

Unintended consequences: Could MLV vaccines be harming reproduction?

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Infectious reproductive diseases have potential to create significant damaging effects on reproductive efficiency and therefore profitability for the beef herd. Pathogens causing reproductive diseases include: Bovine Viral Diarrhea Virus (BVDV), Infectious Bovine Rhinotracheitis Virus (IBRV), *Leptospira*, *Tritrichomonas foetus*, *Campylobacter fetus* subsp. *venerealis* (“vibrio”) and *Neospora caninum*, among others. The prevention and control of these infectious diseases include efforts directed at biosecurity (keeping sources of new diseases out of the herd), environmental control (reducing stress and conditions in which infectious diseases may be transmitted), and for some disease agents, vaccination (Yeager & Holler, 2007; Kelling, 2007; Abbitt & Rae, 2007).

Vaccination has long been employed as a means to increase the resistance of individuals, thereby the group, to infectious agents. For reproductive diseases, the goal is to increase resistance of the female (or bull) toward agents that will adversely affect the female’s ability to become pregnant and carry the pregnancy to term. The focus of many reproductive vaccine programs is on preventing abortions, but attention should also be paid to diseases that have an effect on ovulation rates, fertilization rates, embryonic survival rates, and perinatal survival rates. Many infectious reproductive disease agents have potential effects on several, if not all, of these stages of reproduction.

Perhaps the two most prominent infectious agents implicated in reproductive disease are IBRV and BVDV. Both of these viruses have been implicated in negative effects on bovine reproduction, including ovarian and estrus cycle dysfunction, fetal infection, and pregnancy loss (Kelling, 2007). As a result, vaccines containing IBRV and BVDV have been employed in beef cattle herds in order to prevent these negative effects. These vaccines are widely available as inactivated virus as well as modified live virus preparations, usually in combination with other potential reproductive or respiratory pathogens.

Modified-live virus (MLV) vaccines stimulate the immune system by actively infecting host cells. In general, these types of vaccines are considered to be more cross-reactive and broader in their immune system stimulation (antibody production and cell-mediated immunity), exhibit longer duration of effect and provide more flexibility in timing of administration. Modified live virus vaccines also carry with them the potential to revert to virulence and inflict the damage they are designed to prevent, if used improperly. Inactivated vaccines are safe to use in a wide variety of circumstances, yet carry the general considerations that their effects are less broad and of shorter duration compared to MLV vaccines (Kelling, 2007).

While the effects of these viruses in beef cattle have been well-characterized, the effects of vaccination on reproductive parameters have been studied to a lesser extent. Because of the nature of MLV vaccines, questions have arisen whether they could potentially cause adverse effects on reproduction in the absence of a disease challenge. This presentation will outline research on the effects of pre-breeding MLV reproductive vaccination on reproductive parameters.

Effect of modified live virus IBRV and BVDV vaccines on reproduction in naïve cattle

Several studies have outlined the effects of IBR virus -- both wild strains and MLV vaccine strains -- on the ovaries and reproductive tract of females during and following estrus. In one, live IBRV was inoculated into previously unexposed (seronegative) heifers the day following estrus via intravenous, intramuscular, or aerosol routes. Ovarian necrosis occurred in 7 of 8 heifers exposed to IBRV intravenously or

intramuscularly (Van der Maaten & Miller, 1985). In a similar fashion, investigators then used 4 different live vaccine strains of IBRV to inoculate 8 seronegative heifers intravenously and found mild to marked necrosis and inflammation in the corpora lutea and ovaries (Van der Maaten et al., 1985).

Necrotic and inflammatory ovarian changes were also induced by administering 3 different MLV vaccines to 18 seronegative heifers on day 4 after the 2nd dose of a 2-dose prostaglandin synchronization protocol. In this experiment, it was also notable that IBRV was also isolated from 2 of 4 unvaccinated controls, apparently because they had become infected from vaccine virus excreted by their vaccinated pen mates (Smith et al., 1990).

