

Submission ID #: 70352

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21457408>

Title: Generation of the Early-Gestational Maternal Immune Activation Mouse Model to Assess Prenatal Inflammation on Neurodevelopment

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**

The lab is located on the ground floor and a conference room on another floor. All filming is within the same building but on different floors.

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. **No**

Current Protocol Length

Number of Steps: 20

Number of Shots: 39

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

Videographer has given a detailed mapping of which file belongs to which shot (including the takes). Please see this reference document for AE logging as it might be helpful:

https://review.jove.com/files/ftp_upload/70352/jove-take-list.pdf

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

- 1.1. **Lindsay Hayes:** Our lab studies how prenatal inflammation alters the biology of microglia, the brain's immune cells, contributing to adverse neurodevelopmental outcomes.
 - 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. **Sydney Camfield:** Existing MIA models focus on mid-gestation; however, we present an early gestation protocol that moreover incorporates quality control metrics to improve survival and consistency.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Sydney Camfield:** The model can be applied to any wildtype or genetic mouse model to explore the impact of environmental inflammation on brain development.
 - 1.3.1. 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?~~

- 1.4. **Sydney Camfield**: The protocol allows for reproducible generation of the early MIA model and correlation of the severity of maternal inflammation with offspring experimental outcomes.
 - 1.4.1. 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.~~

- 1.5. **Clara Hibbs**: It is critical to maintain detailed records, track lot numbers of reagents, and evaluate maternal cytokine responses in all dams to ensure reproducible results.
 - 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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Oklahoma

Protocol

Videographer has given a detailed mapping of which file belongs to which shot (including the takes). Please see this reference document for AE logging as it might be helpful:

https://review.jove.com/files/ftp_upload/70352/jove-take-list.pdf

Author's NOTES: The videographer added voice content to the recordings to indicate what shot it was and if it was a deviation or an alternative option for that "shot". There were several scenes that were repeated in the text or shots that needed to be filmed together instead of separate shots because it was one motion during the action. These are indicated within each of the recording files.

2. Generation of Timed Pregnancies in Mice

Demonstrator: Clara Hibbs

2.1. To begin, set up two 8 to 10-week-old C57BL/6 (*C-Fifty-Seven-B-L-Six*) female mice with C57BL/6 breeder males in the late afternoon or evening [1].

2.1.1. Establishing shot of the talent with female and male mice placed in the cage.

2.2. On the next morning, assess the female mice for vaginal plugs and continue checking once daily until plugging occurs [1]. The plugs are temporary and may fall out by the afternoon if not checked in the morning [2].

2.2.1. Talent examining a female mouse to check for the presence of a vaginal plug.

2.2.2. Close-up shot showing a vaginal plug on a female mouse. **Videographer's NOTE:** Take 1 shows positive results, vaginal plug. Take 2 shows negative results with no vaginal plug

2.3. After confirming a vaginal plug, weigh the female mouse and record the weight to monitor pregnancy status by tracking weight gain across gestation [1].

2.3.1. Talent placing the female mouse on a weighing scale.

2.4. Now, separate plugged females into individual cages and maintain them under standard housing conditions with ad libitum access to food and water [1]. Designate plugged females as embryonic day 0.5 and record the date of the vaginal plug to ensure accurate tracking of gestational age [3].

2.4.1. Talent transferring a plugged female mouse into an individual cage provided with food and water.

2.4.2. Talent labeling the cage with embryonic day 0.5 and recording the plug date in

an iPad. **Videographer's NOTE:** Shows recording data on iPad

3. Preparation of Polyinosinic:Polycytidylic Acid [poly(I:C)] for Injections

Demonstrator: Abril Gaona-Gamboa

3.1. To reconstitute lyophilized poly (I:C) (*Poly-I-C*) to a high concentration stock solution, dissolve it in sterile saline or sterile water according to the manufacturer's instructions [1].

3.1.1. Talent adding sterile saline or sterile water to a vial containing lyophilized polyinosinic:polycytidylic acid to reconstitute the powder.

3.2. Now, vortex the high concentration stock solution [1]. Then, dilute the solution in saline to prepare a low concentration stock [2]. To avoid repeated freeze-thaw cycles, aliquot approximately 125 microliters of the low concentration stock into 1.5-milliliter tubes [3] and store the tubes at minus 20 degrees Celsius or minus 80 degrees Celsius [4].

