**Aim 3: Determine the effect of** **early time-restricted feeding in the perinatal period on offspring health.** Pups of dams exposed to time-restricted feeding will be compared to pups of ad libitum fed dams. Survival rates, birthweight, body composition, insulin sensitivity and sensitivity to a high fat diet will be measured.

Methods:

*Animal care and use:*

Upon birth, litters were counted and individual pups weighed within 24 hours. At postnatal day 3, litters were reduced to four (two males and two females, when feasible) to standardize milk supply. At 21 days, pups were weaned by sex and maternal treatment group. Upon weaning, animals are allowed 24-hour access to chow (5% fat, 24% protein, 3.7% sucrose, 32% starch, 2.91 kcal per gram) and water.

*Body composition:*

Body weight was assessed using a scale (). This was assessed at birth, 7, 14, and 21 days of life. At 21 days of life, weekly indirect body composition assessment using EchoMRI; fat mass, lean mass, and free water were determined in addition to body weight.

*Survival:*

Survival of pups to PND 3 was assessed by counting the number of pups in each litter each day until PND 3.

*Food intake:*

Food intake monitoring began at weaning. Weekly food intake was measured in grams for each cage, and food intake in calories was computed by taking the total food intake per week and dividing by number of animals in each cage. At 65 days of age, animals were switched to *ad libitum* feeding with high fat diet (HFD) (45% fat, 20% protein, 17% sucrose, and 7% starch, 4.73 kcal per gram). Animals will remain on HFD for 10 weeks.

*Insulin Sensitivity:*

*Insulin tolerance test:*

After 6-hour fast, blood glucose was taken using a glucometer and tail clip. Animals were given intraperitoneal insulin injections (0.75 units/kg body weight; Humulin U100 in cold, sterile-filtered phosphate buffered saline (PBS)) and blood glucose was tested using a glucometer at 15-minute intervals for 2 hours. If animals began to exhibit moribund behaviors, 300 units of 10% glucose in PBS was administered, the animal was then removed from the experiment, and subsequent blood glucose measurements were omitted from data analysis.

*Statistical Analyses:*

All statistical analyses were completed in R (version \_\_\_\_\_). Repeated measures, such as body weight, body composition, food intake, and insulin tolerance testing utilized mixed linear modeling (LME4 package) with each animal assessed as a random effect. All models were tested for sex-interaction. Models were built bottom up and were tested in pairs using ANOVA. Models where ANOVA p value was <0.05 were considered statistically significant.