

Pregnancy outcomes and calf growth in beef cows given modified-live virus vaccination at synchronization for timed artificial insemination

Jamie Stewart,^a John Currin,^a Sherrie Clark,^a Tracey Redifer,^a Vitor Mercadante^{a,b}

^aDepartment of Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

^bSchool of Animal Sciences, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

Abstract

The objective was to compare reproductive outcomes and calf traits after vaccination of suckled beef cows using a commercial modified-live virus (MLV) vaccine that contained bovine herpesvirus 1 and bovine viral diarrhoea virus (types 1 and 2) at the initiation of a timed artificial insemination (AI) program. In Experiment 1, cows given MLV vaccine in the previous year were enrolled during the fall 2019 and spring 2020 breeding seasons. At initiation of a 7-day CO-Synch + CIDR synchronization protocol (10 days before breeding), cows were given either MLV vaccine or nothing (CONT). Cows were inseminated 60-66 hours after removal of the CIDR insert and subsequently exposed to bulls starting ~ 1 week after AI. All CONT cows received a killed virus vaccine at mid-pregnancy. For Experiment 2, available cows from fall 2019 were reenrolled and given the same vaccination treatment as in the previous season. Treatment with MLV vaccine did not have effects on AI pregnancy rates ($p \geq 0.31$), total season pregnancy rates ($p \geq 0.12$), or AI pregnancy losses ($p \geq 0.41$). There was also no effect of vaccination treatment on calf birth weight ($p \geq 0.27$), weaning weight ($p \geq 0.14$), or average daily gain ($p \geq 0.14$). In summary, cows given MLV vaccinations on day 10 before breeding experienced no deleterious effects on pregnancy outcomes or calf traits. These findings supported the use of MLV vaccination in previously vaccinated cows at synchronization for timed AI.

Keywords: Artificial insemination, cattle, breeding, modified-live virus, synchronization

Introduction

In beef cow-calf herds, exposure to common abortifacients, such as bovine herpesvirus 1 (BoHV-1) and bovine viral diarrhoea virus (BVDV) produces a serious threat to reproductive efficiency and overall production. In addition to well-documented deleterious effects on the mid-pregnancy fetus and pregnancy maintenance, both viruses also have adverse effects on ovulation, corpus luteum formation, and early embryonic development when exposed early in the breeding season.¹⁻⁶ Fortunately, there are various effective approved multivalent vaccines available for use in cattle. These products, frequently referred to as '5-way vaccines,' protect against BoHV-1 and BVD (both types 1 and 2), and common respiratory viruses, parainfluenza 3 and bovine respiratory syncytial virus. Variations of these products also commonly include bacterins to protect against other important reproductive pathogens, namely *Campylobacter fetus* ssp. *venerealis* and several *Leptospira* spp. serovars.

Among the various commercial 5-way vaccine products for cattle, there are 2 general classes: modified live virus (MLV) vaccine or a chemically inactivated/killed virus (KV) vaccine. The MLV vaccines have multiple advantages over the KV vaccines. Authors of a meta-analysis reported that although both vaccine types decreased abortion and fetal infection in cows exposed to BVDV, the relative risk for pregnancy loss was less with the use of MLV vaccines.⁷ Similarly, MLV and KV vaccines against BoHV-1 reduced the risk of abortion in cattle by ~ 60%; however, MLV vaccines had a greater duration of protective immunity and stimulated higher neutralizing antibody titers.⁸ Despite its proven efficacy, notable controversies surrounding the use of MLV vaccines involve their effects on conception rates when given near breeding and on fetal losses when given during pregnancy. It is recommended that commercial multivalent vaccines containing modified-live BoHV-1 and BVDV components be given a minimum of 30 days before breeding or insemination to avoid adverse effects on fertility.⁹

On the contrary, manufacturers of commercial KV vaccines typically recommend giving at 2-4 weeks before breeding, and also state that they are safe to use in pregnant cows and heifers, regardless of vaccination history.

Although KV vaccines are labeled as safe for use in pregnant cows, many MLV vaccine product labels state that they can only be safely given to cows during pregnancy if the cows have been previously vaccinated. A case report calls this claim into question after abortions occurred in a group of 55 heifers vaccinated with a USDA-licensed '5-way' vaccine product at 7-8 months of pregnancy.¹⁰ The heifers had been vaccinated with the same product on 3 earlier occasions prior to breeding. Seven heifers aborted fetuses between 45-55 days after vaccination, of which, BoHV-1 infection was diagnosed in the 6 submitted specimens, based on detection of BoHV-1 antigen and isolation of BoHV-1 from pooled tissue.¹⁰ To err on the side of caution, many practitioners recommend using only KV vaccinations during pregnancy, and reserve the use of MLV vaccines only for prebreeding. Necrotic oophoritis, abnormal estrous cycles, and decreased pregnancy rates are well-documented deleterious outcomes of MLV vaccination near the onset of estrus in naïve heifers,¹¹⁻¹⁴ which is why the label specifies that vaccination should occur no sooner than 30 days before breeding.