3.2.1. Talent vortexing the tube containing the reconstituted poly (I:C) acid.

3.2.2. Talent diluting the solution with saline in a tube. **Videographer's NOTE:** Shows putting solution in big tube

3.2.3. Talent pipetting approximately 125 microliters of the solution into a 1.5 milliliter tube.

3.2.4. Talent placing the labeled tubes into a minus 20 degrees Celsius freezer. **Videographer's NOTE:** This shot shows 'establishing to fridge'

3.3. Immediately before injection on embryonic day 9.5, dilute the low concentration poly (I:C) stock with sterile saline to obtain a working concentration [1].

3.3.1. Talent takes a prealiquoted the low concentration poly(I:C) stock out of the freezer and adding sterile.

3.4. Now, heat the working stock at 65 degrees Celsius for 10 minutes to ensure proper reannealing of the double-stranded ribonucleic acid structure [1]. Then, vortex or pipette the solution to ensure a homogeneous mixture before injection [2].

3.4.1. Talent placing the tube containing the working stock into a heating device set to 65 degrees Celsius. **Videographer's NOTE:** This has different takes with wide and close up versions

3.4.2. Talent vortexing the tube containing the working polyinosinic:polycytidylic acid solution.

4. Injecting Pregnant Mice with Poly(I:C) Solution

- 4.1. To confirm pregnancy, weigh the dam on the morning of embryonic day 9.5 [1]. Calculate the poly (I:C) injection volume for maternal immune activation or MIA (*M-I-A*) and the 0.9 percent saline injection volume for controls [2].
 - 4.1.1. Talent placing the pregnant dam on a weighing scale to measure body weight.
 - 4.1.2. Talent calculating the injection volume using the recorded body weight in an ipad.
- 4.2. Draw the appropriate volume of the prepared poly (I:C) working solution or saline into a sterile 27-gauge or smaller syringe [1].
 - 4.2.1. Talent drawing the calculated volume of solution into a sterile syringe fitted with a 27 gauge or smaller needle. **Videographer's NOTE:** Take 3 shows checking accurate fill
- 4.3. Now, properly restrain the dam to minimize pain and distress [1]. Inject the poly (I:C) acid or saline intraperitoneally in the lower left or right quadrant just above the hip level or at the second set of nipples [2]. Then, dispose of syringes in a biosafety sharps container [3]. Also, record the injection side to maintain consistency across the cohort [3].
 - 4.3.1. Talent restraining the dam securely by hand.
 - 4.3.2. Talent injecting the solution intraperitoneally in the lower abdominal quadrant of the dam.
 - 4.3.3. Talent disposes the syringes.
 - 4.3.4. Talent recording the injection side in a laboratory notebook.
- 4.4. Immediately return the dam to the home cage and monitor for any signs of distress [1-TXT]. Then, supplement the cage with hydrogel for at least 24 hours after injection to prevent dehydration associated with sickness behavior [2].
 - 4.4.1. Talent placing the injected dam back into the home cage and observing the mouse.
 - 4.4.2. Talent placing hydrogel into the cage for post-injection support.
- 4.5. After that, record the poly(I:C) dose, injected volume, injection date and time, gestational age, and poly(I:C) lot number [1].

4.5.1. Talent recording the different parameters in an iPad.

5. Collection of Maternal Blood from Injected Dams

Demonstrator: Sydney Camfield

5.1. Three hours post-injection, observe each mouse individually for 1 minute and assess behavior like reduced activity, hunched posture, facial grimacing, or watery stool using a three-point scale [1-TXT]. Classify mice exhibiting one or more of these behaviors as showing acute sickness behaviors [2].

5.1.1. WIDE: Talent observing an individual mouse in its cage for behavioral assessment.

5.1.2. Talent notes down the acute sickness behavior on the recording sheet.

5.2. Then, using a minimally invasive method such as tail bleed, carefully collect maternal blood from injected dams [1]. Collect approximately 50 microliters of blood [2] and return the dam to the home cage [3].

5.2.1. Talent performing a tail bleed on the dam to collect blood.

5.2.2. Talent collects approximately 50 microliters of blood into a collection tube.

5.2.3. Talent placing the dam back into the home cage.

5.3. Now, allow the collected blood to clot for 30 minutes at room temperature [1]. Centrifuge the sample at 2000 *g* for 5 minutes at room temperature to separate the serum from the clot [2]. After centrifuging the sample once again, carefully collect the clear upper serum layer without disturbing the clot [3-TXT]. Store the serum at minus 20 degrees Celsius until ready for downstream cytokine analysis [4].