Having established that ovarian damage can be caused by MLV vaccines administered around the time of estrus, further work sought to identify the results of this damage in terms of infertility. Modified live IBRV vaccines were given to 8 seronegative heifers 14 days following breeding, resulting in infertility, embryonic death, and return to estrus in 4 of the 8 heifers. This study measured progesterone levels in bred animals and demonstrated a disruption in luteal activity presumably due to the effects of live IBRV (Miller et al., 1989).

In another study, 19 seronegative heifers were synchronized with two doses of prostaglandin and split into MLV vaccine and control groups. All 9 heifers not receiving the vaccine calved after a 35-day breeding period, while only 7 of 10 vaccinated heifers calved (Chiang et al., 1990).

Effects of MLV vaccines on naïve cattle are not limited to those due to IBRV. In a study in which 10 seronegative females were vaccinated with MLV vaccine containing BVDV, cytopathic BVDV was isolated from the ovaries of vaccinated heifers up to 12 days post-vaccination, and was demonstrated to be present via immunohistochemistry from ovaries of cows 10 and 30 days post-vaccination (Grooms et al., 1998).

A larger study examined fertility and abnormal estrus cycles in 59 seronegative crossbred beef heifers vaccinated with either an MLV vaccine or an inactivated vaccine containing IBRV and BVDV at the onset of the synchronization program. Heifers given the MLV vaccine had significantly lower pregnancy rates and significantly higher rates of abnormal estrous cycles. For those heifers experiencing abnormal estrous cycles, those given an MLV vaccine were significantly less likely to conceive at the return estrus (Perry, et al., 2013). Taken together, these studies provide ample evidence that vaccination of naïve heifers with modified-live IBRV vaccines will result in significant negative effects on pregnancy.

Effect of modified live IBRV and BVDV vaccines on reproduction in previously vaccinated cattle

Information regarding effects of MLV vaccines on naïve females is instructive, however many of today's beef operations will raise and select females that have been previously vaccinated for IBRV and BVDV at various time points in their lives. The effect of IBRV and BVDV MLV vaccines on reproduction also must take into account how they influence reproduction in cattle previously vaccinated against those agents.

The effect of modified live IBRV vaccination at the time of estrus in previously-vaccinated animals was examined in a group of 10 heifers. Investigators did not recover IBRV from the ovaries of any of the animals (Spire et al., 1996).

Questions of whether reproductive efficiency would suffer if pre-breeding vaccinations were moved to the onset of a synchronization plan (closer to breeding than vaccine labels permit) drove the initiation of several studies. Yearling crossbred heifers (n = 295) of unknown prior vaccination status were vaccinated with modified live IBRV-BVDV vaccines either 30 or 9 days prior to breeding (at the start of synchronization). There were no differences in pregnancy status or cyclicity between the two groups (Stormshak et al., 1997).

A project that compared two different modified-live IBRV-BVDV vaccines given either 30 or 10 days (to coincide with synchronization onset) prior to breeding showed no differences in reproductive parameters among the groups except for a higher rate of early embryonic death in females vaccinated 10 days prior to breeding compared to those vaccinated 30 days prior (Rosenberg, 2004).

Similarly, the effects of using a modified-live IBRV-BVDV vaccine either 40 or 3 days prior to breeding were examined in 799 previously-vaccinated heifers. There were no differences in conception rates between the two groups (Bolton et al., 2007)

A shortcoming of the projects noted above is that, while the effects of vaccination were measured on well-vaccinated animals, comparisons were made largely only on the basis of vaccine timing relative to estrus. Non-vaccinated control groups were not included, so the effect of modified live vaccination by itself on reproductive parameters could not be elucidated. In recent years, studies including these control groups have been carried out.

