Reproductive consequences of infection with bovine viral diarrhea virus

Daniel L. Grooms, DVM, PhD

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, A100 VTH, East Lansing, MI 48824, USA

Bovine viral diarrhea virus (BVDV) has emerged as one of the most important infectious disease agents in cattle [1,2]. The insidious nature of BVDV has led to substantial economic losses in both the dairy and beef industry on a worldwide level [3,4]. Contrary to its name, BVDV has been associated with pathology in several physiologic systems including the respiratory, hematologic, immunologic, neurologic, and reproductive systems. Reproductive losses may be the most economically important consequence associated with BVDV infection and evidence suggests the incidence of BVDV-related reproductive losses are increasing in the United States [5]. In addition to reduced reproductive efficiency, BVDV uses the reproductive system to maintain and spread itself in the cattle population by inducing immunotolerance following fetal infection, resulting in birth of calves persistently infected (PI) with the virus. Cattle PI with BVDV are the major source of virus spread within and between farms.

Reproductive losses associated with BVDV infection was described by Olafson et al in the first clinical description of BVDV [6]. In this report, pregnant cows subclinically infected with BVDV often aborted 10 to 90 days later. Since that time, it has become evident that BVDV can cause a wide array of reproductive losses that are largely dependent on the time of gestation where infection occurs and the virus strain (Fig. 1). The purpose of this article is to review the effects of BVDV on reproduction.

Infection before conception through the embryonic stage (—9 to 45 days of gestation)

Field and epidemiologic studies suggest that BVDV can have a significant impact on early reproductive performance. In a group of seronegative cattle
accidentally exposed to a PI cow, conception rates in groups that seroconverted to BVDV before, during, or after breeding were 78.6, 44.4, and 22.2% [7]. Those seroconverting to BVDV at breeding or soon after breeding were less likely to conceive than those who had seroconverted before breeding. Similarly, McGowan et al compared BVDV seropositive heifers to heifers that seroconverted between breeding and pregnancy diagnosis at 51 days postinsemination and found the pregnancy rate was significantly reduced in the seroconverting heifers [8]. However, no difference was found with cows under the same circumstances. Houe et al identified and defined a specific risk period for BVDV infection in dairy herds in which cattle PI with BVDV were found. The risk period was defined during the 2-year study period as the period of time previous to when the oldest PI animal was 6 months old. In all herds studied, conception rates were significantly lower during the defined risk period than the postrisk period [9]. In an experimental study looking at BVDV infection around breeding, conception rates in heifers infected intranasally 9 days before insemination was 44% compared with 79% for the control group [8]. The reduction in conception rates was attributed to either failure of fertilization or early embryonic death. In the same report, the conception rate in heifers exposed to a PI cow and calf 4 days following insemination was 60% [8]. However, significant embryo loss was experienced in this group, resulting in a day 77 pregnancy rate of only 33% compared with 79% for the control group.

The mechanism for decreased conception rates is not clear but may depend on the time of infection with respect to the stage of early reproductive events. Virus has been localized in ovarian tissue for prolonged periods of time following acute infection with cytopathic BVDV [10,11], and noncytopathic virus [12]. BVDV has also been isolated from follicular fluid collected from slaughterhouse ovaries [13]. Exposure of developing oocytes

![Fig. 1. Potential clinical reproductive outcomes following infection with bovine viral diarrhea virus. EED = early embryonic death.](image-url)
to BVDV could result in reduced survivability either through direct cell damage or indirectly through changes in the local environment of the oocyte. Following acute infection with cytopathic BVDV, interstitial oophoritis has been described with lesions lasting up to 60 days [10,12]. Significant long-term oophoritis could result in ovarian malfunction with subsequent poor conception rates. Limited information is available on ovarian function following BVDV infection. In a study of cattle being superovulated while undergoing experimental challenge with BVDV, the number of palpable corpora lutea and recovered embryos was significantly reduced when compared with noninfected cows also undergoing superovulation [14]. Infection and subsequent viremia during the preovulatory phase can reduce the rate of follicle growth [15,16]. Similarly, in cows PI with BVDV, ovaries are often hypoplastic and the number of ovarian antral follicles significantly reduced when compared with ovaries from cattle not PI with BVDV [17]. Modulation of ovarian hormone secretion has been demonstrated following acute BVDV infection, and has been postulated as a potential cause of BVDV induced infertility [16,18,19]. Taken together, these studies suggest that ovarian dynamics may be changed in cattle infected with BVDV, and these changes may subsequently lead to transient or long-term decreases in fertility.

