

# A survey of Southeast U.S. beef veterinarians regarding methods for bull breeding soundness evaluation

Todd G. Gunderson, DVM; W. Isaac Jumper, DVM, PhD, DACVPM; E. Heath King, DVM, MS, DACT; Carla L. Huston, DVM, PhD, DACVPM (Epidemiology); \*David R. Smith, DVM, PhD, DACVPM (Epidemiology)

Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University

\*Corresponding author: Dr. David Smith [DSmith@cvm.msstate.edu](mailto:DSmith@cvm.msstate.edu)

## Abstract

The purpose of this survey was to describe veterinarians' methods for performing bull breeding soundness evaluations (BSEs). Veterinarians in Mississippi, Louisiana and Arkansas were emailed a survey regarding their experience, demographics, opinions/practices and perceptions of client preferences regarding BSEs. Logistic regression was used to test respondent characteristics for associations with dichotomous outcomes including evaluating semen morphology as part of every BSE and detecting the diadem defect. Of 3,757 veterinarians solicited, 204 (5%) responded and 83 (2%) qualified for analysis. Of these, 10/73 (14%) indicated they do not evaluate morphology during every BSE. When shown an image of the diadem defect, 18/73 (25%) indicated seeing it often or sometimes, and 55/73 (75%) indicated rarely or never. The only factor associated with evaluating morphology as part of every BSE, was indicating belief that morphology was most predictive of bull fertility vs. believing motility was most predictive, or being unsure (OR = 11.2, 95% C.I. = 1.3-94.1). Compared to respondents who do not, respondents who always evaluate morphology with bright field microscopy at 1,000X (OR = 4.1, 95% C.I. = 1.2-13.3) or with phase contrast microscopy at  $\geq$  400X (OR = 5.4, 95% C.I. = 1.1-27.7) had higher odds of indicating they detect the diadem defect sometimes/often. Among respondents who only use bright field microscopy, there was an interaction between always using 1000X magnification, or not, and always using 400X, or not, on the reported detection of the diadem defect. Veterinarians' perceptions of which aspects of the BSE are most predictive of fertility influence their methods for performing BSEs, and these methods influence the frequency of detecting diadem defects.

**Key words:** Breeding Soundness Veterinary Survey

## Introduction

Bull breeding soundness evaluation (BSE) is an important service offered by beef cow-calf practitioners. Whereas the accuracy of pregnancy diagnosis is usually self-evident (i.e., cows either calve or do not, and if a veterinarian misdiagnoses a pregnancy, there is a high probability the producer will find out eventually), the accuracy of breeding soundness evaluation, at least in multi-sire herds, is more difficult for producers to assess because empirically we recognize that producers rarely know the parentage of calves in multi-sire breeding groups. Guidelines for evaluating breeding soundness in bulls have been established in the United States by the Society for Theriogenology (SFT) in their current format since 1992, with recent updates published in 2018.<sup>1,2</sup> Even before these updates, guidelines for the breeding soundness evaluation (BSE)

had been published in some form by SFT and its precursor, The Rocky Mount Society for the Study of Breeding Soundness of Bulls, since the 1950s.<sup>1,3,4</sup> The most significant shift in BSE guidelines occurred with the 1992 update, which transitioned the BSE from an aggregate scoring system wherein a bull could score high enough on one aspect of the exam to compensate for a low score on a different aspect, to a system where the bull was required to meet the minimum requirements in all sections of the exam to be classified as satisfactory.<sup>1</sup> Under the current guidelines, for a bull to be classified as a satisfactory potential breeder he must be free of physical defects that would preclude him from breeding, must meet minimum scrotal circumference measurements, have no less than 30% progressively motile semen, and no less than 70% morphologically normal semen. Furthermore, the most recent guideline update explicitly recommends that sperm morphology and differential counts be conducted using an eosin-nigrosin stained semen smear evaluated at 1,000X magnification.<sup>2</sup> It has been suggested that these standards should be intended to assess the likelihood that a bull will sire  $\geq$  25 calves in a 65-70-day-breeding window, though to the best of the authors' knowledge this is not the official position of SFT.<sup>5-8</sup> Veterinarians are not required to be members of SFT to perform BSEs, but SFT only distributes its official test form to its members.<sup>9</sup>