However, the reproductive effects of MLV vaccine in previously vaccinated cattle have been inconsistent. In the largest field study to date, our research group reported no deleterious effects of MLV vaccine given on day 10 before timed artificial insemination (AI) compared to cows receiving KV vaccine.¹⁵ However, the biggest limitation of our previous study was the lack of a true negative control. Authors of a study reported a decrease in AI pregnancy rates when previously vaccinated cattle were given either a MLV or chemically altered/inactivated (CA/IA) vaccine between 27-29 or 30-37 days before breeding (52%), compared to those vaccinated with either product at 46-89 days before breeding (64%).¹⁶ There is, therefore, a need to critically evaluate the timing of our prebreeding cow-calf vaccination protocol with a true negative control to better understand its effects on fertility.

Timed AI is a tool that beef cow-calf producers can use to improve calving distribution, enhance pregnancy rates, and increase subsequent calf value.¹⁷ Vaccination of cows > 30 days before breeding may not be practical for well-managed farms that wish to implement timed AI and maintain a 365 days calving interval. Synchronization of ovulation for AI already requires a minimum of 3 trips through the chute. Prebreeding vaccination would add an additional handling event, increasing both cattle stress and producer costs. Vaccination at synchronization could reduce stressors on cattle by decreasing the number of trips through the chute. Although we demonstrated that MLV vaccination at CIDR insertion (10 days before AI) did not have adverse effects on reproductive outcomes or calf traits compared to KV vaccine,¹⁵ there is still a need to compare the use of MLV vaccine to a control group receiving no prebreeding vaccine. Therefore, the objective was to assess the reproductive outcomes (AI and breeding season pregnancy rates and AI pregnancy losses) and subsequent calf traits (birth and weaning weights) of previously vaccinated suckled beef cows in commercial cow-calf operations vaccinated with MLV vaccine 10 days before breeding. We hypothesized that reproductive outcomes and calf traits do not differ between MLV-vaccinated and control cows vaccinated with KV vaccine at mid-pregnancy.

Materials and methods

Cows

All cows utilized were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.¹⁸ Since data collected from routine farm visits were used, animal care and use committee approval was not required, and the study is exempt. Suckled Angus-cross beef cows (n = 2,912) housed at 11 locations during Fall of 2019 (8 locations; n = 1,107), spring of 2020 (5 locations; n = 911), and fall of 2020 (7 locations; n = 894) were enrolled in this study. All cows belonged to commercial cow-calf operations managed by the Virginia Department of Corrections Agribusiness program. Replacement heifers in these cow-calf operations are raised internally and vaccinated with an MLV vaccine at weaning and then again before breeding each season. Experiment 1 included cows bred in the fall 2019 and spring 2020 seasons. These enrolled cows had all been vaccinated the previous year (fall 2018/spring 2019) with a MLV vaccine (Pyramid 10, Boehringer Ingelheim, Duluth, GA, USA) given at CIDR insertion (10 days before timed AI). Experiment 2 included cows bred Fall 2020 that were also used in Experiment 1.

Vaccination, estrus synchronization, and AI

For Experiment 1 (fall 2019/spring 2020), cows were blocked within herd by breeding status from the previous breeding season (AI versus bull bred) and age (primiparous versus multiparous), then randomly assigned to either of 2 study groups: 1. modified-live virus vaccine (MLV, n = 1,003; Pyramid 10, Boehringer Ingelheim, Duluth, GA, USA) given subcutaneously at the labeled dose at initiation of a 7-day CO-Synch + CIDR synchronization protocol¹⁹ or 2. unvaccinated (CONT, n = 1,015). For Experiment 2 (fall 2020), each cow received the same treatment (MLV, n = 406; versus CONT, n = 439) that they were assigned to in Experiment 1. A total of 145 cows (57 in fall 2019, 39 in spring 2020, and 49 in fall 2019) were excluded from AI due to calving late in the previous breeding season but were still given vaccination and remained in the breeding group for natural service. At treatment (10 days before AI), cows were assigned a body condition score (BCS) and received 100 µg of intramuscular GnRH agonist (Factrel, Zoetis, Parsippany, NJ, USA) and an intravaginal insert containing 1.38 g of progesterone (Eazi-Breed CIDR, Zoetis). The CIDR insert was removed after 7 days, and 25 mg of intramuscular prostaglandin F_{2α} analog (dinoprost tromethamine, Lutalyse, Zoetis) was given. At CIDR removal, cows were also fitted with an estrus-detection patch (EstroTECT Breeding Indicator, Rockway, Inc, Spring Valley, WI, USA). At 60-66 hours after CIDR removal and prostaglandin treatment, cows received intramuscular GnRH agonist and were inseminated by trained AI technicians (12 technicians total) using commercially obtained frozen semen from 16 proven bulls selected for breeding based on phenotype and pedigree. Estrus detection patches were observed at AI, and a cow was determined to be in estrus if it had an activated (color change from gray to red), lost, or partially activated (≥ 50% color change) estrus patch. Drugs and vaccinations were given by trained veterinarians, technicians, or veterinary students using single-dose syringes with a new needle for each cow.

