Influence of bovine viral diarrhea virus infections on AI conception and breeding season pregnancy success in vaccinated beef herds

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Abstract

Bovine viral diarrhea virus (BVDV) causes reproductive and economic losses in cattle. The objective of this study was to evaluate the influence of BVDV infections on reproductive success in previously vaccinated herds. Vaccinated cows (n=370) and heifers (n=528) from 9 herds were synchronized and artificially inseminated (AI). On d 28 following insemination, blood samples were collected and pregnancy status was determined. Non-pregnant animals were resynchronized and inseminated a second time. Blood samples were tested for the presence of BVDV antigen. Animals that tested positive were considered infected with BVDV. Herds with at least 1 positive animal were determined to be BVDV-infected (n=4 infected, n=5 non-infected). Statistical analyses were performed using the GLIMMIX procedure of SAS with herd as a random variable. Herds with BVDV infections at d 28 had significantly decreased ($P < 0.01$) AI conception rates compared to non-infected herds ($34 \pm 2.3\%$ vs $56 \pm 2.3\%$). Breeding season pregnancy rates were also decreased ($P < 0.01$) in BVDV-infected herds compared to non-infected herds ($68 \pm 3.1\%$ vs $88 \pm 6.9\%$). In conclusion, BVDV infections in previously vaccinated herds had a negative impact on AI conception rates and breeding season pregnancy success.

Key words: bovine viral diarrhea virus, pregnancy success, vaccination

Introduction

Bovine viral diarrhea virus (BVDV) is a major reproductive pathogen in cattle and is responsible for costly reproductive and other economic losses in the beef industry. Evidence of exposure to BVDV is widespread throughout cattle herds in the United States and the world. It is reported that calves born persistently infected (PI) with BVDV represent as much as 1 to 2% of the cattle population and serve as sources of viral shedding through the duration of their lives.[17] When considering the rise of BVDV-related reproductive loss in cattle,[8] this area of BVDV-mediated loss may pose the greatest economic concern compared to losses incurred through respiratory, immune, and neurological dysfunction caused by BVDV.[13] Infections of females in the breeding herd can result in a variety of consequences, depending on which stage of gestation infection occurs. The most commonly observed effects are poor conception rates, abortion, congenital defects, or birth of PI calves. Infections with BVDV in seronegative cows during the breeding season resulted in a 56.4% reduction in conception rates compared to cows that seroconverted prior to the breeding season.[27] In addition, viremia in previously non-vaccinated animals at time of artificial insemination (AI) significantly reduced first-service conception rates.[30] These studies report the impact of BVDV on reproductive performance in naïve animals. Little is known, however, about the reproductive consequences of BVDV infections in previously vaccinated animals. Therefore, the objective of this study was to evaluate the influence of BVDV infections on reproductive success after AI, and at the end of the breeding season in previously vaccinated animals.

Materials and Methods

Experimental Design

All procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee. This study utilized previously vaccinated beef cows and heifers from 9 different herds in South Dakota (n = 370 cows, n = 528 heifers). All animals utilized had received vaccinations for BVDV (Table 1) as heifers, and as cows were given yearly booster vaccinations. The most recent vaccination was administered a minimum of 30 d prior to the first artificial insemination (AI). Four of these herds (3 groups of heifers

Table 1. Records of herd size, age, most recent vaccination, and number of days the most recent vaccination was administered before AI.

Herd	Herd size	Age*	Most recent vaccine**	Vaccination days pre-breeding
1	135	heifers	Vista 5 VL5	30+ d prior
2	91	heifers	Bovi-Shield Gold FP5 VL5	30+ d prior
3	151	heifers	Vista 5 VL5	30+ d prior
4	79	cows	CattleMaster Gold FP5 L5	30+ d prior
5	83	heifers	Bovi-Shield Gold FP5 VL5	45 d prior
6	43	cows	Bovi-Shield Gold FP5 VL5	45 d prior
7	97	cows	PregGuard 9	30+ d prior
8	151	cows	Bovi-Shield Gold FP5 VL5	30+ d prior
9	68	heifers	Bovi-Shield Gold FP5 VL5	30+ d prior

*Cows were between 2 and 12 years old, and all cows were a minimum of 30 days postpartum at the start of the synchronization protocol. All heifers were between 14 and 16 months of age at synchronization.

