Pregnancy and perinatal losses: general concepts

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Abstract

Determining and, by extension, preventing loss of pregnancy is of utmost importance to a reproductive medicine practioner. However, determining the cause of fetal loss can be a frustrating experience to the clinician and the diagnostician. In general, diagnostic rates are very low amongst any domestic animal species and the phrase 'the cause of fetal loss was not apparent' is considered a failure of the diagnostic approach. However, the owner, clinician and diagnostician should consider the value of negative findings in any abortion investigation in light of the clinical circumstances, condition of fetal tissues, diagnostic tests performed, and the gross and histologic findings. In contrast to pregnancy loss, performing postmortem diagnostics to perinatal mortality cases can be a rewarding endeavor depending on the species and the circumstances. Whereas complete postmortem examination can be intimidating to the clinician, it is a technical skill that can be easily mastered with practice. A general practitioner should be aware that portions of postmortem diagnostics (primarily the postmortem examination) can be performed 'in house' and may provide benefits such as timely results and increased revenue to the practice.

Keywords: Abortion, fetal loss, perinatal mortality, necropsy, diagnostic pathology

Introduction and definitions

Pregnancy loss is roughly categorized into:

1. Embryonic death
2. Midpregnancy fetal death followed by resorption
3. Fetal death characterized by maceration, mummification or abortion
4. Stillbirth

Definition of embryonic death varies among species and this variation is justified given the variable period of pregnancy in domestic animals.

Species	Embryonic death
Equine	< 42 days[1]
Bovine	< 42 days[2]
Canine	< 15 - 17 days
Feline	< 21 days
Ovine	< 45 days[3]
Caprine	< 45 days[4]

Definition of the perinatal period varies in the veterinary literature. In fact, multiple definitions are accepted in human pediatric medicine. These include infant deaths that occur prior to an interval from 7 to 28 days of age and fetal deaths > 20 - 28 weeks of pregnancy.[5] Definitions of perinatal death in veterinary medicine are inconsistent. Some refer to deaths occurring only during midpregnancy (more traditionally referred to as abortion) whereas others align more with human definitions. For the purpose of this review, perinatal death is defined as a fully formed term-animal that was delivered dead or an animal that died within 48 hours after delivery.

Approach to recurrent fetal loss and perinatal loss in humans as it relates to veterinary medicine

Recurrent fetal loss (RPL) is defined as the loss of ≥ 2 pregnancies. Since majority of pregnancy loss in humans occurs before 10 weeks, the diagnostic focus is on classifying causes of miscarriage. The classification of potential causes is well described; however, there are variations in these guidelines among international standard organizations.[6] A critical feature in the generation of these guidelines is the discussion of all conditions that may cause recurrent fetal loss, with recommendations for follow up additional testing or treatment.

The value of standardizing criteria for RFL is clear. Standardization has generated diagnostic algorithms for clinicians to follow to advise and perhaps optimize fertility in patients. The criterion of most comparable importance in veterinary medicine is arguably the anatomic category, similar lesions in the endometrium of domestic animals. Adenomyosis, chronic endometritis and endometriosis are anatomic risk factors for RFL in humans.[7] Endometriosis is only observed in primates. Adenomyosis is a common lesion of canine uterus; however, it is typically has little clinical relevance. For example, adenomyosis was an associated finding in only 1.5% of 399 infertile bitches[9] whereas, chronic inflammation is a finding associated with infertility in the mare[8] and the bitch.[9]

Efforts have been taken to understand the categories and pathogenesis of RFL (Table 1). Epigenetic modifications such as DNA methylation have a role and abnormal occurrences of DNA methylation are associated with RFL and fetal developmental abnormalities.[10] Inflammatory mediators such as TNF-α are implicated in RPL when there is an inappropriate shift towards Th-1 mediated inflammatory responses.[11]

Table 1. Commonly accepted categories associated with recurrent fetal loss

Category	Additional notes
Cytogenetic	Robertsonian translocation, trisomy
Anti-phospholipid syndrome	Associated with vascular thrombosis, lupus anticoagulant, anticardiolipin or antiβ_2 glycoprotein antibody
Anatomic	Mullerian uterine abnormalities, uterotubal patency, uterine fibroids
Hormonal or metabolic	Hypothyroidism, hyperprolactinemia,
Hereditary thrombocytopenia	Investigation only recommended if a family history is present
Male Factors	DNA fragmentation, sperm aneuploidy

Women with a higher incidence of miscarriage do have a higher incidence of uterine malformations;[12] however, there is little evidence that corrective surgery results in a reduction of infertility.[6] Most efforts in veterinary medicine revolve around identification of infectious agents and more effort should be directed at establishing specific categories as documented with human RFL.

