Flinders University

Safe Work Method Statement

Rat – Time Mating 18/06/19

College of Medicine and Public Health Animal Facility

SWMS Number	RA Number	RA Score	
SWMS- 2.4	RA- 2.4	MEDIUM	
Contact Person	**SWMS prepared by**	**AWC Approval Date**	**Review Date**
Roxanne Collingwood	Roxanne Collingwood	18/06/2019	June 2021

Contents

The SWMS **Rat – Time Mating** contains the following sections:

Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed.*
- *Animal Welfare Act 1985*
- *Animal Welfare Regulations 2012*
- *Gene Technology Act 2000* (the Act)
- *Gene Technology Regulations 2001*
- *Work Health and Safety Regulations 2012*

University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

Local Policy

Use of the College of Medicine and Public Health Animal Facilities by all staff and researchers of the College of Medicine and Public Health, Flinders University, is subject to awareness of, and adherence to the following:

Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Committee. No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Research and Teaching can be found on the Flinders University website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc
  http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

  - **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

Safe Work Method Statement

Refer to Risk assessments, Safe Work Method Statement for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager.

SWMS 2.0 Rat- Sexing, Handling, Restraint and Ear Notching
RA 2.0 Rat- Sexing, Handling, Restraint and Ear Notching
SWMS 2.6 Rat -Transportation
RA 2.6 Rat -Transportation
SWMS 7.0 Compliance - Emergency Contingency
RA 7.0 Compliance - Emergency Contingency

Personal Protective Equipment Required

- **Gloves**
- **Gown**
- **Mask**
- **Hair Net**
- **Shoe Covers**
- **Goggles are to be worn during slide preparation**

Hazards and Controls

- **Animal bites- training, demonstrate competency, adhere to SWMS**
- **Animal Scratches- training, demonstrate competency, adhere to SWMS**
- **Animal Allergies- wear PPE when handling animals or handling dirty cages to stop the potential development**
- **Chemical exposure- wear PPE and goggles**

Before Work Commences

Ensure that you are aware of the locations of the following:

- **Spill Kit**
- **Fire Extinguisher**
- **Eye Wash**
- **Exits**

Risk Assessment and SDS (Safety Data Sheet) - Ensure that you have read and understood for all the substances being used.

Equipment:

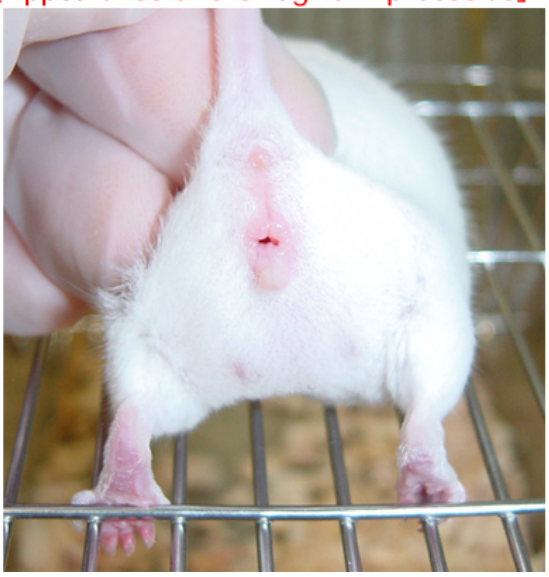
- **Ensure that you have read and understood the Risk Assessment and Safe Work Method Statement**
- **Obtain training before using any equipment**
- **Plastic 1ml disposable pipettes**
- **0.9% Saline**
- **Microscope slides**
- **Slide dryer**
- **Fixative and stains**
- **Microscope**

General Information

- **All procedures are to be performed by trained competent staff.**
- **Training is available from senior animal house staff or Animal Welfare Officer.**
- **Evidence of training is available in on request.**

Time Mating Procedure

[Appearance of the vagina in proestrus]



Mate with vasectomized male

[Vaginal plug]

NOTE: Plug/ smear day is considered 0 dpc (days post coitus). The presence of a plug does not guarantee that the mating will result in a pregnancy. Females are required beyond E10, individuals can be gently palpated to ensure animals are pregnant. If animals are not pregnant, the researcher must be contacted prior to issue.

