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TIMING OF VACCINATIONS IN ESTROUS SYNCHRONIZATION PROGRAMS

Dr. W. Dee Whittier, Professor
Heidi Kara Baitis, BS
VA- MD Regional College of Veterinary Medicine
Virginia Tech, Blacksburg, VA

Introduction

Bovine Viral Diarrhea (BVD) virus and Infectious Bovine Rhinotracheitis (IBR) virus are two of the major viral pathogens responsible for respiratory disease in cattle. These viral agents can be associated with pathology in the respiratory, immunologic, neurologic, hematologic, lymphatic, and reproductive organ systems (Grooms et al., 1998). Infections of cattle with BVD virus and/or IBR virus are associated with reduced conception rates, early embryonic mortality, and reduced pregnancy rates. Proper vaccination protocols are a key part of the prevention and control of losses due to these agents.

Current recommendation for vaccination of cattle against respiratory and reproductive disease with modified live virus (MLV) BVD and IBR vaccines is 30 d prior to the onset of the breeding season (Stormshak et al., 1997). Pre-breeding vaccination is practiced to protect both cows and calves against respiratory antigens, protect the fetus against infection, and reduce economic losses associated with diseases. When compared to killed viral product vaccines, MLV vaccines are typically accepted to stimulate adequate immunity without requiring a second inoculating dose. However, the use of MLV vaccines have been reported to result in decreased conception rates, congenital anomalies, or abortion of pregnant animals.

Estrous synchronization reduces the labor necessary to implement an effective artificial insemination protocols in beef cattle. Programs that decrease or eliminate heat detection (Fixed-time artificial insemination) are increasingly used in beef cattle to achieve pregnancies.

In order to vaccinate cattle with a MLV vaccine 30 d prior to synchronization, animals must be handled at least one additional time. Concurrent vaccination at the initiation of an estrous synchronization protocol has the potential to reduce the labor efforts associated with these two managerial herd practices. Conversely, if vaccination with ML V BVD and IBR antigens at the start of estrus synchronization results in significantly lower pregnancy rate, cattlemen should avoid this management practice.

Effects on Female Fertility

The reproductive effects of BVD infection result in reduced fertility, early embryonic death, abortions, and various congenital anomalies. Decreased fertility in cattle is the most significant complaint in herds where BVD has been diagnosed (Houe et al., 1993). There are several current hypotheses for the mechanisms of decreased fertility including failure of fertilization, early embryonic loss, and ovarian dysfunction.

A study conducted in Switzerland assessed the gestational failures in animals that were both seronegative and immune to BVD to animals that were acutely infected with field strain of BVD. Cows that were seropositive prior to the breeding season did not exhibit significantly greater gestational failures compared to cows that were sero-negative throughout the study. Return to estrus following first insemination, and first stage gestation pregnancy losses were not different between the groups. However, BVD infection between days 46 and 210 of gestation had a significant impact on the number fetal deaths (Rufenacht et al., 2001).

In contrast to the aforementioned study, McGowan and associates reported pestivirus infection during estrus and near the time of insemination significantly decreases conception rates and increases early embryonic loss. These results were also based on natural exposure occurrences that resulted from contact of sero-negative animals with animals persistently infected with BVD virus. Calves that were born to all animals in this study had no indication of BVD infection (1993).

BVD virus has been isolated from the ovaries of infected cattle. In addition, chronic oophoritis has been described after acute infection with BVD virus (Sentongo et al., 1980). BVD virus was isolated from the corpora lutea (CL) of 2 animals approximately 7 days following experimental acute infection (Grooms et al., 1998). The presence of BVD virus in the CL of infected animals is consistent with the highly vascularized tissue found in this structure. In this experiment no BVD virus could be isolated from the follicular fluid collected from any of the infected animals. Previous investigators have isolated BVD from the follicular fluid of abattoir-derived animals. However, the history of these animals was unknown and it is possible that these animals were persistently infected (Bielanski et al., 1993). BVD positive cells were found in association with primary follicles, secondary follicles, and antral follicles (Grooms et al., 1998a).

Another study conducted in 1998 assessed the impact of acute infection with BVD on ovarian follicles. Both the growth rate and diameter of the ovulatory and dominant anovulatory follicle were reduced in animals acutely infected with BVD virus. Additionally, there was a significant decrease in the number of subordinate follicles associated with each follicular wave (Grooms et al, 1998b).

