Assessing bull breeding soundness exam parameters following vaccination with modified-live or killed vaccine

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Abstract

Breeding bulls are often placed on a similar herd health plan and vaccinated for the same pathogens as the mature cow herd. The timing of the yearly vaccination is impacted by labor and time constraints, with some bulls receiving vaccinations at the time of annual, complete breeding soundness exams or at the time of turnout prior to the breeding season. However, the potential impact of vaccination on spermatogenesis, if any, is unknown. Therefore, the study objective was to determine the impacts on normal sperm morphology through a complete spermatogenic cycle after vaccination of mature beef bulls against bovine herpesvirus 1 and bovine viral diarrhea virus using commercially available modified-live virus (MLV) or killed virus (KV) vaccine. Semen was collected from *Bos taurus* bulls (n = 11), which were randomly assigned prior to the first breeding soundness exam to vaccine treatment groups: 1) modified-live virus; or 2) inactivated/killed virus. Breeding soundness exams were completed at days -7, 0, 5, 7, 17, 28, 61 following vaccination. No differences were found between vaccine treatment groups MLV and KV ($P = 0.46$) on the percentage of normal sperm over time (d = -7 through d = 61) as evaluated by 2 boarded theriogenologists. In conclusion, we found no detrimental effect of the administration of multivalent MLV or KV vaccines on percentage of normal sperm morphology of mature bulls over a 61-day period.

Key words: bull, sperm morphology, modified-live vaccine, killed vaccine

Introduction

Breeding bulls are often purchased as yearlings or 2-year-olds in most production systems in North America. Following purchase, it is the general recommendation that bulls are placed on a similar herd health plan and vaccinated against the same pathogens as the mature cow herd.[1,2] Consequently, bulls receive vaccines at the same time as the mature cow herd, or alternatively, it may be requested that bulls receive their annual vaccinations at the time of routine breeding soundness exams if they are declared a satisfactory potential breeder. These core vaccines often include bovine viral diarrhea virus (BVDV), bovine herpes virus 1 (BHV-1), bovine parainfluenza -3 virus (BPI3V), and bovine respiratory syncytial virus (BRSV). BVDV and BHV-1 vaccines most certainly can have reproductive impact while BRSV and BPI3V have little reproductive importance, but may take part in any immune response that occurs post vaccination that may affect spermatogenesis.[3,4] Type of vaccine, whether it is a modified-live vaccine (MLV) or killed virus vaccine (KV), often depends on what the cow herd traditionally receives, the number of doses needed to vaccinate the bull battery in question, biosecurity concerns for the herd, and/or what product the producer or veterinarian has available at the time of processing. It could be conjectured that vaccination at the time of breeding soundness exam is favored among some owners due to the time and labor involved in gathering and processing bulls at a separate time.

The approach of vaccinating bulls at the time of breeding soundness exams, which may occur close to or right at the start of the breeding season, has often led to questioning of the impact of vaccination on spermatogenesis in the bull. More specifically, it has raised the questions among veterinarians of how long before the breeding season should bulls be vaccinated and does the type of vaccine utilized (MLV or KV) have an impact on spermatogenesis?

These questions of reproductive impacts on the bull stems from the concerns of the safety of MLV in pregnant animals and the consequences of necrotic oophoritis in heifers.[5-7] This has led to controversy of the use of MLV vaccines prior to breeding. Current label precautions for MLV vaccine often include: "Do not use in pregnant cows (abortions can result) unless they were vaccinated according to label direction with any MLV vaccine within the past 12 months"[a]; "do not use in calves nursing pregnant animals unless their dams were vaccinated within the past 12 months as described above"[a]; and "vaccination should occur approximately 1 month prior to breeding"[a]. However, no statements are currently available regarding vaccination of breeding bulls.

