Chapter 1
General Approach to Fetal and Neonatal Loss

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Introduction

Determining a definitive diagnosis for abortion or neonatal death is frequently a frustrating and unrewarding exercise. Aborted fetuses are often autolyzed at the time of submission limiting the ability to isolate significant pathogens or identify diagnostically relevant gross or histologic lesions. Even if the fetus and associated membranes are fresh, a diagnosis with a specific etiology is rarely achieved. Diagnosticians and diagnostic laboratories spend an enormous amount of resources and time on these cases, with the frequent result of the ever-frustrating final diagnosis of "idiopathic abortion" or abortion of unknown cause. Despite such poor odds, a consistent, systematic approach must always be employed in order to arrive at the oft-elusive definitive diagnosis. Submitting veterinarians and owners must be made aware that cases that yield "negative findings" do not equate with a diagnostic failure. Rather, the test negative results for the most common causes of abortion and neonatal loss indicate that the diagnostic focus should be directed toward other possible causes such as genetic abnormalities, nutritional deficiencies or excesses, environmental factors, and management practices.

Effective, improved vaccines and vaccination protocols, a better understanding of infectious diseases, and proven management procedures have greatly reduced the frequency and severity of contagious abortion outbreaks. In livestock, the majority of abortion and neonatal losses are often attributable to deficiencies in management. The diagnostician needs to keep in mind that reproduction is just one specific phase of "production," and when the animal's overall state of health is compromised, infertility, fetal death and subsequent abortion, or losses due to neonatal death are a natural outcome. Such submissions will often be unrewarding but may represent a harbinger of further losses and the possible need for management alternatives. Thus, the

diagnostician must be well aware of livestock management procedures, as well as regionally distinct variations, in order to ask the correct questions and be able to effectively work with the owner and referring veterinarian in identification of the genesis of the problem. Although an answer may be forthcoming from a single submission, more often, losses of several offspring with negative findings prompts laboratory staff, along with the referring veterinarian and owner, to pursue less common causes.

Fetal and neonatal losses involving companion animals are equally challenging. In addition to the same pressures of confirming a diagnosis, owners of this group of animals are much more emotionally attached. The spectrum of ownership ranges from "accidental" breeders to professional breeders and breed fanciers, and with this variance comes a broad range of background knowledge. Coupled with myriad Web sites that chronicle anecdotal and rare instances along with factual, science-based accounts of causes, the diagnostician must confront these situations with an added measure of patience. Yet, a systematic and methodical approach is the only way that a specific etiology can be discovered and confirmed.

Background prediagnostics

This general diagnostic approach to fetal and neonatal loss represents a general introduction to the topic to ensure that veterinarians and diagnosticians approach these diagnostic dilemmas in a prototypic, ordered, and organized manner. Specific etiologies will be addressed in the following chapters as they pertain to the spectrum of animal species covered in this book. At the end of this chapter, a section compares and contrasts key concepts specific to abortion diagnostics.

History

A detailed and comprehensive history is paramount for diagnostic success. Over the years, many if not most diagnostic laboratories have borrowed or created questionnaires that provide key prompts when tackling fetal or neonatal losses. Without one, specific questions may be overlooked or forgotten, which can delay or impede the investigation. The outline below is suggested to be used as a starting point. In addition, many North American diagnostic labs have questionnaires posted on their Web sites.

Animal information

- Age of affected dams: Older animals? First pregnancy animals? Mixtures of ages?
- Stage of pregnancy abortion is occurring: first, second, or third trimester? Term? Immediately at or after birth?
- Percentage of animals pregnant that have aborted?

- Are any of aborting animals otherwise clinically ill (beyond the abortion event)?
- Are the abortions clustered by time of year or age group of dams?
- Are the abortions sporadic (that is, a relatively rare event) or are there many in rapid succession (that is, is it an "abortion storm").
- Is the herd/kennel/flock "closed" (that is, no new introductions) or is it maintained "open" with continuous new introductions?
- If new animals were introduced, when?
- Is there other evidence of disease, besides fetal or neonatal losses?
- Is there history of similar problems in previous years? If so, what were the findings and were management changes made?
- Have congenital defects been reported before with this breeding pair?
- In purebred animals, are there known genetic anomalies? Are any animals tested by the breed registry?