5.3.1. Talent placing the blood collection tube on the bench to clot at room temperature. **Videographer's NOTE:** 2 takes are establishing alternatives

5.3.2. Talent placing the tube into a centrifuge. **Videographer's NOTE:** 5.3.1-5.3.2 combined and an alternative wide shot for 5.3.2 is at the end. 5.3.2 take 2 shows hitting start button with settings

5.3.3. Talent pipetting the clear upper serum layer from the tube while leaving the clot undisturbed. **Videographer's NOTE:** 5.3.3a shows a clear shot of separation

5.3.4. Talent places the samples at -20°C.

6. Monitoring Injected Dams and Validating the Immune Response

Demonstrator: Clara Hibbs

6.1. After the injection, weigh the dams daily for 3 days to monitor the response to poly (I:C)

acid [1-TXT].

6.1.1. WIDE: Talent placing a dam on a weighing scale for daily post-injection monitoring.

6.2. On the day of expected parturition, record litter outcomes including litter size, pup viability, or no litter to indicate an unsuccessful pregnancy [1-TXT]. Promptly remove any deceased pups and dispose of them according to institutional biosafety guidelines [2].

6.2.1. Talent observing the cage and recording litter size, pup viability, or absence of a litter.

6.2.2. Talent disposes the dead pups.

6.3. Use ELISA (*E-lie-za*) or multiplex assays to quantify inflammatory cytokines such as IL-6 and TNF- α in maternal serum after MIA or control injections following the manufacturer's instructions [1-TXT].

6.3.1. Talent pipetting the reagent into a vial with all the required reagents in the frame. **Videographer's NOTE:** One of the takes has pull focus and another has standard

6.4. After quantification, use the results to establish a representative threshold for the MIA response [1]. Finally, perform a statistical analysis using R software to assess the relationship between maternal serum IL-6 and TNF- α levels [2].

6.4.1. Talent reviewing the quantified cytokine results to establish the maternal immune activation response threshold.

6.4.2. Talent in front of the computer with the window showing some related data.

Results

7. Results

7.1. Following poly (I:C) administration at embryonic day 9.5, MIA dams displayed significantly increased IL-6 levels relative to control dams [1]. TNF- α levels showed a strong positive correlation with maternal IL-6 levels [2].

7.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the red box plot labeled "MIA,"*

7.1.2. LAB MEDIA: Figure 2B. *Video editor: Show the scatter plot and emphasize the upward sloping line.*

7.2. Between embryonic day 0.5 and embryonic day 9.5, dams that successfully delivered litter gained significantly more weight than dams that failed to produce litter [1].

7.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the blue box plot labeled "Litter."*

7.3. One day after injection, MIA dams lost weight while saline-treated control dams gained weight [1], but by embryonic day 12, both groups showed similar weight gain [2].

7.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the box plots at embryonic day 10 where the red "MIA" group is below the white "CON" group.*

7.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the box plots at embryonic day 12 where the red "MIA" and white "CON" groups are at similar heights.*

7.4. Pre-pregnancy weight did not affect the litter efficacy in either saline-treated control or MIA dams [1].

7.4.1. LAB MEDIA: Figure 4A.

7.5. However, C57BL/6 mice sourced from Jackson Labs had more successful litters than C57BL/6 mice sourced from Charles River during the MIA procedure [1]. In contrast, the source of the poly (I:C) did not affect litter efficacy. [2].

7.5.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the Jackson Labs bar*

7.5.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the "LMW" and "Sigma" bar*

7.6. Moreover, animals sourced from Jackson Labs had a more robust maternal inflammatory response as measured by serum IL-6 compared to animals sourced from Charles River [1].

7.6.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the Jackson Labs bar*

7.7. In contrast, the maternal inflammatory response was the same between different sources of poly (I:C) [1].

7.7.1. LAB MEDIA: Figure 4D.

1. C57BL/6
Pronunciation link: <https://www.merriam-webster.com/dictionary/C57BL%2F6>
IPA: /ˌsiː fɪf.ti ˈsev.ən biː el ˈsɪks/
Phonetic Spelling: see·fif·tee·sev·uhn·bee·el·siks
2. Vaginal
Pronunciation link: <https://www.merriam-webster.com/dictionary/vaginal>
IPA: /vəˈdʒaɪ.nəl/
Phonetic Spelling: vuh·jai·nuhl
3. Embryonic
Pronunciation link: <https://www.merriam-webster.com/dictionary/embryonic>
IPA: /ˌem.briˈɑː.nɪk/
Phonetic Spelling: em·bree·aa·nik
4. Gestation
Pronunciation link: <https://www.merriam-webster.com/dictionary/gestation>
IPA: /dʒeˈsteɪ.ʃən/
Phonetic Spelling: jeh·stay·shuhn
5. Polyinosinic
Pronunciation link: No confirmed link found
IPA: /ˌpɑː.li.ɑɪ.noʊˈsm.ɪk/
Phonetic Spelling: paa·lee·eye·noh·sin·ik
6. Polycytidylic
Pronunciation link: No confirmed link found
IPA: /ˌpɑː.li.sai.təˈdaɪ.lɪk/
Phonetic Spelling: paa·lee·syeh·tuh·dye·lik
7. Lyophilized
Pronunciation link: <https://www.merriam-webster.com/medical/lyophilize>
IPA: /laɪˈɑː.fəˌlaɪzd/
Phonetic Spelling: lye·aa·fuh·lyzed
8. Reconstitute
Pronunciation link: <https://www.merriam-webster.com/dictionary/reconstitute>