The administration of MLV vaccination to naïve heifers near the onset of estrus resulted in negative effects on the corpus luteum[5,7] and pregnancy outcome[6] in several research trials. In a large trial, pregnancy rates of females that received either a MLV or chemically altered/inactivated vaccine were reported as not different. However, within each vaccination type, the authors reported that conception rates to artificial insemination (AI) increased when cattle were vaccinated 46-89 days prior to breeding compared to those that were vaccinated closer to the time of AI.[3] Those authors hypothesized that the vaccine impacted follicular development and/or oocyte quality pre-breeding and consequently vaccination intervals greater than 42 days would have little to no impact on fertility due to the timeline of folliculogenesis. This proposed impact to the follicle and future oocytes is thought to be due to the immune response and inflammation secondary to the vaccine (MLV or KV) and release of increased concentrations of cytokines, similar to what occurs in cows with acute mastitis.[3,8]

Currently, there is a lack of available knowledge on how a vaccine that contains BVDV and BHV-1 affects spermatogenesis in the bull. It has been previously demonstrated that BVDV and BHV-1 can be transmitted via semen.[9] Bovine herpesvirus 1 can cause infectious balanoposthitis and can be associated with detrimental spermatozoa development.[10-13] The herpes virus replicates in the mucosa of the penis, prepuce and urethra and has been found to be present in the seminal plasma of infected bulls. Sperm quality of infected bulls has been diminished but is likely due to generalized illness rather than direct effect on the spermatozoa.[11,14-16] Bovine viral diarrhea virus replicates throughout the bull including within the seminal vesicles, prostate gland and epididymis as well as being associated with the Sertoli cells, spermatogonia and epithelial cells of the urethra.[17-19]

Despite the evidence that we have in cows and heifers in regard to impacts on the estrous cycle and pregnancy rates, no published reports have been recognized that explore the impact of commercially available multivalent MLV or KV vaccines containing BHV-1, BVDV1 and BVDV2 on the testicle and consequently spermatogenesis. Insults to spermatogenesis can be recognized by evaluation of sperm morphology as soon as 3-5 days following an impactful event.[2,20-23] The study aimed to determine how multivalent vaccines (modified-live and killed) affect spermatogenesis in bulls, measured by the percentage of normal sperm morphology and progressively motile sperm over a complete spermatogenic cycle (61 days) in mature bulls.

Materials and methods

This study was performed at a private feedlot in Bushland, Texas. All animals and procedures were approved through the Texas Tech University International Care and Use Committee 2022-1177

Animals

Eleven *Bos taurus* bulls of English or Continental (Angus, Charolais, Hereford) breeding were utilized in the study all were tested and found to be BVD PI-negative by antigen capture ELISA. The study occurred from March-May 2023. Bulls ranged in age from 3-6 years of age and were all scored a body condition score of 6 out of 9. All bulls were commingled at one location, in a single paddock for 10 months leading up to the initiation of the current study. Bulls were fed a total-mixed ration daily consisting of hay and soybean meal mixed with mineral package which was formulated to meet or exceeded maintenance requirements for an adult bovine. The vaccination histories of the bulls were unknown at the time of acquisition. However, the bulls did not receive any vaccinations for 10 months prior to study enrollment.

Vaccination

Bulls were randomly assigned to a treatment group prior to the first breeding soundness exam using the random function in Microsoft Excel (Microsoft Corporation Redmond, WA, USA) to decrease the potential for bias following results of the initial breeding soundness exam. Vaccine treatment groups include: 1) modified-live virus (MLV[a]) or 2) inactivated/killed virus (KV[b]). There were 6 bulls assigned to MLV treatment (n = 6) and five bulls assigned to KV treatment groups (n = 5) respectively. Bulls were vaccinated seven days after initial breeding soundness exam which was deemed Day 0 of the study. Treatment groups were commingled immediately after vaccination and returned to their home pen to prevent any disruption of the social hierarchy.

Bull breeding soundness exam

All bulls underwent a complete breeding soundness exam -7 days prior to vaccination (day 0). In short, bulls were restrained in a cattle squeeze chute. The physical exam included a rectal exam to keep in line with current breeding soundness exam standards.[24] Scrotal circumference was measured with a scrotal tape[c]. Semen was collected by electroejaculation[d]. Semen motility was observed at 400X in the field utilizing a microscope with a heated stage and semen morphology slides were made in duplicate using eosin-nigrosin stain. Semen morphology slides were read at 1000X under oil immersion by 2 board-certified theriogenologists. One of the semen evaluators was aware of vaccine treatment allocation while the second reviewer was blinded to vaccine treatment group. Each reviewer counted 100 cells in line with current SFT standards with normal, head, midpiece and principal piece defects counted. Additionally, detached abnormal heads, detached normal heads, proximal droplets and acrosome defects were also enumerated. Breeding soundness exams were repeated at days 0, 3, 7, 17, 28 and 61 post vaccination for the duration of the study.