**Vista® 5 VL5, Merck Animal Health, Madison, NJ; Bovi-Shield Gold® FP® 5 L5, CattleMaster® Gold FP™ 5 L5, and PregGuard 9®, Zoetis Animal Health, Florham Park, NJ.

and 1 group of cows) were housed at a common commercial facility for the entire breeding season. Animals at this facility were inadvertently exposed to BVDV by purchased animals (1 group of heifers) that were brought to the facility and then either allowed to commingle or have fence-line contact with other animals without having been tested and/or quarantined. The other 5 herds were all housed at different facilities, and had no exposure with this facility or the herds at this facility. The herds in this study consisted of Angus, Red Angus, Hereford, and commercial crossbred animals. Each herd was managed by its own protocols for nutrition and vaccination (Table 1). All herds were synchronized and inseminated in the spring of the year (between May and July) in eastern South Dakota. The authors have worked with all of these herds for several years on reproductive management protocols, and all herds had previously responded similarly in the past. All herds were synchronized and inseminated using the same protocol, and the same technicians inseminated all animals. All 9 herds (including the 4 at 1 location) were inseminated at different times between the 1st of May and the 15th of July). Time of insemination did not impact pregnancy success as the 4 herds were not inseminated in order. Herds that were not exposed were inseminated around the same time and in between herds that were exposed. Multiple sires were used in all herds to be able to account for variations in sire.

Animals were synchronized using the 7-day CO-Synch + CIDR protocol and AI as part of ongoing reproductive research efforts. All cows were a minimum of 30 days postpartum at the start of the synchronization protocol, and all heifers would have been between 14 and 16 months of age. In brief, animals were administered gonadorelin hydrochloride (GnRH)[a] on d -10, and progesterone as a vaginal insert.[b] On d -2 vaginal inserts were removed and dinoprost tromethamine (prostaglandin $F_{2\text{-alpha}}$ [PGF])[c] was administered. On d 0, heifers were bred 52 to 56 h and cows 60 to 66 h after dinoprost tromethamine injection,[c] and gonadorelin hydrochloride (GnRH)[a] was administered at time of AI (AI 1). On d 21 following the first insemination, the animals were resynchronized using

the 7-day CO Synch + CIDR protocol. At this time all animals received an injection of gonadorelin hydrochloride (GnRH)[d], and half of the animals received a progesterone vaginal insert[b] while the other half did not as part of the aforementioned reproductive research efforts. On d 28, vaginal inserts were removed, blood samples were collected, and all animals were examined by transrectal ultrasonography for pregnancy. Those determined not pregnant via ultrasound and a commercially available blood pregnancy test[e] were administered dinoprost tromethamine (prostaglandin $F_{2\text{-alpha}}$ [PGF])[f] and animals were artificially inseminated 52 to 56 h and 60 to 66 h later (heifers and cows, respectively) and GnRH[d] was administered at time of AI (AI 2). Estrus activity was evaluated at the time of AI 1 and 2 by visualizing an estrus detection aid patch[g] and assigning a value according to the degree surface ink had been removed. A 1 to 4 value scoring system was used, with patches that were 0 to 25%, 25 to 50%, 50 to 75%, and 75 to 100% rubbed off having a score of 1, 2, 3, and 4, respectively. Animals with a patch score of 1 to 2 were considered not in estrus, while animals with patch scores 3 to 4 were considered in estrus. Bulls remained separated from heifers and cows for a minimum of 10 d after AI 2.

Animals and Vaccinations

Blood Sampling and Pregnancy Detection

On d 28 after AI 1, all animals were examined by transrectal ultrasonography by a skilled technician. Presence or absence of a fetus was used for pregnancy diagnosis. Additionally, blood was collected from the jugular or tail vein into 10-mL vacuum blood tubes[h] for immediate whole blood analysis via a commercial blood pregnancy test.[e] Samples and controls were pipetted into the provided plates and the test was carried out according to the manufacturer's instructions. Plates were evaluated and scored according to color using the method described by Northrop et al.[23] Remaining whole blood samples were centrifuged at 3,000 x *g* for 30 min at 39.2°F (4°C) for immediate plasma collection. Harvested

plasma samples were then stored at -4°F (-20°C) until further analysis using a commercially available laboratory BVDV test kit.[i] A final pregnancy diagnosis was conducted solely with transrectal ultrasonography between 33 to 80 days following the first pregnancy diagnosis.