Diagnostic modalities to consider

During postmortem evaluation of adult animals, it is not uncommon to recognize entities that render further diagnostic unnecessary. Examples of this include gastric dilatation volvulus (dogs), strangulating mesenteric lipomas (horses), and hepatic lipidosis (cats). However, pathognomonic gross lesions are uncommon in abortion cases and a uniform approach to sampling and test selection is critical to maximize diagnostic yield. Infectious disease is often of primary concern in the evaluation of fetal tissues and fetal membranes and perinatal losses. Detection of infectious disease relies on appropriate selection, sampling and storage of tissue during this examination. A few entities causing fetal or neonatal loss can be recognized grossly. For example, canine herpesvirus-1 is often associated with multifocal renal cortical hemorrhage and hemorrhage in the liver and lungs. However, bacterial septicemia can also be associated with this lesions and polymerase chain reaction (PCR) detection of viral nucleic acid is a useful method to confirm the diagnosis. Similarly, cotyledonary mineralization and necrosis have been associated with ovine toxoplasmosis whereas round target-shaped regions of hepatic necrosis are associated with *Campylobacter fetus* ssp. *fetus.* In general, pathognomonic lesions are not common and should not be relied on exclusively for a diagnosis.

Postmortem examination

The technical expertise required to perform a postmortem examination should be separated from the expertise required to interpret gross tissue changes. Whereas it can take years to master interpretation of gross tissue changes, the good news is that utilizing a uniform technical approach will negate the need for meaningful gross interpretation and maximize diagnostic yield. Equipment should be the first consideration. Regardless of species, some items will be used in common. Most of these items are related to tissue collection and storage for additional diagnostic testing. Tissue collected for histopathology are stored in 10% neutral buffered formaldehyde (formalin). In most circumstances, this tissue can be stored indefinitely; however, this can impede the results of immunohistochemical (IHC) stains. It should be noted that IHC is rarely of use in these types of cases.