Weather

Weather data was gathered during the study to consider any concerns of environmental stress due to extreme weather changes or adverse weather such as blizzards, extreme, prolonged cold, or extreme heat. Historical temperature data was garnered from weatherundergound.com and the weather station closest to the research facility located in Bushland, Texas.

Statistical analysis

Bull was used as the experimental unit in this study as the treatment was applied to each individual bull. Analysis was performed in R programming version 4.2.2.[25] Descriptive data were summarized utilizing the tidyverse package 2.0.0.[26] Data were originally fit into a mixed effects logit model from a Poisson family and log canonical link. The model used the number of normal sperm as the outcome of interest while conditioning on treatment group (MLV or KV) and day of collection (Day) as independent variables and an offset of log transformed total number of sperm cells counted. A random intercept for bull was utilized to account for lack of independence and repeated measures of bull by date of sample collections. The current study utilized day -7 as a negative control comparison for each bull. The interaction of vaccine by day was assessed and deemed non-significant at $P = 0.99$. The Poisson model was found to be over dispersed (dispersion ratio = 1.960, Pearson Chi-squared = 103.855, $P < 0.001$) and thus comparison of negative binomial models with different canonical links (nbinom1 and nbinom2) were made and found that the nbiom1 performed better (AIC 566 vs 576, respectively) at $P < 0.01$. Thus, the nbinom1 family link negative binomial mixed effects model were utilized as the final model with the glmmTMB package version 1.1.5.[27] Post hoc comparisons were performed with the emmeans package version 1.8.3.[28] to show model estimates by treatment and day. Pairwise comparisons were made by treatment group across days of collection and a Tukey-Kramer adjustment was applied for multiple comparisons. Statistical significance was determined a prioi at an $\alpha < 0.05$.

Representation of the final model:

Normal sperm cells count ~ vaccine treatment + day +
random effect (1|bull),
offset = log (total sperm count),
family = nbinom1 (link= '1og'),
data = data

Results

Scrotal circumference measurements for each bull did not change throughout the duration of the study from day -7 to 61. Scrotal circumference ranged from 35.5 cm to 42 cm with an average of 38.45 cm among all bulls.

Table 1 reports the study data summarized by treatment and day of collection respectively. No differences were found between vaccine treatment groups MLV and KV ($P = 0.46$) on the number of normal sperm count or motility over time (d = -7 through d = 61). Table 1 shows the normal sperm counts and motility by treatment group (MLV vs KV) split out by collection date. Figures 1 and 2 show the summary of vaccine treatment effect on normal sperm morphology across study timeframe by treatment group and individual animals. There were no differences between reviewers on counts of normal sperm ($P = 0.99$).

Post hoc comparisons of model estimated rate of normal sperm per 100 counts were found to be no different between treatment groups (MLV or KV). Pairwise comparisons resulted in no difference between treatment groups or day when comparing to controls (d-7, $P > 0.9$). No differences were found between treatment groups over all collection periods ($P > 0.9$).

Morphologic defects noted by the reviewers throughout the study varied by bull. Head defects noted included acrosome defects, nuclear vacuoles, pyriform heads, detached normal heads and detached abnormal heads. Midpiece defects encountered included proximal droplets, distal midpiece reflexes, and mitochondrial sheath defects. Principal piece defects primarily consisted of tightly coiled tails.

No adverse weather occurred during the study period. In March, the average temperature was 48 °F with a max average high of 62.23 °F and minimum average low of 35.55 °F. The average temperature in April was 56.99 °F with an average high of 71.27 °F and an average low of 42.53 °F. May had an average temperature of 78.35 °F an average high of 78.35 °F and an average low of 55.45 °F.

