

Selected diseases and conditions associated with bovine conceptus loss in the first trimester

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Abstract

The outcomes of insults to the bovine conceptus depend on the predilection of the insulting agent for the gravid reproductive tract, the virulence of the insult, and the developmental maturity/immune competence of the conceptus at the time of the insult. Agents that are lethal at one time during gestation may be harmless at another, or may have completely different effects (some not so harmless) at different gestational ages. This review discusses some of the known physical–mechanical, physiological, and infectious causes of first trimester bovine conceptus losses, including three infectious agents that have been the subject of recent studies for their potential to transmit disease via embryo transfer.

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1. Introduction

The bovine conceptus is frequently the victim of a broad array of physiologic, metabolic, environmental, infectious and iatrogenic insults, such that we sometimes have to wonder how the species survives. In the best of circumstances, when a fertile bull breeds a fertile cow, or when an experienced AI technician inseminates a fertile estrous cow at the appropriate physiologic moment with semen from a fertile bull, the statistical probability of obtaining a live calf from such a breeding is 60–70%. These values are the product of the proportion of oocytes fertilized $\times$ the proportion of fertilized oocytes that survive to maternal recognition of pregnancy $\times$ the proportion of maternal recognition “survivors” that remain alive through the period of the embryo to the beginning of the period of the fetus (i.e.,

about 42 days) $\times$ the proportion of fetuses that survive to parturition. There is attrition at each of these milestones. Some of this so-called “background” attrition we may influence, though rarely positively, it seems. Although a thorough discussion of all of the categories of insults to bovine pregnancy would require several volumes, this discussion will be limited to a small number of conditions and diseases germane to the embryo transfer industry, and whose importance has emerged or re-emerged, yielding useful new information.

In terms of the physiology of bovine pregnancy, the term “trimester” does not have particular meaning, in that there are few significant physiological or medical “landmarks” that denote an important change from one trimester to the next. Rather, the major changes, from a free-floating zygote to a well-attached fetus occur in the first 40–50 days. Whereas the placenta gradually increases its role in support of the pregnancy, providing increasing amounts of progesterone from the fifth month to term, most of its metabolic machinery is used

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to support fetal growth. However, the word “trimester,” probably borrowed from human obstetrics, seems to be here to stay, and does have some usefulness, especially when it is associated with early abortion and infertility. Several agents, processes and procedures have their most negative consequences in the first 90 days of pregnancy.

This review will discuss known and proposed mechanisms of the “background” losses mentioned above, as well as the risk of iatrogenic loss, and lastly a “short list” of the more troublesome infectious causes resulting in the demise of the conceptus during the first 90 days of gestation. Another presentation at this meeting, given by Dr. Mark Anderson, will discuss conceptus death after the first trimester in cattle.

2. Attrition of bovine pregnancy

Many studies have documented the “normal” or “background” losses of the conceptus in cattle, following mating or AI. From these studies, three things are clear: (a) simply surviving to the time of maternal recognition of pregnancy is no assurance that a calf will be delivered; (b) intensively managed cattle, usually dairy cows, tend to have higher fetal attrition than extensively managed cattle; and (c) infectious causes get a great deal of attention, not so much because they routinely contribute on a scale equal to the background losses, but because they represent some of the few causes that we can do something about.

The modest “term pregnancy” rate following natural service or AI is probably not due to fertilization failure. In matings between healthy estrous cows and breeding-sound bulls, most studies agree that fertilization is the rule, and fertilization failure is the exception, with 85–95% of ovulated oocytes being fertilized [1]. From fertilization at Day 1 (Day 0 = standing estrus) through Day 14, approximately 30% of embryos are lost, most of them before Day 8 [2,3]. It appears that most of the Days 5–14 losses occur during the transition from morula to blastocyst. Another 5–10% of embryos die during maternal recognition of pregnancy (approximately Days 14–19). During the first part of the “late embryonic” period, i.e., from maternal recognition of pregnancy to the earliest development of the placentomes, Days 18–28, an additional 5–10% may die. As the complex interlocking structures of the placentome develop from 28 to 42 days, as many as 5–10% of embryos from lactating dairy cows will be lost [4,5]. By Day 42, all organ systems of a surviving conceptus are in place, such that the conceptus is now a fetus. Fetal attrition varies considerably from region to region, and with the type and age of animal (dairy or beef; lactating

Table 1

Attrition of bovine pregnancy from ovulation to term, according to lower and upper estimates of embryonic and fetal death at common milestones

Gestational age (days)	Low range embryo/fetal death (%)	High range embryo/fetal death (%)
0 (Preovulatory oocyte)	(Not known)	(Not known)
1	5 (95% fertilization)	10 (90% fertilization)
8	25	30
14–28	5	10
29–42	5	10
43–term	5	12
Total attrition	45	72

Adapted from [2,4,5].

or dry; heifer or cow), and to some extent, between intensively and extensively raised cattle. Although many authors describe background fetal losses (abortions) of 2–5% for extensively managed beef cattle, North American and European researchers routinely report that as many as 9–12% of dairy cows diagnosed pregnant at 30–45 days do not calve to the breeding responsible for the diagnosed pregnancy [5–7] (Table 1). The proportion of pregnant non-lactating females that abort is typically less than for lactating cows [6,8].

2.1. Relationship of folliculogenesis and early luteal function to embryonic death

At least part of the embryonic death story can be told in terms of the pathophysiology of folliculogenesis. The ovulation of “old oocytes” from persistent follicles is an example. It has been shown that the follicle that ovulates following a two-wave cycle is older and larger than the follicle that ovulates after a three-wave cycle. Thus, the oocyte from the “two-wave cow” ages, a process that includes resumption of meiosis, and perhaps apoptosis, while it waits to ovulate [4]. Also, ovulations early in the postpartum period may produce a CL with low progesterone production. In this low progesterone environment, the dominant follicle persists, allowing the aging mentioned above, often in an environment of prolonged, elevated serum estradiol concentrations. The aging process may not interfere with fertilization, but it may render the embryo incapable of developing beyond the 16-cell stage [9].

2.2. Iatrogenic causes of poor luteal function

Another type of low-progesterone environment may occur when progestin-based synchronization is

attempted. Apparently the progestin type and dose does not sufficiently suppress pituitary pulses of LH, such that continued growth of the dominant follicle is supported. The continued growth allows for continued secretion of estradiol. Recall that LH stimulates the elaboration of androgens from the thecal layer of the follicle, and that these androgens are the precursors for estradiol production by the granulosa. Other factors besides a primary progesterone insufficiency can cause damage to the oocyte and/or follicle, with the same effect on oocyte aging. The insult to the follicle that renders it persistent and its oocyte developmentally disabled often occurs two cycles before the damaged oocyte is ovulated. Rapid body condition loss, heat stress, trauma, high fever, severe lameness, retained placenta, and, common metabolic diseases can all negatively influence the final development of the oocyte and its follicle [10].

Inskip and Dailey's thorough review of the subject of folliculogenesis and embryo loss also includes a discussion of the possibility that ovulatory follicles can be too *small* [4]. They cite others who showed that undersized follicles that are forced to ovulate during a timed AI (TAI) produce less estradiol, yield lower conception rates and exhibit reduced luteal function after ovulating [11–13]. These authors and others suggest that, in addition to compromising the competence of the oocyte, ovulation of smaller follicles also reduces the luteal function of the subsequent CL, which may be manifest as an inadequate upward slope of the progesterone profile in early diestrus. The result is a decreased conception rate and an increase in late embryonic/early fetal mortality. These observations highlight the importance of developing TAI programs that induce ovulation of appropriately sized follicles. However, since spontaneously ovulating cows in these studies had significantly less relationship between ovulatory follicle size and conception/embryo survival, we must consider that follicle diameter at ovulation is an imperfect proxy for follicular competence in the spontaneously cycling cow. To summarize bluntly and perhaps simplistically, bad follicles (persistent, too big, too small, etc.) make bad oocytes, bad CLs and bad embryos.