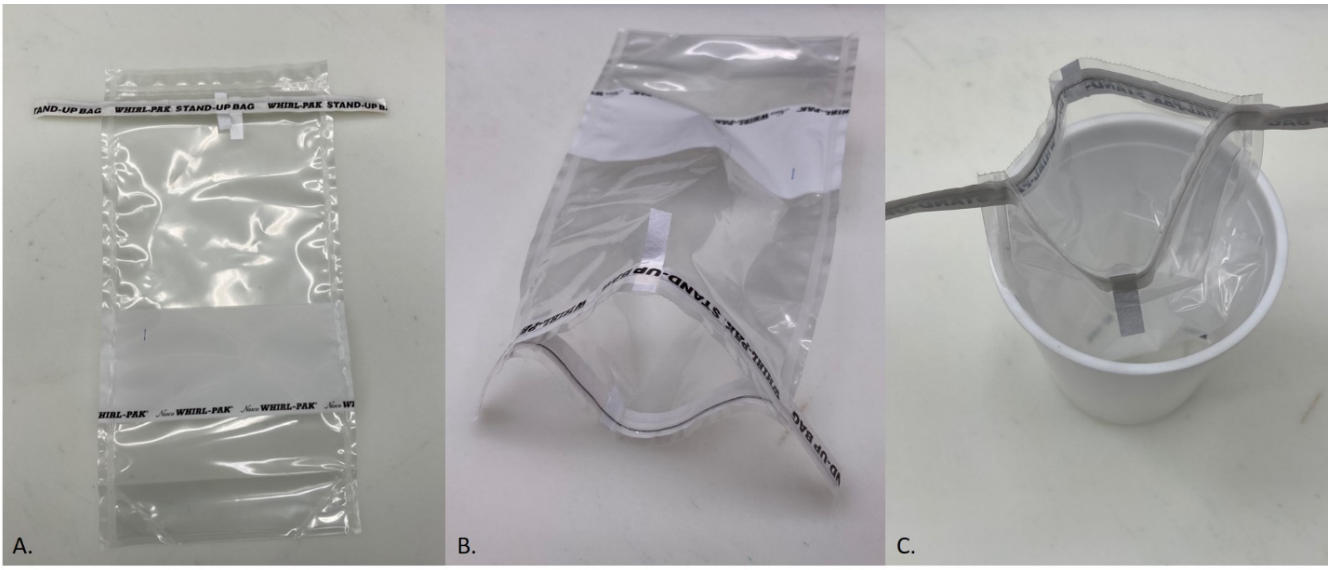

Figure 1. Whirl-pak bag and convenient configurations to prepare them for tissue collection

Sterile collection containers are critical for fresh tissue collection. Whirl-pak bags are an ideal container. They are light weight, inexpensive, and easy to use. Bags should be labeled prior to starting the postmortem and as the postmortem is being performed, the perforated top is torn off and the bag can be propped open with wire supports at the edge of the opening or by opening the base so the bag stands on its own (Figure 1). Note that there are tags on the outside of the bag that are used to open the Whirl-pak bag without contaminating the inner surface. These bags should not be physically opened by inserting fingers or instruments into the inside of the bag.

Prosection equipment varies depending on the species examined. For small animals, a scalpel blade, Mayo scissors and forceps are typically sufficient. An older pair of scissors is preferable as they can be used to cut costochondral junction as well as sutures of the calvarium. For ruminant and equine fetuses, a sharp knife and cutters are necessary. A skilled prosector can perform a majority of the postmortem examination with these implements. A cleaver, handsaw or Stryker saw is typically necessary for brain removal.

Culture swabs and collection of fluids is seldom helpful for microbiology and molecular diagnostics in postmortem diagnostics. Tissues collected in a semisterile or sterile fashion is ideal. Collection of the required solid organs as soon as the thoracic and abdominal cavities are open: 1. minimizes the chance of contamination and 2. reminds the prosector to collect tissue in the first place. The only exception to this is stomach or abomasal contents. In many species, this material represents an uncontaminated source of amniotic fluid contents. This material is best stored in a small glass or plastic cylindrical container such as a red top blood tube in a sterile fashion near the end of the prosection. All containers should be sealed properly prior to shipment. Packages containing formalin jars should also contain absorbent material in case of leakage and Whirl-Pak bags should be contained in a second sealed plastic bag or container.

Actual postmortem procedure varies among prosectors; however, in general, are performed in either dorsal or left lateral recumbency, with the former being practical for fetal dogs and cats. Skin incisions are made from the ventral aspect of the mandible to the pubis and the skin is reflected dorsally (and bilaterally if a ventral incision is made). At this point, the abdominal wall is incised at the linea alba and additional incisions are made at the body wall just caudal to the ribcage, allowing the abdominal contents to be exposed completely (Figure 2). Depending on the approach, the thoracic cavity is opened by cutting the sternum and the dorsal aspect of the ribs in a linear fashion (with a lateral approach) or the dorsal aspect of the ribs in a linear fashion bilaterally.

At this point in the postmortem, it is most prudent to collect the fresh tissues required for ancillary diagnostic testing. A set of clean scissors and forceps are helpful at this point. Immersing the tips of the instruments in ethanol is helpful prior to tissue collection; however, this step can be omitted if the prosector uses careful technique. Most microbiology laboratories will heat sterilize the outside of the tissue prior to plating the sample. Liver, lung, kidney and spleen are ideal for collection at this point and should be collected without puncturing the gastrointestinal tract. Although contamination is less of an issue with a stillborn animals and aborted fetuses, this guideline should be adhered to for animals that have nursed. Stomach content collection can be reserved until the gastrointestinal tract is examined and should be stored in a separate container than the other fresh tissues. In general, liver, lung, kidney, and spleen can be pooled together in 1 container for submission. Fresh tissue can be frozen until testing is performed. Tissue should be frozen at - 70˚C if being stored for long intervals but can be stored at - 20˚C if testing will take place soon after collection.