All cows, regardless of treatment, were commingled immediately after vaccination within their assigned breeding groups to be exposed to bulls starting 1 week after AI. Intact Angus

bulls that had successfully passed a breeding soundness examination were turned in with each breeding group at a ratio of 1:20-1:40 for ~ 65 days during each season. Cows in the CONT group were subsequently vaccinated at 2-5 months pregnancy with a KV vaccine (Virashield 6L5, Elanco, Greenfield, IN, USA).

Pregnancy diagnosis

Percentage of cows pregnant by AI was determined by transrectal ultrasonography performed by experienced veterinarians (blinded to treatment) between 40-80 days after AI. Presence of a fetal heartbeat was used to determine fetal viability and crown-rump length of the fetus or embryo was used to determine age (AI versus bull bred). Confirmation of pregnancy status was performed at 1-2 months after initial pregnancy detection and additional pregnancies from natural service were similarly recorded. Cows that were diagnosed as pregnant by AI at first pregnancy diagnosis, and were no longer pregnant at second pregnancy diagnosis, had confirmed abortions, or did not calve were designated AI pregnancy losses. Pregnancy losses to those bred by the bull were not included in analyses. The total season pregnancy rate for each herd was determined using a combination of pregnancy diagnosis results and final calving data provided by each farm and included both AI and bull-bred cows. Calving day was determined for each cow by calculating the difference between the recorded calving date and day 0 of the calving season. Day 0 was established to be 283 days after timed AI for AI-bred cows or 283 days after the first day of bull exposure for cows that were only bred by natural service. Calf birth weights and weaning weights were also recorded and provided by farm personnel. Calves were weaned between 5-9 months, at an average of 230 ± 0.7 days for calves born to fall-bred cows in 2019, 228 ± 0.8 days for calves born to spring-bred cows in 2020, and 236 ± 0.7 days for calves born to fall-bred cows in 2020. Average daily gain (ADG) at weaning was calculated for each calf by subtracting the birth weight from the weaning weight and dividing the age (in days) at weaning.

Data analyses

Cow was the experimental unit in this study as the treatment was applied to each individual cow. All statistical models included farm as a random variable to allow for its use as an error term for interpretation across all farms. Days postpartum (DPP), BCS at CIDR insertion, percentage of cows detected in estrus, calving day within each herd's calving season, calf birth weights, calf weaning weights, and calf ADG at weaning were

assessed by ANOVA in R, with treatment and season included as fixed variables for Experiment 1 and just treatment for Experiment 2. The model for calf weaning weights and ADG also included calf birth weight and calf sex as independent covariates, and the model for calving day included DPP and cow age as independent covariates. The binomial outcomes of AI pregnancy rates, total season pregnancy rates (AI + bull bred pregnancies), and AI pregnancy losses were analyzed using a generalized linear mixed-effects model (GLMER procedure) in R with treatment, season, and DPP included as fixed variables. The mixed effect model for AI pregnancy rates also contained the effects of AI technician, BCS at CIDR insertion, and estrus expression as independent covariates. The mixed effects model for season pregnancy rates also contained the effects of BCS at CIDR insertion and breeding groups as independent covariates. The 145 cows that were excluded from AI were not included in the model for AI pregnancy rates or AI pregnancy losses, but were included in all other analyses. Significance was set at $p \leq 0.05$, and tendencies were discussed between $p = 0.06$ and 0.10 .

Results

Prebreeding variables, BCS at CIDR insertion, DPP at AI, and percentage of cows having activated or lost estrus-detection patches at AI for Experiments 1 and 2 are summarized (Table 1). In Experiment 1, BCS, DPP, and estrus expression differed ($p \leq 0.05$) between seasons, but did not differ ($p \geq 0.38$) between treatment groups. There was also no treatment ($p \geq 0.51$) by season interaction for any of these variables. Similarly, none of these variables differed ($p \geq 0.56$) between treatment groups for cows in Experiment 2.