3. Mechanical insult: does transrectal palpation put the pregnancy at risk?

Over the past 30 years, several groups have wrestled with the problem of determining the risk of transrectal palpation. A common problem for many of these studies was the definition of the control group. Clearly

in order to determine the damage – if any – done by palpating, palpated groups of cows must be compared with non-palpated groups; but how do we know that the non-palpated group is pregnant if we do not palpate them? From the late 1970s to the mid 1990s, a few papers were published describing the relative risk of late embryonic or early fetal death associated with different palpators [14], or different early stages of pregnancy [15] or the use of different criteria for pregnancy [14,16], but this approach did not address the fundamental question; does any transrectal palpation increase the risk of embryonic or early fetal death? Some groups used milk progesterone to develop a single group of presumed pregnant cows, and then palpated approximately half of those cows in the early stages of pregnancy at which most veterinarians feel competent, for example 35–45 days [17–20]. Although milk progesterone concentration is highly accurate for a diagnosis of not pregnant, it is notoriously inaccurate as a positive pregnancy diagnosis, with a typical predictive value of a positive test of about 80% [20,21]. This large error confounded our ability to detect small but significant differences between the palpated and non-palpated groups. Recently, ultrasound technology has provided a tool for more precisely studying the effects of palpation on early pregnancy. The assumption is that ultrasound itself does not harm the pregnancy, or that if it does, it does so equally between palpated and control animals. The few papers published to date would seem to indicate that there is either no risk or an acceptably minute risk to the pregnancy when an experienced diagnostician uses the membrane slip method of pregnancy diagnosis during this early stage of pregnancy [22]. One paper examined the outcomes of tens of thousands of early pregnancy diagnoses, and concluded that the relative risk of conceptus loss actually *increased* as the gestational day of palpation increased, between Day 35 and 42, then declined again [15].

Conclusions from these somewhat varied studies include: (a) some palpators can diagnose pregnancy at 35–45 days without appreciably increasing the background embryo–fetal death rate; and (b) for the rest of us, until a detailed quantitative study is available, we will assume that the risk of conceptus death is smaller than the benefit of the information provided by pregnancy diagnosis. As diagnostic ultrasound becomes more commonly employed for routine dairy reproductive work, the issue may become moot. Regardless, we have a tool for more accurate studies of embryonic and early fetal death in cattle.

4. Selected infectious causes of first trimester losses

Tritrichomonas foetus, *Leptospira borgpetersenii* serovar *hardjobovis*, and BVD virus. These agents are discussed as first trimester abortifacients, because they *can* cause disruption of pregnancy before the conceptus is a true fetus (i.e., before Day 42), or later, after the placentomes are well-established. Some are causes of second or third trimester abortions as well. Each has been studied with reference to its potential for transmission of disease via embryo transfer [23–29].

4.1. Obligate venereal parasite, *Tritrichomonas foetus*

This venereal disease is still prevalent in North America, and becomes an issue in some ET programs when non-pregnant heifers are exposed to bulls after unsuccessful transfers. Historically, trichomoniasis was always considered with another venereal disease, namely “vibriosis” (Campylobacteriosis), because its epidemiology is identical to trichomoniasis. However, this discussion will exclude *C. fetus venerealis*, except to say that efficacious vaccines are available for the cow, and there is convincing evidence that the bull can be protected, and even cured, by vaccination (for review see [23]). The efficacy of female and male vaccination has, in the author’s experience, resulted in reduced attempts to diagnose the presence of *C. fetus venerealis*. Instead, many practitioners simply vaccinate for “campy” as inexpensive insurance.

Tritrichomonas foetus, a motile flagellated protozoan, is an obligate venereal pathogen, i.e., found only in the genitalia of cattle, living in a low oxygen tension environment [23]. It does not invade through the genital epithelium to other organs/tissues. Instead, it remains a lumen-dwelling, extracellular organism [24,25]. It is carried on the non-keratinized, stratified squamous epithelium of the bull’s penis and prepuce. The bull, as he ages, provides deeper and deeper epithelial crypts where these organisms can thrive. As a consequence, the older a bull is when he becomes infected, the more likely he is to become a permanent carrier [23,25].

After infection at coitus, bulls older than 3 years can become lifetime carriers of *T. foetus*. Conversely, the cow mounts a somewhat effective immune response, and, typically can shed *T. foetus* from her uterus in about 8–12 weeks [30–32], although in some cases, infection can persist for more than 20 weeks, and in rare cases, through gestation and into the postpartum period [31,33].

4.1.1. Clinical signs and impact of venereal disease with *T. foetus*

Trichomoniasis is insidious. Typically, there are no observable signs in either the bull or the cow following infection. Fertilization usually occurs in spite of infection, and the preimplantation embryo survives amongst the pathogens for some time, often beyond the period of maternal recognition of pregnancy. Consequently, few cows return to estrus 21–24 days following breeding to an infected bull and such cows are often presumed to be pregnant. In beef cattle, very often venereal disease is not even suspected until the time of pregnancy examination, well after the bulls have been removed. In the large number of dairies that use bulls, an observant herdsman or veterinarian may notice that the interval from placement of cows in the “bull string” to conception, as detected by pregnancy diagnosis, is widening, with the result that Mature Equivalent milk production is depressed by as much as 7% [34,35]. In beef or dairy cattle, substantial economic loss often occurs before the disease is recognized [36–38].

4.1.2. Transmission and pathogenesis

Except for the rare case of AI with contaminated semen or gross violation of hygienic AI practices [36], transfer of organisms during heterosexual coitus is the only known form of transmission, and when older bulls are involved, transmission can be quite efficient [39–46]. There is little evidence to support the often-expressed concern for bull-to-bull transmission that supposedly could occur when bulls mount each other. Older literature suggested that young bulls, which frequently mount each other, may leave *T. foetus* on the skin and haircoat of the “mountee’s” rump. As a precaution, personnel at AI centers attempt to minimize opportunities for transmission by routinely wiping the rear quarters of the teaser animal with disinfectant. Whether such “hind-quarter exposure” occurs in a natural mating setting is not known, but seems unlikely due to the susceptibility of *T. foetus* to desiccation and to ultraviolet light [25]; hence, its survival time on the surface of the hair coat of a bull is probably very brief, and the opportunity for transmission between males is therefore very limited. Personal observations of large numbers of cohabiting infected and non-infected bulls would indicate that bull-to-bull transmission happens rarely, if ever [42].

Recent work has shown that even when in vitro fertilized oocytes are co-incubated with *T. foetus*, there is little damage to the embryo through the hatching stage. These organisms are in the uterine lumen at the time that the blastocyst is traversing the uterotubal

junction and entering the uterus. The embryo is thus surrounded by trichomonads. Consequently, the amniotic membrane, which presumably forms by an out-pouching from the ventral side of the fetus [47], will entrap trichomonads within the developing sac, and the fetus will ultimately inhale and/or swallow them. This presumably occurs well before the fetus is immunologically competent against *T. foetus*. As a result, we may see in the aborted fetus huge numbers of parasites in the lungs or abomasum, or below the lamina propria of the gut.

4.1.3. Immunity to *T. foetus*

In natural infections of the female both systemic and local antibodies are generated against *T. foetus* antigens. Specific IgA, IgG1, IgG2, and IgE have been detected in the lumen of the uterus and/or vagina following infection [33,48,49]. In general, it takes about 3–5 weeks before a rise in cervicovaginal mucus antibody can be detected. In vitro work has identified protective surface antigens of *T. foetus* by demonstrating protective functions for monoclonal antibodies to these antigens [48,50]. Both IgA and IgG1 are effective in clearing the parasite from the vagina, and presumably from the uterus as well, although we found that IgG1 predominates in the uterine secretions [51].