BVDV Antigen Testing

Frozen plasma samples were analyzed using a commercially available laboratory BVDV test kit[i] after the end of the breeding season. This test was selected as it is USDA licensed and has superior sensitivity (97% relative to RT-PCR) and specificity (100% relative to RT-PCR) for detection of the BVDV E[rns] antigen.[18] As stated from the manufacturer, to determine persistent infections a second confirmation test is necessary as this test will also detect animals that are transiently infected. In the current study, all animals that tested positive with the day 28 blood sample and were pregnant at the end of the breeding season were tested again after the breeding season (all pregnant animals were negative for antigen in the second test). Controls and samples were pipetted into the provided plates, and plates were handled according to the manufacturer's instructions. The results from the BVDV test[i] were analyzed on a microtiter plate reader. Optical density values obtained from the plate reader from the samples and controls were used to calculate the BVDV ratio according to the manufacturer's instructions. Positive samples were indicative of BVDV infections. Furthermore, herds were classified as infected based on the presence of at least 1 (as many as 8) positive animal for BVDV antigen.

Statistical Analysis

All statistical analyses were completed using commercially available statistical software.[j] The effect of herd BVDV infection status on AI 1, AI 2, AI 1 and 2, and breeding season pregnancy rates, as well as estrus expression at each AI, were evaluated using the GLIMMIX procedure of SAS. Additionally, the effect of herd BVDV infections on embryonic loss, and percent of embryonic loss that became rebred was performed using the GLIMMIX procedure of SAS. The generalized linear mixed model (GLIMMIX) in SAS allows for the modeling of data that is both continuous (i.e. animal age) and categorical (pregnant vs open). This allows for all data to be analyzed together to account for factors that might impact fertility that were not part of the study. In all analyses, herd was utilized as a random variable to account for differences in management that could not be controlled but that might impact fertility. Differences were considered to be significant when $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$. All data are reported as LS means ± SE of the mean.

Results

Detection of BVDV from Blood Samples

In using the d 28 blood samples with the BVDV test,[i] 4 herds were identified as having been exposed to BVDV. Utilizing the d 28 blood samples, an active infection of BVDV was identified in 18 animals (animals that had antigen for BVDV in their blood on the day of sample collection), thus subjecting the entire commercial facility to BVDV infections during the breeding season due to exposure through shared facilities and fenceline contact. Additionally, these 4 herds were the only herds in the study which added and commingled recently purchased animals around the start of the breeding season.

Estrus Expression

Herd estrus expression prior to AI 1 was significantly decreased by BVDV infection status ($P = 0.04$). BVDV infected herds had an estrus expression rate of 54 ± 2.3%, while the non-infected herds had a rate of 62 ± 2.9%. However, herd BVDV infection status did not significantly influence estrus expression prior to AI 2 ($P = 0.30$) on day 30 or 31. At this time, infected herds had an estrus expression rate of 56 ± 2.9% and 61 ± 3.9% for non-infected herds (Table 2).

Pregnancy Success

First-service AI conception rate was influenced by BVDV infections ($P < 0.01$). Herds which were infected with BVDV had a significantly decreased AI 1 conception rate compared to herds with no BVDV infections (34 ± 2.3% vs 56 ± 2.3%, respectively). Additionally, there was a tendency ($P = 0.06$) for pregnancy success to be reduced in BVDV infected herds after AI 2 compared to non-infected herds (37 ± 4.4% vs 51 ± 9.5%, respectively). When conception rates for AI 1 and 2 were analyzed collectively, a similar response was observed. Infected herds had significantly decreased conception rates after 2 rounds of AI compared to non-infected herds (51 ± 2.3% infected vs 68 ± 2.3% non-infected, $P < 0.01$). Overall breeding season pregnancy success was influenced by herd BVDV infection status ($P < 0.01$). Herds with evidence of BVDV infections had decreased breeding season pregnancy

Table 2. Influence of BVDV infections on estrus expression and pregnancy rates.

