Investigating Fetal Calf Loss

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Take Home Messages

- Fetal calf loss (abortion) is economically significant to the dairy industry. A background reproductive loss of approximately 1% is expected. A rate higher than this may warrant investigation.
- Abortion diagnosis can be frustrating and costly. The chance of diagnosing the cause of a single abortion is relatively low (25–50%). However, the likelihood of diagnosis is increased if there are multiple cases and if the cause is infectious.
- The chance of diagnostic success is maximized if the producer, veterinarian and diagnostic laboratory collaborate. The most important part of this is collection and submission of correct tissue samples.
- Tissue sampling as part of an abortion investigation is not haphazard. Collection of an inadequate tissue set or preserving samples inappropriately can potentially cost a diagnosis.
- Any abortion investigation should begin with collection and examination of the aborted fetus and placenta. The placenta should always be included in a diagnostic submission. The diagnostic value of the placenta is equal to or greater than that of the fetus, yet it is frequently overlooked.
- Producers can play an important role in collecting data about aborted fetuses and placentas that may otherwise be lost. This is not difficult or time-consuming, and is explained in this paper.
- Abortion investigation is a complex, detailed and systematic procedure. Knowing what it involves will help producers understand its inherent limitations.

General Introduction

Fetal calf loss is an important cause of economic loss in the dairy cattle industry. The significance of a cow aborting a calf is not always clear. It may be part of the normal expected background reproductive loss of 1%, a sporadic case of disease, or the start of an abortion outbreak or storm. Each may require a different investigative approach (Holler, 2012; Taylor and Njaa, 2012).

Determining a specific cause for abortion can be difficult, costly and frustrating. Despite spending several hundred dollars working up a case, the final diagnosis may be 'abortion; cause undetermined.' This should not discourage investigations of later abortions, especially when fetal losses are unexpectedly high in number, clustered in time, and at a similar stage of gestation. Identification of a specific cause may allow implementation of management strategies that reduce or prevent further losses.

This paper discusses: a) the common causes of bovine abortion in Western Canada, b) what information can be obtained from examination of the fetus and placenta, c) when to contact the veterinarian, and d) how the producer and veterinarian can work together to maximize the chance that a cause of abortion can be determined.

Is Fetal Calf Loss Different from Abortion or Stillbirth?

For the purposes of this paper, fetal calf loss is not different from abortion or stillbirth. I chose the term 'fetal calf loss' for my title because it encompasses both abortion and stillbirth. Abortions happen in early gestation and stillbirths happen in late gestation. Specifically, abortion refers to fetal death and expulsion from the uterus before the fetus is viable. Even if it is alive at the time of expulsion, an aborted calf is not developed enough to survive outside the uterus. In contrast, stillbirth refers to delivery of a fully formed, but dead, calf. Had the calf been born alive it stood a chance of surviving (Holler, 2012). For practical purposes there is very little difference in the diagnostic workup of an aborted calf versus a stillborn calf. Most producers and veterinarians use the term 'abortion' in a wider sense to mean death and expulsion of a calf any time during gestation. This is the sense in which I use this term for the remainder of this paper.

What are the Common Causes of Bovine Abortion?

The simplest way to subdivide bovine abortion is into infectious and non-infectious categories. Infectious causes of abortion are typically easier to diagnose through laboratory testing than are non-infectious causes because diagnostic laboratories are well set up to screen tissue samples for viruses, bacteria, protozoa and other infectious agents. Identification of non-infectious causes such as faulty management practices, toxins in feed, or genetic abnormalities typically requires a more in-depth investigation. Although there are numerous causes of abortion in cattle, very few of them leave tell-tale gross lesions. Identification of a specific cause, therefore, usually requires a detailed investigation by a veterinarian working in conjunction with a diagnostic laboratory. This does not mean that there is no value in gross examination of an aborted calf and placenta, just that this is only one step in a larger work-up (more on this later).

Infectious Causes of Bovine Abortion

How Infectious Agents Reach the Fetus

During pregnancy it is relatively uncommon for infectious agents to reach the uterus by ascending through the cervix. Instead, the majority of infectious agents that reach the pregnant uterus arrive hematogenously, via the cow's bloodstream (Anderson, 2012; Holler, 2012).

