When the Lab Reports Come Back, "Abortion: Cause Undetermined" - What Next? An Overview of "Non-Infectious" Causes of Bovine Abortion

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Abstract

How frustrating and how far too common it is that after a "full" work-up of a case of bovine abortion one ends up with a final report saying, "Abortion: Cause Undetermined". Unfortunately, we frequently think of abortions as the outcome of *in utero* microbial infections and that submission of samples to a lab should result in determination of cause. In most labs, testing normally will consist of bacterial, viral or fungal culture, serology and gross and histopathology to scrutinize fetal and maternal tissues for clues of cause for the abortion. Endocrinology, toxicology or genetic testing of submitted samples are rarely done and, too frequently, thorough medical examinations of the dam or herdmates are lacking.

Solutions for complex clinical problems usually involve a "team effort" approach and failures can result anywhere along this chain. In practical terms laboratory testing is not perfect and our ability to clinically investigate many aspects of pregnancy is limited, if not impossible!

When reports come back with no cause determined, the question, "what next?" quickly becomes "what are we missing?"

Lists of differential diagnoses for non-infectious causes tend to be shorter, less clear-cut, and less often considered than differentials for infectious bovine abortion. We must, however, also include the possibility that some abortions are caused by infectious agents which are missed. This paper addresses non-infectious conditions that potentially could account for some of the missed, or cause-unknown abortions. Six subject areas are discussed.

- Failure to detect infectious causes.
- Conditions that disrupt essential supporting endocrine systems.
- Unrecognized endometrial or cervical diseases.
- Direct or indirect effects of toxic compounds.
- Underlying genetic abnormalities.
- Potential immune-mediated conditions.

Introduction

Let us start from the self evident, but often overlooked basic premise that, "Every cow that aborts does so because of a specific failure has occurred". How often are diagnostic laboratories unable to detect a specific cause for abortion cases they are sent? This question is easy to ask, but really only gets at one part of the clinical challenge abortion cases present. I would suggest that we include two additional questions: 1) How often are submissions and information submitted to labs really complete or adequate? 2) What could be causing all the abortions for which we don't have answers?

Taking this a little further, suppose we did know what some causes of the many undiagnosed cattle abortions were. Would we be able to identify them, given the technological and financial limitations under which practitioners and diagnosticians work in the real world? This paper suggests areas we should consider exploring, with the hope for a renewal in interest and focused dialogue that addresses undiagnosed causes.

Clinical and diagnostic approaches to bovine abortion have changed some, but have they been enough? New diseases have emerged; neospora abortions plague farms around the world and new technologies move from research labs into diagnostic labs; PCR procedures have become available for many infectious diseases within the last few years, and more are added each month. A PubMed computer search done in April, 2004 for keywords "bovine" and "abortion" had 1,623 manuscripts from the scientific literature listed for a period covering about the last 50 years. As one would expect, because its recent emergence and importance has generated a large body of new information, a search using the terms "bovine", "abortion" and "neospora" had 219 "hits" since 1989, when the first papers were listed.

In a paper published recently,[13] the "normal annual abortion frequency" was suggested to be between 2 and 5% for "observed abortions", or 5 to 8% for "both unobserved and observed abortions". These "background levels" are very costly. Are we willing to accept losses of even 5% before concerted efforts are made to seek their cause? How and where do you set the threshold parameter, above which you begin diagnostic testing? An abortion in a herd of 60 would likely evoke a different response from a farmer or his veterinarian than an abortion in a cow in a herd of 2,000, but each abortion represents failure that occurred for some specific reason. Each abortion involved a disease process that was related to some causal event. Costs associated with these losses are huge. Some of the most recent figures are based on California abortions which were estimated to cost $200 million dollars per year.[7] Understanding why a single animal aborts is key to herd health and management decisions.

How are we doing with regard to establishing causes of abortion? Large clinical data sets are not available, but we can consider results from established state diagnostic labs. When we do so, we find the results are quite similar. In 1992 and 1993, Kirkbride published summaries of bovine abortion diagnostic testing done in North Dakota covering a 10-year period.[14,15] Of 8,962 abortion cases he reports that "a suitable diagnosis" was made in 32.8%. Infectious causes were recognized in 30.3%, of which 14.5% were bacterial, 10.5% viral, and 5.3% fungal. Additionally, in 1,554 fetuses and/or placentas, recognizable lesions were found that suggested an infectious process, but no specific agent was diagnosed. One concludes from this that during that time period, of the 8,962 submissions received by this highly regarded lab, the cause of 6,020 was "not determined".[15]

A more recent report of the experience of diagnostic labs in California describes the results from about 600 submissions.[10] Of those, an infectious cause was determined in 37%, and non-infectious causes attributed to 5.5%. The remaining 57.3% were of undetermined cause.