- IPA: /,ri:'kɑ:n.stə,tu:t/
Phonetic Spelling: ree·kaan·stuh·toot
9. Vortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔ:r'teks/
Phonetic Spelling: vor·teks
 10. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'æl.ɪ.kwɑ:t/
Phonetic Spelling: al·ih·kwaat
 11. Reannealing
Pronunciation link: No confirmed link found
IPA: /ri:ə'ni:.lɪŋ/
Phonetic Spelling: ree·uh·nee·ling
 12. Ribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic%20acid>
IPA: /,raɪ.boʊ.nu:'kli:.ɪk/
Phonetic Spelling: rye·boh·noo·klee·ik
 13. Intraperitoneally
Pronunciation link: <https://www.merriam-webster.com/medical/intraperitoneal>
IPA: /,ɪn.trə'per.ə.tə'ni:.əl.i/
Phonetic Spelling: in·truh·peh·ruh·tuh·nee·uh·lee
 14. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /,baɪ.oʊ'seɪf.ti/
Phonetic Spelling: bye·oh·sayf·tee
 15. Dehydration
Pronunciation link: <https://www.merriam-webster.com/dictionary/dehydration>
IPA: /,di:.haɪ'dreɪ.ʃən/
Phonetic Spelling: dee·hye·dray·shuhn
 16. Grimacing
Pronunciation link: <https://www.merriam-webster.com/dictionary/grimace>
IPA: /'grɪm.əs.ɪŋ/
Phonetic Spelling: grim·uh·sing
 17. Cytokine
Pronunciation link: <https://www.merriam-webster.com/medical/cytokine>
IPA: /'saɪ.tə,kɑɪn/
Phonetic Spelling: sye·tuh·kyne
 18. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sen.trə'fju:dʒ/
Phonetic Spelling: sen·truh·fyooj
 19. Serum
Pronunciation link: <https://www.merriam-webster.com/dictionary/serum>

- IPA: /'sɪr.əm/
Phonetic Spelling: seer·uhm
20. Parturition
Pronunciation link: <https://www.merriam-webster.com/medical/parturition>
IPA: /,pɑːr.tʃə'ɹɪʃ.ən/
Phonetic Spelling: par·chuh·rish·uhn
21. ELISA
Pronunciation link: <https://www.merriam-webster.com/medical/ELISA>
IPA: /i:'laɪ.zə/
Phonetic Spelling: ee·lye·zuh
22. Multiplex
Pronunciation link: <https://www.merriam-webster.com/dictionary/multiplex>
IPA: /'mʌl.tə'pleks/
Phonetic Spelling: mul·tuh·pleks
23. Inflammatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/inflammatory>
IPA: /ɪn'flæm.ə'tɔːr.i/
Phonetic Spelling: in·flam·uh·tor·ee
24. Cytokines
Pronunciation link: <https://www.merriam-webster.com/medical/cytokine>
IPA: /'saɪ.tə'kaɪnz/
Phonetic Spelling: sye·tuh·kynz
25. Maternal
Pronunciation link: <https://www.merriam-webster.com/dictionary/maternal>
IPA: /mə'tɜː.nəl/
Phonetic Spelling: muh·tur·nuhl
26. TNF- α
Pronunciation link: No confirmed link found
IPA: /,tiː.ən'ef'æl.fə/
Phonetic Spelling: tee·en·ef al·fuh
27. Gestational
Pronunciation link: <https://www.merriam-webster.com/dictionary/gestational>
IPA: /dʒe'steɪ.fən.əl/
Phonetic Spelling: jeh·stay·shuh·nuhl
28. Efficacy
Pronunciation link: <https://www.merriam-webster.com/dictionary/efficacy>
IPA: /'ef.ɪ.kə.si/
Phonetic Spelling: ef·ih·kuh·see
29. Robust
Pronunciation link: <https://www.merriam-webster.com/dictionary/robust>
IPA: /roʊ'bʌst/
Phonetic Spelling: roh·bust