After reaching the pregnant uterus, many agents colonize and proliferate in the placenta, causing a placentitis (inflammation of the placenta), without necessarily spreading further downstream into the fetus. Placentitis alone may be enough to cause abortion because an inflamed placenta is less effective at the exchange processes needed to support a fetus. A fetus aborted because of a primary placentitis may lack gross or histologic lesions, and infectious agents may not be detectable; therefore, the placenta, if available, should always be submitted for testing along with the fetus.

After colonizing and proliferating in the placenta, infectious agents may spread secondarily to the fetus. Some agents do this by entering the amniotic fluid around the fetus, where they are ingested and inhaled, leading to lesions in the fetal lungs, digestive tract, and skin. Other agents enter the fetus via the umbilical blood vessels, leading first to lesions in the liver and later to widely disseminated lesions in many organs, including the brain. The distribution of fetal lesions provides some information about the route of pathogen entry and may hint broadly at a cause (e.g., viral versus fungal).

Viral Agents of Bovine Abortion

Viral agents include bovine herpesvirus type 1 (BHV-1) and bovine viral diarrhea virus (BVDV). Both agents are familiar to producers and should be controlled with an appropriate vaccination program. Bluetongue virus (BTV) and bovine parainfluenzavirus type 3 (PI-3) are less common viral causes of bovine abortion.

Bacterial Agents of Bovine Abortion

Bacterial agents that cause bovine abortion are numerous, and most are opportunistic environmental or mucosal microbes that infect the cow, gain entry to the maternal bloodstream and spread hematogenously to the pregnant uterus. The cow may or may not show signs of illness, and abortions tend to be sporadic rather than occur as outbreaks. Opportunistic bacteria include *E. coli, Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*), *Listeria monocytogenes*, *Pasteurella* species, and *Histophilus somni*. Species of bacteria that are more commonly associated with maternal illness or have a predilection for the reproductive tract include *Salmonella* species, *Leptospira* species, *Ureaplasma diversum*, and *Campylobacter fetus*. Neither list is exhaustive and determination of a specific bacterial cause for abortion always requires testing of samples by a diagnostic laboratory.

Fungal Agents of Bovine Abortion

Like bacterial agents of abortion, most abortigenic fungal agents are opportunistic environmental microbes that gain entry to the maternal bloodstream (and hence, uterus) via the respiratory or digestive tracts. They include *Aspergillus* and *Zygomyces* species.

Protozoal Agents of Bovine Abortion

The most important abortigenic protozoal species in dairy cattle are *Tritrichomonas foetus* and *Neospora caninum*. *T. foetus* is spread venereally and is therefore more common in herds where cows are bred naturally rather than by AI; bulls may be inapparent and lifelong carriers. *N. caninum* (spread by canids such as dogs and coyotes, or acquired congenitally *in utero* without canid involvement) may cause lifelong, inapparent infection in cattle. Infection in cows is efficiently spread to the fetus resulting variably in abortion, a live calf with neurologic disease (rare), or a live, infected calf with no clinical signs that may go on to pass the parasite to its own offspring.

Non-Infectious Causes of Bovine Abortion

Non-infectious causes of bovine abortion are also numerous, but are less easily diagnosed than infectious causes. The majority consist of chromosomal abnormalities that cause early fetal death and resorption; these typically go undiagnosed. A number of ingested plant and fungal toxins may also cause abortion. These include ergot toxins derived from the grass fungus of the genus *Claviceps*, an increasing cause of abortion in Western Canadian cattle. Other recognized abortigenic plant toxins are derived from pine needles, juniper plants and locoweed (*Oxytropis* species). Toxins in hemlock (*Conium* species) can cause fetal malformation and deformity. Management issues, including vitamin A, vitamin E or selenium deficiency, may play an underappreciated role in bovine fetal loss.

In general, non-infectious causes for bovine abortion are more difficult to identify than infectious causes. An abortion investigation typically rules out common infectious causes first, and then focuses on a more complex investigation, which may include feed sample analysis, fetal and maternal tissue and blood mineral level testing, genetic analysis, farm walk-throughs, and observation of management and handling procedures.

How often is the Specific Cause of Bovine Abortion Determined?