The reproductive outcomes in MLV versus CONT cows from Experiments 1 and 2 are summarized (Figure). In Experiment 1, there were effects of estrus expression ($p < 0.01$), BCS ($p < 0.01$), DPP ($p < 0.01$), and AI technician ($p < 0.01$) on AI pregnancy rates, but no treatment ($p = 0.31$), treatment by season ($p = 0.94$), or treatment by DPP ($p = 0.54$) interaction (Figure). Similarly, there were effects of estrus expression ($p < 0.01$), BCS ($p = 0.04$), and DPP ($p = 0.04$), but no effect of treatment ($p = 0.93$) or treatment by DPP ($p = 0.11$) interaction on AI pregnancy rates in Experiment 2 (Figure). In Experiment 1, there was a main effect of BCS ($p < 0.01$) on total season pregnancy rates, but there were no treatment ($p = 0.37$), season ($p = 0.60$), or treatment by season ($p = 0.71$) interaction (Figure). In Experiment 2, BCS tended ($p = 0.08$) to have an effect on total season pregnancy rates, but there was also no effect of treatment ($p = 0.12$; Figure). There were

[AQ1]

Table 1. Prebreeding variables; body condition score (BCS) at CIDR insertion, days postpartum (DPP) and estrus expression at AI; modified-live virus (MLV) group were vaccinated 10 days before timed AI and unvaccinated (CONT) group received KV vaccine at mid-pregnancy

Season	BCS		DPP		Estrus expression	
	CONT	MLV	CONT	MLV	CONT	MLV
Fall 2019	5.5 ± 0.03	5.5 ± 0.03	78 ± 0.7	78 ± 0.7	57% (303/534)	55% (283/516)
Spring 2020	5.2 ± 0.04	5.3 ± 0.04	83 ± 0.8	83 ± 0.9	65% (282/431)	65% (289/443)
p-value	0.38		0.63		0.59	
Fall 2020	5.5 ± 0.04	5.5 ± 0.04	74 ± 0.8	73 ± 0.7	59% (259/439)	57% (231/405)
p-value	0.69		0.81		0.56	