Nutrition

- Are animals fed a balanced ration? Home grown or prepared? Who is the nutritionist?
- What was the quality of the previous years' harvest?
- What additional supplements are provided?
- Have you had any of the feed tested for nutrients? Toxins?
- Is the plane of nutrition increased during periods of extreme weather? As pregnancy progresses?
- Do any of the hay bales have visible or evident mold? Spoilage?
- What is the water source? Has the water been tested for possible toxins? Mineral excesses?

Preventative and treatment therapies

- What therapies have been used without success?
- What vaccines have been used and what is the protocol? Modified live versus killed? A combination of the two?
- What are the serial number and expiration date of any vaccines used?
- How were vaccines handled at time of vaccination (storage, refrigeration, etc. prior to, and at actual time of usage)?
- Is there any modification of the vaccination protocol depending on age of animals in the herd/kennel?
- What is the deworming history? Which products were used?

Environmental influences

- Has the herd or kennel been exposed to any unauthorized visitors? People? Wildlife or feral animals?
- What are the biosecurity measures employed?

- Is there any possibility of exposure to abortifacient plants? Abortifacient or teratogenic pharmaceuticals?
- Is the pasture or area deficient in particular micronutrients (such as, vitamin E or selenium deficient areas)?
- How severe has the weather been? Do abortions or neonatal losses correspond with recent surges in severe weather (that is, typically very cold weather)?

Site visit

With the more vague and difficult abortion cases, an invited formal visit may be a productive exercise that provides a useful addendum to the history. Another "set of eyes" will often pick up some important detail that may not have been clarified by the original information from the referring veterinarian or animal owner. If a visit is not yielding much useful information, sometimes it may be helpful to speak with a hired hand, spouse, or older children in the absence of the owner. Important details sometimes are forgotten or overlooked and may surface when this strategy is employed.

Diagnostic investigation

Necropsy and tissue collection procedures

Necropsy technique and procedures are similar whether the carcass is a fetus, neonate, adolescent, or mature animal, with few exceptions. Prior to beginning the necropsy procedure, a crown-to-rump length of fetus is measured in addition to its weight to approximate or verify stage of gestation (Refer to Appendix A for established species specific gestational age estimates). Overall condition of the fetus is noted (degree of autolysis, etc.). The animal is opened, with ample exposure of all three cavities (that is, pericardial, thoracic, and abdominal cavities) in order to thoroughly examine the major organs and tissues. The calvarium is removed to expose the brain within the cranial vault. Some notable exceptions include a general lack of tinctorial variation between the various tissues, higher water content in many of the tissues, and in the case of fetuses, lack of aerated lungs. All tissues tend to be reddish-pink-gray, whether looking at the lung, heart, or liver, and this lack of distinction worsens as the tissues become autolyzed. Fetal and early neonatal brains tend to lack tissue integrity due to very high water content. As a result, brain tissue is often poured out of the skull into the collection containers.

Gross lesions are rarely seen in aborted fetal tissues. When present, the types of lesions seen are usually small grayish-pink or yellow pinpoint foci on the surface of the liver, lung, or kidney or a combination. These will often translate into systemic infection of viral or bacterial origin. Plaques found on the skin, fetal membranes, or both may be an indication of mycotic infection. Other lesions may be seen such as anatomical anomalies associated with a

Figure 1.1 Contaminated bovine placenta. Fetal membranes are commonly contaminated at the time of collection for submission. (Courtesy of Dr. BL Njaa, Oklahoma State University.)

variety of genetic disorders or exposure to poisonous plants. Finally, lesions may be observed in the fetal membranes that may be an indication of feto-maternal interface disease, either the result of systemic maternal disease or ascending disease processes.