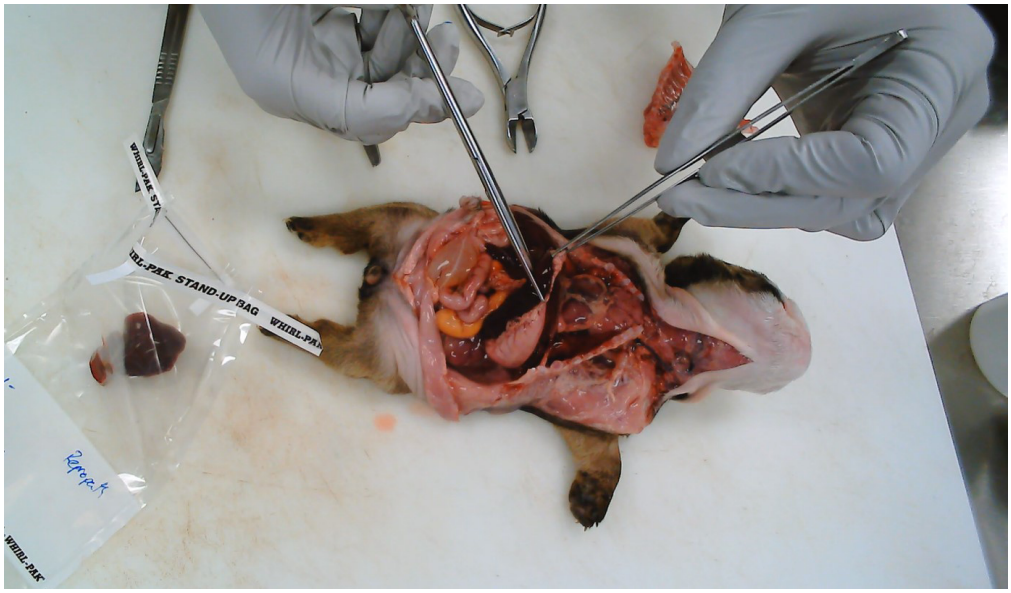

Figure 2. Sampling of fresh tissue and storage of pooled tissue into a Whirl-pak immediately after opening the peritoneum and thorax

Following collection of fresh specimens, tissue collection for histopathology (formalin fixation) can take place. Most diagnostic laboratories will collect an exhaustive set of tissues for histopathology. This may be difficult to perform out in the field with equine and food animal perinatal deaths; however, it is important to remember that a very small percentage of the formalin fixed tissue is evaluated histologically (~ 3 µm tissue thickness). Large amounts of tissue are not necessary for histologic assessment. It is more effective to provide tissue representative of lesions and multiple small representative areas of organs. All tissue submitted in formalin should be ≤ 0.5 cm in thickness. Eye and brain are the exceptions and can be submitted whole. If the organs are already < 0.5 cm in width (or close to this thickness), then they can be submitted whole. Most canine or feline tissues can be submitted whole and it is unnecessary to extensively dissect most tissues in these species (heart, kidney, etc.) aside from sectioning them to a thinner width. The order in which organs are collected is less important, although generally, the gastrointestinal tract should be reserved until after solid organs have been sampled.

Fetal and perinatal tissues can exhibit different physical characteristics than those observed in adult animals, leading to misinterpretation of nonlesions. The most common of these is the appearance of fetal brain. Due to incomplete myelination and increased water content, the brain will be soft and difficult to handle without traumatizing the tissue. In aborted fetuses, the brain can be poured out of the open calvarium. The soft nature of the tissue does not impede histologic evaluation as long as it is adequately formalin fixed. Fetal pulmonary parenchyma tends to be reddish-purple and will often sink in formalin when obtained from stillborn animals or aborted fetuses. This is due to fetal atelectasis. If lungs consistently sink from perinates that died after parturition, this may be an abnormal finding. Fetuses examined after an extended postmortem interval may have an accumulation of red opaque watery fluid in the peritoneal and pleural cavity. This change should generally be ignored particularly if the animal has been frozen and thawed before examination. Due to the number of misleading postmortem findings, a uniform and consistent collection of tissues in the manner outlined above should take priority over identification of potential causes during the examination.

