**Specific Aim 1: Determine the effects of maternal psychological stress on placental function.**

We will expose pregnant dams to the synthetic glucocorticoid dexamethasone and then collect pre-term placentae in order to evaluate nutrient transport and endocrine function. In separate cohorts we will monitor how gestational dexamethasone exposure affects offspring metabolic health.

**Aim 1.1:** How does maternal GC exposure affect placental, fetal IUGR, and offspring survival?

**Aim 1.2:** How does maternal GC exposure affect placental endocrine function (specific hormones: lactogen,IGF2 , GDF15…) look at qPCR mRNA expression – will not use ELISA yet since ELISA is expensive and we may not see a difference in qPCR/mRNA expression initially

**Aim 1.3:** Is placental mTORC1 signaling altered after maternal GC exposure? Western blot for 4EBP, S6, PS6, AKT

**Aim 1.4:** How does maternal time-dependent GC exposure affect the expression of placental nutrient transporters? qPCR of transporters, not flux until we see a change in nutrient transporters

**Aim 1.5:** Is offspring metabolic health survival, wt, mri, if they survive after Dex exposure during gestation only (no 1 week preconception)

**Aim 1.6:** Does a placental GR-KO model rescue the placental and fetal effects of GC exposure?

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

We will expose dams to dexamethasone during pregnancy and lactation then assess mammary gland development, milk volume and composition. We will then monitor the growth and health of the offspring of the exposed dams.

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

**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?

**Specific Aim 3: Elucidate the consequences of placental nutritional stress on placental role.** To model excessive nutritional stress, such as that of maternal obesity we will use a genetic model of mTORC1 hyperactivation in the placenta using a trophoblast-specific driver. With this model, we will determine placental hormone production and evaluate nutrient transport, along with offspring metabolic health.

**Aim 3.1:** How does placental mTORC1 activity affect placental and fetal growth (macrosomia)? Describe ko from grant- mice may die, use from dave’s ratio of het, ko, WT 1:1:5

**Aim 3.2:** How does mTORC1 signaling affect placental endocrine function (specific hormones: lactogen, IGF2 or other hormones you know are important,GDF15…) same as aim 1

**Aim 3.3:** How does placental mTORC1 hyperactivation affect the expression of placental nutrient transporter expression?

**Aim 3.4:** Is offspring metabolic health altered in response to placental nutritional stress? If we use the het we need to ensure mtroc is truly hyperactivated in placenta if the mice survive

**Specific Aim 4: Detect the effects of nutritional stress on lactation***.* Milk output, composition and offspring health from dams with adipocyte mTORC1 hyperactivation will be measured.

As a result of these studies we will learn how maternal stressors influence placental and mammary gland function along with the interplay between the two organs. We will also determine how early life exposures, demonstrated by conceptional and lactational periods, affect offspring health.

**Aim 4.1:** Is mammary gland development altered after maternal adipocyte mTORC1 hyperactivation?

**Aim 4.2:** How does adipocyte mTORC1 hyperactivation affect milk volume and composition?

**Aim 4.3:** How is milk macronutrient composition altered in maternal mTORC1 hyperactivity?

**Aim 4.4:** Is offspring metabolic health altered after maternal mTORC1 adipocyte activation?