Kafi and associates (1997) did not report similar differences in follicle size or number associated with a superovulation protocol in BVD infected animals however, many ovulatory follicles followed by transrectal ultrasonography failed to ovulate following behavioral estrus. Ultrasonographic examination 48 h after AI revealed follicles that failed to ovulate on the ovaries of infected heifers.

Assuming BVD triggers changes in ovarian endocrine function, alteration of normal hormone concentrations would negatively affect the viability of an embryo (Smith et al., 1990). Decreased embryo viability would inevitably lead to increased embryonic mortality, an increase in percent of animals returning to estrus and an overall decline in reproductive efficiency.

Histologically, scattered interstitial lymphocytes were evident 8 days post infection with BVD. Lymphocytic infiltrations increased up to 18 days post infection and were evident up to 60 d after initial exposure. Eosinophils and vacuolation of the ovarian stroma suggested the presence of edema 2-3 weeks post infection (Grooms et al., 1998). These results agree with previous research models where an immune mediated ovaritis was

documented from day 15 to 61 post infection. These ovaries had varied lesion severity however; a vasculitis of the small and medium arterioles and a lymphocyte infiltrate was present (Ssentongo et al., 1980). Prolonged ovaritis is consistent with slow clearance of viral antigens, which could help explain the ovarian dysfunction recognized during BVD infection.

The early stage of pregnancy is very sensitive to BVD infection (Moennig and Liess, 1995). High BVD titers prior to first breeding, seroconversion to BVD during pregnancy, and increasing BVD titers during pregnancy were associated with fetal loss in some herds (Thurmond et al., 1990; Michel et al., 1993; Rufenacht et al., 2001). Herd BVD infection results in a higher percentage of cows that have a late return to service (versus a 3 week or less return to service) indicating a higher rate of embryonic loss after the first month of pregnancy (Robert et al., 2004).

BVD virus infection of the dam in the pre-implantation period may result in a high incidence of embryo of fetal mortality (McGowan et al., 1993), while infections that occur from implantation to the fourth month of pregnancy (day 40 to 125) are characterized by fetal death, abortion, mummification and teratogenesis and the birth of persistently infected calves (Moenning and Leiss, 1995). Abortion rates up to 40% have been reported in naive cattle following experimental infection on the 100th day of gestation (Done et al., 1980) although lower losses result from field infection (Duffell et al., 1986).

IBR Reproductive Consequences

IBR virus is an important cause of reproductive disease in cattle. During the last two decades more information has been discovered concerning the effect of IBR on the reproductive tract of the bovine. Infection with IBR can cause inflammation throughout several body systems in cattle, including the reproductive tract. Infertility and shortened estrous cycles have been observed when cows were bred with semen containing IBR virus. The most consistent IBR lesion in the reproductive tract is endometritis, however cysts have also been reported on the ovaries of infected cattle (Parsonson and Snowdon, 1975).

In 1983, Miller and Van Der Maaten inoculated 12 IBR negative heifers 24 h after insemination with one of three strains of IBR infective tissue culture fluid. Following intrauterine inoculation, acute necrotizing endometritis was observed in the uterine body or caudal aspect of the uterine horns. Lymphocytic aggregations with numerous mitotic figures suggested that lymphoproliferation is an inflammatory response to the virus and not simply an infiltration of lymphocytes from a systemic effect. The cranial uterine horns were minimally affected which would not likely interfere with blastocyst attachment beginning about 21 days after conception.

Kendrick and McEntee suggested intrauterine IBR infection stimulated cystic CL formation (1967). IBR has since been associated with a variety of ovarian lesions at or near the time of estrus (VanDer Maaten et al., 1985).

Heifers in a 1983 study had luteal cysts with necrotic walls bordered by a zone of proliferating mononuclear cells when intrauterine inoculation of IBR virus occurred 24 h post insemination. Focal necrosis and lymphoid proliferation were common in the parenchyma of both cystic and non-cystic luteal tissue. IBR virus was isolated from the cystic CL in some animals. Intramuscular inoculation with IBR results in multifocal necrotizing lesions in the CL similar to intrauterine inoculation. Intravenous inoculation with IBR virus however, causes a more diffuse necrosis of luteal tissue and necrosis of the

follicles. Ovarian lesions have also been reported in animals following aerosol exposure. Lesions resulted in reduced plasma progesterone levels in the estrus cycle after inoculation. The detrimental effects to the ovary by IBR virus inoculation were temporary and there was little adverse effect on the subsequent estrus cycles of luteal function (Miller and Van Der Maaten, 1984).