Fetal membranes

Fetal membranes, commonly referred to as the placenta, should always be submitted. They are handled separately from other fetal tissues and reflect the maternal environment. As it is almost always contaminated with straw, manure, shavings, or dirt (Figure 1.1), it should be washed off, then spread out and examined grossly. When there is an obvious surface exudate, remove coarse debris and then sample for microbiological and histological evaluation. Depending on the type of placenta, gravid and non-gravid horns (equine) should be examined; cotyledenal and noncotyledenal (ruminant) portions of fetal membranes should be examined and sampled; and in cats and dogs, zonary and nonzonary placenta should be examined, including the marginal hematoma. Those that lack experience examining fetal membranes should seek advice from more experienced colleagues, include digital images of the purported lesions at the time of submission, or include most or all of the fetal membranes as part of the submission to the diagnostic laboratory. Placentitis is often somewhat focal or "spotty" in its distribution, therefore, multiple sections should be collected and submitted for histologic evaluation. Since different animal species are represented in this text, we will leave the specifics of this part of the examination to the experts in the chapters that follow.

Table 1.1 Fresh specimens collected for fetal and neonatal diagnostics.

Test	Storage	Specimens	Special Collection Notes
Bacteriology/ mycology (via culture, PCR)	Keep refrigerated, NOT frozen	Stomach contents, liver, lung, brain, placenta	• Collect stomach contents in a syringe with a large gauge needle • Package each specimen separately in sterile containers
Virology (via VI, PCR)	May be frozen at −70°C, if necessary	Lung, liver, kidney, heart blood, placenta	• Package each specimen separately in sterile containers
Nutrition, toxicology	May be frozen	Liver, kidney, ocular fluid	
Fetal/ maternal fluids	Keep refrigerated	Dam serum/ fetal fluids	• Paired (acute and convalescent), if possible

Proper collection and handling of fresh tissues

Collection of a comprehensive set of tissues and material is paramount for effective abortion diagnostics. An outline is provided above that may be used as a guideline for any species (Table 1.1). Species-specific and pathogen-specific sampling is further described in later chapters. Tissues samples should be refrigerated for short-term storage when pursuing bacterial or fungal etiologies if the sample cannot be processed immediately. Even if a toxic or nutritional problem is not initially suspected, it is advisable to place liver, kidney, and fetal fluids in frozen storage for possible future testing. If samples are collected for future virological testing, it is a much better idea to freeze the samples at −70°C rather than −20°C. Fetal and maternal fluids can be stored frozen. With blood samples, however, to minimize effects of lysed erythrocytes, serum should be separated before freezing the sample.

A good submission requires the use of proper primary and secondary containers. Fetal fluids and maternal blood samples should be collected in sterile red-top glass vials and then placed into padded, protective containers for shipping. Fresh samples should be collected into plastic, sealable, freezer bags; properly sealed bags are those with a locking seal mechanism as opposed to Whirlpak® plastic bags that are more prone to leakage. When applicable, feed samples should be included in sealable containers. Fixed samples, discussed in more detail later, should be included in jars that have properly sealing lids. Tape can be used to provide an additionally seal between the lid and jar. Alternatively, once the tissues have fixed for at least 24 hours, formalin-fixed tissues can be placed in sealable plastic bags for shipment as long as the excess air is removed

from the bags, thus preventing the tissues from drying out. As a precaution for accidental leakage or breakage during the shipping process, include absorbent materials in the package in order to minimize contamination of other packages. Absorbent materials can be purchased specifically for this purpose. Alternatives to specific packaging absorbent material are disposable diapers, which are made of similar material and designed to absorb large quantities of fluid.

Fixed specimens for histologic evaluation

A full spectrum of specimens should be collected and preserved or fixed in neutral-buffered 10% formalin. Formalin can be made in clinics from raw materials or purchased from commercial companies or from your local diagnostic laboratory. Formalin solutions prepared in clinics are typically inferior due to a lack of proper buffering resulting in excess amounts of artifactual pigmentation of tissue sections, especially fetal and heavily congested tissues. When fixing collected specimens, ensure a proper ratio of formalin to tissues of 10:1. This ratio has proven the test to time, and filling a container with tissues without the proper amount of formalin fixative results in incomplete tissue fixation, continued autolysis, and, overall, a poor quality submission. Increasing the concentration of formalin, such as 30% may hasten fixation of the tissues but can lead to artifactual changes in the fixed tissues (i.e., darkened neurons).