Diagnosis of bovine abortion can be challenging: a cause for abortion is found in only 25–50% of cases (Table 1). The chance of achieving a specific diagnosis is decreased if: 1) the abortion is a single case, rather than an outbreak, 2) its cause is non-infectious, or 3) incorrect samples are submitted to the diagnostic lab (Foster, 2012). The converse is true—the chance of successfully obtaining a specific diagnosis is increased in an outbreak situation caused by an infectious agent and where appropriate diagnostic samples are collected and submitted.

 Table 1: Causes of sporadic failure of pregnancy in cattle (modified from Table 18-3 from Foster, 2012)

Cause	%
Unknown; no diagnosis	60
Non-infectious	3
Infectious	37

• How can the Producer Increase the Chances of Diagnostic Success?

Abortion investigation is a team effort with six players: 1) the cow, 2) the fetus, 3) the placenta, 4) the producer, 5) the veterinarian, and 6) the diagnostic laboratory. The producer can and should play an active role in abortion investigation in the ways described below.

Decide When to Call the Veterinarian

The term 'abortion storm' is not precisely defined. In theory it should reflect a certain percentage of abortions above an expected background level of 1%; for example, 4% of the herd aborting in a short time period. In practice it tends to be three abortions, regardless of herd size. However, there are no firm rules about what constitutes an abortion storm or how many abortions need to occur before calling the veterinarian.

Prepare for the Veterinarian's Arrival

Preparing in advance for the veterinarian's arrival saves time and money.

- The affected cow should be easily accessible for physical examination and blood sampling.
- The aborted fetus and placenta should be bagged and refrigerated, if possible.
- Information that may be requested by the veterinarian as part of the investigation should be easily accessible. This will include information about the whole herd, the affected cow(s), nutrition, vaccination history, any therapies used, the farm environment and possible toxin exposure.

For example, animal information requested could include the following questions (Holler, 2012; Taylor and Njaa, 2012):

- What is the age of affected cows: Older cows? First pregnancy cows? Mixtures of ages?
- What stage of pregnancy is abortion occurring—first, second or third trimester? Term?
- What percentage of cows pregnant have aborted?
- Are aborting cows otherwise clinically ill (beyond the abortion event)?
- Are abortions clustered by time of year or age group of cows?
- Are the abortions sporadic or are there many in rapid succession (that is, an abortion storm)?
- Is the herd closed or have there been new introductions?
- If new animals were introduced, when?
- Are cows bred naturally or by artificial insemination?
- Is there other evidence of disease in the herd besides abortion or neonatal loss?
- Is there a history of similar problems in previous years? If so, what were the findings and were management changes made?

Have congenital defects been seen before in this herd?

In cases of suspected vaccine failure, some of the requested information will be very specific. It may include vaccine serial numbers and expiration dates, information about storage (refrigeration) prior to use, and any modification to the vaccination protocol. It is helpful to have this information recorded and on hand.

Collect (and Examine) the Aborted Fetus

Necropsy (post mortem) examination of the fetus and collection of specific tissue and fluid samples are integral to the diagnostic investigation. These procedures are generally handled by the veterinarian on site or by the diagnostic lab pathologist after submission. However, the producer has earliest access to the fetus and can collect important information that may otherwise be lost. This is especially true for the first and second abortions in an abortion outbreak, when fetuses may be discarded rather than submitted, and the veterinarian is not called. Photographs, gross inspection findings and crown-rump length measurements of aborted fetuses are easily collected before disposal. The following sections describe what a producer should look for and record. When a fetus is submitted to a diagnostic lab the pathologist will conduct a similar examination along with a more detailed internal examination of the carcass.

Obvious Causes for Abortion

Obvious causes for abortion are rare, and diagnosis generally relies on laboratory testing and histopathology. However, fetal malformations sometimes occur, which are easily seen and photographed. These may suggest a heritable, viral or toxic cause. The more common external malformations to look for include (Foster, 2012; Schlafer and Foster, 2016):

- Cleft palate or lip (palatoschisis or cheiloschisis)
- Overshot or undershot jaw (prognathism or brachygnathism)
- Domed, possibly fluid-filled skull (hydrocephalus)
- Abnormal contraction of the limbs (arthrogryposis)
- Multiple limbs (polymelia)
- Arched back with intestines on the outside of the body (schistosomus reflexus)
- Fetal monster (amorphus globosus)
- Spinal deviation causing a hunchback (scolioisis and kyphosis)
- Incomplete closure of the spine along the midline (spina bifida)
- Distended, fluid-filled fetus (anasarca or ascites)

Crown to Rump Length and Body Weight

The crown to rump (C-R) length is the length of the fetus from the dome of the head, just behind the eyes, to the junction of the sacrum and tail head (Figure 1). The fetus should be straightened out as much as possible before taking this measurement, with the muzzle pointed down. Note that the measurement is NOT 'tip of nose to tip of tail.' The measurement is taken using a flexible tape measure or a length of string and compared against readily available charts to estimate the gestational age of the aborted fetus. Search the internet for terms such as 'bovine fetal age calculator' or 'fetal age assessment for cattle.' An example is included below (Table 2).

