APPENDIX A: BIRTH SPECIMEN COLLECTION PROTOCOL

Cerebrospinal fluid collection: Up to 1.5 mL CSF will be collected by the attending anesthesiologist or fellow at the time of epidural placement in the intrapartum period using a 3mL syringe to draw from the spinal catheter prior to delivering anesthetic. The syringe will be put inside a biohazard bag over ice immediately after collection. If study personnel is available at time of collection and sample does not appear bloody, sample will be distributed evenly into two Eppendorf 2.0mL cryovials, labeled as described in the following section, and stored at -80Celsius. If sample appears bloody, it will be spun in the microcentrifuge prior to aliquotting into Eppendorf 2mL cryovial(s). If sample obtained is no greater than 0.5mL, one 2.0mL cryovial may be used for storage. Should study personnel not be available at time of CSF collection, the sample will be transferred from the syringe to a 5mL freezer safe cryovial, and placed in the transitory freezer at -20Celsius before processing.

Maternal Blood: Up to 18 mL of maternal blood will be collected at any point throughout the hospital admission after informed consent and before discharge. Study personnel will collect one 6mL EDTA purple tube and one 6mL Serum red top tube for all participants. One 6mL SST gold top will be collected for participants who have completed an endothelial function study. Once collected, tube(s) will be inverted 10 times to ensure uniform additive distribution in the sample. The EDTA and Serum tubes will be placed in iced water while transporting to the research lab centrifuge where samples will be spun for 15 minutes at 4 degrees Celsius at 2200 RPM. After the centrifuge step is complete, serum, plasma and buffy coat samples are transferred using a disposable sterile, plastic transfer pipet (e.g. Falcon Cat
into Eppendorf 2 mL cryovials in 1mL aliquots. Samples are stored at -80 freezer immediately following collection.

*Cord Blood:* Using either a syringe method or vacutainer method, as much cord blood as possible is collected after the placenta has been delivered and cord blood gases obtained. A 10 mL syringe attached to a 20G or 23G needle is inserted into a large vein as close to the placental base plate as possible, filled and then injected into a 10 mL purple EDTA tube. The sample will then be processed according to the same protocol outlined for maternal EDTA samples.

*Placenta:* Two methods will be employed when collecting placental samples, outlined below. Processing will always begin with uniform sampling.

1. **Uniform Sampling:** Laying the placenta flat with the fetal membrane and umbilical cord facing up, the placenta is divided into four quadrants (*Figure 2*). The fifth segment of the placenta will be where the four segments meet at the base of the cord. An area with no calcifications is selected from each segment and 2 samples are removed from each segment, first removing the fetal membrane and then using surgical forceps and scissors to employ the “pinch” collection method. One sample will be placed in an empty 2mL Eppendorf cryovial and the other will be placed in a cryovial containing 1.0 mL RNA solution. Once all 10 samples from the fetal side are collected, this process is repeated on the maternal aspect of the placenta, with each collection from the maternal side corresponding to the same segment on the fetal side. Samples are placed in an empty cryovial and stored at -80 Celsius. Samples in RNA are stored at room temperature.
2. Random Sampling: Using a scalpel and tissue forceps, a 1 cubic inch cross section (from the fetal to the maternal side) is cut from a region midway between the umbilical cord and the outer edge of the placenta that has not been altered by uniform sampling. Saline solution (sodium chloride) is poured into a disposable container (urine collection cup) and the sample placed in the solution. Excess blood is gently washed off the sample by stirring the container. Once washed, the sample is cut in half (transversally), creating two segments holding all placental layers. Each sample is enveloped in a separate 5x5 inch sheet of aluminum foil by placing the sample in the center and folding edges towards each opposite end- securing all tissue contents. Samples are labeled with a cryosafe marker and stored at -80 Celsius.