Smith and associates examined the progesterone hormone profiles of heifers inoculated with IBR infected nondiluted cell culture. Five out of 8 heifers examined with severe ovarian lesions has markedly decreased plasma progesterone profiles.

Embryonic Loss

Components of the Bovine Respiratory Disease Complex are documented potent abortifactants. IBR and BVD may infect the reproductive tracts of cattle and fetuses during estrus or gestation thereby resulting in reproductive losses (Fulton et al., 1995). Fetuses may become infected when susceptible animals are infected, develop viremia, and the virus crosses the placenta (Baker, 1995). IBR was first documented as a cause of bovine abortion in the United States in 1957 (Brown et al., 1957). In 1971, 24.4% of 808 documented abortions were caused by IBR from either natural environmental exposure or by vaccination with an IBR MLV vaccine (Kirkbride et al., 1973). Natural BHV-1 infections can cause abortion throughout gestation in cattle. More recently, a survey indicated viruses were associated with 10.58% of bovine abortions and stillbirths from 1980-1990. IBR comprised 5.41 % of the aforementioned diagnoses, while BVD was detected in 4.54% (Kirkbride, 1990).

Pregnant, immunologically naive cows are at risk to BVD infection. Early in gestation (100 days), infection results in abortion whereas mid-gestation (100-125) exposure may cause congenital infections that result in persistently infected calves (Grooms et al., 2002). A persistently infected calf carried to term will be born immunotolerant to the infected BVD strain and will shed virus continually throughout its lifetime (Baker, 1995). The first four months of gestation is associated with the greatest risk of BVD related abortion (Wren, 1999). Additionally, decreased fertilization rates were reported after intrauterine infusion of laboratory strains of BVD at the time of insemination (Grahn et al., 1984). Reduced conception rates occurred as a result of natural infection around the time of insemination (Virakul et al., 1999).

Similarly, IBR can cause infertility and pregnancy loss in cattle. Infectious bovine Rhinotracheitis causes lesions in the reproductive tract that could either prevent conception or result in pregnancy loss. After the sixth month of gestation, IBR will cause abortion in susceptible cattle at a rate of 25-50%. Examination of the aborted fetus due to IBR infection will reveal autolysis (Radostits, 1994).

Herd Health and Vaccination

Health and production management programs for beef cattle herds are implemented to maintain animal health and production efficiency that allow maximum economic return to the producer (Schnurrenberger, 1979). Vaccination programs for cattle in all phases of production are integral components of herd health procedures designed to reduce or eliminate negative effects of infectious disease (Ribble, 1992). More than 150 vaccines are available containing immunogens for BRDC therefore, veterinarians and producers must address individual herd needs when selecting appropriate vaccine products for use in

varying aspects of production (Arrioja, ed., 2001).

Available vaccines may be monovalent containing single immunogens or in combination with other viruses or bacteria. Criteria for consideration include identification of casual agent, ability for the vaccine to elicit protective immune response, and risks associated with use of vaccine product (Tizard, 2000).

Most licensed vaccines contain either killed or modified live whole bacteria or viruses (Roth and Henderson, 2001). Modified live vaccines undergo a process of attenuation that alters the vaccine product to reduce virulence. Relative incompatibility is present amongst the two major prerequisites for an ideal vaccine, which include high antigenicity and relative absence of adverse side effects (Tizard, 2000). Vaccines containing viable infectious agents provide strong and long lasting immunity while requiring few inoculating doses. The most important advantage of this type of vaccine is the variety of administration routes and the ability to combine multiple viruses in one inoculating dose. Adjuvants are unnecessary and there is a reduced risk of hypersensitivity with live vaccines (Radostits et al, 2001). A single vaccination by either intramuscular or intranasal routes will elicit protection as early as 24 hours post-vaccination in adult animals (Sutton, 1980). MLV vaccines cause systemic response resulting in potential mild disease because of residual virulence. Live vaccines therefore stimulate adequate immunity without requiring a second booster dose to provide protection. Additional disadvantages to ML V vaccine use is the possibility of reversion to a virulent state by means of mutation or recombination, perpetuation of the virus in the environment (viral shedding), limited shelf life, increased potential contamination, and abortion in pregnant animals (Radostits et al., 2001).