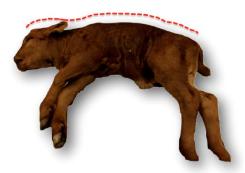


Figure 1: Measurement of fetal crown to rump (C-R) length, as indicated by the dashed line.

Gestational Age (months)	Crown to Rump Length (cm)	Weight (kg)	External Features
3	13–17	0.2–0.4	Hair on lips, chin and eyelids
4	22–32	1–2	Fine hair on eyebrows; claws developed
5	30–45	3–4	Hair on eyebrows and lips; testes in scrotum; teats developing
6	40–60	5–10	Hair on inside of ear and around horn buds, tail tip and muzzle
7	55–75	8–18	Hair on distal extremities and beginning on back; long hair on tip of tail
8	60–85	15–25	Fine short hair all over body; incisor teeth not erupted

State of Fetal Autolysis at Time of Expulsion

Autolysis refers to the softening of tissues that occurs after death (the reason carcasses are hung for a period after slaughter). It occurs as dead cells leak internal enzymes that cause self-digestion. Even tissues kept in a sterile environment will undergo autolysis. Autolyzed fetuses are soft and dark red, and their tissues ooze watery red fluid when incised. Autolysis is distinct from the putrefaction that occurs when dead tissues are colonized by bacteria, producing foul-smelling gas and green discoloration.

The state of autolysis of an aborted fetus is useful information because it may suggest the type of condition responsible for abortion. Note that the state of autolysis must be assessed at the time of expulsion, not after two days in a dead pit when bacterial putrefaction will make assessment inaccurate. This is a good reason for a producer to examine an aborted calf as soon as it is found.

Normal calves initiate their own birth at full term by signalling the cow through hormonal surges. A calf that dies suddenly (acutely or peracutely) does not have time to send these hormonal signals to the cow. As a result, the dead calf will remain undetected in the uterus for some period, during which autolysis will occur; this is accelerated by the cow's core body temperature. When it is eventually expelled, the aborted calf will be in an advanced state of autolysis. Conditions that can cause peracute fetal death include certain overwhelming fetal bacterial and viral infections.

In contrast, fetuses that are stressed for a period and die slowly have time to signal the cow and initiate their own birth. Thus, they are expelled in a fresh, non-autolyzed state shortly after they die. Conditions that cause fetal distress and hormonal signals to initiate birth include non-specific maternal illness,

chronic fetal illness, hypoxia (caused, for example, by placental disease), or maternal hyperthermia.

Indications of Dystocia or Fetal Distress

Sometimes it may not be clear whether a near-term calf was truly aborted, or died during an unobserved difficult delivery. The presence of fluid swelling (edema) of the subcutaneous tissues of the jaw, head and neck can indicate that the calf spent a prolonged period in the pelvic canal while still alive. Edema cannot develop after the heart stops, and an aborted calf will not develop regional edema even if compressed. Similarly, yellow staining of a calf's hair coat by meconium indicates prolonged delivery during which the stressed calf defecated into its own fetal sac. A calf that was already dead before delivery began cannot defecate and will not be meconium-stained.

Other ways to differentiate abortion from death during or after birth rely on a necropsy examination. Specifically, the lungs will be assessed for flotation in fluid, and the internal stumps of the umbilical arteries will be assessed for hemorrhage, both indicating that the calf was alive at the time of birth.

Skin Lesions

Although rare, in utero infection by fungal organisms may result in abortion of a calf with plaque-like skin lesions.