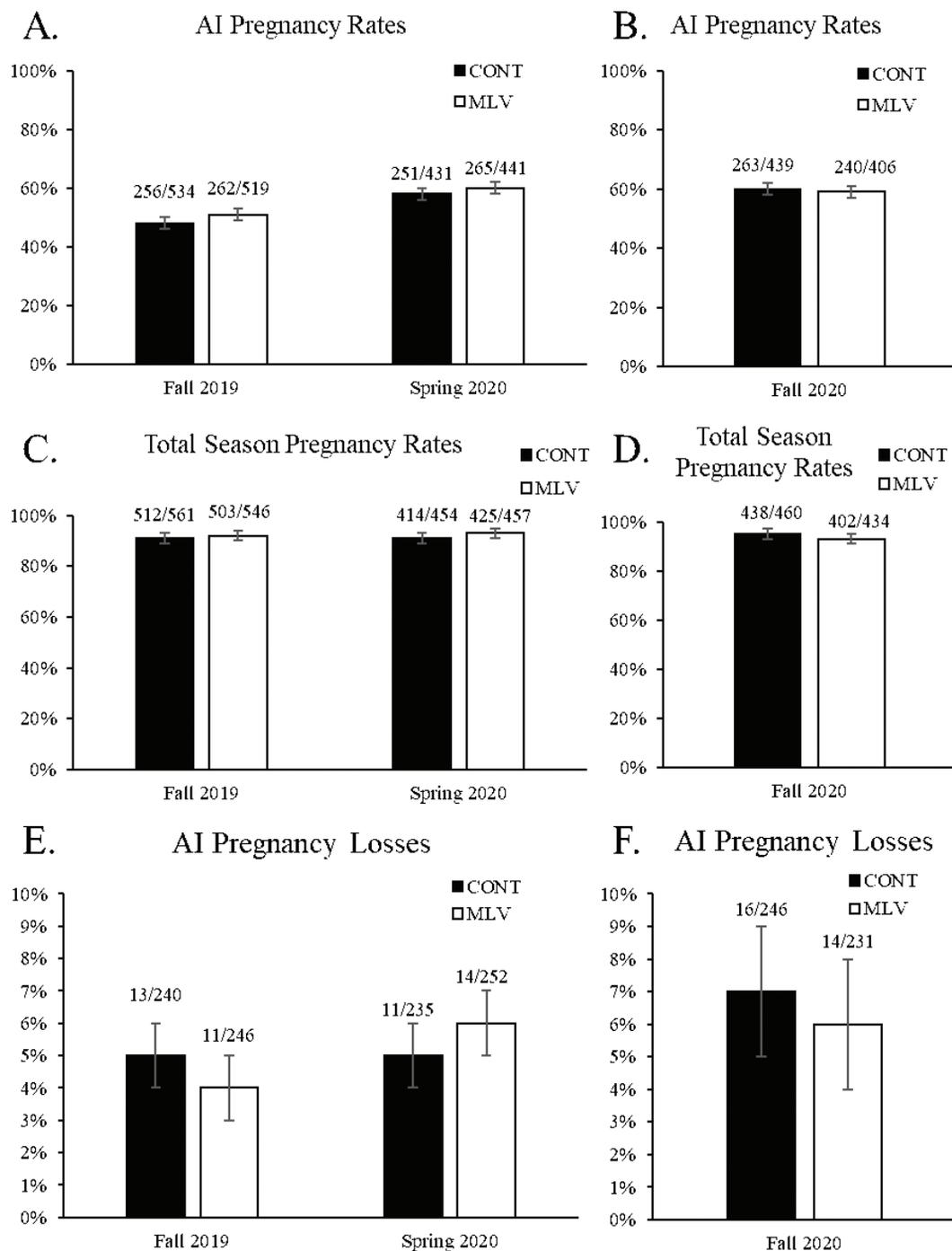


Figure. Reproductive outcomes in cows given MLV vaccine 10 days before timed AI compared to unvaccinated cows (CONT) given KV vaccine at mid-pregnancy in Experiments 1 (A, C, E) and 2 (B, D, F). A. In Experiment 1, there were no treatment ($p = 0.31$), treatment by season ($p = 0.94$), or treatment by days postpartum ($p = 0.54$) interactions (A). B. Similarly, there were no effects of treatment ($p = 0.93$) or treatment by DPP ($p = 0.11$) interaction on AI pregnancy rates in Experiment 2. C. There were no effects of treatment ($p = 0.95$), season ($p = 0.70$), or treatment by season ($p = 0.42$) interaction on total season pregnancy rates in Experiment 1. D. Treatment also did not affect total season pregnancy rates in Experiment 2 ($p = 0.12$). E. AI pregnancy losses did not differ by treatment ($p = 0.95$), season ($p = 0.70$), or treatment by season ($p = 0.42$) in Experiment 1. F. AI pregnancy losses also did not differ between treatments ($p = 0.41$) in Experiment 2.

no effects of treatment ($p = 0.95$), season ($p = 0.70$), or treatment by season interaction ($p = 0.42$) on AI pregnancy losses in Experiment 1 (Figure), nor were there effects of treatment ($p = 0.41$) on AI pregnancy losses in Experiment 2 (Figure).

Calf traits for MLV versus CONT cows in both Experiments 1 and 2 are summarized (Table 2). In Experiment 1, there were effects of season ($p < 0.01$) and DPP ($p < 0.01$) on the average day within the calving season that cows gave birth, but no treatment ($p = 0.59$) or treatment by season ($p = 0.42$) interaction.

Table 2. Summary on calving day, birth weights, weaning weights, weaning age, and average daily gain (ADG) of calves born to cows treated with a modified-live virus (MLV) vaccine 10 days before timed AI and unvaccinated cows (CONT) that were given KV vaccine at mid-pregnancy

Season	Calving day		Birth weight (kg)		Weaning weight (kg)		Weaning age (days)		ADG (kg/day)	
	CONT	MLV	CONT	MLV	CONT	MLV	CONT	MLV	CONT	MLV
Fall 2019	9.9 ± 0.9	9.9 ± 0.9	34.9 ± 0.3	35.1 ± 0.3	244 ± 1.9	248 ± 2.1	230 ± 1.0	229 ± 1.0	0.912 ± 0.008	0.934 ± 0.009
Spring 2020	5.2 ± 0.8	6.0 ± 0.9	34.3 ± 0.3	34.5 ± 0.3	256 ± 2.0	256 ± 1.9	228 ± 1.1	228 ± 1.1	0.976 ± 0.008	0.975 ± 0.008
p-value	0.59		0.58		0.18		0.80		0.14	
Fall 2020	6.4 ± 0.9	5.8 ± 0.9	34.6 ± 0.3	35.1 ± 0.3	250 ± 2.0	253 ± 2.3	236 ± 1.0	236 ± 1.0	0.916 ± 0.008	0.927 ± 0.010
p-value	0.73		0.27		0.32		0.79		0.35	

Similarly, there was an effect of DPP ($p = 0.01$) on calving day in Experiment 2, but no treatment effect ($p = 0.73$). In Experiment 1, there was an effect of season ($p = 0.02$) and calf sex ($p < 0.01$) on calf birth weight, but no treatment ($p = 0.58$) or treatment by season ($p = 0.90$) interaction. In Experiment 2, there was an effect of calf sex ($p < 0.01$) on calf birth weight, but no treatment effect ($p = 0.27$). In Experiment 1, there were main effects of season ($p < 0.01$), calf birth weight ($p < 0.01$), and calf sex ($p = 0.02$) on weaning weight, but no treatment ($p = 0.14$) or treatment by season ($p = 0.23$) interaction. In Experiment 2, there were main effects of calf birth weight ($p < 0.01$) and calf sex ($p < 0.01$) on weaning weights, but no treatment effect ($p = 0.32$). Similarly, there were main effects of season ($p < 0.01$), calf birth weight ($p < 0.01$), and calf sex ($p < 0.01$) on ADG, but no treatment ($p = 0.14$) or treatment by season ($p = 0.17$) interaction in Experiment 1. There were main effects of calf birth weight ($p < 0.01$) and calf sex ($p < 0.01$) on ADG in Experiment 2, but no treatment effect ($p = 0.35$). In both experiments, calf weaning ages did not differ between treatments ($p \geq 0.79$). There tended to be an effect of season ($p = 0.09$) on weaning age in Experiment 1, but no treatment by season interaction ($p = 0.73$).