Multiple studies have shown benefits from utilizing the BSE in beef herds for both improvement of reproductive efficiency and increased profitability.<sup>10-15</sup> However, in spite of the extensive research related to the BSE and its long history of use in beef herds, there are anecdotal indicators that veterinary compliance with the established guidelines is not universal, and that certain practitioners may sacrifice the quality of the exam in favor of speed or higher pass rates.<sup>16,17</sup> Additionally, there is concern that even veterinarians who attempt to follow the established guidelines may be hindered in their ability to detect certain morphological defects if they are using microscopes with insufficient optics.<sup>17</sup> It has been suggested in textbooks devoted to bovine reproduction that a litmus test for whether a microscope, or BSE, is sufficiently sensitive for detection of important defects is the ability of the scope/examiner to detect nuclear vacuoles, with the diadem defect being a typical example.<sup>18,19</sup> Because one of the main goals of the BSE is to detect morphological sperm defects that could have an adverse effect on reproduction, even in the presence of good sperm motility, these factors could compromise the diagnostic sensitivity of the BSE, and decrease the predictive value of the test.<sup>17,20-22</sup> Therefore, the purpose of this study was to describe the methods veterinarians in the states of Mississippi, Louisiana and Arkansas use for BSEs, assess compliance with the SFT guidelines for BSEs, and how veterinarians' perceptions of the BSE influence their methods. Furthermore, we

sought to investigate the frequency of detection for the diadem defect, and assess how veterinarians' methods for performing BSEs influence this outcome.

## Materials and methods

### Target population and survey development

The target population for this survey was beef cow-calf veterinarians who practiced in the southeastern United States. Our sampling frame consisted of the rosters of veterinarians in the states of Mississippi, Louisiana and Arkansas. These rosters contained the names of veterinarians licensed to practice in these states, and their email addresses, and were obtained from the veterinary licensing boards of each state. We created an electronic mailing list composed of every email on all 3 lists in order to distribute links to a web-based survey to all veterinarians on the rosters<sup>a,b</sup>.

The survey did not ask respondents for any identifiable information, and the survey settings were adjusted so that the IP address of the respondents' internet connections were not recorded. As a result, the survey was anonymous and was not human-subjects research. The survey questionnaire was piloted on beef cow-calf veterinarians outside of the sampling frame to evaluate question content and clarity prior to dispersal, but their responses were not included in the survey.

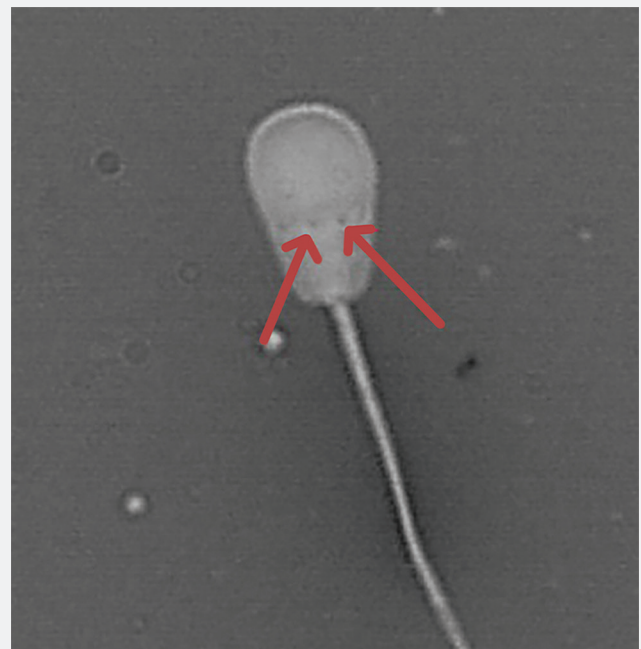
The email messages sent to recipients contained information about the study, contact information for the investigators, and a link to the survey. These messages were distributed through the electronic mailing list on April 21, 25, 29 and May 23 of 2022. After the first email was sent, recipients of subsequent emails were also given instructions for unsubscribing from the mailing list if they no longer wished to receive emails or felt they didn't qualify for the survey.

Respondents who indicated that they did not service beef cow-calf clients, or that they were not in private veterinary practice, were thanked for their participation and precluded from answering further questions. Veterinarians who indicated their primary practice was not located in either Mississippi, Louisiana or Arkansas were excluded from analysis. Respondents were asked questions regarding their employment status, years in practice, gender and percentage of their professional time spent performing cow-calf services.

Veterinarians were asked for their opinion regarding whether percentage of morphologically normal sperm or percentage of progressively motile sperm was most predictive of a bull's ability to sire calves. Veterinarians could also indicate if they were unsure which semen trait was most predictive.