Because of its essential role in fertilization, changes in the oviductal environment could have a detrimental effect on conception rate. BVDV has been detected in oviductal cells [13,20]. Archbald et al isolated BVDV from oviductal tissue and detected evidence of salpingitis for up to 21 days postintratrauterine infusion with cytopathic BVDV [21]. Similar findings have not been reported with noncytopathic BVDV.

Early studies suggested that interruption of normal fertilization or embryonic death may be the mechanism for decreases in conception rates associated with acute BVDV infection. This was concluded following the finding that infusion of cytopathic BVDV into the uterus at insemination of superovulated cows resulted in a significant reduction in the number of fertilized ova found at slaughter 3 and 13 days later [22]. Archibald et al provided evidence that BVDV may interfere with early embryonic development. In superovulated cattle in which BVDV had been infused into one uterine horn, the quality of the embryos collected from the infected horn was significantly reduced compared with those collected from the noninfected horn [23]. In a similar study, the conception rate in seronegative heifers infused with BVDV 2 hours following breeding was 27%, and was significantly reduced compared with sham inoculated cows (67%) [24]. However, in the same study, conception rates of seropositive cows inoculate in utero with BVDV or seronegative cows inoculated orally and intranasally were not significantly different than control cows.

Although it is thought that BVDV has a direct effect on the developing embryo, inflammatory changes in the uterus following BVDV infection may result in an incompatible environment for embryo development. In a study
looking at uterine pathology following intrauterine infusion of cytopathic BVDV, histologic changes in both the uterus and oviduct were evident from day 6 to 21 postinfection [21].

Several in vitro studies have been undertaken to elucidate the effects of BVDV on early reproductive function. Ova exposed to BVDV in vitro can have virus particles attached to the zona pellucida [25]. However, in vitro studies have shown that the intact zona pellucida protects the developing embryonic cells from BVDV infection, allowing normal development to continue [26,27]. In morula and blastocyst stage bovine embryos with the zona pellucida intact or damaged, no cytopathic effects were seen for 48 hours following exposure to cytopathic BVDV [28]. Similarly, zona pellucida intact embryos exposed to noncytopathic BVDV infected bovine oviductal epithelial cells for 7 days showed no adverse effects in their rates of development [29]. In contrast, blastocysts hatched from the zona pellucida (day 8 of gestation) have been shown to decrease viability when exposed to cytopathic BVDV in vitro. In the same study, noncytopathic BVDV did not decrease blastocyst survivability [30]. These studies suggest that the zona pellucida protects the developing embryo from direct effects of BVDV. However, following removal of the zona pellucida, cytopathic BVDV may have detrimental effects on survivability of blastocysts. Noncytopathic BVDV has not been shown to have the same effects. As noncytopathic BVDV is the most common virus isolated in acute outbreaks of BVDV, and has been the biotype associated with reported decreases in conception rates, further characterization of the effect of noncytopathic BVDV on the early stages of the developing embryo is necessary. Interestingly, at day 14 posthatching, BVDV antigen has been detected in embryos inoculated with noncytopathic BVDV at hatching [30]. In contrast to cytopathic BVDV, it is possible that the effect of exposure of embryos to noncytopathic BVDV may be delayed.

**Infection following the embryo stage (45–175 days of gestation)**

Following implantation, transplacental infection of the developing fetus can occur in susceptible cows with either biotype of BVDV. The outcome of the infection is largely dependent on the timing of the infection, the immunocompetence of the developing fetus, the virus biotype involved, and the virulence of the virus. Although the mechanism of fetal infection is not clear, evidence suggests that BVDV may cross the placenta by causing vasculitis on the maternal side of placentation allowing for access to the fetal circulation [31].

**Abortion**

Abortions associated with BVDV infection were first described in 1946 [6]. Early reports linked abortions to epizootics of disease described as BVD, although a definitive cause of the abortion was not established [32–34].
Early studies involving experimental infection with BVDV resulted in abortion, although virus was not isolated from the fetus [35,36]. Subsequently, noncytopathic [37] and cytopathic BVDV [38,39] were isolated from aborted fetuses. Experimental transplacental fetal infection with BVDV was first demonstrated in 1969 [40]. Under experimental conditions, both cytopathic BVDV [41] and noncytopathic BVDV [42–44] can cause fetal death following infection of seronegative dams. In a field investigation where BVDV was introduced into a susceptible herd as a point source, an abortion rate of 21% occurred during the subsequent 6 months [45]. In endemically infected herds without BVDV control programs (vaccination, biosecurity, test, and removal), it has been estimated that 7% of fetal deaths may be attributable to infection with BVDV [46].