Discussion

The results of the current study demonstrated that vaccination of suckled beef cows with a commercial MLV product at initiation of a 7-day CO-Synch + CIDR synchronization protocol (10 days before AI) did not have adverse effects on reproductive outcomes or subsequent calf growth traits. Most notably, AI pregnancy rates were not affected by MLV vaccination on day 10 before breeding in either Experiment 1 (fall 2019: 48-51%; spring 2020: 58-60%) or 2 (fall 2020: 59-60%). This finding was in contrast to our previous study, where AI pregnancy rates were higher in cows given MLV versus KV vaccine at CIDR insertion the fall breeding season only,¹⁵ despite having 2 fall breeding seasons represented in the current study. We previously theorized that since respiratory pathogen exposure is greatest in the cooler fall/winter months in the USA,²⁰ cows given MLV vaccine may have had greater protection against the effects of these pathogens on fertility and early embryonic development.¹⁻⁶ It is worth noting that since both our previous and the current studies were field trials, exposure to pathogens could not be controlled. As a result, pathogen exposure could have been less in the years of our current study compared to those in our previous study. Regardless, the lack

of any deleterious effect of prebreeding MLV vaccination on AI or total season pregnancy rates in both of our studies challenged the notion that MLV vaccination at < 30 days before breeding is detrimental to cow fertility. In addition, there were no effects of prebreeding MLV vaccination on estrus expression or AI pregnancy losses, consistent with findings in prior studies.^{15,16}

The timing of prebreeding MLV vaccinations has been a notable health management controversy, due to inconsistent findings among researchers. Replacement heifers given a combination MLV vaccine containing BoHV-1 and BVDV 9 days before breeding did not differ in estrus expression or pregnancy outcomes (by either AI or natural service) compared to those vaccinated on day 30 before breeding.²¹ In a similar study, beef heifers were vaccinated with either a MLV or KV (with no BoHV-1/BVDV component) vaccine on day 2 after an unsynchronized estrus and then revaccinated with the same product either 10 or 31 days before natural service.²² Neither vaccine type nor timing of vaccination had an effect on the duration of interestrus intervals, estrus expression, total pregnancy rates, or pregnancy losses.²² On the contrary, authors of another study reported decreased AI pregnancy rates in spring-calving beef cows and heifers given MLV vaccine (52%) compared to those given a chemically altered/inactivated (CA/IA) vaccine (60%) on \geq day 27 before breeding.¹⁶ Interestingly, cows vaccinated with either a MLV or CA/IA vaccine between days 27-29 or 30-37 before breeding had lower pregnancy rates (52%) compared to those vaccinated using either product on days 46-89 before breeding (64%).¹⁶ These findings called into question the use of either product close to breeding, as it was suggested that the nonspecific inflammation from vaccination could negatively impact fertility.¹⁶ In contrast, there were no adverse effects on AI or total season pregnancy rates in beef cattle given a CA/IA vaccine (47%; 98%) on days 60 and 30 before breeding compared to saline-treated controls (43%, $p = 0.49$; 96%, $p = 0.14$).²³ Similarly, there was also no reported effect on AI or season pregnancy rates in beef cattle given a MLV vaccine (40%; 95%) on days 60 and 30 before breeding compared to saline-treated controls (43%, $p = 0.21$; 96%, $p = 0.34$)²³ similar to the findings of our current study.

Due to the concerns with CA/IA vaccine given close to AI,¹⁶ we elected to have a control group in the current study that

received no prebreeding vaccine, but instead received a KV vaccine at mid-pregnancy. A controlled study evaluated the efficacy of mid-pregnancy (day 118) CA/IA vaccination to heifers that had received 2 MLV vaccinations previously.²⁴ Heifers received an initial CA/IA vaccine booster during their first pregnancy. During second pregnancy, beef heifers were exposed to BVDV PI animals (days 95-111 of pregnancy), revaccinated with CA/IA vaccine on day 118, and inoculated with BoHV-1 on day 210 of pregnancy.²⁴ The results of that study suggested that this combination MLV/CA/IA vaccine protocol provided adequate protection against fetal infection and loss following controlled exposure to BVD and BoHV-1.²⁴ The cows enrolled in Experiment 2 herein similarly received KV vaccines mid-pregnancy in 2 subsequent pregnancies after having previously received MLV in the years prior with no issues regarding fetal loss. Although these results also supported the use of KV or CA/IA vaccine at mid-pregnancy in multiple pregnancies, it is worth noting that there was no known (or controlled) pathogen exposure in these herds during the current study. Also, the KV vaccine used in our study does not guarantee protection against development of persistently infected BVD calves, whereas most MLV vaccines guarantee this protection.