4.1.4. Control, treatment and prevention of trichomoniasis

Control requires identifying and culling all infected bulls; therefore, most attempts to “clear” a herd start with the bulls. The gold standard test is culture of smegma from all bulls [51], although several diagnostic laboratories have added polymerase chain reaction (PCR) assays as well. Bulls confirmed positive for *T. foetus* should be sold for slaughter only and replaced with virgin bulls that have passed a breeding soundness evaluation. Because the diagnostic test most frequently used (culture in a semi-selective medium) is only about 70–90% sensitive [39], we typically recommend that suspect bulls be sampled three times at weekly intervals, and demand that all samples test as culture-negative, before those bulls are released for breeding. In either the cow or bull, serological tests for *T. foetus* exposure are neither sensitive enough nor specific enough for routine use [40].

There is no legal, efficacious treatment for infected bulls or cows. In the 1980s, the substituted imidazoles (metronidazole, dimetridazole, ipronidazole) were shown to have excellent efficacy against *T. foetus* in bulls [41–43]. However, the entire family of compounds was subsequently outlawed for any food animal use

[45]. Practitioners who use any of these compounds in cattle in the USA may be subject to arrest and imprisonment.

As a herd treatment, it is useful to segregate the females, using a plan similar to the following [23]:

1. A “safe in calf” group that is pregnant ≥ 5 months. These are very unlikely to still be infected or to lose their calves due to *T. foetus*.
2. A group that is pregnant < 5 months. These should be pastured separately, and observed frequently for signs of abortion (elevated tails, sticky discharge on tail hairs, exposed fetal membranes, etc.).
3. Non-pregnant cows and heifers. Ideally, these should be sold for slaughter. If this is not economically appropriate, then divide this group into two further groups:
 - a. Abnormal reproductive tract, based on palpation findings. Cull these immediately.
 - b. Normal reproductive tracts. Keep these, vaccinate them according to label instructions, and rebreed next season.

A killed whole-cell vaccine for trichomoniasis (Trich Guard, Ft. Dodge, IA, USA) is available in North America. If vaccination is to be part of the control program, it is critical that the vaccine for this sexually transmitted disease induce peak immunity at the time that cows and bulls are being joined. Therefore, label instructions call for two inoculations with the killed, whole-cell, product, with the first given approximately 1 month before joining cows with bulls, and the booster inoculation given 5–7 days before exposure to bulls. Informal polling of veterinarians suggests that compliance with this regimen is low, probably because the vaccine is expensive, and because it is difficult to gather cows, who would likely be nursing relatively young calves at this time. Frequently, herdsmen will elect to vaccinate females for “trich” at the time of branding, tagging, etc., and again immediately before joining with the bulls, an interval that can be as much as 6 months. And although some *Campylobacter fetus venerealis* vaccines can protect naïve bulls and even cure infected ones [52,53], the lack of similar data on the efficacy of *T. foetus* vaccine in bulls has further contributed to the apparently low rate of *T. foetus* vaccination. In females, the efficacy of Trich-Guard has not been definitively established, but in one field study vaccinated heifers exposed to infected bulls and then intravaginally infected with an additional 10^7 *T. foetus* organisms had twice the calving rate of non-vaccinated heifers similarly exposed [29]. However, the lack of a

non-challenged control group made it difficult to ascertain what the actual calving rate should have been.

To summarize, trichomoniasis control is based on detection and culling of infected bulls and replacement with virgin bulls, segregation of exposed cows, and proper vaccination of females only.

4.2. *Leptospira*

This family of spirochetes is better known for its ability to induce abortions in the late 2nd to early 3rd trimester. But recent evidence corroborates early observations, and it is now apparent that “Lepto” can be an agent of early conceptus failure as well [54–57].

4.2.1. *Etiologic agent*

There has been a great deal of confusion about the reproductive effects of these organisms, about diagnostic criteria, and about the names of the organisms themselves. For years, the major bovine pathogenic leptospire was presumed to be *L. interrogans* serovar *hardjo*. This serovar was considered the most important of the antigens in the pentavalent vaccines manufactured by several companies. Yet there was also concern that the pentavalent vaccines were not particularly effective, nor did they induce sufficient longevity of protection. Consequently, some practitioners increased the frequency of *L. hardjo* immunizations, nearly always with a pentavalent bacterin, to provide protection against other important serovars, i.e., *pomona*, *canicola*, *icterohemorrhagiae*, and *grippotyphosa*, as well as for *hardjo*. However, according to recent evidence, it appears that we have been blaming the wrong spirochete for our losses, and using the wrong leptospire species to immunize against those losses. The *Leptospira* are now classified into “genome species” based on their genetic sequences. What we called “Lepto *hardjo*” is actually *Leptospira interrogans*, serovar *hardjo* type *hardoprajitno*, an organism found primarily in the British Isles. In contrast, the majority of our cattle are being exposed to a different species of spirochete, *Leptospira borgpetersenii*, serovar *hardjo*, type *hardjobovis*. The two organisms share significant surface antigens used for serological typing; hence, the common serovar names given to both. Regardless, they are quite different genomically. The *L. hardjobovis* genome has been markedly reduced, suggesting that it has lost some of the genetic potential to adapt to new hosts [58]. For simplicity, *L. hardjobovis* will be used to denote *L. borgpetersenii* serovar *hardjo* type *hardjobovis* for the remainder of this discussion.

As with most *Leptospira*, *L. hardjobovis* gains access to the host through mucous membranes. Following bacteremia, the organisms persist primarily in the kidneys and the genital tract. Convincing data has shown that *L. hardjobovis* is responsible for not only frank abortions of established pregnancies, but also for increased infertility (reduced conception rates) associated with carrier cows [54,55,59,60] and bulls [61].

4.2.2. *Diagnosis, treatment, control*

The most successful diagnostic sample is typically urine collected shortly after administration of a diuretic [62], fetal and dam serum, and placental tissues, if available. Laboratory diagnostic tests include fluorescent antibody testing (FAT), culture, PCR, and, for tissue samples, silver staining and immuno-histochemistry. The latter is especially helpful in establishing a diagnosis of frank abortion.

Serology is useful in establishing a diagnosis, but may require some interpretation. Serological assays include ELISA [63,64] and microscopic agglutination testing (MAT) [65]. The latter requires the maintenance of a live stock of cultures of all tested serovars. The classic approach of obtaining “acute” and “convalescent” sera may not be beneficial, as titers generally peak well before abortion. Moreover, cross reaction between serovars is common, although most laboratories interpret the serovar with the highest titer to be the infecting serovar *hardjo* antigens, whether from the infecting strain or from the popular pentavalent vaccines used by practitioners (they typically induce lower titers than the other serovars). Although vaccine titers can be difficult to differentiate from titers induced by natural infection, in general, *L. hardjobovis* titers above 1:200 can be compatible with a diagnosis of leptospirosis if signs and history are also compatible. However, many animals in the same herd may have higher *hardjo* titers without evidence of conceptus losses. By comparison, titers produced by cows aborting due to *Pomona* or other serovars may commonly reach 1:1600 or more [65].

Because the carrier state exists in the kidney, if treatment is desired, its goal should be to clear the carrier state. Older protocols used combinations of penicillin and streptomycin [66,67], a protocol which would be difficult today due to prohibitive milk and meat withdrawal times and the lack of availability of dihydrostreptomycin. The restriction on the use of aminoglycosides dictates that other approaches be made. Recent work has claimed that the following treatments successfully cleared the renal carrier state [68,69]:

1. A single injection of oxytetracycline (20 mg/kg, i.m.); not for use in lactating dairy cows.
2. A single injection of Tilmicosin (10 mg/kg, s.c.); not for use in lactating dairy cows.
3. Multiple injections of ceftiofur (i.e., 5 mg/kg, i.m. once daily for 5 days; or 20 mg/kg, i.m. once daily for 3 days); for withdrawal times, see Food Animal Residue Avoidance Databank [FARAD: <http://www.farad.org/>].
4. Two injections of amoxicillin (15 mg/kg, i.m. twice at a 48 h interval); 96 h milk withholding time and 25 days meat withholding time.