- **Time-mating Rats: Oestrus cycle is 4-5 days.**
- Use females at least 9 weeks of age- set up 1 male to 1 female. The number of positives required will determine the number of matings to set up. On average you should find 1 positive for every 4 rats set up (then set up an extra 4 rats), ie if you require 2 +ves, then set 12 females (1 male: 1 female). Number of matings will vary depend on whether the males are virgins or experienced.
- Smear day (0 days post coitus). Copulation or Vaginal Plugs are sometimes present and formed from coagulated seminal fluid and will remain in the vagina for 8-12hrs.
    1. Fill the required number of 1ml plastic pipettes with 0.9% saline, one per female.
    2. Place stud box on the bench and remove the water bottle. Remove the female rat from the cage and gently put her into the vacant water bottle holder in the cage lid, with her nose towards the rear of the cage. Holding the rats tail between your thumb and finger, use your free fingers to the firmly hold the rats body against the side of the cage (NOTE: A towel can be used to prevent being bitten).
    3. Insert the end of the pipette gently into the vagina; gently pump the bulb approx. 6 times, taking care not to squeeze out the entire contents.
    4. Label a microscope slide with the rats ID, place a drop of the vaginal fluid onto a microscope slide, and disperse the drop slightly to assist with drying.
    5. Continue to smear the remaining female rats using a fresh pipette for each rat to prevent cross contamination between samples.
    6. Prepare and examine the microscope slides using the methods below.
    7. Record either ✓ or ✗ on the stud cage card depending whether the male has mated or not. Replace any studs which have failed to mate as evident by 4 consecutive crosses.
    8. Positive females must be placed into a separate cage. Record on the cage card researcher(s) name, ethics approval, number of +ves (plugs), the mating date, and plug date.
    9. If the required numbers of plugs are not found, set up matings for the following night. Inform the Researcher of the outcome of the mating and the number of plugs found on each day.
    10. Negatives females may be culled or kept for 2 weeks (observing for any pregnancies) then reused.
- **If time-mated females are required beyond day E10, mice can be gently palpated to determine if they are pregnant. If animals are not pregnant, the researcher must be contacted prior to issue. Pregnancy can be difficult to determine in older or obese animals.**

Slide Preparation

1. Plug in and turn on the slide warmer, set the temperature to 21°C. Place the labelled microscope slides onto the slide warmer, and allow to the slides to fully dry.
2. Dip or flood the smear on the microscope slide in the “Quick Dip Fixative”, then place the microscope slipe on a piece of paper towel for 15 seconds. Hold the microscope slide vertically to allow excess Fixative to drain.
3. Dip or flood the smear on the microscope slide in the “Quick Dip 1”, then place the microscope slipe on a piece of paper towel for 15 seconds. Hold the microscope slide vertically to allow excess solution to drain.
4. Rinse the smear thoroughly with deionised water. Hold the microscope slide vertically to allow excess solution to drain.
5. Dip or flood the smear on the microscope slide in the “Quick Dip 2”, then place the microscope slipe on a piece of paper towel for 15 seconds. Hold the microscope slide vertically to allow excess solution to drain.
6. Rinse the smear thoroughly with deionised water. Hold the microscope slide vertically to allow excess solution to drain.
7. Place the slide on the slide warmer and allow to fully dry.

Slide Examination

1. Ensure that the (X 10) magnification lens is clean.
2. Place the slide on to the stage, positioning it beneath the lens.
3. Focus the microscope. If the smear contains cells then the procedure has been performed correctly.
4. Move the slide around on the stage, so you can scan for sperm (*See Figure 2*). A positive smear can contain anywhere from a few sperm to hundreds.

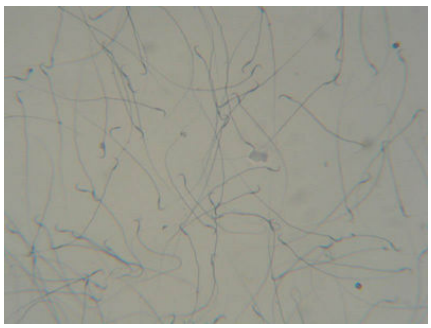


Figure 2. Rat sperm

SWMS Review

This SWMS currently applies to the animals housed in the College of Medicine and Public Health Animal Facility. This SWMS will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change

Position	Name	Contact Details
Manager Animal Facility	Roxanne Collingwood	8204 4380 roxanne.collingwood@flinders.edu.au
Animal Welfare Officer	Lewis Vaughan	0450 424 143 awo@flinders.edu.au

Useful References

http://www.nhmrc.gov.au

http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/home-1

http://www.adelaide.edu.au/ANZCCART/

http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.