	Modified-Live Vaccine	Killed (lnactivated Vaccine
Advantages	─ Robust, long lasting immunity ─ Fewer inoculating doses ─ Effective non-parentrally (oral, intranasal) ─ Decreased incidence of hypersensitivity ─ Interferon stimulation	─ Unlikely to cause disease ─ Stable storage
Disadvantages	─ Systemic reactions/mild disease ─ Reversion to virulent strain (mutation or recombination) ─ Abortion ─ Limited shelf life ─ Contamination	─ Incomplete activation ─ Short-lived immunity ─ Effective only parentrally ─ Multiple doses required ─ Increased cost ─ Hypersensitivity ─ Local sensitivities (local site reactions)

Table 1. Adapted from Radostitis, 1994. Advantages and disadvantages of MLV and Killed vaccines.

The current recommendation for use of ML V vaccines in cows is at least 30 days prior to the initiation of the breeding season (Stormshak et al., 1997). This pre-breeding vaccination should protect the adult animal against viral respiratory disease. Additionally, vaccination at this time will also provide protection to a future fetus.

Bovine Viral Diarrhea virus vaccine usage in the United States is difficult to assess. However, it can be estimated that nearly 60% of dairy farms routinely vaccinate replacement heifers while only about 32% vaccinate dry cows. Only 13% of US beef herds are estimated to routinely vaccinate cows for BVD. This percentage drops to 8% for producers who routinely vaccinate herd bulls (Bolin, 1995). Successful prevention of IBR abortion has been achieved by proper and timely vaccination prior to insemination. Follow-up vaccination with a killed vaccine during pregnancy is also recommended (Cravens et al., 1996).

Several IBR vaccines have been studied to determine whether commercially available IBR vaccines could cause pathologic changes in the ovaries during estrus. Some vaccine strains have a more severe effect on the reproductive tract than other strain (Miller et al., 1989; Smith et al., 1990).

Routine vaccination with the recommended dosage by the approved IM route during estrus has been found to have a profound negative effect on the conception rate of cows inseminated to that estrus or the subsequent cycle (Chiang, 1990). Lesions in the non-luteal tissues of the ovaries can also be associated with other causes of infertility and damage at other phases of the reproductive cycle (Smith et al., 1990). Use of caution has been suggested with the practice of vaccination of breeding stock with MLV virus vaccines (Chiang et al., 1990; Smith et al., 1990).

The use of ML V vaccines may cause fetal infection, death and subsequent abortion when used in pregnant cattle (Kit et al, 1986). Vaccine strains of IBR have been well documented as etiologic agents associated with abortion and infertility when associated with herd vaccination procedures (Kahrs, 1977).

Study to assess safety of vaccination

We conducted a study to assess whether vaccination with two popular modified live vaccines containing IBR and BVD would have an influence on the reproductive outcomes of beef cows if administered at the beginning of an estrous synchronization program.

Postpartum beef cows between ages 2 and 13 years at six correctional centers in Virginia were included in this experiment (n=807). All animals had been vaccinated pre-weaning or at the time of weaning with a ML V vaccine. All heifers used for replacements were vaccinated with a ML V vaccine pre-breeding. Herds had no prior history of abortion due to Vibrio, Neospora, Lepto, or BVD.