Abortion/fading neonate panels

One of the more vital tools in the approach to these cases are standardized panels utilized for the detection of infectious diseases. These panels employ a variety of diagnostic modalities to detect pathogens. In general, panels directed towards food animal and equine abortion/perinatal disease cases are more commonly provided by state diagnostic laboratories. A panel submission brings the advantage of a simpler submission process without the need to select individual tests. However, it is a straightforward process to order the appropriate tests for a panel 'a la carte' that is often the case for canine and feline cases (Table 2).

Table 2. Common ancillary diagnostic tests ordered in canine and feline investigations

Species	Tests
Canine	Aerobic culture, Salmonella spp. culture, *Brucella canis* culture, Leptospirosis spp. darkfield microscopy, Leptospirosis spp. PCR, *Brucella canis* PCR, Toxoplasma PCR, Canine herpesvirus-1 fluorescence antibody (FA), Canine distemper virus FA, Canine adenovirus FA
Feline	Aerobic culture, Feline panleukopenia PCR, Toxoplasma PCR, Feline leukemia virus indirect FA, Feline herpes PCR, Feline calicivirus FA

This does require some working knowledge of testing protocols and the limitations of these modalities. In either circumstance, it is critical to pay attention to the requirements for tissue preservation. For instance, certain tests cannot be performed on formalin fixed tissue (culture and reverse transcriptase PCR). This requirement outlines the need to save a set of fresh tissue separately from the formalin fixed tissue. The most common testing modalities used include: bacteriologic assays, fluorescence antigen assays, viral isolation, and molecular diagnostics.

1. Bacteriologic assays

In general, standard aerobic culture is acceptable in most cases. In most circumstances, tissues are preferable to swabs or fluid collection. Culture for specific pathogens that may require specific bacteriological assays include *Salmonella* spp. and *Brucella* spp.[9] However, the diagnostician must keep in mind that confirmation of brucellosis is often more effectively accomplished through serologic assays.

2. Fluorescence antibody testing

Also referred to as 'direct fluorescence antibody testing,' this testing modality utilizes an antibody labeled with a fluorochrome directed at a specific antigen associated with a pathogen. This needs to be distinguished from 'indirect fluorescence antibody testing,' a test that utilizes a secondary fluorochrome labeled antibody directed at an unconjugated antibody directed at the antigen. These methods can be used to detect a variety of pathogens in research settings but in the context of this discussion, FA is primary utilized to detect viral pathogens.