Fetal death following BVDV infection of susceptible dams can occur at any point during gestation, although they are most common during the first trimester [2,42,45,47–50]. However, BVDV should not be ruled out in cases where late term abortions predominate. In a field investigation of an abortion outbreak in a large dairy operation, BVDV was isolated from 18 fetuses, 14 of which were aborted during the last three months of gestation (Grooms, unpublished data). Depending on the time of infection, fetal reabsorption, mummification, or expulsion can occur [42,47].

In diagnostic lab surveys in the United States, BVDV has been isolated from 0.1% [51], 1.5% [52], 4.54% [53], and 27.2% [54] of submitted abortion cases. BVDV was determined to be the etiology in 4.1% of abortions submitted to the Ontario (Canada) Ministry of Agriculture from 1993–1995 [55]. In the United Kingdom, BVDV was isolated from 27% of examined abortion cases [56,57]. Fetal death usually follows 10 to 27 days postexposure with expulsion of the fetus occurring up to 50 days later [57]. Because of the delay between fetal death and subsequent diagnosis of abortion, fetal and placental lesions seen are usually nondiagnostic, and BVDV virus isolation is not always successful [1]. Under experimental conditions or when aborted fetuses are expelled soon after death, lesions observed include conjunctivitis, peribronchiolar, and interalveolar pneumonia, and nonspecific myocarditis. Placental lesions consist mainly of vasculitis, edema, congestion, and hemorrhage, with some degeneration and necrosis [57,58].

**Immunotolerance**

Fetuses that survive infection with noncytopathic BVDV between 18 and 125 days of gestation invariably develop immunotolerance to the virus and subsequently become PI with BVDV. This phenomenon was first described in an apparently healthy bull [59], and subsequently reproduced experimentally [44,60]. Although the exact mechanism of immunotolerance is unclear, it is generally felt that circulation of virus during the period of gestation when immunocompetence is developing (90–120 days) is a prerequisite for
persistence. Viral proteins are recognized as self-antigens with resulting negative selection of BVDV specific B and T lymphocytes during their ontogeny. Persistent BVDV infection in cattle appears to arise from specific B- and T-lymphocyte immunotolerance [59,60] that results in an absence of neutralizing and nonneutralizing antibodies to the persistent virus [61]. It is not clear when the exact stage of fetal genesis is where infection must occur to cause immunotolerance. Under experimental conditions, persistence occurred in 86% and 100%, of calves derived from cows infected with BVDV at day 18 and 30 of gestation, respectively [62]. Likewise, persistent infections were induced in 100% of fetuses derived from dams challenged at 75 days of gestation [63–65]. Persistent infections are rare following fetal infection after day 100, but have been reported up to day 125 of gestation [66]. Non-cytopathic BVDV is the only biotype that has been observed or been able to experimentally produce persistence [41,67,68]. Experimental infections with cytopathic BVDV have failed to produce PI calves [41,47,60].

**Congenital defects**

Fetal infection between 100 and 150 days of gestation, also referred to as congenital infection, often results in the development of a variety of congenital defects (Table 1). During this stage of gestation, organogenesis is being completed and the immune system is becoming fully functional. Although not clear, the combination of direct cellular damage by virus and inflammatory responses to virus have been proposed as pathogenic mechanisms [69].

Congenital anomalies involving the central nervous system are most common following fetal infection with BVDV. These include microencephalopathy, hydrocephalus, hydranencephaly [70], porencephaly [71], cerebellar hypoplasia [72,73], and hypomyelination [74]. Cerebellar hypoplasia was the first recognized teratogenic effect of BVDV, and has been well documented [40,72,73,75]. At birth, calves that have cerebellar hypoplasia show extreme difficulty in becoming ambulatory. Those that can stand are ataxic, resulting in tremors, wide-based stance, and stumbling gait. The defects are usually

<table>
<thead>
<tr>
<th>Defects involving the central nervous system</th>
<th>Defects involving the ocular system</th>
<th>Other defects</th>
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<tbody>
<tr>
<td>Cerebellar hypoplasia [40,72,73,75,84]</td>
<td>Cataracts [78,79]</td>
<td>Thymic hypoplasia [42]</td>
</tr>
<tr>
<td>Microencephalopathy [70]</td>
<td>Microphthalmia [80,73,84]</td>
<td>Hypotrichosis/alopecia [1,49]</td>
</tr>
<tr>
<td>Hydrocephalus [70]</td>
<td>Retinal degeneration [73]</td>
<td>Deranged osteogenesis [83]</td>
</tr>
<tr>
<td>Porencephaly [71]</td>
<td></td>
<td>Growth retardation [1,42,44]</td>
</tr>
<tr>
<td>Hypomyelination [74]</td>
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</tbody>
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severe enough that compensation does not occur and the calves either die or are euthanized [1]. The changes in the cerebellum have been characterized as a reduction in the number of molecular layer cells and granular layer cells [42,76–78]. Purkinje cell numbers are also reduced and often displaced [76]. Fetal cerebellar effects have been seen following infection as early as the 79th day and as late as the 150th day of gestation [76]. Severity of cerebellar lesions increase with the age of the fetus when infected [76].

