

Harvest of Fetal and Adult Tissues from Cattle

Collection: November 28, 2018 at University of Idaho

*This protocol was adapted from tissue collection protocols written by Emily Clark (April 13, 2016) and Alisha Massa (April 26, 2016).

Purpose/Introduction

To collect fetal and adult tissues from L1 Hereford cattle for the Bovine FAANG project and subsequent RNA/DNA assays. Fetuses were 78 days of gestation. Harvest was conducted at a United States Department of Agriculture (USDA) inspected facility.

Equipment/Reagents/Materials

- Cryotubes for snap freezing tissues (2 mL)
- Liquid nitrogen dewars filled with liquid nitrogen
- Sterile scalpel handles and blades
- Sterile forceps
- Sterile scissors
- Sterile 1X PBS in liter bottles
- Sterile beakers
- Wet ice
- Sterile table covers
- Sterile metal trays
- Sharps disposal

Procedure

1. All surfaces were cleaned and disinfected prior to collection.
2. Participants wore gloves to process samples, and changed gloves and sterile instruments, including trays, between tissues and animals.
3. Euthanasia was accomplished by captive bolt and exsanguination in a USDA inspected facility.

4. Tissue processing
 - a. Each tissue was processed by cutting into pieces of approximately 0.5 cm diameter in all directions. Sterile scalpel blades were changed between tissues and animals.
 - b. Tissue aliquots were snap frozen in liquid nitrogen in 2 mL cryovials and transferred to storage at -80°C, and one fresh aliquot was used to isolate nuclei and nuclei were then slow-frozen to -80°C and stored at -80°C.
 - c. All tissues, including fetal tissues, were processed and snap frozen within 20 minutes of exsanguination of the adult animal.
5. Following evisceration and inspection, the adult pancreas and mammary gland were collected.
6. Dissection of the uterus, placenta, and fetus
 - a. The uterus was removed from the adult animal, and the fetus was quickly removed and sexed.
 - b. The placenta was removed, and placentomes were separated into cotyledons and caruncles for processing.
 - c. The head was removed from the fetus, and the fetal brain was removed from the skull.
 - d. The umbilical cord was removed and cut into four sections.
 - e. The abdominal cavity was opened, and gonads were removed first. Gonads were cut in half.
 - f. Both left and right kidneys were removed and cut in half.
 - g. The liver and spleen were then removed and cut into eight pieces (liver) and three pieces (spleen).
 - h. The thoracic cavity was opened, and heart was removed and cut into four pieces.
 - i. Both left and right back legs were removed, and fetal biceps femoris and vastus lateralis were isolated and cut into eight pieces.
 - j. The entire fetal intestine was removed.
 - k. The entire fetal rumen/reticulum/omasum/abomasum was removed.
 - l. Both left and right lungs were removed.