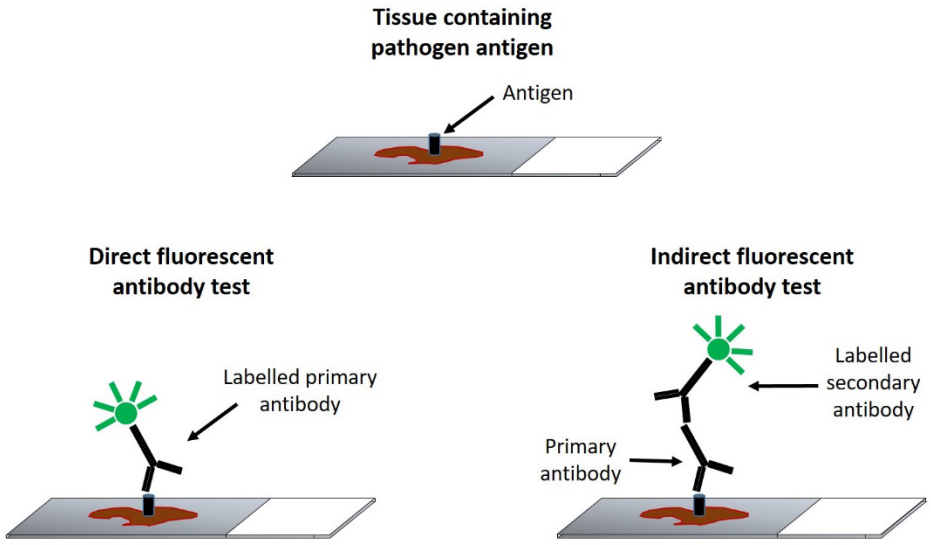

Figure 3. Illustration of fluorescent antibody testing in tissue

3. Viral isolation

Viral isolation is the general term used for cultivation of virus particles as methods vary depending on the type of virus. Cell cultures, embryonated eggs or live animal subjects can be utilized but, in most circumstances, cell culture is used. Cell cultures can be primary (derived directly from an animal and propagated in media) or continuous immortalized cell culture lines derived from neoplasms. Either can serve as a suitable media to propagate and identify viral organisms. The type of cell line chosen for cell culture largely depends on the tropism of the virus. Cell lines of epithelial, mesenchymal and hematopoietic origin are available for this purpose.

Following propagation of a virus, multiple subsequent steps can be taken to assist in identification (electron microscopy, hemagglutinin inhibition assays, complement fixation assays, and immunofluorescence assays). Viral isolation from tissue samples can be more complex that from a swab stored in viral isolation media, so it is critical that as soon as possible fresh tissue be prevented from degrading. Either immediate submission for testing or freezing tissue as an intermediate step should take place.

4. Molecular diagnostics

This category covers a wide variety of testing modalities but PCR is the most cited in this discussion. A wide variety of PCR modalities are available, but an exhaustive discussion is beyond the scope of this work. In general, DNA viruses are best detected using standard PCR assay whereas RNA viruses are detected using reverse transcriptase PCR. Additional modalities such as multiplex PCR and nested PCR can maximize the amplification yield if there is less viral nucleic acid in the specimen. Techniques such as quantitative PCR or RT-PCR can determine the viral load in given sample but may not be critical in the diagnostic process. It is important to recall that any viral nucleic acid can be detected in PCR assay, even if intact viral particles are not present or are not the primary cause of the disease.

Serology

Whereas fetal serology can be performed, it is of little use diagnostically unless the fetus is immunocompetent. Fetal serum can be collected in circumstances with mild postmortem degradation; however, it will be difficult in smaller species. Maternal serology is more convenient to obtain and more likely to provide usable diagnostic information. Paired serum samples are ideal and a necessity when certain pathogens are suspected (e.g. *Leptospira* spp.). The first sample is ideally collected at abortion and the second is collected after 2 - 3 weeks.

Toxins and nutritional deficiencies

Exposure to toxins is certainly associated with teratogenesis, abortion and stillbirth. Any toxin that induces severe maternal illness can result in secondary fetal loss. However, when referring to toxins that primarily have teratogenic effects or induce fetal loss, ruminants and horses are primarily affected. Toxins range from plant based (Ponderosa pine, locoweed), plant associated fungal products (ergot alkaloids) to pharmaceutical agents. The ideal tissues for toxicology testing can vary depending on the toxin; however, the general tissues that fulfill most test requirements include liver, spleen, and kidney.

Conclusion

Fetal and perinatal loss is a difficult scenario for most veterinarians to resolve for multiple reasons. In general, we have limited information regarding the specific causes of genetically related embryonic loss in domestic animals.[14] In ruminants, horses, and pigs, at least nutritional deficiencies, toxicities and select infectious agents are well established causes.[15,16] However, more work is necessary to identify specific causes of chromosomal abnormalities and DNA damage, particularly in dogs and cats.

In general, investigating fetal and perinatal loss may not be rewarding in all cases but the technical aspect of investigating these cases can be handled in an adequate manner by most practitioners with equipment that is available in most veterinary practices. Whereas submission of a whole fetus to a diagnostic laboratory is optimal in most cases, the cost associated with the postmortem examination and ancillary diagnostic testing can be prohibitive. The option to perform the diagnostic examination 'in house' can provide a lower cost option to the client and increase the number of investigations in these scenarios.

Conflict of interest

The author has no conflict of interest. No funding was received for this publication.

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