One such study on 60 beef heifers included groups that began seronegative and then were vaccinated with two doses of either a modified live IBRV-BVDV vaccine or an inactivated vibrio-lepto vaccine. Each of these groups was split so that half received their final vaccine dose 10 days prior to breeding and half received their final vaccine dose 30 days prior to breeding. Numbers were too small to demonstrate statistical significance, but heifers vaccinated with MLV vaccine had a 78% (31/40) calving rate at the end of the study, compared to 95% (19/20) in the inactivated vaccine group (Walz et al., 2015). Investigators performed a larger study that looked at reproductive parameters in 686 primiparous dairy cows, all of which had been previously vaccinated with 4 doses of modified-live IBRV-BVDV vaccine as calves or heifers. Half of the cows were vaccinated with MLV versions and half were vaccinated with inactivated versions of IBRV-BVDV vaccines 45 days prior to timed artificial breeding. There were no differences in conception rates between vaccine groups. In that study, the authors noted a less-than ideal rate of vaccine response overall, however (Walz et al., 2015).

The effect of inactivated and modified live vaccination on reproductive parameters in beef cows

To continue expanding the knowledge base of the effects of vaccines on reproduction in beef cows, a two-year multi-herd study was undertaken (Perry et al., 2016). While one previously mentioned study in particular (Walz et al., 2015) had detected differences in pregnancy rates between MLV and inactivated vaccine groups, animal numbers were small. Such studies have the ability to only detect large differences in reproductive parameters among groups. In modern beef cattle operations, a small percentage difference in pregnancy rates or conception to AI can be extremely significant. Therefore, this study was designed with sufficient power to detect a difference of less than 10% in pregnancy success between groups.

Cows utilized for this study were those in the South Dakota State University and North Dakota State University herds. These animals are managed similarly to most commercial cow herds. Over 2 years, 7 unique herds totaling 1436 females were included in the study. Cows and heifers in these herds had been vaccinated annually with a modified live IBRV-BVDV vaccine prior to each breeding season.

Within herds, cows were blocked by parity and calving date and randomly assigned to one of three groups: 1) modified-live multivalent vaccine containing IBRV and BVDV; 2) inactivated multivalent vaccine containing IBRV and BVDV; and 3) saline (non-vaccinated control). Vaccines were administered per label (MLV group = 30 days pre-breeding [plus 60 days pre-breeding for heifers]; inactivated group = 30 days pre-breeding [plus 60 days pre-breeding in year 1 of the study]; saline control group = 30 and 60 days pre-breeding).

All females were synchronized with the 7-day CO-Synch + CIDR protocol and inseminated (AI) at the appropriate time after CIDR removal (cows 60 to 66 hrs.; heifers 52 to 56 hrs.). Cows remained separated

from bulls for at least 10 days after AI. Pregnancy success and fetal age were determined by transrectal ultrasonography on day 28 day after AI (year 1 and 2), 56 days after AI (year 1), and > 30 days after the end of the breeding season (year 1 and 2).

Data were analyzed using the GLIMMIX procedure in SAS and included treatment, day postpartum, and the treatment by day postpartum interaction in the model, using the treatment by herd interaction as the error term. Some cows were sold prior to calving for non-reproductive reasons (age, health, and disposition; n = 132) and were removed from the analysis, leaving pregnancy data from 1304 cows for analysis.

Reproductive parameters for all three study groups are shown in Table 1. The researchers found numerical, but not statistically significant, differences in conception rates to AI between MLV and control groups ($P = 0.21$; $40.0 \pm 4\%$ vs $43.3 \pm 4\%$) and between inactivated and control groups ($P = 0.49$; $46.5 \pm 4\%$ vs $43.3 \pm 4\%$). Rates tended to differ between MLV and inactivated groups ($P = 0.055$).

Table 1. Impact of Vaccine on Pregnancy Success.

Vaccine	AI Conception (%)	Day 56 Pregnancy Success (%)	Breeding Season Pregnancy Success (%)	Early Embryo Loss (%)
Modified Live	40.0 ± 4^a	88.9 ± 2^c	95.2 ± 2^c	2 ± 1
Inactivated	46.5 ± 4^b	93.2 ± 2^d	98.0 ± 1^d	2 ± 1
Saline	43.3 ± 4^{ab}	92.5 ± 2^d	96.4 ± 1^{cd}	2 ± 1

Means within a column having different superscripts are different ^{ab} $P = 0.055$, ^{cd} $P \leq 0.01$

At 56 d after AI, MLV animals ($88.9 \pm 2\%$) had decreased pregnancy success compared to both the inactivated ($93.2 \pm 2\%$) and control groups ($92.5 \pm 2\%$, $P \leq 0.01$).