Of the four treatments, ceftiofur would have the least impact on milk or meat withdrawal. However, since the dose recommended is above the labeled dose, ceftiofur, like the other three choices of these protocols would require milk withdrawal, the cost of which must be factored into the decision to treat. It is logical to simultaneously attempt to protect the herd by vaccinating. It has recently been shown that monovalent vaccines can provoke cell-mediated Th1-type responses that produce a robust, effective defense against not only abortion or the reestablishment of the carrier state, but in many cases against infection itself [70,71]. Interestingly, two different companies' antigen preparations are successful in inducing the responses mentioned above, although only one of these bacterins is from a *L. borgpetersenii* source. Both are offered as monovalent vaccines. Multivalent vaccines do not induce this type of response. However, the two monovalent vaccine antigens will cross react with serovar *grippytyphosa* [72].

4.3. Bovine virus diarrhea (BVD): effects on first trimester pregnancy

Several viruses are capable of destroying the conceptus if they can gain access to the fetus and/or the placenta. For example, IBR virus is notorious for causing rapid death of the conceptus at essentially any stage of gestation. Because highly efficacious vaccines are available and can be incorporated into routine herd health programs, easily no further discussion of IBR is included in this review. Only one viral agent, namely bovine virus diarrhea virus (BVDV), is on the list for this discussion of selected first trimester abortifacients. But even with this limitation, a discussion of all the effects on bovine pregnancy of this single agent alone could fill volumes of text. More than any other agent, BVDV demonstrates the concept that the outcome of infection of the conceptus is a function of the predilection of the agent for feto-placental tissues, its

virulence, and the developmental state (including immune system development) of the conceptus at the time of infection.

4.3.1. Natural history of BVDV infection

Bovine virus diarrhea virus is notorious for establishing itself permanently in the host. The “persistently infected” (PI) animal is the reservoir of infection for the herd. The PI animal is the result of in utero exposure to a BVD virus at less than 125 days of gestation, before the fetus has developed a competent immune system. There are two major *biotypes* of BVDV, based on their behavior in vitro cell cultures. These are the so-called non-cytopathic and cytopathic biotypes (NCPB and CPB). Both biotypes are capable of damaging or destroying the conceptus almost anytime between Days 0 and 125, but only NCPB are represented amongst PI animals. These animals are generally born viremic, and because they are infected before they are immuno-competent, they have no antibodies to the NCPB strain of virus they are carrying.

In addition to the two biotype categories, BVDV is also described as having two major families of *genotypes*, i.e., the so-called BVDV I and BVDV II genotypes. Both genotypes are further subdivided into BVDV Ia, BVDV Ib, and BVDV IIa, BVDV IIb, respectively [73,74] and newer subtypes are being described frequently [75]. The genotype II BVDs are less prevalent [76–78], but have been associated with high morbidity/high mortality outbreaks of a disease characterized by thrombocytopenia and hemorrhage in calves and adults [79]. Both genotypes can be of either the CPB or NCPB biotypes.

Animals that are PI are susceptible to mucosal disease (MD), an acute and lethal gastrointestinal and respiratory inflammatory disease. The “trigger” that apparently sends a PI animal into MD is the conversion of non-cytopathic biotype to cytopathic biotype by RNA recombination of NCPB viruses [80–82].

4.3.2. Timing of infection

Reproductive tract infection with BVDV can begin even before there is a conceptus. For example, it is possible to infect the gametes' environment in either the bull or the cow [83,84]. Within the ovary, the virus has been located in interstitial, luteal, granulosa and thecal cells, as well as follicular fluid. Several reports exist of bulls with BVDV-positive semen. In most but not all cases, the bull is a PI animal infected with NCPB BVD virus [85,86]. There are, however, examples of bulls acutely (i.e., transiently) infected with either NCPB or CPB [83]. Very few quantitative studies are available,

but the few that are available would suggest that BVDV-contaminated semen decreases conception rates [86,87]. Small but well-controlled studies showed that either CP or NCP biotypes of virus, when inoculated into cows at approximately 30 days of gestation, induced the loss of the conceptus in every case [88]. In another study, animals that were viremic with NCPB at the time of AI had conception rates that were similar to controls, but significantly reduced embryonic survival to Day 77 [89]. Within- and between-biotype and genotype strain differences in these effects have been reported [90,91].

As in the bull, the cow's gonads can become infected. Wild-type virus from acutely infected animals [92] and vaccine virus from animals recently vaccinated with modified live virus preparations [93] have been found associated with oocytes; both lesions (oophoritis) and virus have been detected in the follicles and oocytes of PI animals [94–96]. Virus could be found in primordial, primary and secondary follicles. Interestingly, endocrine capability of the follicle was also affected, with reduced estradiol output by the follicle, and in one study, reduced progesterone production by the resulting CL [97,98] (see earlier discussion of “bad follicles”). Apparently, acute infections at these very early stages of development have an all or none response, i.e., infected embryos are destroyed; uninfected embryos become uninfected fetuses and survive to term without ill effects [99]. This all or none effect is in contrast to the situation where BVDV infection of the conceptus occurs after the period of the embryo, i.e., from Day 42 to about Day 125. By this time, the placentomes are well established, such that fetal infection is likely to be the result of placentome infection and associated vasculitis following a period of maternal viremia. Infection of the fetus from Days 42 to ~130 with BVDV can lead to abortion, developmental defects, particularly but not exclusively defects of the derivatives of the embryonic neurotrophectoderm or in the case of NCP BVD only, to the PI calf born viremic and without circulating antibodies to the infecting strain.

A further detrimental effect attributed to BVD infection is the apparent suppression of immune responses to other pathogens [100]. Although few specific interactions have been studied, it is known that calves acutely infected with NCPB BVD virus have a markedly diminished response in lymphocyte proliferation and gamma interferon production in response to *Mycobacterium bovis* infection [101].

4.3.3. BVD and ET

Because of the possibility of infection of gametes and embryos, BVD is a particular concern for the ET industry. Although virus can be seen associated with

oocytes even before ovulation, several studies have failed to transmit BVDV to recipients of in vivo-produced morulae and blastocysts, processed according to the International Embryo Transfer Society (IETS) protocol, i.e., washed 10 times and trypsin treated (for review see [28]). This is in spite of the fact that in some cases, uterine tubal cells (UTCs) could be infected by incubation with sonicated embryos [102]. However, at least two reports of international transmission of BVDV via embryo transfers exist; in one, recipients seroconverted following transfer, and in the other, an ET calf was born persistently infected [103,104]. As is true for several potential pathogens, well-washed embryos with intact zonae pellucidae reduce the risk of disease transmission, but they do not reduce that risk to zero. It is worth noting that in both in vivo and in vitro embryo production systems, fetal bovine serum (FBS) is featured prominently. It may appear in oocyte maturation medium, embryo culture medium, flushing medium, etc. Because of the high prevalence of BVD virus, is estimated that as much as 20% of FBS lots in North America are contaminated with BVDV [105].

Infection with BVD is even more problematic in the in vitro ET industry. Washing and trypsin treatment alone will not provide the assurance of minimal risk demanded by importing countries. A detailed discussion of measures taken to increase this assurance is beyond the scope of this presentation, but excellent reviews are available [28,106].

4.3.4. Diagnosis, treatment, control

In some northern European countries, where prevalence of BVD virus infection is low, a test-and-slaughter program is employed [107]. Complete eradication is within sight in Norway and Sweden. In North America, where prevalence is much higher, a more complex approach to control is needed. Certainly identification of all BVD-infected animals is a fundamental requirement of any serious control program. Most programs are directed at detecting antigen. Several laboratory tests are available, including virus isolation, PCR, or immunofluorescent assays from buffy coats, immunohistochemistry (IHC) of skin taken from an ear notch, or antigen capture ELISA of homogenates of ear notch tissue, buffy coats, or serum. In general, many assays are sensitive enough to identify PI animals, but few have the sensitivity necessary to detect transiently (acutely) infected animals. Of the above named tests, virus isolation is the gold standard. However, for screening purposes, the antigen capture systems will generally suffice. Positive results with the screening tests should be confirmed by virus isolation.

