Sampling Protocol for Bovine Abortions

1. Examine the placenta for any abnormalities
   a. Collect multiple sections of cotyledonal and intercotyledonal areas for histopathology (formalin fixed), bacteriology (fresh), and virology/PCR (fresh-frozen). Critical in the diagnosis of some mycotic and bacterial infections!

2. External examination of the fetus for any outward congenital malformations, meconium staining, or skin lesions

3. Estimate/verify the gestational age
   a. Weigh the fetus
   b. Measure the crown to rump length
   c. Note fetal characteristics
   d. Refer to the chart on aging

4. Determine the state of preservation
   a. Fresh, autolyzed, mummified, macerated

5. Classify the fetal death
   a. Abortion, stillbirth, non-viable neonate (lungs partially inflated)

6. Perform a routine necropsy and note any gross abnormalities
   a. Section femur longitudinally to look for growth disturbances

7. Collect the following tissues for ancillary testing. Remember to maximize sampling initially. Samples can always be discarded later!
   a. Histopathology and immunohistochemistry (10% neutral buffered formalin; 10:1 formalin to tissue ratio)
      i. Eyelid, ear notch, parotid salivary gland, tongue, thyroid, thymus, lung, heart (t-section), diaphragm, liver, kidney, adrenal gland, spleen, ileum, colon, mesenteric lymph node, skeletal muscle, half of brain, placenta, any lesions
      ii. IHC is available for many infectious agents
   b. Bacteriology/mycology via culture or PCR (fresh)
      i. 5 ml abomasal content, lung, liver, placenta
      ii. Collect stomach content in a syringe with a large gauge needle
      iii. Package each specimen separately in sterile containers
   c. Virology and molecular techniques (PCR) (fresh-frozen)
      i. Lung, liver, kidney, spleen, placenta, brain
      ii. Package each sample separately in sterile containers
   d. Nutrition/Toxicology (fresh-frozen)
      i. Liver (2-5 grams of tissue required)
   e. Serology (refrigerated or frozen)
      i. Fetal fluids - heart blood, thoracic fluid, abdominal fluid
      ii. Collect in sterile red top tubes
### Gestational age estimates for bovine fetuses

<table>
<thead>
<tr>
<th>Gestational Age (months)</th>
<th>Crown to Rump Length (cm)</th>
<th>Fetal Weight (kg)</th>
<th>Placentome Diameter (cm)</th>
<th>Fetal Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6-8</td>
<td>0.008-0.03</td>
<td>&lt;1.0</td>
<td>Claw buds present; small scrotum visible in males; no hair present</td>
</tr>
<tr>
<td>3</td>
<td>13-17</td>
<td>0.2-0.4</td>
<td>1.0-1.5</td>
<td>Few hairs on lips, chin, and eyelids</td>
</tr>
<tr>
<td>4</td>
<td>22-32</td>
<td>1-2</td>
<td>1.5-2.5</td>
<td>Fine hairs on eyebrows; claws developed; amniotic epithelial plaques present</td>
</tr>
<tr>
<td>5</td>
<td>30-45</td>
<td>3-4</td>
<td>2.5-4.0</td>
<td>More abundant hair on eyebrows, lips and chin; testes in scrotum; teats developing</td>
</tr>
<tr>
<td>6</td>
<td>40-60</td>
<td>5-10</td>
<td>4.0-5.0</td>
<td>Hair on the inside of the ear, around the horn pits, on the tail tip and on the muzzle</td>
</tr>
<tr>
<td>7</td>
<td>55-75</td>
<td>8-18</td>
<td>5.0-7.5</td>
<td>Hair on the metatarsal, metacarpal, and phalangeal regions of the limbs; hair beginning on dorsal aspect of back; long hairs on tail tip</td>
</tr>
<tr>
<td>8</td>
<td>60-85</td>
<td>15-25</td>
<td>6.0-9.0</td>
<td>Fine short hair present all over body; incisor teeth present, but not erupted</td>
</tr>
<tr>
<td>9</td>
<td>70-100</td>
<td>20-45</td>
<td>8.0-12.0</td>
<td>Hair coat is complete with long guard hairs; incisor teeth are erupted</td>
</tr>
</tbody>
</table>
Figure 1. The cervical region of a newborn calf dissected to reveal important structures for sampling.