Other teratogenic effects that have been associated with BVDV infection include cataracts [78,79], microophthalmia [72,73,80], optic neuritis [78], retinal degeneration [73], thymic hypoplasia [42], hypotrichosis/alopoeia [1,49], curly hair coat [81], hyena disease [82], deranged osteogenesis [83], mandibular brachygnathism [39], and growth retardation [1,42,83].

Infection late in gestation (125–285 days of gestation)

In the later stages of gestation, immunocompetence and organogenesis are usually complete. Although abortions and the birth of weak calves have been attributed to infection with BVDV late in gestation [40], fetuses infected during this time period are normally able to mount an effective immune response to BVDV and effectively clear the virus. These calves are usually normal at birth and have precolostral neutralizing antibodies to BVDV [47,49,85–87]. However, calves congenitally infected with BVDV may be at more risk for experiencing a serious postnatal health event. In a study attempting to define the impact of congenital BVDV infections on large dairy farms, Munoz-Zanzi et al showed that calves born with BVDV neutralizing titers were twice as likely to experience a severe illness during their first 10 months of life compared with calves born free of BVDV neutralizing titers [88]. Further studies are needed to determine if detrimental effects may continue long term.

Fetal protection

A key component in controlling BVDV is prevention of fetal infections that result in the birth of calves PI with the virus. In addition, preventing other reproductive losses attributed to BVDV is of obvious economic importance. Preventing fetal infection and the subsequent sequelae involves controlling virus exposure and enhancing BVDV specific immunity in susceptible dams. Cattle PI with BVDV are the primary source of virus spread within and between farms. Identifying and eliminating PIs should be a major focus when attempting to control and prevent BVDV. By eliminating PIs, the major source of virus capable of causing transient infections in pregnant dams and subsequent fetal infections is eliminated.

An important issue is immune response stimulated by one virus strain to crossprotect against heterologous BVDV strains and prevent fetal infection.
Understanding the strengths and limitations of vaccines is important as we gain an understanding of BVDV antigenic diversity and try to design programs capable of controlling this diversity. Early vaccines were developed with little knowledge of their ability to provide fetal protection. Currently, efficacy data on fetal protection is not required for approval of vaccines for BVDV in the United States [89]. Several field studies suggest that immunologic protection against heterologous BVDV challenge may be incomplete with respect to fetal protection [90–93]. Experimental studies attempting to answer this question are limited, and have focused primarily on immunity developed following vaccinations. Results of vaccine fetal protection studies have been mixed and are often dependent on the challenge model (Table 2). Most reported trials have involved killed vaccines, and efficacy has ranged from 25% to 100%. In studies evaluating the fetal protection efficacy of a modified-live vaccine, Cortese and Brock demonstrated 88% and 58% fetal protection in heifers immunized one time with a commercially available type 1 modified-live BVDV vaccine and challenged at 75 days in gestation with type 1 or type 2 BVDV, respectively [63,64]. Except for different challenge viruses, these studies were

<table>
<thead>
<tr>
<th>Study</th>
<th>Vaccine characteristics</th>
<th>Challenge virus(s)</th>
<th>Route of exposure</th>
<th>Day of challenge</th>
<th>Fetal protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>McClurkin et al, 1975 [94]</td>
<td>Killed type 1</td>
<td>1 CP type 1</td>
<td>Intranasal</td>
<td>165–255</td>
<td>86%</td>
</tr>
<tr>
<td>Harkness et al, 1985 [95]</td>
<td>4 Killed viruses</td>
<td>9 CP type 1</td>
<td>Intranasal</td>
<td>80</td>
<td>64%</td>
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<tr>
<td>Meyling et al, 1987 [96]</td>
<td>Killed</td>
<td>1 NCP type 1</td>
<td>Oral</td>
<td></td>
<td></td>
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<tr>
<td>Brownlie et al, 1995 [97]</td>
<td>MLV type 1</td>
<td>NCP type 1</td>
<td>Intranasal</td>
<td>75</td>
<td>88%</td>
</tr>
<tr>
<td>Brock and Cortese, 2001 [63]</td>
<td>MLV type 1</td>
<td>NCP type 2</td>
<td>Intranasal</td>
<td>75</td>
<td>58%</td>
</tr>
<tr>
<td>Patel et al, 2002 [98]</td>
<td>Killed type 1</td>
<td>NCP type 1</td>
<td>PIs</td>
<td>~83</td>
<td>100% b</td>
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<tr>
<td>Zimmer et al, 2002 [99]</td>
<td>2 Killed viruses</td>
<td>3 NCP type 1</td>
<td>Intranasal</td>
<td>82</td>
<td>32%</td>
</tr>
<tr>
<td>Zimmer et al, 2002 [99]</td>
<td>2 Killed viruses</td>
<td>3 NCP type 1</td>
<td>Intranasal</td>
<td>82</td>
<td>40%</td>
</tr>
<tr>
<td>Dean et al, 2003 [100]</td>
<td>MLV type 1</td>
<td>NCP type 1</td>
<td>IV</td>
<td>55–100</td>
<td>92%</td>
</tr>
</tbody>
</table>