BVDV status	Herds (n)	# Hd	Estrus expression		Pregnancy			
			AI 1	AI 2	AI 1	AI 2	AI 1 and 2	Breeding season
Infected	4	456	54 ± 2.3%[c]	56 ± 2.9%	34 ± 2.3%[a]	37 ± 4.4%[e]	51 ± 2.3%[a]	68 ± 3.1%[a]
Non-infected	5	442	62 ± 2.9%[d]	61 ± 3.9%	56 ± 2.3%[b]	51 ± 9.5%[f]	68 ± 2.3%[b]	88 ± 6.9%[b]

Values within a column having different superscripts are different [ab]$P < 0.01$, [cd]$P = 0.04$, [ef]$P = 0.06$

rate compared to non-infected herds (68 ± 3.1% vs 88 ± 6.9%, respectively). These results are summarized in Table 2.

Embryonic Loss

Following AI 1, a total of 25/158 and 29/245 females lost their pregnancy in BVDV-infected and non-infected herds, respectively. There was no significant difference in the percentage of animals which experienced embryonic loss after the first insemination between BVDV-infected and non-infected herds (16 ± 2.7% infected vs 12 ± 2.2% non-infected, *P* = 0.23). Furthermore, there were no differences in the percentage of animals which lost a pregnancy from AI 1 and that were able to be subsequently rebred by bull exposure by the end of the breeding season (28 ± 9.6% infected vs 43 ± 10.5% non-infected, *P* = 0.30).

Discussion

Infertility, or the failure of females to become pregnant in a defined breeding period, stands as the most costly reproductive condition for beef cattle.[3] Reproductive conditions and diseases cost 3.6% of the value of beef production, resulting in expenditures of $441 to $502 million annually.[3] It is well established that BVDV is an infectious disease of both economic and physiologic importance for the beef industry. In particular, its ability to cause decreased conception rates, higher incidence of abortion, and development of PI calves due to exposure in naïve or non-vaccinated animals remains an area of concern for the breeding herd.[20,27,30]

Despite research reports available that evaluate the negative effects of BVDV on reproductive performance in naïve animals, little research has examined the effects of BVDV exposure on reproductive success in previously vaccinated animals. In the present study, evidence of BVDV infections was associated with significantly decreased first, second, and overall breeding season pregnancy rates in previously vaccinated heifers and cows. These results are contrary to the current dogma on vaccinated animals. However, these results are supported by recent research that has investigated the efficacy of vaccines to prevent PI calves. A recent study completed by Walz and co-authors[28] compared reproductive protection between heifers vaccinated with 2 different vaccination programs (both a modified-live and a combination vaccine program), and all animals were administered boosters prior to BVDV exposure. A challenge with BVDV (via PI animals) during d 95 to 111 of gestation resulted in an abortion rate of 13% in the modified-live vaccine treatment group and 5% in the combination vaccination group (25% of the aborted fetuses tested positive for BVDV). Additionally, 1 live calf born in the modified-live group tested positive for BVDV, and was later determined to be PI.[28] Another study compared the level of protection provided by 3 different multivalent vaccines with inactivated BVDV components. Although animals were vaccinated with their respectively assigned treatment twice prior to exposure to PI animals (d

33 to 91 of gestation), between 48% and greater than 90% of animals in any treatment group were positive for virus isolation from white blood cells between d 6 and 10 of exposure. Additionally, abortion rates ranged from 9.7% to 22.7%, and between 43% and 93% of fetuses were infected with BVDV.[29] Furthermore, a recent meta-analysis investigated the efficacy of BVDV vaccination to prevent reproductive disease.[22] This meta-analysis indicated that the risk of fetal infections in vaccinated animals was one-seventh the risk of unvaccinated animals, and that vaccination reduced the abortion risk by approximately 40% compared to unvaccinated animals.[22] These results further indicate the ability of BVDV to surpass the immune system and interfere with pregnancy, despite animals having been administered multiple vaccinations prior to exposure (all heifers had received between 2 and 3 vaccinations prior to the breeding season, and all cows were vaccinated as heifers and were receiving annual boosters prior to breeding).

In a production situation, animals can be exposed to BVDV through contact with an infected animal (transient infection or PI). In the current study, animals were exposed to BVDV by animals being purchased and then either allowed to commingle or have fenceline contact with other animals without being tested and/or quarantined. This type of exposure can result in a transient infection in animals exposed. It has been suggested that the most influential manifestations of BVDV are through acute transient, postnatal infections.[6] Several reports verify the consequences of acute transient infection of BVDV in causing fever, diarrhea, oral ulcerations, abortions, and mortality.[7,10,19,24] Acutely infected animals may display differing levels of severity in clinical signs, as well as differences in the duration of infection. However, regardless of the nature of BVDV infection, costly reproductive consequences can result for the breeding herd.