Table 2
Overview of diagnostic tests for identifying cattle persistently infected with BVD

Test	Target analytes	Sample analyzed	Comment
Antigen capture	BVD protein	1. Skin extracts; 2. Buffy coats; 3. Serum	Requires two tests to find acutely infected vs. PI animals
Immunohistochemistry (IHC)	BVD antigens	Ear notch skin	Can be formalin-preserved. Somewhat subjective
Virus isolation	Replicating virus	Buffy coats preferred	Slow turnaround (5 days). Retest to find acutely infected animals.
IPMA ^a	Replicating virus	Serum	Good for large-scale screening. Slow (4+ days). Retest to find acutely infected animals.
RT-PCR	Viral nucleic acids	1. Serum; 2. Bulk tank milk	Needs validation for type of sample. Retest to find acutely infected animals.

^a Immunoperoxidase monolayer assay. From [109].

Advantages of the skin-punch IHC approach include the simplicity of sample collection, its efficacy in formalin-preserved tissues, which simplifies tissue preparation and storage, and its high sensitivity as a screening test. The test employs a monoclonal antibody against BVDV that can detect a diverse array of isolates, and significantly, can do so in paraffin-embedded, formalin-fixed tissues, which is often not the case with other monoclonal antibody-based assays. Agreement between IHC and virus isolation methods is very high, reportedly 97.5% [108] (Table 2).

Serology can be used as a surveillance tool, in order to assess vaccine efficacy or client compliance, or to confirm exposure after observation of clinical signs suggestive of BVD. It is particularly useful when applied to sentinel animals (seronegative calves after their colostral titers have waned). Another important use of serology is to document acute infections in non-PI animals. In this case, an ELISA that can detect BVD-specific IgM antibody will provide evidence of recent exposure [109]. In addition, a (pre-colostral seropositive, virus-negative) calf born to a dam acutely infected in late pregnancy, is unlikely to be a threat to other animals, but is a sign that the virus is active in the herd.

4.3.5. Immunization

In North America, immunization remains a fundamental part of protection against BVD. In an ideal situation, maintenance of a “closed herd,” followed by identification and culling of PI animals and the use of serology in sentinel animals for surveillance can eliminate the need for vaccination. But the same geographical realities that make trichomoniasis control difficult in the American west also make BVD control difficult. The use of common grazing land in the western half of the continent makes the closed herd concept difficult to achieve. Because the virus is maintained in a herd via PI animals, it is unlikely that

vaccination alone will achieve the protective state desired. However, in combination with identification and removal of PI animals, vaccination can severely limit the negative impacts of this virus on productivity. There is no lack of choice for BVD vaccines. As new information has become available about the diversity of biotypes, genotypes, and strain differences in the population of BVD viruses, vaccine companies have developed new products to combat the constantly changing pathogen. In North America alone, there are at least 180 licensed BVD immunizing products [110]. These include modified live and killed vaccines, vaccines containing various combinations of Type I and II genotypes, vaccines containing only CP biotypes or only NCP biotypes, and some with combinations of the above. Several studies have noted a tendency for BVD vaccines containing genotype I antigens to protect against type I and to a slightly lesser extent, against type II BVD challenge strains as well [111–113]. Interestingly, this cross-over protection to date has only worked in one direction. For example, immunization with type II virus only does not protect against type I challenge.

Before choosing an immunogen, it is worthwhile taking some time to consider where vaccination fits in the greater picture of disease control. Infectious diseases are controlled by intervention that increases the resistance of the host to the agent, and/or that minimizes or eliminates the reservoirs of the agent, or if neither of these is achievable, that prevents contacts between infected and susceptible animals [114]. Since the main reservoir for BVDV in a herd is the PI animal, it is reasonable to begin a control program here, by identifying as many PI animals as possible. Vaccination alone with even the best immunogens will not address the first requirement, and so is almost certain to cause disappointment. Identifying PI animals is a substantial undertaking, since the national prevalence is thought to be 0.5–2.0%, which means one has to check nearly 200

animals to find the one that needs action. Various pooling strategies have been devised, whereby individual animal samples can be pooled in limited numbers and examined in one of the diagnostic assays discussed earlier. Of the tests previously discussed, antigen capture of extracted ear notch samples, buffy coats or sera, or PCR of buffy coats lend themselves to pooling, whereas the skin punch IHC assay does not. However, the IHC method will yield the necessary information on single test. Assays that detect whole virus or viral nucleic acid must be run twice to distinguish PI from transiently infected [109].

In evaluating BVD vaccines, a critical factor to be considered is not only the ability of the vaccine to protect the host, but also its ability to protect the conceptus throughout gestation. It is likely that such fetal protection involves “intercepting” BVDV before viremia and or population of the placentomes can occur. Cell-mediated immunity and humoral immunity undoubtedly are both active in preventing the virus from infecting the placentome.

5. Discussion

Infectious diseases rightfully deserve much of our professional attention when considering potential causes of abortion, but non-infectious causes probably contribute more to the total pregnancy attrition, and tend to occur in the first trimester of pregnancy. As we learn more about the pathobiology of folliculogenesis and its relationship to luteal function, fertilization and embryo development, we may be able to target specific elements of that relationship, and thus at least partially reverse some of the so-called “background” losses of conceptuses. For the moment, we do not need to blame our transrectal palpation as an important cause of conceptus loss, although we must remember that there are documented differences in apparent iatrogenic loss of early pregnancies among palpators, suggesting that there is room for improvement for many of us.

Regarding infectious diseases as first trimester abortifacients, the protozoal, bacterial, and viral examples given here can each arrive at the site of fertilization and/or implantation very soon after mating/insemination. Vaccines exist for all of these agents, and would probably be listed in decreasing order of efficacy (which we have defined as protection of the conceptus) as BVDV, *Leptospira*, and *T. foetus*. However, carrier animals represent a major challenge to controlling each of these diseases by vaccination alone. Thus, another common thread amongst all three is the need for more stringent surveillance and biosecurity. In the case of

trichomoniasis, where the bull is the carrier, much of the world has eliminated this disease by eliminating the bull through the widespread adoption of AI as the major means of breeding dairy and beef cattle. Since the carrier state of *L. borgpetersenii hardjobovis* can be in either the male or female, control may be more complex, and directed towards clearing the carrier state with appropriate antibiotics, and immunizing with an apparently potent monovalent vaccine. Bovine virus diarrhea virus, because it is maintained in a herd by vertical transmission that produces a PI calf, calls for the most stringent of biosecurity measures. The difficulty in establishing a true “closed herd” approach to the breeding of cattle, especially beef cattle in western North America, will limit the success of attempts to eradicate BVD from a given herd, or from the entire cattle community.