PARITY	
1	n = 216
2	n = 112
3+	n = 479

BREED	
Angus	n = 473
Angus Cross ..	N = 171
Other	N =163

Primiparous (n = 216) and multiparous (n = 591) cows received one of two vaccines 30 d prior to insemination or at the initiation of estrous synchronization in a 2 x 2 factorial design. Prior to experiment initiation, cows were assessed for general health. Twenty days prior to synchronization cows were blocked according to age and days post partum, then randomly assigned within blocks to treatment groups la, 1b, 2a, 2b using Microsoft Excel@ (Windows Copyright@ Microsoft Corporation 1985-1999 File Version 9.0.0.2719). Cows received 2ml of physiological saline subcutaneously as a placebo and a label dose of ML V vaccine product at one of two time treatments. Group 1 received physiological saline on d 0 and either vaccine 1 (V AC1; subgroup 1a) or vaccine 2 (VAC2; subgroup 1b) on d 20. Group 2 received either V AC1 (subgroup 2a) or V AC2 (subgroup 2b) on d 0 and physiological saline on d 20. All cows received a 2 ml dose (per label instructions) of Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza$_3$, Respiratory Syncitial Virus- *Leptospira Canicola-Grippotyphosa-Hardjo Icterohaemorrhagiae-Pomona* Bacterin (Groups la and 2a; Pyramid@9 (V ACl), Fort Dodge, Fort Dodge, Iowa; Groups 1b and 2b; Bovi-Shield GoldTMFPTM 5L5@ (V AC2), Pfizer, NY, New York). Two MLV vaccine products utilized contained identical antigens. Vaccine diluents contained different adjuvants. Between treatments animals were separated by group, but not by subgroup to prevent unintentional exposure of Group 1 animals to shed BVD Types I and II virus and IBR virus.

Cows were synchronized with an injection of GnRH (Cystorelin@, 200Jlg, Lm., Merial, Iselin, NJ) and an intravaginal controlled drug-releasing device (EAZI-BREED^{TM} CIDR@, Pfizer, InterAg, Hamilton, New Zealand), containing 1.38 g of progesterone, followed by CIDR removal and an injection ofPGF2a (Lutalyse, 25 mg, Lm., Pharmacia-Upjohn, Kalamazoo, MI) 7 d later. At the time of CIDR removal, cows were fitted with a Kamar Heatmount@ Detector (Kamar Inc., Steamboat Springs, CO) to assist with detection of standing events associated with estrus. Thirty-six hours following PGF2ά administration, cows received a second injection of GnRH. Cows were observed twice daily for signs of behavioral estrus following CIDR removal and PGF2 ά administration. Timed artificial insemination was performed 12-18 h following the second GnRH administration. Clean-up Angus bulls were turned out 10 d post insemination.

At all locations, conception rate to a single artificial insemination was determined via transrectal ultrasonography (Aloka 500V, Cormetrics Inc., Wallingford, CT) on d 28-45 post insemination. To measure early embryonic mortality, transrectal ultrasonography was performed using a B-mode ultrasound machine (Aloka 500V, Cormetrics Inc.,

Wallingford, CT) on d 28, d45, and d60 post TAlon cows at two locations. Due to logistics and funding, only cows at two experimental locations underwent intensive monitoring for early embryonic death. Transrectal ultrasonography was performed at day 45 on all cows. All experimental animals were subjected to per-rectal palpation examination on day l00 to confirm pregnancy. Figure 1 depicts the timelines used in the study.

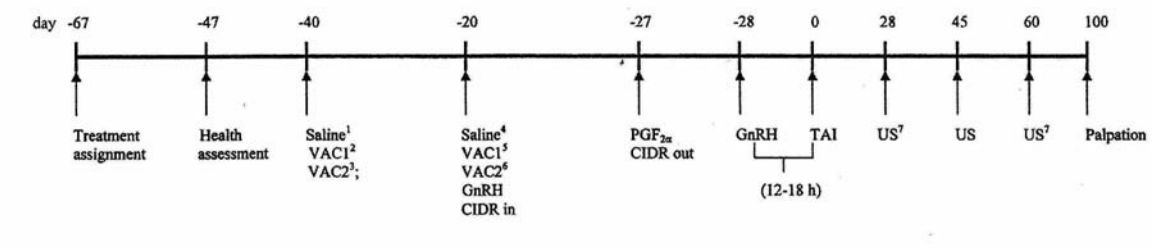

Figure. Diagram of experimental treatments with d -40 or d -20 vaccination, estrous synchronization, and pregnancy diagnostic measurements. [1] Group 1;[2] Subgroup 2a (n=199);[3] Subgroup 2b (n=198);[4] Group 2;[5] Subgroup 1a (n=209);[6] Subgroup 1b (n=201);[7] Cows at 2 intensive locations.

Conception rate (CR) is here defined as the proportion of cattle diagnosed pregnant to the single timed insemination. Pregnancy rate (PR) is defined as the proportion of cows that were confirmed pregnant at the end of the breeding season.