A complete set of tissues should be collected. This would include fetal membranes, brain, lung, heart, liver, kidney, pancreas, adrenal gland, thyroid gland, thymus, lymph node, spleen, and skeletal muscle. In general, alimentary samples are of limited utility for diagnosis of most causes of abortion, stillbirth, or neonatal death, but to ensure that proper tissues are collected, regardless of age, multiple sections of the alimentary tract can be collected and saved. As is the case in non-neonatal or fetal animals, the prosector should pay particular attention to any tissues that appear to have lesions. It is advisable to collect at the margin of normal and abnormal for large lesions. For smaller lesions, collect sections with enough surface area so that several lesions are available for examination. Record any observed lesions in the history in order to alert the pathologists and laboratory staff of your findings. This will ensure proper sampling at the time tissues are processed for histologic evaluation and hasten the return of meaningful results.

As previously mentioned, fetal brain samples often appear as undesirable samples for histologic evaluation due to the high water content and "soupy" consistency. However, for most species, it is advisable to examine multiple sections histologically in order to look for evidence of fetal septicemia or protozoal infections (Figure 2.20).

Feed and water samples

Because compromised nutrition may lead to abortion as a means of survival by the dam, it is always prudent to evaluate both feed and water for quality. A comprehensive feed and water analysis may be useful information and offer insights into deficiencies or excesses that may play a role in fetal or neonatal loss as well as impaired fertility and ongoing herd losses.

Fetal and maternal serology

Fetal fluids, such as heart blood, thoracic fluid, and abdominal fluid, should be routinely collected at the time a fetus or neonate is necropsied for the purpose of serologic testing. It is advisable to collect serum from the aborting dam at the same time in order to compare serology results between dam and offspring. In addition, blood should be collected from herd mates that are unaffected and from those that have aborted during the same time period in order to provide a baseline for the group and evidence for a possible cause, respectively. Many references describe the importance of collecting and analyzing paired serum samples from females in the herd, at the time of abortion and 2 to 3 weeks later. In reality, the dam has typically seroconverted by the time she has aborted and therefore, the convalescent sample may show evidence of declining titers. That is why comparing mean antibody titers of affected with unaffected herd or kennel mates is a much better alternative.

A single sample is of limited diagnostic utility. It cannot differentiate if the antibody levels are due to recent vaccination, an endemic condition in the group, or recent exposure and thus the cause of the abortion. Each laboratory and each individual test method has a broad range. Therefore, determining the significance of a titers magnitude can be very difficult. Certain viruses, such as Parvovirus in swine and BVD in cattle herds, tend to be endemic, and the presence of antibodies to these viruses is more of a reflection of the herd than a cause of neonatal or fetal loss.

Samples reflective of the maternal environment

Fetal membranes represent the actual interface between dam and fetus. When carefully collected directly from the dam during a premature delivery prior to becoming contaminated, it is probably the best sample to assess the maternal environment. However, this is extremely unrealistic and impractical. Instead, stomach contents and portions of fetal lung are specimens that are collected to assess the maternal environment. In a fresh or autolyzed, intact fetus, the lung and stomach contents represent uncontaminated samples reflective of the amniotic environment. Infectious agents that either ascend through the cervix or are transmitted through maternal blood will infect the developing fetus. Swallowed or "inhaled" amniotic fluid during development represents a portal of entry. Thus, bacteria or viruses isolated from these samples should be considered to be significant and likely causative.

Topics of special consideration

Sporadic abortions versus abortion storms

The number of animals that abort due to a particular cause is one way of classifying abortigenic disease. When few animals in a group are affected, they are classified as sporadic abortions. Either a small number of animals abort in

a short, defined period of time or the abortions occur throughout the entire reproductive cycle of the group. They can be caused by infectious agents that primarily target unprotected dams in the group or may be due to feed contaminants, such as *Aspergillus* spp. overgrowth in hay.