Discussion

Bull breeding soundness exams consist of 4 equally weighted components including physical exam, scrotal circumference, sperm motility as derived by estimation of progressively motile cells, and evaluation of sperm morphology. According to the Society for Theriogenology (SFT) standards, a bull must have 70% morphologically normal sperm and have a minimum of 30% progressively motile sperm to be considered a satisfactory potential breeder. Semen motility and morphology have been considered important factors in assessing potential fertility of a bull.[22,29-31] Sperm morphology has been likened to a testicular biopsy as the presence of normal or abnormal sperm are directly related to the health and function of the testes and epididymis.[2,20,21] Abnormal sperm morphology is the most common reason that a bull is classified as an unsatisfactory potential breeder.[32-34]

Table 1: Summary table of vaccine treatment group (MLV vs Killed) on bull breeding soundness exams of normal sperm cell counts and semen motility from day -7 to day 61.

			Semen morphology				Semen motility			
Treatment	Day	Observations	Average[1]	SD[2]	Minimum	Maximum	Average	SD[2]	Minimum	Maximum
Killed	-7	5	74	12.3	55	90	68	7.9	60	80
Killed	0	5	77	15.6	49	90	68	7.9	60	80
Killed	5	5	74	24.9	28	92	69	12.7	50	80
Killed	7	5	73	28.9	16	96	68	12.3	50	80
Killed	17	5	70	29.3	14	95	69	11.7	50	80
Killed	28	5	72	23.3	26	94	70	6.7	60	80
Killed	61	5	81	6.9	69	89	70	6.7	60	80
MLV	-7	6	64	17.6	39	91	62	12.7	40	70
MLV	0	6	67	23.2	27	90	63	13.4	40	75
MLV	5	6	65	24.7	28	90	68	9.4	60	80
MLV	7	6	61	26.5	26	94	67	9.9	50	80
MLV	17	6	64	23.7	30	96	70	10.9	50	80
MLV	28	6	65	21.7	35	91	70	10.0	50	80
MLV	61	6	65	22.3	30	91	70	10.0	50	80

[1] Average number of normal sperm cells counted per 100 sperm cell counts. Value averaged between reported normal counts from board-certified veterinary theriogenologists.

[2] SD = standard deviation of average count

Figure 1: Model estimated mean rate of normal sperm and SE per 100 sperm count (vertical axis) of bulls (n = 11) vaccinated with MLV (red) or Killed (black) vaccines over time (days; horizontal axis). Letters denote post hoc treatment comparisons with significance at *P* = 0.05. Differing letters denotes significantly different; no differences were reported.