For the total breeding season, there were numerical but not statistically significant, differences in pregnancy success between MLV and control groups ($P = 0.34$; $95.2 \pm 2\%$ vs $96.4 \pm 1\%$) and between the inactivated and control groups ($P = 0.14$; $98.0 \pm 1\%$ vs $96.4 \pm 1\%$). Breeding season pregnancy success was significantly greater for the inactivated group compared to the MLV group ($98.0 \pm 1\%$ vs $95.2 \pm 2\%$; $P = 0.01$).

When cumulative calving distribution was evaluated, the proportion of females that calved by day 12 and 30 of the calving season was similar between MLV and control groups ($P > 0.30$) and between the inactivated and control groups ($P > 0.30$). However, this proportion in the Inactivated group tended ($P = 0.09$) to be greater compared to that of the MLV group. As expected, the researchers found that days postpartum influenced conception rates, with heifers and short postpartum cows having decreased conception rates compared to cows that were further postpartum ($P < 0.05$).

To summarize this particular study, measures of reproduction were numerically, and sometimes statistically significantly, poorer in cattle that had received a modified-live IBRV-BVDV vaccine when compared to cattle that had received an inactivated vaccine. Interestingly, the cattle groups receiving saline were intermediate between the two vaccine groups in terms of reproductive measures. When taken individually, each particular vaccine group performed similarly to a saline control, indicating that reproductive measures did not significantly suffer when either vaccine was used. These herds did not experience known infectious reproductive disease challenges during the study period; had exposure to these agents occurred, results may have been different. The differences in reproductive parameters observed between well vaccinated cattle receiving MLV vaccine and receiving inactivated vaccine at a labeled interval prior to breeding in this study were noteworthy and merit further examination.

Conclusions

Vaccines against infectious reproductive disease agents are valuable tools in the prevention of these diseases, outbreaks of which can be potentially devastating to beef cattle herds. However, evidence exists that MLV versions of these vaccines can create demonstrable negative effects when given to naïve animals. Further evidence exists to demonstrate the importance of proper vaccination of female beef cattle before they enter the breeding herd, as the negative effects of MLV vaccines appear to be mitigated in previously-vaccinated animals. Studies utilizing different pre-breeding vaccination intervals indicate that in well-vaccinated females, MLV vaccines may be used closer to breeding than directed by product labels, without significant negative effect. However, an increasing level of evidence is also emerging that indicates that MLV vaccines, even when given at labeled pre-breeding intervals, may negatively affect reproductive parameters compared to cattle vaccinated with inactivated vaccines.

In light of research presented, it appears the choice of pre-breeding vaccine product type and timing may or may not significantly affect reproductive parameters in a given herd. Importantly, these studies were not carried out in the face of disease challenge and do not address the question of protection in the face of an infectious reproductive disease exposure. Future research will help determine how to strike the best balance between appropriate disease protection and minimizing harmful effects from the vaccines themselves. It is reasonable to expect that striking this balance will be different for each individual cattle operation, making it imperative that cattle producers consult their veterinarian and weigh all available information when making decisions about pre-breeding vaccinations in their herds.