References

- [1] Hawk HW. Sperm survival and transport in the female reproductive tract. *J Dairy Sci* 1983;66:2645–60.
- [2] Diskin MG, Sreenan JM. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J Reprod Fertil* 1980;59:463–8.
- [3] Dunne LD, Diskin MG, Sreenan JM. Embryo and foetal loss in beef heifers between day 14 of gestation and full term. *Anim Reprod Sci* 2000;58:39–44.
- [4] Inskeep EK, Dailey RA. Embryonic death in cattle. *Vet Clin North Am Food Anim Pract* 2005;21:437–61.
- [5] Santos JE, Thatcher WW, Chebel RC, Cerri RL, Galvao KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci* 2004;82/83:513–35.
- [6] Labernia J, Lopez-Gatius F, Santolaria P, Lopez-Bejar M, Rutllant J. Influence of management factors on pregnancy attrition in dairy cattle. *Theriogenology* 1996;45:1247–53.
- [7] Thurmond MC, Picanso JP, Jameson CM. Considerations for use of descriptive epidemiology to investigate fetal loss in dairy cows. *J Am Vet Med Assoc* 1990;197:1305–12.
- [8] Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci* 2002;85:2803–12.
- [9] Inskeep EK. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J Anim Sci* 2004;82(E-Suppl):E24–39.
- [10] Peter AT, Bosu WT. Relationship of uterine infections and folliculogenesis in dairy cows during early puerperium. *Theriogenology* 1988;30:1045–51.
- [11] Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, Macneil MD, et al. Relationship between follicle size at insemination and pregnancy success. *Proc Natl Acad Sci* 2005;102:5268–73.
- [12] Perry GA, Smith MF, Roberts AJ, Macneil MD, Geary TW. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J Anim Sci* 2007;85:684–9.

- [13] Mussard ML, Burke CR, Behlke EJ, Gasser CL, Day ML. Influence of premature induction of an LH surge with GnRH on ovulation, luteal function and fertility in cattle. *J Anim Sci* 2007;85:937–43.
- [14] Abbitt B, Ball L, Kitto GP, Sitzman CG, Wilgenburg B, Raim LW, et al. Effect of three methods of palpation for pregnancy diagnosis per rectum on embryonic and fetal attrition in cows. *J Am Vet Med Assoc* 1978;173:973–7.
- [15] Thurmond MC, Picanso JP. Fetal loss associated with palpation per rectum to diagnose pregnancy in cows. *J Am Vet Med Assoc* 1993;203:432–5.
- [16] Vaillancourt D, Bierschwal CJ, Ogwu D, Elmore RG, Martin CE, Sharp AJ, et al. Correlation between pregnancy diagnosis by membrane slip and embryonic mortality. *J Am Vet Med Assoc* 1979;175:466–8.
- [17] Franco OJ, Drost M, Thatcher MJ, Shille VM, Thatcher WW. Fetal survival in the cow after pregnancy diagnosis by palpation per rectum. *Theriogenology* 1987;27:631–44.
- [18] Thompson JA, Marsh WE, Calvin JA, Etherington WG, Momont HW, Kinsel ML. Pregnancy attrition associated with pregnancy testing by rectal palpation. *J Dairy Sci* 1994;77:3382–7.
- [19] Lopez-Gatius F, Santolaria P, Yaniz J, Rutllant J, Lopez-Bejar M. Factors affecting pregnancy loss from gestation day 38–90 in lactating dairy cows from a single herd. *Theriogenology* 2002;57:1251–61.
- [20] Bulman DC, Lamming GE. The use of milk progesterone analysis in the study of oestrus detection, herd fertility and embryonic mortality in dairy cows. *Br Vet J* 1979;135:559–67.
- [21] Bulman DC, Lamming GE. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *J Reprod Fertil* 1978;54:447–58.
- [22] Romano JE, Thompson JA, Kraemer DC, Westhusin ME, Forrest DW, Tomaszewski MA. Early pregnancy diagnosis by palpation per rectum. influence on embryo/fetal viability in dairy cattle. *Theriogenology* 2007;67:486–93.
- [23] BonDurant RH. Venereal diseases of cattle. natural history, diagnosis, and the role of vaccines in their control. *Vet Clin North Am Food Anim Pract* 2005;21:383–408.
- [24] Corbeil LB, Campero CM, Rhyan JC, BonDurant RH. Vaccines against sexually transmitted diseases. *Reprod Biol Endocrinol* 2003;1:118.
- [25] BonDurant RH, Honigberg BM. Trichomonads of veterinary importance. In: Krier JP, editor. *Parasitic protozoa*. San Diego, CA: Academic Press; 1994. p. 168–77.
- [26] Bielanski A, Ghazi DF, Phipps-Todd B. Observations on the fertilization and development of preimplantation bovine embryos in vitro in the presence of *Tritrichomonas foetus*. *Theriogenology* 2004;61:821–9.
- [27] Bielanski AB, Surujballi O. *Leptospira borgpetersenii* serovar *hardjo* type *hardjobovis* in bovine embryos fertilized in vitro. *Can J Vet Res* 1998;62:234–6.
- [28] Givens MD, Waldrop JG. Bovine viral diarrhoea virus in embryo and semen production systems. *Vet Clin North Am Food Anim Pract* 2004;20:21–38.
- [29] Kvasnicka WG, Hanks D, Huang JC, Hall MR, Sandblom D, Chu HJ, et al. Clinical evaluation of the efficacy of inoculating cattle with a vaccine containing *Tritrichomonas foetus*. *Am J Vet Res* 1992;53:2023–7.
- [30] Corbeil LB, Duncan JR, Schurig GD, Hall CE, Winter AJ. Bovine venereal vibriosis variations in immunoglobulin class of antibodies in genital secretions and serum. *Infect Immun* 1974;10:1084–90.
- [31] Skirrow SZ, BonDurant RH. Induced *Tritrichomonas foetus* infection in beef heifers. *J Am Vet Med Assoc* 1990;3–15(196):885–9.
- [32] Anderson ML, BonDurant RH, Corbeil RR, Corbeil LB. Immune and inflammatory responses to reproductive tract infection with *Tritrichomonas foetus* in immunized and control heifers. *J Parasitol* 1996;82:594–600.
- [33] Skirrow S, BonDurant RH. Immunoglobulin isotype of specific antibodies in reproductive tract secretions and sera in *Tritrichomonas foetus*-infected heifers. *Am J Vet Res* 1990;45(51):645–53.
- [34] Akhtar S, Riemann HP, Thurmond MC, Franti CE. The association between antibody titres against *Campylobacter fetus* and milk production efficiency in dairy cattle. *Vet Res Commun* 1993;17:183–91.
- [35] Gaines JD. Trichomoniasis in a dairy herd. Control by artificial insemination and prostaglandin F2 alpha treatment. *Theriogenology* 1988;29:1367–74.
- [36] Goodger WJ, Skirrow SZ. Epidemiologic economic analyses of an unusually long epizootic of trichomoniasis in a large California dairy herd. *J Am Vet Med Assoc* 1986;189:772–6.
- [37] Rae DO. Impact of trichomoniasis on the cow-calf producer's profitability. *J Am Vet Med Assoc* 1989;194:771–5.
- [38] Villarreal A, Carpenter TE, BonDurant RH. Development of a simulation model to evaluate the effect of vaccination against *Tritrichomonas foetus* on reproductive efficiency in beef herds. *Am J Vet Res* 2004;65:770–5.
- [39] BonDurant RH. Diagnosis, treatment, and control of bovine trichomoniasis. *Comp Cont Educ Pract Vet* 1985;7:S179–88.
- [40] BonDurant RH, Van Hoosear KA, Corbeil LB, Bernoco D. Serological response to in vitro-shed antigen(s) of *Tritrichomonas foetus* in cattle. *Clin Diagn Lab Immunol* 1996;3:432–7.
- [41] Kimsey PB, Darien BJ, Kendrick JW, Franti CE. Bovine trichomoniasis. Diagnosis and treatment. *J Am Vet Med Assoc* 1980;177:616–9.
- [42] Skirrow S, BonDurant R, Farley J, Correa J. Efficacy of ipronidazole against trichomoniasis in beef bulls. *J Am Vet Med Assoc* 1985;187:405–7.
- [43] Guest GB. Extra label policy update. *FDA Vet* 1988;5–6.
- [44] Clark BL, Dufty JH, Parsonson IM. The effect of *Tritrichomonas foetus* infection on calving rates in beef cattle. *Aust Vet J* 1983;60:71–4.
- [45] Christensen HR, Clark BL. Spread of *Tritrichomonas foetus* in beef bulls in an infected herd [letter]. *Aust Vet J* 1979;55:205.
- [46] Clark BL, Dufty JH, Parsonson IM. Studies on the transmission of *Tritrichomonas foetus*. *Aust Vet J* 1977;53:170–2.
- [47] Roberts S. Gestation period—embryology, fetal membranes and placenta—teratology. In: *Veterinary obstetrics and genital diseases* 2nd ed., Woodstock, VT: Published by the author; 1971.
- [48] Hodgson JL, Jones DW, Widders PR, Corbeil LB. Characterization of *Tritrichomonas foetus* antigens by use of monoclonal antibodies. *Infect Immun* 1990;58:3078–83.
- [49] Corbeil LB, Campero CM, Rhyan JC, Anderson ML, Gershwin LJ, Agnew DW, et al. Uterine mast cells and immunoglobulin-E antibody responses during clearance of *Tritrichomonas foetus*. *Vet Pathol* 2005;42:282–90.
- [50] Ikeda JS, BonDurant RH, Campero CM, Corbeil LB. Conservation of a protective surface antigen of *Tritrichomonas foetus*. *J Clin Microbiol* 1993;31:3289–95.