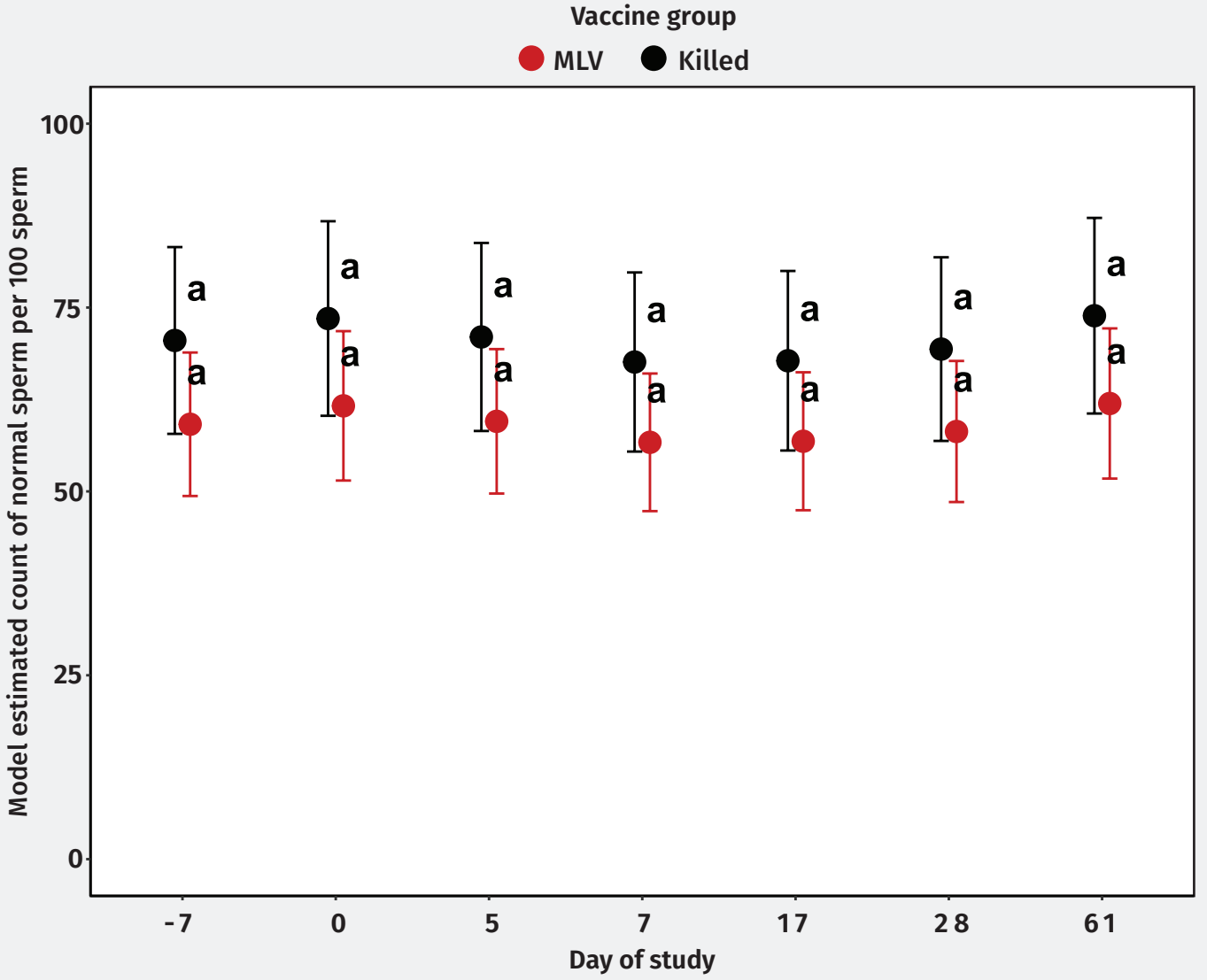
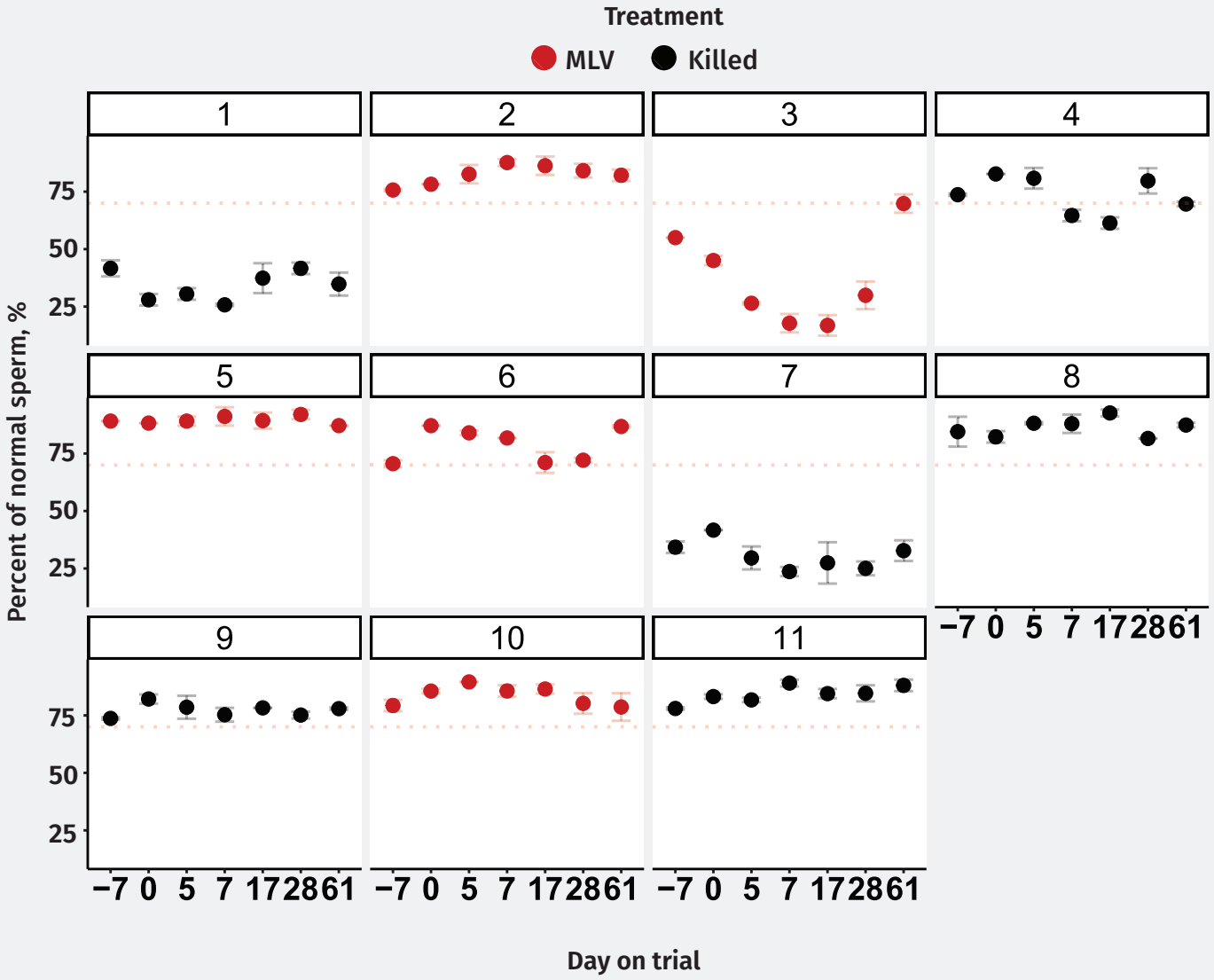