Abortions storms are more difficult to define. Although it should represent a certain percentage of animals, such as 1% of a group, affected in a short period of time, in reality, an abortion outbreak becomes defined as a "storm" when three or more animals abort! Three animals in a group of 10 is very different from 3 in a group of 1,000, yet most owners and veterinarians sound the alarm at 3, regardless of herd size. Many infectious agents can cause severe outbreaks of fetal losses, especially when the herd immunity is poor or inapparent for a particular pathogen (i.e., BVD virus outbreak in an unprotected group of pregnant cattle). Although determining the cause of an abortion storm becomes paramount during the event, many times, producers and veterinarians are helpless to prevent it. For some pathogens, such as Equine Herpes Virus, vaccinating in the midst of a storm may be effective. Some pathogens have the potential to cause abortions that occur sporadically or as an outbreak, dependent on the dose and herd immunity.

Acute versus progressive fetal loss

Typically, the ideal submission for diagnostic purposes is an animal that is examined immediately after death or a morbid animal that is euthanatized specifically for diagnostic purposes. When dealing with fetal carcasses, the time and manner of death has even greater implications in terms of quality of the submission. Regardless of cause, various hormonal stimuli in the dam are required to induce physical changes necessary for the aborted fetus to be delivered. If the inciting cause first leads to illness of the dam, fetus, or both, most often these same causes lead to induction of parturition. Typically, so long as the abortus is identified promptly, the fetus and fetal membranes tend to be fresher. However, if the cause leads to abrupt cessation of fetal viability, induction of parturition occurs as a secondary event and may take many hours to a few days. In these instances, the abortus and fetal membranes can be extremely autolyzed and often have limited utility for determining a definitive diagnosis. However, the condition of the fetus and its approximate age can help rule in possible causative categories (i.e., Brucellosis in second trimester bovine abortions). Thus, in cases of death *in utero*, peracute to acute death typically results in fetal retention for many hours leading to severe autolysis of the fetus and associated membranes.

Periparturient fetal viability and stress

During parturition, fetuses are forcibly extricated from a highly controlled, protective womb into a more varied and potentially hostile ambient environment by a combination of powerful, coordinated uterine and abdominal muscular contractions. Delivery of a live neonate is dependent on the fetus entering the pelvic canal in a normal position and in a timely manner so that the newborn can transition to breathing on its own. Prolonged parturition due to fetal malposition,

Figure 1.2 Meconium-stained ovine fetus. Two fetuses are present in the image. The fetus in the lower left is stained yellow-brown with flecks of brown meconium over its hair coat. This is an indication of *in utero* stress, likely the result of fetal hypoxia. The twin lamb has a whiter hair coat, with no evidence of meconium staining. (Courtesy of R Irvine, University of Glasgow.)

small pelvic diameter, or possible nutritional deficiencies can result in fetal stress or eventually death. Presumably as a response to fetal hypoxia, the best indication of periparturient fetal stress is relaxation of the anus with release of meconium. This results in orange-brown to orange-yellow discoloration to the fetus (Figure 1.2). In fetuses that die, similar material can be found in the lungs, histologically, indicating aspiration of the meconium during the periparturient period.

Differentiating a stillborn fetus, defined as born dead, from a neonate that was born alive but died in the postparturient period requires necropsy evaluation. Lung inflation is the best method for differentiation. Dark, red-purple lungs in fresh fetuses indicate congenital atelectasis (Figure 1.3); however, this can be a little more difficult in autolyzed lungs. Inability of a section of fetal lung to float in water is another test to confirm lack of antemortem lung aeration.