Collect (and Examine) the Placenta

As with the aborted fetus, gross examination of the placenta and collection of tissue samples are integral to the diagnostic investigation. Although the placenta is often the most important piece of diagnostic material it is frequently overlooked and rarely submitted. I would like to emphasize here that if the placenta is available it should always be collected and submitted. It may look irrelevant as it lies in the manure spreader but please collect and save it. Its diagnostic value is equal to, or greater than, that of the fetus. Placental examination and sampling are generally handled by the veterinarian on site or by the diagnostic lab pathologist after submission. However, the producer's early access to the placenta means that it can be collected and refrigerated as soon as it is found, reducing the chance that it will be lost or decompose further.

Placental Anatomy and Gross Examination

Gross examination of the placenta is often unrewarding, since very few diseases leave tell-tale pathognomonic lesions. Its diagnostic value comes from microbial testing and histopathologic examination. Nevertheless, gross placental examination can provide some basic information if the appearance of the placenta is abnormal. Appreciating this requires an understanding of normal placental anatomy (Figure 2). The placenta consists of two of the three so-called fetal membranes: the fused chorion and allantois, or chorioallantois. (The third fetal membrane, the amnion, is the translucent whitish membrane covering the calf at birth; it is not collected or examined). The several dozen, circular, raised tufts on the chorioallantois are the cotyledons. These are the site of exchange between the fetus and the cow's uterus. The thin, translucent areas between cotyledons are called the intercotyledonary zones. No exchange between the fetus and cow takes place here (Dyce et al., 2009).

Healthy intercotyledonary zones are translucent; as a rule of thumb, it should possible to read a page of printed text through the intercotyledonary zones when the placenta is draped over it. Bacterial, protozoal or fungal placentitis may result in exudate (pus and fibrin) covering the placenta, giving it a grey-yellow, thickened, leathery appearance and making the intercotyledonary zones opaque. Although this change is non-specific, it does suggest that the abortion was caused by a non-viral infectious microbe (bacterial, fungal or protozoal) rather than other non-infectious causes.

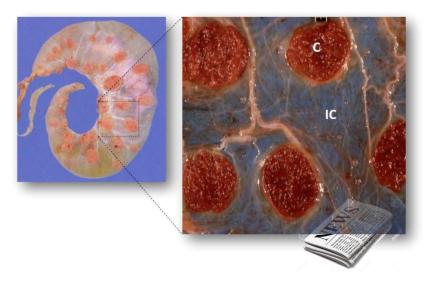


Figure 2: Normal bovine placenta. Exchange between the fetus and cow takes place at specialized tufts called cotyledons (C). No exchange takes place between cotyledons in the intercotyledonary (IC) zones, which should be nearly translucent; you should be able to read a newspaper through them. Both sites—cotyledonary and intercotyledonary—should be sampled and submitted (Modified from figure 5-67 in Dyce et al., 2009).

• How do the Veterinarian and Diagnostic Laboratory Work Up Abortion Cases?

It should be emphasized once again that any abortion workup should always begin with the collection, examination and storage of appropriate samples from the aborted fetus and placenta. These procedures can be conducted by the producer, as described in previous sections. Even if producer-collected samples are eventually discarded without being examined by a veterinarian or submitted to a diagnostic laboratory, they still provide important information that can and should be saved for later. At the very least, every aborted fetus should be weighed, measured and photographed.

It is frustrating for veterinarians and diagnostic laboratories to receive samples from a fourth abortion case accompanied by a history stating that nothing was recorded about the first three. Were the aborting cows clinically ill? Were the fetuses at a similar stage of gestation? Were the fetuses fresh or autolyzed? Were there lesions or malformations? Did the placentas look normal? Were any tissues from the first three cases stored and frozen? It is worth re-emphasizing that the likelihood of diagnosis of an abortion submission is fairly low but is increased by the submission of multiple abortions. If earlier tissues are still frozen and available, and the producer has collected information that suggests a common gestational age and cause, it is possible to pool tissues from multiple cases. This increases the chance of successful detection of microbes, toxins, mineral deficiencies, etc. by the diagnostic laboratory, generally at no extra cost.

Subsequent steps in an abortion investigation generally occur in the diagnostic laboratory. A pathologist conducts a necropsy examination of the calf during which internal organs and fluids are sampled for further testing. Sampling is very detailed and different samples have different destinations and testing utility. For example, a cube of fetal liver that has been placed in formalin to preserve it is ideal for microscopic examination but is worthless for bacterial and fungal culture (formalin kills bacteria and fungi). The converse is true; a cube of fresh liver that has been frozen is ideal for bacterial and fungal culture, but is not suitable for microscopic examination (freezing causes cells to expand and rupture, destroying microscopic architecture). In fact, four separate cubes of liver must be collected, with one placed in formalin (for histopathology), one kept chilled (for fungal and bacterial culture), and two frozen (one for

virology and molecular techniques and the other for nutritional/toxicological testing).