It has been suggested that the current focus of vaccine research is centered on improving fetal protection by preventing birth of PI calves and abortions following BVDV infection.[21] The adverse effects noted in the current study, however, illustrate the consequences of BVDV infection early in the breeding season. It also further supports the idea that vaccination, as the sole method of controlling BVDV, has not resulted in elimination or reduction of this disease in the US cattle industry.[11,26] Therefore, vaccination should be considered a tool along with biosecurity and testing to identify and eliminate infected cattle to decrease the adverse impacts of BVDV exposure to a breeding herd.[21] Although vaccination remains an important consideration, this method alone is not capable of eliminating the risk of BVDV-associated reproductive and economic loss. Because BVDV remains a contributor to infertility, the significance of its ability to remain a reproductive barrier for vaccinated herds should be carefully considered. The illustrated ability of BVDV to hinder reproductive function and subsequently decrease pregnancy success in the present study draws attention to the need for biosecurity measures and routine BVDV testing

for PI animals to be included in herd management practices. Thus, recommendations for effective reproductive management of beef herds include regular vaccination for aid in control of detrimental infectious reproductive diseases, and testing to decrease the possible exposure to infectious reproductive diseases.

Conclusions

Bovine viral diarrhea virus poses reproductive and economic risks to beef producers. The results from this study exemplify the negative consequences of BVDV infection during the breeding season on pregnancy success, despite appropriate vaccination prior to the breeding season. Although vaccination programs assist in providing protection against BVDV, other methods of herd health management such as biosecurity and testing for PI animals are necessary practices to prevent BVDV-mediated reproductive loss.

Endnotes

Acknowledgements

References

1. Archbald LF, Fulton RW, Seger CL, Al-Bagdadi F, Godke RA. Effect of the bovine viral diarrhea (BVD) virus on preimplantation bovine embryos: A preliminary study. *Theriogenology* 1979;11:81-89.

2. Archbald LF, Gibson CD, Schultz RH, Fahning ML, Zemjanis R. Effects of intrauterine inoculation of bovine viral diarrhea-mucosal disease virus on uterine tubes and uterus of nonpregnant cows. *Am J Vet Res* 1973;34:1133-1137.

3. Bellows DS, Ott SL, Bellows RA. Review: Cost of reproductive diseases and conditions in cattle. *Prof Anim Sci* 2002;18:26-32.

4. Bielanski A, Loewen KS, Del Campo MR, Willadsen S. Isolation of bovine herpesvirus-1 (BHV-1) and bovine viral diarrhea virus (BVDV) in association with the in vitro production of bovine embryos. *Theriogenology* 1993;40:531-538.

5. Booth PJ, Stevens DA, Collins ME, Brownlie J. Detection of bovine viral diarrhea virus antigen and RNA in oviduct and granulosa cells of persistently infected cattle. *J Reprod Fertility* 1995;105:17-24.

6. Brodersen BW. Bovine viral diarrhea virus infections: Manifestations of infection and recent advances in understanding pathogenesis and control. *Vet Pathol* 2014;5:453-464.

7. Carman S, van Dreumel T, Ridpath J, Hazlett M. Severe acute bovine viral diarrhea in Ontario, 1993-1995. *J Vet Diagn Invest* 1998;10:27-35.

8. Evermann JF, Ridpath JF. Clinical and epidemiologic observations of bovine viral diarrhea virus in the northwestern United States. *Vet Microbiol* 2002;89:129-139.

9. Fray MD, Mann GE, Clarke MC, Charleston B. Bovine viral diarrhea virus: Its effects on estradiol, progesterone and prostaglandin secretion in the cow. *Theriogenology* 1999;51:1533-1546.

10. Friedgut O, Rotenberg D, Brenner J, Yehuda S, Pas R, Alpert N, Ram A, Yadin H, Grummer B. Description of the first acute bovine diarrhea virus-2 outbreak in Israel. *Vet J* 2011;189:108-110.

11. Fulton RW, Whitley EM, Johnson BJ, Ridpath JF. Prevalence of bovine viral diarrhea virus (BVDV) in persistently infected cattle and BVDV subtypes in affected cattle in beef herds in south central United States. *Can J Vet Res* 2009;73:283-291.