An important distinction among many of these studies with contradictory results is that the MLV and KV vaccines used were from different manufacturers. The KV vaccine product used^{16,24} contained chemically altered strains of BoHV-1 and parainfluenza 3 viruses, modified live bovine respiratory syncytial virus, and a liquid, adjuvanted preparation of inactivated BVD viruses (Type 1 and 2), which is why we have denoted it as a CA/IA, and is the only 'KV' vaccine product known to guarantee protection against the development of persistently infected BVD calves. The KV vaccine product used in our current and previous¹⁵ studies contained all inactivated viruses but did not provide protection against the development of persistently infected BVD calves. In addition to different KV vaccine products, MLV vaccines among these studies also came from various manufacturers and contained different adjuvants. An earlier study compared the use of 2 commercial MLV vaccines in postpartum beef cows (n = 807) at 2 intervals before AI (day 30 versus day 10); authors reported no effect of vaccine product or time of treatment on AI (53-56%) or seasonal (85-89%) pregnancy rates.²⁵ Data are still lacking on a more recent and thorough comparison of commercial MLV and KV cattle vaccine products at various intervals before breeding.

The mean calving day did not differ between cows receiving prebreeding MLV vaccines versus KV vaccination at mid-pregnancy. In our previous study, MLV-vaccinated cows calved, on average, 2 days sooner than KV-vaccinated cows (~ 8.4 versus ~ 10.3 days), regardless of the season.¹⁵ There was a difference between seasons in Experiment 1, with decreased mean calving day in the spring (5.2-6 days) versus the fall (9.9 days), but no interaction with the vaccine treatment. Several factors may have roles in these differences, including cow nutrition, weather, pathogen exposure, and bull genetics. Pregnancy length has been reported to be negatively correlated (genetically) with all growth traits except birth weights, suggesting that faster growing fetuses may trigger earlier parturition.²⁶ Pearson's correlation test determined a moderately negative correlation between the mean calving day and weaning weights ($r = -0.30$; $p < 0.01$) of the current study and from data collected in the previous study ($r = -0.35$; $p < 0.01$) (author communication). Neither calf birth weights, ADG, nor weaning weights were affected by vaccine treatment in the

current study or previously.¹⁵ Therefore, it appeared unlikely that calf growth traits may have previously affected pregnancy length and mean calving day. More likely, the greater fall AI pregnancy rates observed in the previous study could have a role in the observed differences on mean calving day.¹⁵ Future studies that evaluate the downstream effects of vaccinations on calf growth traits with controlled pathogen exposure are needed to better understand these findings.

In conclusion, there were no differences in reproductive outcomes or calf traits between suckled beef cows given MLV vaccine 10 days before AI compared to those given KV at mid-pregnancy. Pregnancy rates observed were consistent with industry standards of ~ 45-55% AI pregnancy rates and ~ 90-95% total seasonal pregnancy rates. However, based on contradictory results among several large field trials, there is still a need to more thoroughly investigate the effects of vaccine protocols on pregnancy outcomes based on factors such as manufacturer, season, geographical location, pathogen exposure risks, and farm goals. Understanding how these factors impact production systems can help veterinarians provide up-to-date and practical vaccination recommendations to cow-calf producers.

Acknowledgments

Authors thank all the farm managers and workers at the Virginia Department of Corrections for their valuable assistance and partnership throughout this study. In addition, we acknowledge clinical-year veterinary students and technicians at the Virginia-Maryland College of Veterinary Medicine, who assisted in synchronization, vaccinations, and pregnancy diagnosis. We extend our gratitude to Zoetis and Estroject for their donation of synchronization products.

Conflict of interest

Authors have no conflict of interest to declare.

References

1. Miller JM, van der Maaten MJ: Experimentally induced infectious bovine rhinotracheitis virus infection during early pregnancy: effect on the bovine corpus luteum and conceptus. *Am J Vet Res* 1986;47:223-228. PMID: 2420240
2. Miller JM, van der Maaten MJ: Early embryonic death in heifers after inoculation with bovine herpesvirus-1 and reactivation of latent virus in reproductive tissues. *Am J Vet Res* 1987;48:1555-1558. PMID: 3434897
3. Miller JM, Van der Maaten MJ, Whetstone CA: Effects of a bovine herpesvirus-1 isolate on reproductive function in heifers: classification as a type-2 (infectious pustular vulvovaginitis) virus by restriction endonuclease analysis of viral DNA. *Am J Vet Res* 1988;49:1653-1656. PMID: 2847601
4. Grooms DL, Brock K, 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. doi: 10.1177/104063879801000201
5. Archbald LE, Gibson CD, Schultz RH, et al: 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.