- [51] BonDurant RH, Campero CM, Anderson ML, Van Hoosear KA. Detection of *Tritrichomonas foetus* by polymerase chain reaction in cultured isolates, cervicovaginal mucus, and formalin-fixed tissues from infected heifers and fetuses. *J Vet Diagn Invest* 2003;15:579–84.
- [52] Bouters R, De Keyser J, Vandeplassche M, Van Aert A, Brone E, Bonte P. *Vibrio fetus* infection in bulls. Curative and preventive vaccination. *Br Vet J* 1973;129:52–7.
- [53] Clark BL, Dufty JH. The duration of protection against infection with *Campylobacter fetus* subsp. *venerealis* in immunised bulls. *Aust Vet J* 1982;58:220.
- [54] Dhaliwal GS, Murray RD, Dobson H, Montgomery J, Ellis WA. Reduced conception rates in dairy cattle associated with serological evidence of *Leptospira interrogans* serovar *hardjo* infection. *Vet Rec* 1996;139:110–4.
- [55] Dhaliwal GS, Murray RD, Ellis WA. Reproductive performance of dairy herds infected with *Leptospira interrogans* serovar *hardjo* relative to the year of diagnosis. *Vet Rec* 1996;138:272–6.
- [56] Dhaliwal GS, Murray RD, Dobson H, Montgomery J, Ellis WA. Presence of antigen and antibodies in serum and genital discharges of cows from dairy herds naturally infected with *Leptospira interrogans* serovar *hardjo*. *Res Vet Sci* 1996;60:163–7.
- [57] Ellis WA, Songer JG, Montgomery J, Cassells JA. Prevalence of *Leptospira interrogans* serovar *hardjo* in the genital and urinary tracts of non-pregnant cattle. *Vet Rec* 1986;118:11–3.
- [58] Bulach DM, Zuerner RL, Wilson P, Seemann T, McGrath A, Cullen PA, et al. Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. *Proc Natl Acad Sci* 2006;103:14560–5.
- [59] Dhaliwal GS, Murray RD, Dobson H, Montgomery J, Ellis WA. Effect of *Leptospira interrogans* serovar *hardjo* infection on milk yield in endemically infected dairy herds. *Vet Rec* 1996;139:319–20.
- [60] Guitian J, Thurmond MC, Hietala SK. Infertility and abortion among first-lactation dairy cows seropositive or seronegative for *Leptospira interrogans* serovar *hardjo*. *J Am Vet Med Assoc* 1999;215:515–8.
- [61] Heinemann MB, Garcia JF, Nunes CM, Gregori F, Higa ZM, Vasconcellos SA, et al. Detection and differentiation of *Leptospira* spp. serovars in bovine semen by polymerase chain reaction and restriction fragment length polymorphism. *Vet Microbiol* 2000;73:261–7.
- [62] Nervig RM, Garrett LA. Use of furosemide to obtain bovine urine samples for leptospiral isolation. *Am J Vet Res* 1979;40:1197–200.
- [63] Yan KT, Ellis WA, Mackie DP, Taylor MJ, McDowell SW, Montgomery JM. Development of an ELISA to detect antibodies to a protective lipopolysaccharide fraction of *Leptospira borgpetersenii* serovar *hardjo* in cattle. *Vet Microbiol* 1999;69:173–87.
- [64] Bercovich Z, Taaijke R, Bokhout BA. Evaluation of an ELISA for the diagnosis of experimentally induced and naturally occurring *Leptospira hardjo* infections in cattle. *Vet Microbiol* 1990;21:255–62.
- [65] Grooms DL, Bolin CA. Diagnosis of fetal loss caused by bovine viral diarrhoea virus and *Leptospira* spp. *Vet Clin North Am Food Anim Pract* 2005;21:463–72.
- [66] Gerritsen MJ, Koopmans MJ, Dekker TC, de Jong MC, Moerman A, Olyhoek T. Effective treatment with dihydrostreptomycin of naturally infected cows shedding *Leptospira interrogans* serovar *hardjo* subtype hardjobovis. *Am J Vet Res* 1994;55:339–43.
- [67] Ellis WA, Montgomery J, Cassells JA. Dihydrostreptomycin treatment of bovine carriers of *Leptospira interrogans* serovar *hardjo*. *Res Vet Sci* 1985;39:292–5.
- [68] Smith CR, Corney BG, McGowan MR, McClintock CS, Ward W, Ketterer PJ. Amoxycillin as an alternative to dihydrostreptomycin sulphate for treating cattle infected with *Leptospira borgpetersenii* serovar *hardjo*. *Aust Vet J* 1997;75:818–21.
- [69] Alt DP, Zuerner RL, Bolin CA. Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar *hardjo*. *J Am Vet Med Assoc* 2001;219:636–9.
- [70] Naiman BM, Alt D, Bolin CA, Zuerner R, Baldwin CL. Protective killed *Leptospira borgpetersenii* vaccine induces potent Th1 immunity comprising responses by CD4 and gamma-delta T lymphocytes. *Infect Immun* 2001;69:7550–8.
- [71] Naiman BM, Blumerman S, Alt D, Bolin CA, Brown R, Zuerner R, et al. Evaluation of type 1 immune response in naive and vaccinated animals following challenge with *Leptospira borgpetersenii* serovar *hardjo*. Involvement of WC1(+) gamma-delta and CD4 T cells. *Infect Immun* 2002;70:6147–57.
- [72] Brown RA, Blumerman S, Gay C, Bolin C, Doby R, Baldwin CL. Comparison of three different leptospiral vaccines for induction of a type 1 immune response to *Leptospira borgpetersenii* serovar *hardjo*. *Vaccine* 2003;21:4448–58.
- [73] Ridpath JF. BVDV genotypes and biotypes. Practical implications for diagnosis and control. *Biologicals* 2003;31:127–31.
- [74] Ridpath JF. Practical significance of heterogeneity among BVDV strains. Impact of biotype and genotype on U.S. control programs. *Prev Vet Med* 2005;72:17–30.
- [75] Vilcek S, Durkovic B, Kolesarova M, Paton DJ. Genetic diversity of BVDV. Consequences for classification and molecular epidemiology. *Prev Vet Med* 2005;72:31–5.
- [76] Fulton RW, Hessman B, Johnson BJ, Ridpath JF, Saliki JT, Burge LJ, et al. Evaluation of diagnostic tests used for detection of bovine viral diarrhoea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot. *J Am Vet Med Assoc* 2006;228:578–84.
- [77] Harding MJ, Cao X, Shams H, Johnson AF, Vassilev VB, Gil LH, et al. Role of bovine viral diarrhoea virus biotype in the establishment of fetal infections. *Am J Vet Res* 2002;63:1455–63.
- [78] Fulton RW, Ridpath JF, Ore S, Confer AW, Saliki JT, Burge LJ, et al. Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions. distribution of BVDV 1a, 1b, and 2a subgenotypes. *Vet Microbiol* 2005;111:35–40.
- [79] Dean HJ, Leyh R. Cross-protective efficacy of a bovine viral diarrhoea virus (BVDV) type 1 vaccine against BVDV type 2 challenge. *Vaccine* 1999;17:1117–24.
- [80] Neill JD, Ridpath JF. Recombination with a cellular mRNA encoding a novel DnaJ protein results in biotype conversion in genotype 2 bovine viral diarrhoea viruses. *Virus Res* 2001;79:59–69.
- [81] Ridpath JF, Neill JD, Vilcek S, Dubovi EJ, Carman S. Multiple outbreaks of severe acute BVDV in North America occurring between 1993 and 1995 linked to the same BVDV2 strain. *Vet Microbiol* 2006;5–31(114):196–204.
- [82] Tautz N, Meyers G, Thiel HJ. Pathogenesis of mucosal disease, a deadly disease of cattle caused by a pestivirus. *Clin Diagn Virol* 1998;10:121–7.
- [83] Kirkland PD, Richards SG, Rothwell JT, Stanley DF. Replication of bovine viral diarrhoea virus in the bovine reproductive