Given what we know, what is causing so many of these undiagnosed abortions in cattle? Let us consider some possibilities.

1. Failure to Recognize Infectious Causes

The two studies described above reported that no diagnosis was found for 67.2% or 57% of the cases.[10,15] The surge in neospora cases in California may account for this slight difference, as that study occurred later and neospora had become a significant problem in the area, but regardless, the percentages are remarkably similar.

Given the reality that about two-thirds of cases cannot be explained, what are the possible causes of all the others?

Are some of these causes by infectious diseases - either those well recognized but not able to be cultured, or recognized by histopathology or serology are there infectious agents causing abortions we currently just don't recognize? Dr. Kirbride's summary from his 1992 publication of 8,962[15] abortion submissions noted that 1,554 fetuses and/or placentas (17.3%) had lesions, many of which suggested an infectious etiology, but that "no infectious agent could be demonstrated". These data are strong evidence that based solely on the presence of damage done to the bovine fetus or placentas, we are missing perhaps 17.3% or more of infections caused by microbial diseases. There are many tenable explanations, and they include:

- Testing error: laboratory tests are never 100% accurate
- Some microbial infections are missed:
  - Samples submitted may not be fresh – most bovine fetuses are held *in utero* for up to a day after the fetus dies, before they are expelled;
  - Samples available to work with are incomplete;
  - Specific microbes are difficult or impossible to grow;
  - Some microbes are very fragile and do not survive in tissues after fetal death;
  - Lack of knowledge about the agent and the pathogenesis of the disease;
  - The infectious disease may affect maternal systems, so fetus or placenta samples have no lesions and there are no tests using submitted samples from them that would be diagnostic;
  - Presence of antibodies generated by the fetus as a result of the infection can interfere with test systems;
  - Expertise, experience and interest of laboratory personnel

Because there are no "gold standards" from which we can know how often infectious agents really cause abortions, we have to look for indirect evidence. As the bovine fetal immune system becomes competent (somewhere around 90 to 130, days depending on the epitopes of the antigen), exposure to infectious agents can induce a fetal immune response. This may result in elevation of immunoglobulins which can be detected in fetal serum. This is used as a crude screening tool – elevation suggests that a fetal infection has occurred. Testing to see if these antibodies for any specific known fetal pathogen can be used to arrive at a presumed specific diagnosis. Two studies reported in the veterinary literature - one from the United States, the other from Argentina - contain surprising findings.

A study done some years ago on sera from 486 bovine fetuses from a slaughterhouse in Minnesota revealed that many fetuses had immunoglobulins in their sera at levels that suggested *in utero* exposure.[22] Further evaluation of sera from the 27% of fetuses that had elevated immunoglobulins was done looking for specific antibodies for bovine viral diarrhea (BVD), bovine parvovirus, para influnza -3 (PI-3), infectious bovine rhinotracheitis (IBR), or leptoirosis (5 different serovars), resulting in detection of antibodies in sera from 4, 31, 18 , 0.9 and 0%, respectively. Given that these were "normal" fetuses, albeit from cattle selected for shipment, the data suggests that indeed, the bovine fetus is commonly exposed to a variety of infectious agents.

The second study was done using both dairy and beef cattle. Testing fetal samples from 95 fetuses, antibodies to one or more of the following known abortifacient infectious agents were found in 65 (68.5%): BVD,1BR, Neospora, or Leptospira (45, 30, 27, and 5% respectively).[18] Even more surprising was the determination that 32 of the 95 fetuses had antibodies to more than one of these agents. Again, these were from slaughterhouse samples, not aborted fetuses! This also underscores another important fact: mere presence of antibodies in fetal serum does not necessarily implicate an agent as the cause of an abortion.

Limitations on sampling also represent a major problem in detection of microbial infections. One is usually limited to working with aborted material, or limited samples from the dam. Impending abortions, or evaluation of herdmates in situations where abortion storms are occurring, require thorough examination of pregnant animals at risk. Under ideal conditions, collection of fetal samples for testing would be helpful, but require extreme measures. A recent study reported collection of fetal fluids from 169 pregnant cows by percutaneous, intrauterine placement of a spinal needle (ultrasound-guided) with culture for BVD.[1] One fetus was positive for virus, but 12 pregnancies were lost within a three-week follow-up. Development of practical, inexpensive fetal monitoring (or even sampling?) techniques would greatly improve our ability to understand the pathogenesis of bovine abortions, but for now these are only practical in research settings.

