The SWMS Rat – Zucker Breeding and Colony Maintenance contains the following sections:

- Legislation
  - University Policy
  - Local Policy
  - Safe Work Method Statement
  - Personal Protective Equipment Required
  - Hazards and Controls
  - Before Work Commences
  - General Information

- Environmental Conditions
- General Rat Biological Data
- Zucker Characteristics
- Room Entry and Exit
- Inbred Breeding - Traffic Light System
- Pedigree
- Cleaning IVC, Open Top Cages, and Colony Maintenance
- Feeding
- Water Bottles
- Floor and Walls
Legislation

- Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed.
- 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 (via the Animal Ethics Review Sub-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

- 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 Statements for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager

RA 2.0 Rat Sexing, Handling and Restraint
SWMS 2.0 Rat Sexing, Handling and Restraint
SWMS 7.0 Compliance - Emergency Contingency
RA 7.0 Compliance - Emergency Contingency
SWMS 7.1 Compliance - Transportation
RA 7.1 Compliance - Transportation

Personal Protective Equipment Required

- Gloves – to be worn throughout entire procedure
- Gown
- Mask
- Hair Net
- Shoe Covers
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 or handling dirty cages to stop the potential development
- Manual handling- IVC cages weigh approximately 10kg, attend manual handling training, adhere to SWMS

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

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 the “Training Needs Analysis”.

Environmental Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>18 – 24°C</td>
</tr>
<tr>
<td>Humidity</td>
<td>40-70%</td>
</tr>
<tr>
<td>Photo period</td>
<td>12hr light: 12hr dark</td>
</tr>
<tr>
<td>Light intensity</td>
<td>350 lux (max)</td>
</tr>
<tr>
<td>Room Ventilation</td>
<td>10-20 air changes/ hr</td>
</tr>
</tbody>
</table>

General Rat Biological Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Span</td>
<td>2.5 - 3.5 year</td>
</tr>
<tr>
<td>Average Weights</td>
<td>Newborn: 5g</td>
</tr>
<tr>
<td>Adult Male</td>
<td>300-500g</td>
</tr>
<tr>
<td>Adult Female</td>
<td>200-400g</td>
</tr>
<tr>
<td>Oestrus frequency</td>
<td>4-5days</td>
</tr>
<tr>
<td>Duration of oestrus</td>
<td>13-15hrs</td>
</tr>
<tr>
<td>Gestation period</td>
<td>20 – 22days</td>
</tr>
<tr>
<td>Ave litter Size</td>
<td>7 – 11</td>
</tr>
<tr>
<td>Weaning Age</td>
<td>21 days</td>
</tr>
<tr>
<td>Sexual maturity</td>
<td>65 – 110days</td>
</tr>
<tr>
<td>Breeding Life Span</td>
<td>1.5 years</td>
</tr>
<tr>
<td>Body temperature</td>
<td>38-39°C</td>
</tr>
<tr>
<td>Heart rate</td>
<td>250-600per min</td>
</tr>
<tr>
<td>Respiration rate</td>
<td>66 – 144 per min</td>
</tr>
<tr>
<td>Daily Food Consumption</td>
<td>15 – 30g</td>
</tr>
<tr>
<td>Daily Water Consumption</td>
<td>24-60mls</td>
</tr>
</tbody>
</table>
Zucker Characteristics

- **Zucker (fa/fa)** – *fa* is an autosomal-recessive mutation on chromosome 5.
  - Rats express a missense mutation in the leptin receptor, this leads to virtually a complete loss of leptin receptor function resulting in the loss of response to leptin.
  - This leads to increased food intake, altered lipid and carbohydrate metabolism, including development of obesity, a fatty liver, and decreased energy expenditure.

- **Fa/Fa** Wild Type Phenotype, No mutations.

- **Fa/fa** Heterozygous - Lean Phenotype - One allele normal, one allele mutated.

- **fa/fa** Homozygous mutation - Fat Obese phenotype - Both alleles mutated, exhibit obesity from 4 weeks of age.
  - Coat Colour - Black and white.

- **Male obese (fa/fa)** (11 – 45 weeks, 460 – 910g) and lean heterozygous (FA/fa) rats (11 – 30 weeks weighing 430 – 600g).
  - Obese animals are prone to pressure sores. Contact the Animal Welfare Officer if pressure sores are detected.

Room Entry and Exit

1. Mop the square in the room entrance with F10sc.
2. Step into the square, and put on mask, hair net, and gown.
3. Put one shoe cover on, step over the square, then put on the other shoe cover.
4. Put on gloves. If they develop a hole at any time whilst in the room, replace the glove.
5. To Exit the room, mop the square with F10sc, remove one shoe cover, then step into the square and remove the other shoe cover (NOTE: Shoe covers can be reused if re-entering the room).
6. Remove and label gown if required to re-enter the room. Discard cap, mask, and gloves.
Inbred Breeding - Traffic Light System

- Inbred strains within the Animal Facility may be maintained using the Traffic Light System, depending on the size of the colony. The Zucker colony consists of Pedigree Breeders only.
  - Pedigree – Brother / Sister mating.
  - Green Breeders – offspring from Pedigree.
  - Yellow Breeders – offspring from Green Breeders.
  - Red Breeders – offspring from Yellow Breeders - progeny of Red Breeders MUST NOT be used for further breeding.