- tract and excretion of virus in semen during acute and chronic infections. *Vet Rec* 1991;128:587–90.
- [84] Vanroose G, Nauwynck H, Van Soom A, Vanopdenbosch E, de Kruif A. Replication of cytopathic and noncytopathic bovine viral diarrhoea virus in zona-free and zona-intact in vitro-produced bovine embryos and the effect on embryo quality. *Biol Reprod* 1998;58:857–66.
- [85] Kirkland PD, Mackintosh SG, Moyle A. The outcome of widespread use of semen from a bull persistently infected with pestivirus. *Vet Rec* 1994;135:527–9.
- [86] Paton DJ, Brockman S, Wood L. Insemination of susceptible and preimmunized cattle with bovine viral diarrhoea virus infected semen. *Br Vet J* 1990;146:171–4.
- [87] Fray MD, Paton DJ, Alenius S. The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control. *Anim Reprod Sci* 2000;60/61:615–27.
- [88] Carlsson U, Fredriksson G, Alenius S, Kindahl H. Bovine virus diarrhoea virus, a cause of early pregnancy failure in the cow. *Zentralbl Vet A* 1989;36:15–23.
- [89] McGowan MR, Kirkland PD, Rodwell BJ, Kerr DR, Carroll CL. A field investigation of the effects of bovine viral diarrhoea virus infection around the time of insemination on the reproductive performance of cattle. *Theriogenology* 1993;39:443–9.
- [90] Dubovi EJ. Genetic diversity and BVD virus. *Comp Immunol Microbiol Infect Dis* 1992;15:155–62.
- [91] Waldrop JG, Stringfellow DA, Riddell KP, Galik PK, Riddell MG, Givens MD, et al. Different strains of noncytopathic bovine viral diarrhoea virus (BVDV) vary in their affinity for in vivo-derived bovine embryos. *Theriogenology* 2004;62:45–55.
- [92] Grooms DL, Brock KV, Ward LA. Detection of bovine viral diarrhoea virus in the ovaries of cattle acutely infected with bovine viral diarrhoea virus. *J Vet Diagn Invest* 1998;10:125–9.
- [93] Grooms DL, Brock KV, Ward LA. Detection of cytopathic bovine viral diarrhoea virus in the ovaries of cattle following immunization with a modified live bovine viral diarrhoea virus vaccine. *J Vet Diagn Invest* 1998;10:130–4.
- [94] Grooms DL, Ward LA, Brock KV. Morphologic changes and immunohistochemical detection of viral antigen in ovaries from cattle persistently infected with bovine viral diarrhoea virus. *Am J Vet Res* 1996;57:830–3.
- [95] Brownlie J, Booth PJ, Stevens DA, Collins ME. Expression of non-cytopathogenic bovine viral diarrhoea virus (BVDV) in oocytes and follicles of persistently infected cattle. *Vet Rec* 1997;141:335–7.
- [96] Fray MD, Prentice H, Clarke MC, Charleston B. Immunohistochemical evidence for the localization of bovine viral diarrhoea virus, a single-stranded RNA virus, in ovarian oocytes in the cow. *Vet Pathol* 1998;35:253–9.
- [97] Fray MD, Mann GE, Bleach EC, Knight PG, Clarke MC, Charleston B. Modulation of sex hormone secretion in cows by acute infection with bovine viral diarrhoea virus. *Reproduction* 2002;123:281–9.
- [98] Fray MD, Mann GE, Clarke MC, Charleston B. Bovine viral diarrhoea virus. Its effects on ovarian function in the cow. *Vet Microbiol* 2000;77:185–94.
- [99] Dubovi EJ. Impact of bovine viral diarrhoea virus on reproductive performance in cattle. *Vet Clin North Am Food Anim Pract* 1994;10:503–14.
- [100] Potgieter LN. Immunology of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 1995;11:501–20.
- [101] Charleston B, Hope JC, Carr BV, Howard CJ. Masking of two in vitro immunological assays for *Mycobacterium bovis* (BCG) in calves acutely infected with non-cytopathic bovine viral diarrhoea virus. *Vet Rec* 2001;149:481–4.
- [102] Waldrop JG, Stringfellow DA, Galik PK, Riddell KP, Riddell MG, Givens MD, et al. Infectivity of bovine viral diarrhoea virus associated with in vivo-derived bovine embryos. *Theriogenology* 2004;62:387–97.
- [103] Lindberg A, Ortman K, Alenius S. Seroconversion to bovine viral diarrhoea virus (BVDV) in dairy heifers after embryo transfer. In: *Proceedings of the 14th International Congress on Animal Reproduction*; 2000 [Abstract No. 20].
- [104] Drew TW, Sandvik T, Wakeley P, Jones T, Howard P. BVD virus genotype 2 detected in British cattle. *Vet Rec* 2002;151:551.
- [105] Bolin SR, Ridpath JF. Prevalence of bovine viral diarrhoea virus genotypes and antibody against those viral genotypes in fetal bovine serum. *J Vet Diagn Invest* 1998;10:135–9.
- [106] Gard JA, Givens MD, Stringfellow DA. Bovine viral diarrhoea virus (BVDV): epidemiologic concerns relative to semen and embryos. *Theriogenology* 2007;68:434–42.
- [107] Sandvik T. Progress of control and prevention programs for bovine diarrhoeal virus in Europe. *Vet Clin North Am Food Anim Pract* 2004;20:151–69.
- [108] Brodersen BW. Immunohistochemistry used as a screening method for persistent bovine viral diarrhoea virus infection. *Vet Clin North Am Food Anim Pract* 2004;20:85–93.
- [109] Saliki JT, Dubovi EJ. Laboratory diagnosis of bovine viral diarrhoea virus infections. *Vet Clin North Am Food Anim Pract* 2004;20:69–83.
- [110] Kelling CL. Evolution of bovine viral diarrhoea virus vaccines. *Vet Clin North Am Food Anim Pract* 2004;20:115–29.
- [111] Brock KV, McCarty K, Chase CC, Harland R. Protection against fetal infection with either bovine viral diarrhoea virus type 1 or type 2 using a noncytopathic type 1 modified-live virus vaccine. *Vet Ther* 2006;7:27–34.
- [112] Fairbanks K, Schnackel J, Chase CC. Evaluation of a modified live virus type-1a bovine viral diarrhoea virus vaccine (Singer strain) against a type-2 (strain 890) challenge. *Vet Ther* 2003;4:24–34.
- [113] Paton DJ, Sharp G, Ibata G. Foetal cross-protection experiments between type 1 and type 2 bovine viral diarrhoea virus in pregnant ewes. *Vet Microbiol* 1999;64:185–96.
- [114] Smith DR, Grotelueschen DM. Biosecurity and biocontainment of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 2004;20:131–49.