12. Grahn TC, Fahning ML, Zemjanis R. Nature of early reproductive failure caused by bovine viral diarrhea virus. *J Am Vet Med Assoc* 1985;185:429-432.

13. Grooms DL. Reproductive consequences of infection with bovine viral diarrhea virus. *Vet Clin North Am Food Anim Pract* 2004;20:5-19.

14. Grooms DL, Brock KV, Pate JL, Day ML. Changes in ovarian follicles following acute infection with bovine viral diarrhea virus. *Theriogenology* 1998;49:595-605.

15. Grooms DL, Brock KV, Ward LA. Detection of bovine viral diarrhea virus in the ovaries of cattle acutely infected with bovine viral diarrhea virus. *J Vet Diagn Invest* 1998;10:125-129.

16. Grooms DL, Brock KV, Ward LA. Detection of cytopathic bovine viral diarrhea virus in the ovaries of cattle following immunization with a modified live bovine viral diarrhea virus vaccine. *J Vet Diagn Invest* 1998;10:130-134.

17. Houe H. Epidemiological features and ecnomical importance of bovine virus diarrhoea virus (BVDV) infections. *Vet Microbiol* 1999;64:89-107.

18. IDEXX Laboratories Inc. IDEXX BVDV PI X2 Test. Validation Data Report., 2012.

19. Liebler-Tenorio E, Ridpath J, Neill J. Distribution of viral antigen and tissue lesions in persistent and acute infection with the homologous strain of noncytopathic bovine viral diarrhea virus. *J Vet Diagn Invest* 2004;16:388-396.

20. McGowan MR, Kirkland PD, Richards SG, Littlejohns IR. Increased reproductive losses in cattle infected with bovine pestivirus around the time of insemination. *Vet Rec* 1993;133:39-43.

21. Newcomer BW, Chamorro MF, Walz PH. Vaccination of cattle against bovine viral diarrhea virus. *Vet Microbiol* 2017;206:78-83.

22. Newcomer BW, Walz PH, Givens MD, Wilson AE. Efficacy of bovine viral diarrhea virus vaccination to prevent reproductive disease: A meta-analysis. *Theriogenology* 2015;83:360-365.

23. Northrop EJ, Rich JJJ, Rhoades JR, Perry GA. Comparison of two bovine serum pregnancy tests in detection of artificial insemination pregnancies and pregnancy loss in beef cattle. *PLoS ONE* 2019;14:e0211179.

24. Olafson P, MacCallum AD, Fox FH. An apparently new transmissible disease of cattle. *Cornell Vet* 1946;36:205-213.

25. Ssentongo YK, Johnson RH, Smith JR. Association of bovine viral diarrhoea-mucosal disease virus with ovaritis in cattle. *Australian Vet J* 1980;56:272-273.

26. USDA. Beef 2007-08, Prevalence and control of bovine viral diarrhea virus on US cow-calf operations, 2007-08 In: USDA:APHIS:VS C, ed. Fort Collins, CO, 2010.

27. Virakul P, Fahning ML, Joo HS, Zemjanis R. Fertility of cows challenged with a cytopathic strain of bovine viral diarrhea virus during an outbreak of spontaneous infection with a noncytopathic strain. *Theriogenology* 1988;29:441-449.

28. Walz PH, Givens MD, Rodning SP, Riddell KP, Brodersen BW, Scruggs D, Short T, Grotelueschen D. Evaluation of reproductive protection against bovine viral diarrhea virus and bovine herpesvirus-1 afforded by annual revaccination with modified-live viral or combination modified-live/killed viral vaccines after primary vaccination with modified-live viral vaccine. *Vaccine* 2017;35:1046-1054.

29. Walz PH, Riddell KP, Newcomer BW, Neill JD, Falkenberg SM, Cortese VS, Scruggs DM, Short TH. Comparison of reproductive protection against bovine viral diarrhea virus provided by multivalent viral vaccines containing inactivated fractions of bovine viral diarrhea virus 1 and 2. *Vaccine* 2018;36:3853-3860.

30. Yavru S, Kale M, Gulay MS, Yapici O, Levent O, Ata A. Effects of bovine viral diarrhoea virus on the fertility of cows. *Acta Veterinaria Hungarica* 2013;61:281-289.