6. Archbald LF, Fulton RW, Seger CL, et al: Effect of the bovine viral diarrhea (BVD) virus on preimplantation bovine embryos: a preliminary study. *Theriogenology* 1979;11:81–89. doi: 10.1016/S0093-691X(79)80020-5
7. Newcomer BW, Walz PH, Daniel Givens M, et al: Efficacy of bovine viral diarrhea virus vaccination to prevent reproductive disease: a meta-analysis. *Theriogenology* 2015;83:360–365. doi: 10.1016/j.theriogenology.2014.09.028
8. Newcomer BW, Cofield LG, Walz PH, et al: Prevention of abortion in cattle following vaccination against bovine herpesvirus 1: a meta-analysis. *Prev Vet Med* 2017;138:1–8. doi: 10.1016/J.PREVETMED.2017.01.005
9. Callan RJ: Fundamental considerations in developing vaccination protocols. *Proc Am Assoc Bovine Pract* 2001; p. 14–22. doi: 10.21423/aabppro20015171
10. O'Toole D, Miller MM, Cavender JL, et al: Pathology in practice. *J Am Vet Med Assoc* 2012;241:189–191. doi: 10.2460/JAVMA.241.2.189
11. Chiang BC, Smith PC, Nusbaum KE, et al: The effect of infectious bovine rhinotracheitis vaccine on reproductive efficiency in cattle vaccinated during estrus. *Theriogenology* 1990;33:1113–120. doi: 10.1016/0093-691X(90)90071-Z
12. Smith PC, Nusbaum KE, Kwapien RP, et al: Necrotic oophoritis in heifers vaccinated intravenously with infectious bovine rhinotracheitis virus vaccine during estrus. *Am J Vet Res* 1990;51:969–972. doi: 10.2460/ajvr.1990.51.07.969
13. Van Der Maaten MJ, Miller JM: Ovarian lesions in heifers exposed to infectious bovine rhinotracheitis virus by non-genital routes on the day after breeding. *Vet Microbiol* 1985;10:155–163. doi: 10.1016/0378-1135(85)90017-3
14. Perry GA, Zimmerman AD, Daly RE, et al: The effects of vaccination on serum hormone concentrations and conception rates in synchronized naive beef heifers. *Theriogenology* 2013;79:200–205. doi: 10.1016/j.theriogenology.2012.10.005
15. Stewart JL, Currin J, Clark SG, et al: Assessing pregnancy outcomes in cow-calf operations after administration of modified-live or killed virus vaccinations at the initiation of synchronization for fixed-time AI. *Theriogenology* 2023;200:43–48. doi: 10.1016/J.THERIOGENOLOGY.2023.01.027
16. Perry GA, Geary TW, Walter JA, et al: Influence of vaccination with a combined chemically altered/inactivated BHV-1/BVD vaccine or a modified-live BHV-1/BVD vaccine on reproductive performance in beef cows and heifers. *Bovine Practitioner* 2018;52:53–58. doi: 10.21423/bovine-vol52no1p53-58
17. Lamb GC, Mercadante VRG: Synchronization and artificial insemination strategies in beef cattle. *Vet Clin North Am Food Anim Pract* 2016;32:335–347. doi: 10.1016/j.cvfa.2016.01.006
18. FASS: Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 4th edition. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Champaign, IL: 2020.
19. Larson JE, Lamb GC, Stevenson JS, et al: Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. *J Anim Sci* 2006;84:332–342. doi: 10.2527/2006.842332X
20. Lubbers BV, Renter DG, Hesse RA, et al: Prevalence of respiratory viruses and *Mycoplasma bovis* in U.S. cattle and variability among herds of origin, production systems and season of year. *Bovine Practitioner* 2017;51:159–164. doi: 10.21423/bovine-vol51no2p159-164
21. Stormshak E, Tucker CM, Beal WE, et al: Reproductive responses of beef heifers after concurrent administration of vaccines, anthelmintic and progestogen. *Theriogenology* 1997;47:997–1001. doi: 10.1016/S0093-691X(97)00056-3
22. Walz PH, Edmondson MA, Riddell KP, et al: Effect of vaccination with a multivalent modified-live viral vaccine on reproductive performance in synchronized beef heifers. *Theriogenology* 2015;83:822–831. doi: 10.1016/j.theriogenology.2014.11.015
23. Perry GA, Larimore EL, Crosswhite MR, et al: Safety of vaccination with an inactivated or modified live viral reproductive vaccine when compared to sterile saline in beef cows. *Jacobs J Vet Sci Res* 2016;2:1–6. doi: 10.21423/aabppro20163477
24. Walz PH, Givens MD, Rodning SP, et al: 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. doi: 10.1016/J.VACCINE.2017.01.006
25. Whittier WD, Baitis HK: Timing of vaccinations in estrous synchronization programs. *Proceedings, Applied Reproductive Strategies in Beef Cattle* 2005; p. 147–156.
26. Bourdon RM, Brinks JS: Genetic, environmental and phenotypic relationships among gestation length, birth weight, growth traits and age at first calving in beef cattle. *J Anim Sci* 1982;55:543–553. doi: 10.2527/JAS1982.553543X