Figure 2: Normal sperm cell counts of bulls vaccinated with either Killed (red) or MLV (black) vaccines over study period (-7 to day 61). Sperm were evaluated by 2 board-certified veterinary theriogenologists. The dotted red line represents the normal cutoff for acceptable percentage of normal sperm morphology of 70% per Society for Theriogenology bull breeding soundness exam standards.



The bovine testicle is sensitive to adverse influences including heat and stress. Stress typically elevates systemic cortisol concentrations, profoundly decreasing release of LH and ultimately testosterone.[35-37] Stress has many origins, including environment (weather, heat, humidity, nutrition, and social hierarchy) illness, or injury; all causing changes in the spermiogram similar to those induced by disruption of testes thermoregulation. Disruption of critical maturation processes for spermatids, and recently released spermatozoa in the testes and epididymis leads to the appearance of morphologic abnormalities in the spermiogram.[22,38] Mild disturbances may only affect spermatids or epididymal spermatozoa with a transient period of increased numbers of morphological abnormalities. A study characterizing the sequential appearance of morphologic abnormalities noted that there were differences between animals in the overall degree of response to the negative stimulus reflected in the proportion of morphologically abnormal sperm and the proportion of certain types of defects.[23] More severe or prolonged disturbances cause destruction of the spermatocytes and spermatogonia.