Blood samples were collected prior to synchronization, at a 7-14 d interval via jugular venipuncture to determine progesterone presence. Only cows at two intensively monitored locations underwent blood collection. A validated (Holt et al., 1989), solid-phase RIA procedure (Coat-A-Count, Diagnostic Products) was used to determine serum progesterone concentrations. Samples were analyzed in duplicate by two assays. The intra-assay coefficients of variation were 8 and 10 % respectively.

Data were analyzed with a statistical software program (SAS Version 9.1 for Windows, SAS Institute, Cary, NC, USA). Logistic regression analysis (Genmod procedure of SAS) was used to examine the effects of treatment. Models (binary logit function and Fisher's scoring optimization technique) were created with a multivariate logistic regression model. The outcomes measured were timed AI pregnancy rate and over-all pregnancy rate. Variables included in the model were vaccine (1 vs. 2), vaccination time (1 vs 2), location 1 to 6), parity (1, 2, 3 and >3), postpartum days at insemination (<60 days, 60 to 80 days and > 80 days), body condition score at the initiation of synchronization program (< 4, 5 to 6 and > 6), breed (Angus vs others), Kamar activation at AI (white, partial and red). All possible two-way interaction effects were also tested. Appropriate variables were retained in the model after manual backward elimination. Odds ratio and 95% confidence intervals were calculated for all variables in the final model and included in the results listed in Tables 1 and 2. The pregnancy rates for treatment groups were given in Table 2.

Table 2 shows the outcomes of the study including probabilities shown by the model. Table 3 shows numerical data for embryonic loss observed during the study.

Table 2. Over-all and Fixed Time AI pregnancy rate for treatment, parity, postpartum days at AI, farm, breed, body condition score, Kamar activation at AI.

Parameter	Classifi-cation	Outcome		Significance in the model FTAI	Significance in the model Overall PR
		FTAI PR	Over-all PR		
Vaccine	1	55.4 (226/408)	85.6 (350/408)	0.79	0.13
	2	54.9 (215/399)	89.2 (356/399)		
Vacc. time	0	53.1 (211/397)	86.9 (345/397)	0.40	0.57
	1	56.1 (230/410)	88.0 (361/410)		
BCS	< 4	47.6 (59/124)	87.9 (109/124)	0.51	0.86
	5 - 6	56.3 (343/609)	87.2 (531/609)	0.79	0.70
	> 6	52.7 (39/74)	89.2 (66/74)		
Parity	1	60.8 (132/217)	87.6 (190/217)	0.09	0.50
	2	33.0 (37/112)	82.1 (92/112)	0.0009	0.29
	3	53.4 (31/58)	86.2 (50/58)	0.03	0.12
	> 3	57.4 (241/420)	89.0 (374/420)		
Farm	1	47.9 (57/119)	83.2 (99/119)	0.99	0.96
	2	53.3 (65/122)	88.5 (108/122)	0.65	0.60
	3	62.4 (58/93)	93.5 (87/93)	0.008	0.03
	4	59.8 (67/112)	96.4 (108/112)	0.0001	0.002
	5	59.8 (91/211)	84.8 (179/211)	0.91	0.91
	6	48.0 (72/150)	83.3 125/150)		
Breed	Angus	54.5 (258/473)	85.8 (406/473)	0.33	0.32
	Other	54.8(183/334)	89.8 (300/334)		
Kamar	Red	51.8 (317/612)	86.3 (528/612)	0.03	0.08
	Partial	68.3 (43/63)	90.5 (57/63)	0.96	0.50
	White	61.4 (81/132)	91.7 (121/132)		
Postpartum days @ AI	< 60	56.2 (212/377)	86.0 (166/193)	0.32	0.86
	60 – 80	58.9 (104/236)	90.3 (213/236)	0.86	0.21
	> 80	48.8 (184/377)	86.7 (327/377)		

Table 3. Early Embryonic Death based on treatment category from 2 farms.

Parameter	Classification	Outcome 28 – 45	Over-all
Treatment	1	1/53	3/82
	2	0/51	3/75
	3	0/48	1/70
	4	1/46	4/72
Cyclicity	Yes	0/110	4/182
	No	2/87	7/116

Conclusion

Neither vaccine used nor timing of administration had an influence on either the conception rate to timed artificial insemination nor overall pregnancy rate during the breeding season. There were farm differences for both parameters. Embryonic loss rates were very low throughout the study.