a PIs—exposure through controlled contact with cows persistently infected with BVDV.
b Two vaccinated cows aborted post challenge, but no BVDV identified.
c Different vaccine virus combination than used in Zimmer reference below.
d Different vaccine virus combination than used in Zimmer reference above.
conducted similarly suggesting that the vaccine was less likely to stimulate
a fetal protective immunity against type 2 viruses compared with type 1
viruses. Other studies have not fully evaluated protection against multiple
viruses.

**Effect of bovine viral diarrhea virus on bull fertility**

Virus has been isolated from the semen of bulls PI [59,101–104] and
acutely infected [105–108] with BVDV. In bulls experimentally infected with
noncytopathic BVDV, virus was isolated from the semen between 7 and
14 days postinfection at a titer ranging from 5 to 75 CCID50/mL [107].
Immunohistochemical studies of tissues collected from these same bulls
suggested that virus replication was confined to the seminal vesicles and
prostate glands. No changes in semen quality were observed. Similar results
were reported in bulls experimentally infected with cytopathic BVDV [105].
A virus titer range of 10^4 to 10^7 CCID50/mL of semen has been reported in
PI BVDV bulls [101,107]. BVDV isolation from raw semen may be less
successful than from extended semen [103]. This is presumably due to the
documented virucidal effects of semen [109]. However, the semen remains
infective, which is evident by the demonstration that susceptible cows can
become infected following artificial [104,110] or natural insemination
[111,112].

PI bulls can successfully sire calves; however, their breeding efficiency is
generally low [102,104,110]. Semen from a PI bull was released for sire
evaluation to 97 dairy farms. Analysis of breeding records indicated a first
service conception rate of 38% in cows inseminated with this semen
compared with 66% for cows bred during the same period on the same
farms with different semen [104]. Semen quality is variable in PI bulls
ranging from acceptable [101,107] to abnormal, with various defects
predominantly involving the head of the spermatozoa [101,103,111]. Poor
reproductive efficiency following the use of bulls PI with BVDV is likely
attributable to a combination of several factors including low-quality semen,
il thrift, and viral effects on the reproductive tract and conceptus in sus-
ceptible females.

Persistent infection with BVDV localized only in the testes of an
immunocompetent bull has been documented [113]. It is hypothesized this
bull became acutely infected with BVDV at puberty when the blood–testes
barrier forms, thus trapping the virus in gonadal cells away from the animals
immune response. Bovine viral diarrhea virus could not be isolated from
blood, white blood cells, or other tissues at necropsy, but could be isolated
continually from semen samples. Semen from this bull was shown to be
capable of transmitting BVDV infection to susceptible females at insemina-
tion [114]. These findings suggest that screening bulls for persistent
infection with BVDV using serum or white blood cells may not be adequate
in assuring BVDV free semen.
Summary

Reproductive efficiency is imperative for the maintenance of profitability in both dairy and cow-calf enterprises. Bovine viral diarrhea virus is an important infectious disease agent of cattle that can potentially have a negative effect on all phases of reproduction. Reduced conception rates, early embryonic deaths, abortions, congenital defects, and weak calves have all been associated with BVDV infection of susceptible females. In addition, the birth of calves PI with BVDV as a result of in utero fetal exposure is extremely important in the perpetuation of the virus in an infected herd or spread to other susceptible herds. Bulls acutely or PI with BVDV may be a source of viral spread through either natural service or semen used in artificial insemination. Management practices including elimination of PI cattle, biosecurity measures and strategic use of vaccination can be implemented to reduce the risk of BVDV related reproductive losses. Development of vaccines and vaccine strategies capable of providing better protection against fetal infection would be of benefit.

References