Not surprisingly, but a reality, is that the experience and interests of the diagnosticians in individual labs will affect the diagnoses made, to some extent. Regional differences certainly explain some differences in diagnosis, as many diseases do have regional distributions. Examples are foothill abortion in the West, some plant toxicoses, infectious diseases foreign to the United States. Different regions experience outbreaks of different diseases, and data for abortion diagnostic rates reflect this. However, sometimes interest and experience do impact the ability to make a diagnosis. An example of this from my own area is a relatively high abortion diagnostic rate for Ureaplasma abortion in cattle in southern Canada, where "approximately 10% of submissions" from the region were attributed to this agent.[17] Yet our own lab, within a few hundred miles, has a much lower rate of detection. The personnel in the Canadian lab had been engaged in ureaplasm[a] research for many years. They had special interest and expertise that translated into higher detection rates for that particular infectious disease.

Sampling is also critically important. It is important to know the specific pathogenesis for individual diseases to help understand what samples can best serve to help arrive at a diagnosis. We routinely recommend a complete set of samples that cover most conditions, but even so, may be inadequate. For example, some 30 years ago research on IBR-induced abortion showed that time from inoculation with virus until abortion ranged from 18 days to three months. In one study, culture of tissues from fetuses and placentas of 13 experimentally infected cows revealed that virus could be detected in placental cotyledons, but not fetal tissues, from four cows.[12] Such cases would easily be missed if field submissions did not include placental tissues.

Some infectious agents are most easily detected using serology. Initial infections of the dam may occur weeks earlier (for example, leptospirosis) and there may be few or no lesions identifiable in fetal tissues.[3] Culture is difficult, and molecular diagnostic approaches are not in wide use, so if a submission does not include paired maternal sera or fetal blood of sera, diagnosis of leptospira abortions can be a problem.

2. Conditions that Disrupt Essential Endocrine Systems

Bovine reproductive endocrinology during pregnancy is, not surprisingly, complex, with interplay between the dam's ovaries, pituitary, endometrium, placenta (primarily the binucleate trophoblast cells) and the fetal pituitary and adrenal glands. Instrumentation of the bovine fetus is difficult, but the fetal lamb has been an important experimental animal for several decades. Extrapolations have been made between these ruminants, though not always with sufficient recognition that cattle and sheep likely have many differences. The sheep work provides helpful information about fetal placental pathophysiology.

The maternal-placental-fetal endocrine interplay is relatively delicate. Examples of this can be drawn from research literature. A recent study using chronically instrumented fetal lambs found that mechanical compromise to blood flow through the umbilicus for only three days resulted in increased relative weight of the fetal adrenal glands and changes in placentomal weights and shapes.[6] The number of binucleated trophoblast cells in the placenta can be experimentally controlled by either removing the fetal adrenal glands or by injecting cortisol,[24] which simulate *in utero* fetal stress.

Fetal stress accompanies fetal or placental infections, mechanical cord compression, periods of hypoxia caused by maternal illness, and compromise to uterine blood flow (partial uterine torsion). Severe stress by itself can mediate changes in placenta structure and function. Initiation of prostaglandin cascades, luteolysis, and uterine contractures may follow.

Maternal systems that regulate the antiluteolytic systems can also fail. Although this is studied more in the context of early pregnancy loss,[21] " luteal failure" has long been suggested as one cause of non-infectious abortions. Experimental studies have shown that even nutritional factors can affect progesterone production during pregnancy.[16] Factors that influence ovulation of a normal dominant follicle can ultimately result in "inadequate" corpora lutea development with suboptimal progesterone production.[25] We have no knowledge how often luteal failure results in pregnancy loss.

3. Unrecognized Underlying Endometrial or Cervical Diseases

Low-grade endometrial inflammation can lead to prostaglandin release, followed by luteolysis and increased myometrial contractions. Some infections associated with postpartum placental retention and endometritis are never completely cleared. Trauma to the cervix and uterus during delivery can result in compromise to the functional cervical barrier. Cervical mucous has some antibacterial properties. Presence of bacteria in the cervix might change cervical mucous composition, resulting in ascension of infection through the cervix during pregnancy. Postmortem examination of uteri from cattle during necropsy examination, or from tissues collected from meat packing plants, commonly reveals chronic healed lacerations, eversions of the outer cervical ring, presence of cystic remnants of the mesonephric ductal system, and other lesions that could affect the mechanical cervical barrier allowing infectious agents to traverse the cervix. Insemination of pregnant animals probably occurs much more commonly than is appreciated.