The necropsy procedure is fairly specialized, both in terms of the tools required and the experience needed to interpret tissue changes. Similarly, sampling is laborious and time-consuming and requires a large number of separate sterile containers, as well as access to formalin and freezing. For these reasons, few veterinarians conduct this part of the examination themselves although this forms part of their training. Generally, the veterinarian will submit the frozen or chilled intact fetus and placenta to the diagnostic laboratory. This is not always the case, however, and diagnostic labs sometimes receive a 'necropsy in a bottle,' where the veterinarian has performed the necropsy him or herself and collected tissue samples. As long as tissues are sampled and packaged appropriately this can save the producer money.

The chart below shows how veterinary students at the University of Calgary are taught to sample bovine abortion cases. This is too much information to memorize and we encourage students (and practicing veterinarians) to bring this chart with them as they sample. It is available for download from the University of Calgary's Diagnostic Services Unit (DSU). Most diagnostic labs produce similar guidelines. It is worthwhile for producers to understand what samples are collected and how complex an abortion investigation can be. As frustrating as it is never to receive samples from abortion cases, it is equally frustrating for diagnostic laboratories to receive inappropriate or inadequate samples, or samples that have been preserved incorrectly (e.g., formalin-fixed or frozen tissues only rather than both).

Sampling Protocol for Bovine Abortions

Reproduced with permission from Dr. Jennifer Davies, Director of the University of Calgary Diagnostic Services Unit. (https://vet.ucalgary.ca/dsu)

- 1. Examine the placenta for any abnormalities
 - a. Collect **multiple** sections of <u>cotyledonary</u> and <u>intercotyledonary</u> areas for histopathology (formalin fixed), bacteriology (fresh-frozen), 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 (refer to aging chart)
 - a. Weigh the fetus
 - b. Measure the crown to rump length
 - c. Note fetal characteristics
- 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 (or send to lab) 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, 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 the brain, placenta, any lesions
- ii. IHC is available for many infectious agents
- b. Bacteriology/mycology via culture or PCR (fresh-frozen)
 - 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, half of the brain, ear notch
 - 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

What Happens if I Receive a Diagnosis of 'No Diagnosis'?

Submitting veterinarians and producers should be aware that cases that yield negative findings do not equate with diagnostic failure. Rather, negative test results for the most common infectious causes of abortion 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.

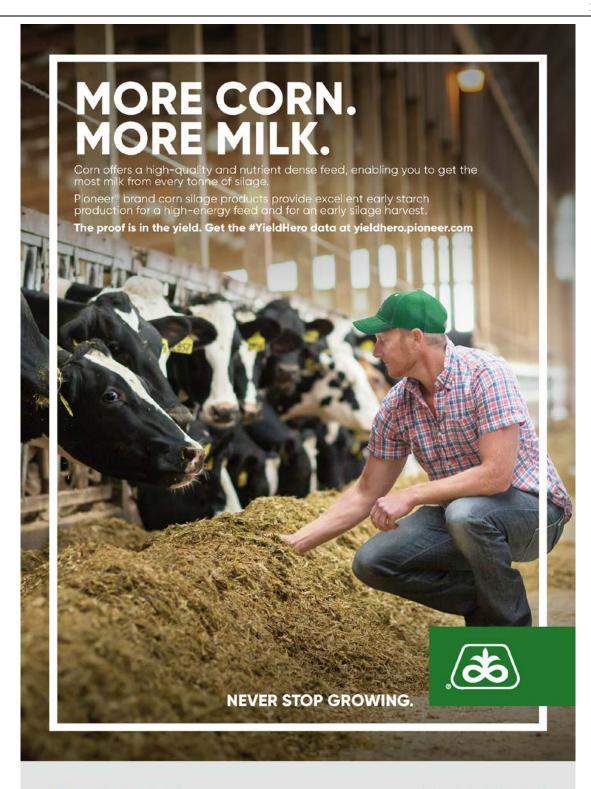
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