Through the 68-day characterization of the semen quality of these bulls, there was no significant change in the percentage of morphologically normal sperm produced by any of the bulls nor motility among individuals following vaccination with either MLV or KV product. While no statistical difference was observed among the KV cohort, Bull 3 presumptively was going through a period of testicular degeneration at the start of the project. This period of degeneration was followed by testicular regeneration as noted in the improvement of semen quality by the end of the 61-day study period with day 7 post vaccination being the nadir point of the degenerative process. While outside the period of this study, the bull in question did return to > 70% morphologically normal sperm by day 75. The reason for the period of testicular degeneration was transient and the cause was unknown. The bull was beginning to recover by the end of the study which caused the average percentage of normal cells among the KV group to appear numerically different. While teratozoospermia is commonly observed in cases of testicular degeneration, no single morphological aberration is exclusive to degeneration.[31] This particular bull produced a large number of vacuole defects in congruence with mitochondrial sheath defects of the midpiece at the height of the testicular degeneration episode. Differential counts were performed at every BSE time point; however, we only reported the normal sperm cells. Due to the declining sperm morphology and the subsequent recovery, the authors agree that this decline in normal sperm morphology was not likely secondary to the vaccine. To further corroborate the transient nature of the testicular degeneration, the scrotal circumference did not change during this period of infertility. Furthermore, as discussed previously, the nadir was 7 days post vaccination. In general, one would expect the highest number of sperm morphologic defects to occur approximately 21 days post insult due to insult on sensitive cells late in spermatogenesis followed by a period of epididymal transport prior to ejaculation.[21] As this was the only bull in the study that was going through testicular degeneration at the time of the study, no definitive answer can be given whether vaccination hastened or magnified the testicular degeneration response, but likely the bull was already in a period of testicular degeneration based on the morphologic pattern presented.

Bull 7 in the MLV group consistently had high levels of head abnormalities, specifically acrosome defects. The number of acrosome defects noted consistently by both reviewers during the entirety of the study did not significantly vary. This bull likely had a genetic predisposition to the production of knobbed acrosomes which is a known genetic defect.[39] Bull 1 also in the MLV group consistently demonstrated poor morphology throughout the study. Distal midpiece reflexes were the most consistent midpiece abnormality noted with this bull which had a range of 41-59% midpiece abnormalities throughout the study. Distal midpiece reflexes occur secondary to low testosterone levels in the epididymis.[22] Upon necropsy at 30 days post the end of the study, there were significant adhesions between the parietal vaginal tunic and the visceral vaginal tunic. The adhesions can be correlated with the increase distal midpiece reflexes seen in this bull as they likely reduced the ability of the testicles to properly thermoregulate.

The reviewers of the sperm morphology did not detect any patterns or upticks of certain defects following vaccination such as distal midpiece reflexes or proximal droplets that would indicate a temporary reduction in testosterone following a transient inflammatory event.[20] The authors conclude that there were no significant consequences secondary to the administration of the vaccination that negatively impact the testicles and resulting spermatogenesis and consequently the spermiogram as described by the sperm morphology.

Authors acknowledge that there are potential limitations in the current study. Previous vaccination status of the bulls utilized was unknown at the time of purchase along with a smaller sample size. In addition, no antigen seroconversion was determined prior to or after administration of vaccine and no juvenile bulls (< 2 years old) were included in the study. The authors can only hypothesize that a larger sample size would increase confidence in the results found in the manuscript. Additionally, the authors cannot say with justification that juvenile bulls that are just reaching puberty will have a similar outcome to the mature bulls in this trial.

Conclusion

No detrimental effect associated with the use of multivalent MLV or KV vaccine on the sperm morphology of mature bulls over a 61-day period or a full spermatogenic cycle was noted in this study. This lack of evidence of testicular impacts suggests that vaccination of mature bulls following a routine breeding soundness exam or at the time of turnout could be performed with limited risk. However, bull breeding soundness exams should be performed 30-60 days before the breeding season starts to recognize any bulls classified as deferred or unsatisfactory potential breeders. It remains adventitious to continue to advocate for these routine health management practices to occur in advance of the start of the breeding season.

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Conflicts of interest

The authors have no competing interests to report.

Author contributions

Conception and design of the work, acquisition, analysis and interpretation of the data, drafting and revising the manuscript and final approval of the manuscript to be submitted for publication, J.K.; analysis and interpretation of the data, revising the manuscript, and final approval of the manuscript to be submitted for publication, B.J.; conception and design of the work, analysis of the data, revising the manuscript and final approval of the manuscript, C.A.; acquisition of data and review of manuscript before submission, D.B. and D.D.

End notes

[a] Bovi-shield® Gold 5, Zoetis, Kalamazoo, MI

[b] Vira Shield® 6, Elanco, Greenfield, IN

[c] Reliabull®, Lane Manufacturing, Denver, CO

[d] Pulsator V, Lane Manufacturing, Denver, CO

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