4. Direct or Indirect Effects of Toxic Compounds

Plant toxins can cause either teratogenic changes of the developing fetus, or abortion.[11] These tend to be recognized as regional problems. One classic example of a regional plant that causes pregnant cattle to abort is exposure to ponderosa pine needles.[11,19] Endotoxins from gram-negative bacterial infections likely can cause bovine abortion[5] by initiating inflammatory cytokine cascades.

In addition to plant toxins and bacterial products, other administered chemical compounds or medications can be involved. Iatrogenic-induced abortions are rarely documented, but an incidental observation during a study being conducted in a large Florida dairy with 1800 cows was quite revealing. The research was being done to study PGF-2∝ administration and its effects on return to estrus. Progesterone profiles were being done for cattle that the herdsman had selected for PGF-2∝ administration as part of his reproductive management program. Retrospective analysis of the progesterone data for 103 cows the herdsman elected to inject, revealed that 17 cows had been pregnant at the time of injection. All later were in estrus, having been inadvertently aborted by the herdsman. How often does this error occur?

5. Underlying Genetic Defect

Of the abnormally developed fetuses commonly found, some defects have resulted from exposure to toxic plants, or viral or chemical teratogens, but most are associated with inherent genetic defects. Most embryos with genetic anomalies are probably lost during the delicate early embryonic period, as is also true of embryonic and fetal loss associated with abnormal placentation in cloned calves,[9] which also likely has a genetic basis. The frequency of chromosomal abnormality in *in vitro*-produced embryos is significantly higher than in embryos in bred cattle,[23] but embryos derived either naturally or by IVF have surprisingly high incidences of genetic abnormalities. The authors of this 2001 paper note that chromosome abnormalities are reported to occur from 9 to 56% in early cattle embryos. Clearly, not all of these are "lethal genes," but the numbers should attract our attention. Rarely do we undertake genetic analysis of aborted fetuses. This is in marked contrast to standard practice in human medicine, and an area that should be further investigated.

6. Immune Mediated Conditions

A major paradox in mammalian immunology has been, and still is, why the dam does not immunologically recognize the fetus as an allograft and initiate its rejection. Mechanisms purported to be in play have been recently reviewed.[4] If immunological rejection were to be the basis for a bovine abortion, what kinds of lesions might we find, or how would one identify an immunologically rejected fetus or embryo? Maternal lymphocytic invasion of placental tissues is rarely found. There may be no lesions in either the fetus or placenta that would suggest an immunological cause.

We have shown that unlike other species, cattle trophoblast cells do express MHC Class 1 antigens on some trophoblast cells[2] and that some cloned embryos are aborted because they prematurely express Class 1 antigens and evoke a maternal immune reaction, with associated inflammatory cell infiltration in the endometrium.[2,8] Current diagnostic testing would likely routinely miss any immune-mediated bovine abortions.

Are cattle that have aborted likely to have failed pregnancies in the future? Abortion or delivery of growth-retarded or premature infants constitute a huge problem in human medicine. Their experience, albeit involving a very different species, could and should be studied by those of us interested in, and contending with, abortion in cattle on a regular basis. In a recent paper in the *New England Journal of Medicine*,[20] the authors addressed the association of abortion, small size for gestational age and prematurity and they suggest that these forms of reproductive failure likely have similar causes. Study of the pathogenesis of any one would likely result in valuable information that would help clarify our understanding of the others. The authors of the NEJM paper found that women who give birth to abnormally small babies are more likely to have a stillbirth in their next pregnancy. The more severe the growth retardation in the initial pregnancy, the greater the likelihood of subsequent pregnancy stillbirth. Do some cattle also have subtle failures of systems required for pregnancy support we are not fully aware of, or have not considered?

It seems clear that unless we expand the breadth and depth of our approaches to bovine abortion diagnostics–unless we invest in, and do more research on, normal bovine gestation, fetal - placental physiology, bovine genetics, pathogenesis and detection of infectious diseases affecting the cow–our success rates in identifying specific causes of bovine abortion will not improve much, and "abortion - cause undetermined" will continue to frustrate us all. Perhaps a first step is to generate more active discussion of what we might be missing, and how we as a profession should proceed.

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