A second method of determining periparturient fetal viability involves examination of the intra-abdominal umbilical arteries. At the time of parturition, the umbilical connection between dam and fetus is severed. In the live fetus, severed umbilical vessels trigger a combination of vascular smooth muscle spasms and contractions, localized release of mediators of inflammation, as well as continuous delivery of platelets and clotting factors. The result is vascular luminal narrowing, periarteriolar hemorrhages, and thrombosis of these umbilical arteries (Figure 1.4). In stillborn fetuses, severing of the umbilical cord does not result in thrombosis or hemorrhages due, at least in part, to the lack of fetal blood flow.
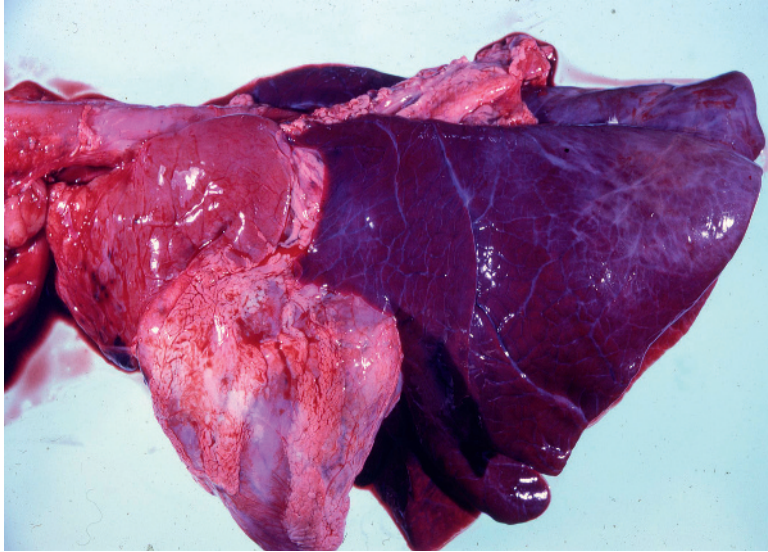
Figure 1.3 Fetal bovine pluck. The lungs from this fetus are dark red to purple indicative of congenital atelectasis. The heart and thymus are visible in this image. (Courtesy of Dr. RJ Panciera, Oklahoma State University.)
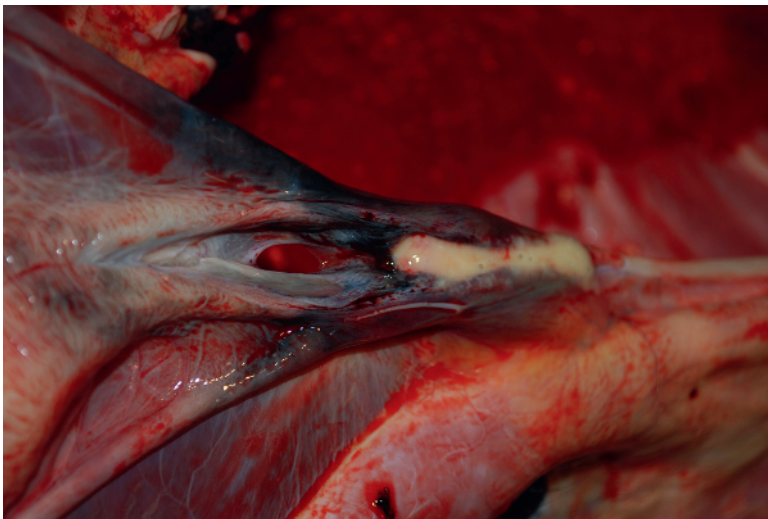
Figure 1.4 Neonatal bovine umbilical arteries. Flanking the urinary bladder are two thrombosed umbilical arteries with peripheral areas of hemorrhage within the abdomen of early neonatal foal. In this case, the foal also had urachal inflammation, with pus evident in the region of an opened urachus. (Courtesy of Dr. SD Cramer, Oklahoma State University.)

Summary

In summary, abortion diagnostics are often challenging and frustrating. Even with continued improved techniques, the broad range of possible causes for fetal and neonatal death prevents diagnosticians and diagnostic labs from confirming definitive diagnoses most of the time. One or two abortions likely represent isolated events that do not warrant further investigation. When the number of dead fetuses or neonates begins to increase, more effort should be put into determining a cause. In order to maximize the return on effort, labs try to minimize the cost and effort by pooling tissues and search for a select number of known, more common pathogens during initial screens. If these tests return negative results, other less common causes are explored. Coupled with a thorough, comprehensive, and systematic approach that may include obtaining a more in-depth history, scheduling a site visit, and gaining a broader set of samples that may include maternal serum, water and feed samples, or special genetic analytical tests, a diagnostician has the best chance of arriving at a definitive diagnosis now than ever before.