Pedigree

- The pedigree breeders consist of Het x Het brother / sister matings, and recorded on the Pedigree Chart/ Family Tree.
- Zuckers are maintained with 3 to 5 breeding boxes depending on the requirement of the colony.
- Breeders are mated at 8 - 10 weeks of age with 1 male to 1 female per cage, and kept for a period up to 12 months.
- Terminate breeders if they fail to produce pups or wean a viable litter for 2 months, cross the breeder box number off pedigree chart.
- Save Reserve Breeders from the 3rd litter, as this will allow you to assess which breeders consistently produce large litters and equal ratios of males: females.
- When the litter is ready to be weaned, house brother and sister offspring separately. The cage must have food and water.
- Record the number of males or females on the Reserve Breeder cage cards, strain, Parent’s breeder Id number, date of birth, and ethics number on the cage card.
- Join reserve breeders when they are 8 - 10 weeks old: 1 male: 1 female, record mating date, new box number (next consecutive number), parent’s box number, and strain.
- Insert the new number on to the pedigree chart, and connect with a line to its parent’s box number.
- Before pedigrees are terminated, ensure that new breeders have been saved 8 weeks prior to termination, to continue the line.
- Remove the old males from the parent box only after the new breeders have been paired and produced their first litter. The female can be culled when the last of the litters have been weaned.

Cleaning IVC, Open Top Cages and Colony Maintenance

NOTE: Rat IVC cages are approximately 10kgs; care must be taken when handling them.

1. All supplies and consumables must be autoclaved.
2. IVC Cages are to be cleaned each fortnightly, unless the cage is excessively dirty or wet and may require to be cleaned weekly. Open top cages must be cleaned weekly.

3. Add sufficient corn cob bedding (IVC) and Fibre Cycle to thinly cover the base of the cage, add one handful of shredded autoclaved paper towel, and 1 handful of Food.

4. Remove water bottles and place them onto the sink. Remove cage by supporting the base of the cage, and releasing the locking lever with your right hand. Place cage on the cage change station.

5. Release the red latches on the cage to remove the lid.

6. Identify and label any rats that are heavily pregnant, as these must be checked daily and the births recorded on the cage card. Pups do not need to be counted at this stage (handling new born pups may result in mothers eating them). Euthanize any pregnant females that are experiencing birthing difficulties, and record on the “Health Report”.

7. Count and record the number of pups for any new born litters (record number on the cage card, Rat production Sheet, and the Production Sheet).

8. Wean all litters that will be 21 days of age on the Saturday of that week. If pups are small, check to see if they are within the correct weight range for that particular strain.

9. Record the number of male and females weaned, plus any pre-weaning deaths on the breeder cage card, the Rat Production Sheet for corresponding breeder box number, and on the Weekly Production Sheet.

10. Sex and separate weaners into either the male or female stock boxes (up to 4 per box). Complete cage card including researcher, ethics approval, department, sex, number, strain, weaning date, and parent details.

11. Ensure all cages have food on the bottom of their cages as well as in the food hopper, and all have water bottles, and the weaners can reach the sipper tubes and that the sipper tubes are operating properly and not blocked.

12. Place the clean IVC base on the cage change station and transfer the rats. Top up food if necessary. Dust the metal lid with a damp cloth to remove any accumulated dust. Transfer the metal lid to the clean cage, replace the plastic lid and secure with the red clips.

13. Repeat the above process until all of the breeding boxes for that strain have been cleaned.

14. Tally the total number of births, pre-weaning deaths, and males and females weaned on the Weekly Production Sheet.

15. Transfer all of the above information on to the Breeding Statistics sheet, including the number of litters born, plus any males and females culled at wean.

16. If any animals require culling due to illness, euthanize immediately and record on the “Health Report”.
Feeding

- “Gordon’s Autoclavable Premium Rat and Mouse Pellets” must be used for all cages in this room.
- Feed must be autoclaved, and topped up Mondays and Thursday, or as required.
- Some food is to be placed in the bottom of the cage as well as the hopper when cleaning.
- The feed bin must be kept stocked at all times.

Water Bottles

- Water bottles are to be checked daily. All are to be emptied and refilled on cleaning days. Fresh water is to be available to the animals each day.
- Water Bottles are to be scrubbed fortnightly.
- Water bottles in cages are to be checked and changed as required daily. If the water level does not change, check the sipper tube as it may be blocked.
- Ensure that all sipper tubes are operating properly by shaking them; ball bearings should move freely. Sipper tubes should be checked whenever the bottles are filled.

Floor and Walls

- To be swept daily to remove any food on the floor, reducing the chance of slipping.
- Swept and mopped with F10sc (8ml/1Litre water) on the day of cleaning.
- Walls and racks are to be wiped over at this time.

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.

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manager Animal Facility</td>
<td>Roxanne Collingwood</td>
<td>8204 4380</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:roxanne.collingwood@flinders.edu.au">roxanne.collingwood@flinders.edu.au</a></td>
</tr>
<tr>
<td>Animal Welfare Officer</td>
<td>Lewis Vaughan</td>
<td>0450 424 143</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:awo@flinders.edu.au">awo@flinders.edu.au</a></td>
</tr>
</tbody>
</table>

Useful References

http://www.nhmrc.gov.au


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

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