spelling: or·thuh·DON·tik
6. Pulpitis
Pronunciation link: <https://www.merriam-webster.com/medical/pulpitis>
IPA: /pəlˈpaɪtəs/
Phonetic Spelling: puhl·PYE·tuhs
7. Intraperitoneal
Pronunciation link: <https://www.merriam-webster.com/medical/intraperitoneal>
IPA: /ˌɪntrəˈperətəˈniːəl/
Phonetic Spelling: in·truh·pair·uh·tuh·NEE·uhl
8. Ketamine
Pronunciation link: <https://www.merriam-webster.com/dictionary/ketamine>
IPA: /ˈkiːtəˌmiːn/
Phonetic Spelling: KEE·tuh·meen
9. Xylazine
Pronunciation link: <https://www.merriam-webster.com/medical/xylazine>
IPA: /ˈzaɪləˌziːn/
Phonetic Spelling: ZYE·luh·zeen
10. Stereomicroscope
Pronunciation link: No confirmed link found
IPA: /ˌsteriəʊˈmaɪkrəˌskoʊp/
Phonetic Spelling: stair·ee·oh·MY·kruh·skohp
11. Maxillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/maxillary>
IPA: /ˈmæksəˌlɛri/
Phonetic Spelling: MAK·suh·lair·ee
12. Mandibular
Pronunciation link: <https://www.merriam-webster.com/dictionary/mandibular>
IPA: /mænˈdɪbjələr/
Phonetic Spelling: man·DIB·yuh·ler
13. Incisors
Pronunciation link: <https://www.merriam-webster.com/dictionary/incisor>
IPA: /ɪnˈsaɪzərz/
Phonetic Spelling: in·SYE·zerz
14. Molar
Pronunciation link: <https://www.merriam-webster.com/dictionary/molar>
IPA: /ˈmoʊlər/
Phonetic Spelling: MOH·ler
15. Endodontic
Pronunciation link: <https://www.merriam-webster.com/dictionary/endodontic>
IPA: /ˌɛndəʊˈdɒntɪk/
Phonetic Spelling: en·doh·DON·tik

16. Zymosan
Pronunciation link: No confirmed link found
IPA: /'zəməsən/
Phonetic Spelling: ZYE·muh·san
17. Histological
Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>
IPA: /ˌhɪstəˈlɒdʒɪkəl/
Phonetic Spelling: his·tuh·LOJ·ih·kuhl
18. Euthanasia
Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanasia>
IPA: /ˌjuːθəˈneɪzə/
Phonetic Spelling: yoo·thuh·NAY·zhuh
19. Decalcify
Pronunciation link: <https://www.merriam-webster.com/dictionary/decalcify>
IPA: /diːˈkælsəˌfaɪ/
Phonetic Spelling: dee·KAL·suh·fye
20. Formic acid
Pronunciation link: <https://www.merriam-webster.com/dictionary/formic%20acid>
IPA: /ˈfɔrmɪk ˈæsɪd/
Phonetic Spelling: FOR·mik AS·id
21. Occlusal
Pronunciation link: <https://www.merriam-webster.com/medical/occlusal>
IPA: /əˈkluːzəl/
Phonetic Spelling: uh·KLOO·zuhl
22. Sagittal
Pronunciation link: <https://www.merriam-webster.com/dictionary/sagittal>
IPA: /ˈsædʒətəl/
Phonetic Spelling: SAJ·uh·tuhl
23. Palatal
Pronunciation link: <https://www.merriam-webster.com/dictionary/palatal>
IPA: /ˈpælətəl/
Phonetic Spelling: PAL·uh·tuhl
24. Cusps
Pronunciation link: <https://www.merriam-webster.com/dictionary/cusp>
IPA: /kʌspz/
Phonetic Spelling: kuspz
25. Paraffin
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraffin>
IPA: /ˈperəfɪn/
Phonetic Spelling: PAIR·uh·fin
26. Microtome
Pronunciation link: <https://www.merriam-webster.com/dictionary/microtome>
IPA: /ˈmaɪkrəˌtoʊm/
Phonetic Spelling: MY·kruh·tohm

- 27. Histomorphological**
Pronunciation link: No confirmed link found
IPA: /ˌhɪstəʊˌmɔːfəˈlɒdʒɪkəl/
Phonetic Spelling: his·toh·mor·fuh·LOJ·ih·kuhl
- 28. Inflammatory**
Pronunciation link: <https://www.merriam-webster.com/dictionary/inflammatory>
IPA: /ɪnˈflæməˌtɔːri/
Phonetic Spelling: in·FLAM·uh·tor·ee
- 29. Extravasation**
Pronunciation link: <https://www.merriam-webster.com/dictionary/extravasation>
IPA: /ɪkˌstrævəˈseɪʃən/
Phonetic Spelling: ik·strav·uh·SAY·shuhn
- 30. Necrosis**
Pronunciation link: <https://www.merriam-webster.com/dictionary/necrosis>
IPA: /nəˈkroʊsɪs/
Phonetic Spelling: nuh·KROH·sis
- 31. Edema**
Pronunciation link: <https://www.merriam-webster.com/dictionary/edema>
IPA: /ɪˈdiːmə/
Phonetic Spelling: ih·DEE·muh
- 32. Necrotic**
Pronunciation link: <https://www.merriam-webster.com/dictionary/necrotic>
IPA: /nəˈkrɒtɪk/
Phonetic Spelling: nuh·KROT·ik