References

- Abbitt, B., & Rae, D. O. (2007). Protozoal Abortion in Cattle. In R. S. Youngquist, & W. R. Threlfall (Eds.), *Current Therapy in Large Animal Theriogenology* (2nd ed., pp. 409-413). St. Louis, Missouri, USA: Saunders Elsevier.
- Bolton, M., Brister, D., Burdett, B., Newcomb, H., Nordstrom, S., Sanders, B., & Shelton, T. (2007, Fall). Reproductive safety of vaccination with Vista 5 L5 SQ near breeding time as determined by effect on conception rates. *Veterinary Therapeutics*, 8(3), 177-82.
- Chiang, B. C., Smith, P. C., Nusbaum, K. E., & Stringfellow, D. A. (1990, May). The effect of infectious bovine rhinotracheitis vaccine on reproductive efficiency in cattle vaccinated during estrus. *Theriogenology*, 33(5), 1113-1120.
- Grooms, D. D., Brock, K. V., & Ward, L. A. (1998, Apr). 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*, 10, 130-4.
- Kelling, C. L. (2007). Viral Diseases of the Fetus. In R. S. Youngquist, & W. R. Threlfall (Eds.), *Large Animal Theriogenology* (2 ed., pp. 399-408). St. Louis, Missouri, USA: Saunders Elsevier.
- Miller, J. M., Van der Maaten, M. J., & Whetstone, C. A. (1989, Apr). Infertility in heifers inoculated with modified-live bovine herpesvirus-1 vaccinal strains against infectious bovine rhinotracheitis on postbreeding day 14. *Am J Vet Res*, 50(4), 551-4.
- Perry, G. A., Larimore, E. L., Crosswhite, M. R., Neville, B. W., Cortese, V., Daly, R. F., . . . Dahlen, C. R. (2016). Influence of Vaccination with an Inactivated or Modified Live Viral Reproductive Vaccine on Reproductive Parameters in Beef Cows. *J. Anim. Sci.*, 94(Suppl. 2), 152.
- Perry, G. A., Zimmerman, A. D., Daly, R. F., Buterbaugh, R. E., Rhoades, J., Scholz, D., . . . Chase, C. C. (2013). The effects of vaccination on serum hormone concentrations and conception rates in synchronized naïve beef heifers. *Theriogenology*, 79, 200-205.

- Rosenberg, J. B. (2004). Vaccination and Reproductive Efficiency. Southeast Dairy Herd Management Conference, (pp. 76-82). Macon, GA.
- Smith, P. C., Nusbaum, K. E., Kwapien, R. P., Stringfellow, D. A., & Driggers, K. (1990, Jul). Necrotic oophoritis in heifers vaccinated intravenously with infectious bovine rhinotracheitis virus vaccine during estrus. *Am J Vet Res*, 51(7), 969-72.
- Spire, M. F., Edwards, J. F., Leipold, H. W., & Cortese, V. S. (1996). Absence of ovarian lesions in IBR seropositive heifers subsequently vaccinated with a modified live IBR virus vaccine. *Agri-Practice*, 16(7), 33-8.
- Stormshak, F., Tucker, C. M., Beal, W. E., & Corah, L. R. (1997, Apr). Reproductive responses of beef heifers after concurrent administration of vaccines, anthelmintic, and progestogen. *Theriogenology*, 47(5), 997-1001.
- Van der Maaten, M. J., & Miller, J. M. (1985, January). Ovarian lesions in heifers exposed to infectious bovine rhinotracheitis virus by non-genital routes on the day after breeding. *Vet Microbiol*, 10(2), 155-63.
- Van der Maaten, M. J., Miller, J. M., & Whetstone, C. A. (1985, Sept.). Ovarian lesions induced in heifers by intravenous inoculation with modified-live infectious bovine rhinotracheitis virus on the day after breeding. *Am J Vet Res*, 46(9), 1996-9.
- Walz, P. H., Edmondson, M. A., Riddell, K. P., Braden, T. D., Gard, J. A., Bayne, J., . . . Givens, M. D. (2015). Effect of vaccination with a multivalent modified-live viral vaccine on reproductive parameters in synchronized beef heifers. *Theriogenology*, 83, 822-31.
- Walz, P. H., Montgomery, T., Passler, T., Riddell, K. P., Braden, T. D., Zhang, Y., . . . Zuidhof, S. (2015). Comparison of reproductive performance of primiparous dairy cattle following revaccination with either modified-live or killed multivalent viral vaccines in early lactation. *J. Dairy Sci.*, 98, 8753-63.
- Yeager, M. J., & Holler, L. D. (2007). Bacterial Causes of Bovine Infertility and Abortion. In R. S. Youngquist, & W. R. Threlfall (Eds.), *Current Therapy in Large Animal Theriogenology* (2nd ed., pp. 389-399). St. Louis, Missouri, USA: Saunders Elsevier.