**Specific Aim 2: Identify the relationship between time-dependent glucocorticoid exposure and mammary function.**

**Aim 2.1:** Is mammary gland development altered after maternal GC exposure during gestation and/or lactation?

To assess the effects of glucocorticoids on milk production and milk volume, we will obtain 8-week old C57Bl6/J mice from Jax laboratories. Mice will be given one week to acclimatize with *ad libitum* access to normal chow diet and water. After acclimatization, one cohort of dams will be assigned to control or experimental arms. The control group will be on a normal chow diet with access to water. The experimental group will have *ad libitum* access to normal chow diet with dexamethasone administration in the drinking water at a dose of 1mg/kg/day. After one week of treatment, both groups in this cohort will be mated with age-matched male mice. The dexamethasone exposure will last all throughout gestation and lactation until postnatal day (PND) 16.5. In another cohort, mice will be assigned to a treatment or control group after 1 week of acclimatization and at the beginning of mating with age-matched males. Both groups in the second cohort will have *ad libitum* access to normal chow diet and water throughout gestation. The treatment group will be given dexamethasone in the drinking water at a dose of 1mg/kg/day during lactation until PND16.5.

In both cohorts, the dams will undergo body mass assessment three times a week using magnetic resonance all throughout the study to determine body composition in response to dexamethasone exposure. Body composition of dams will be assessed right after delivery using echoMRI. We will measure dam food and water intake weekly. The offspring will be weighed at PND0.5, PND7.5, 14.5 and at 21.5.

At PND10.5, we will determine milk output volume. To determine milk volume, we will use the weigh-suckle-weigh technique (Boston *et al.*, 2001). We will weigh the dam and determine the aggregate weight of the pups. The dam and pups will then be separated for two hours. The pups will be placed in a new cage and will be kept warm using a heating pad placed under the cage, while the dam will remain in its initial cage with *ad libitum* access to normal chow diet and water or dexamethasone-water based on its assigned group. After the two-hour separation period, the dam will be weighed again and the aggregate weight of the pups will be measured. The pups will then be returned to the dam’s cage and will be allowed to nurse for one hour. At the end of the nursing timepoint, the dam will be weighed and the aggregate weight of the pups will be determined. After the one-hour nursing period, milk volume will be determined as the weight change of the dam and the pups.

On PND16.5, we will collect milk samples (~1ml) from the nursing dams. Briefly, we will separate the dam and pups for 2 hours. We will anesthetize the dam using Ketamine (0.1275g/kg body weight) intraperitoneal injection. Once the mouse is under anesthesia, we will inject the forelimb with oxytocin (2U/dam) to induce milk production. The dam’s nipples will be manually squeezed to promote milk ejection, and the milk will be collected into a 1.5 ml tube via suction. After milking is complete, the dam will immediately be sacrificed using isoflurane drop jar. We will then dissect the dam by a midline incision of the skin, extract thoracic, abdominal and inguinal mammary glands. The pads will be weighed and a section will be embedded in formalin for histology while the rest will be snap frozen in liquid nitrogen to determine mTORC1 expression as previously discussed via Western blotting. Milk macronutrient composition will be analyzed using milk gels and creamatocrit measurement.

Methods:

Expected Results:

Potential Limitations and Alternate Approaches:

**Aim 2.2:** How does maternal time-dependent GC exposure affect milk output and macronutrient composition?

**Aim 2.3:** Is maternal + offspring metabolic health altered after maternal GC exposure during gestation and/or lactation?

We will assess body composition of offspring of dams who will be sacrificed on PND16.5, then they will undergo glucose tolerance testing. After the glucose tolerance test, offspring will be sacrificed for tissue collection of inguinal and epididymal fat pads.