Respondents who indicated they performed BSEs on bulls were asked a series of questions regarding their practices and techniques for performing BSEs. These included the number of bulls they tested per year, their method of collecting semen, which physical exam criteria they *always* evaluated as part of *every* BSE, which semen criteria they *always* evaluated as part of *every* BSE, how they kept records of BSEs, whether they read stained semen slides at the time of the BSE or at a later time, and how often they collected preputial scrapings for *Tritrichomonas foetus* testing at the time of BSE. Respondents were shown a picture of a spermatozoa with arrows pointing to nuclear vacuoles arranged in the diadem defect (Figure 1). They were not told what the defect was, but were asked to indicate whether they saw this defect often, sometimes, rarely or

**Figure 1:** Nuclear vacuoles arranged in the diadem defect as indicated by the arrows. Adapted from *Bovine Reproduction*



never. For inferential analysis, the outcomes of this variable were aggregated into binary categories; those who reported seeing the diadem defect sometimes/often vs. those who reported seeing this defect rarely/never. Veterinarians were also provided 3 sets of 3 criteria that producers might consider when choosing a veterinarian to perform BSEs, and asked to estimate which criteria were most important and least important to their clients. Veterinarians were asked to do this separately for commercial cow-calf and seed-stock producers. Also, veterinarians were asked to consider the general service categories of access to preventive herd health services, access to prescription veterinary drugs, and access to emergency services, and rank these categories according to which they felt were most and least important to their clients (i.e., which were most and least important from their clients' point of view), and which they felt were most and least valuable to their clients (i.e., which provided the most and least utility to the client from their point of view as veterinarians).

### Data collection and analysis

Results of the survey received prior to June 27, 2022 were downloaded into spreadsheet software and, if necessary, reformatted for use in a statistics software program<sup>c</sup>. Multivariable logistic regression was performed to test associations between binary outcome variables and potential explanatory variables<sup>d</sup>. Variable selection was accomplished using manual forward multivariable modeling. The Tukey-Kramer test was used to adjust for multiple comparisons. In the first step of manual forward selection, each explanatory variable was evaluated in a univariable model, and the variable with the most significant association was chosen for subsequent multivariable analysis. If none of the other explanatory variables had significant associations in multivariable models, then the univariable model was reported. An  $\alpha$  of 0.05 was used for all analyses.

To test factors for association with the outcome of whether veterinarians adhered to SFT guidelines, we used evaluation of morphology by any method as part of every BSE as the outcome variable (i.e., yes if veterinarian evaluated an eosin nigrosin-stained slide at 400X and/or 1,000X, and/or phase contrast at 400X or higher, no if not). We then developed logistic regression models with veterinarian years of practice, gender, number of bulls tested per year, percentage of time spent in cow-calf practice, and opinions regarding which aspect of the BSE was most predictive of fertility as explanatory variables. To test factors for association with the probability of veterinarians indicating they see diadem defects, we developed logistic regression models with seeing diadem defects sometimes/often vs. rarely/never as a binary outcome variable. Explanatory variables were years of practice, gender, number of bulls tested per year, percentage of time spent in cow-calf practice, evaluating semen morphology on stained slides at 1,000X and/or 400X, evaluating morphology on phase contrast at  $\geq 400X$ , and evaluating semen morphology by any method. Some of the respondents indicated they always evaluate morphology using bright field microscopy at 400X, but not 1,000X, and also indicated that they evaluate morphology using phase contrast microscopy at  $\geq 400X$ . Therefore, to assess the effect of using the higher bright field magnification on the reported frequency of observing the diadem defect, we developed a logistic regression model that excluded respondents who reported always using phase contrast. This model tested the interaction between always evaluating morphology on bright field microscopy at 1,000X (yes/no), or at 400X (yes/no).

## Results

Out of 3,757 veterinarians who were sent emails advertising the survey, a total of 204 respondents opened the link to the survey. Of these, 83 qualified for study inclusion and analysis. Table 1 shows the demographic data collected from these respondents. The percentage of time respondents spent in cow-calf practice is displayed in Figure 2.

Thirty-nine out of 83 (47%) respondents indicated they felt morphology was most predictive of a bull's ability to sire calves, 35 out of 83 (42%) indicated they felt motility was most predictive of a bull's ability to sire calves, and 9 out of 83 (11%) indicated that they were unsure. Of 83 qualifying respondents, 73 indicated they perform BSEs on bulls. Figure 3 shows the distribution of respondents by number of bulls tested per year. All 73 of these respondents indicated that their primary method for collecting semen from bulls was electroejaculation (the alternative method offered was manual massage of accessory sex glands). The physical exam criterion evaluated most consistently was palpation of the testicles/epididymis, followed by measurement of scrotal circumference. Table 2 shows the percentage of respondents who evaluated specific physical examination and semen criteria as part of every BSE they performed. Table 3 shows the percentage of respondents who use SFT forms, either digital and/or paper, and the percentage of respondents who keep stained slides as part of their medical record; this table also shows the percentage of respondents who read stained slides at the chute vs. at a later time, or not at all. Of 73 respondents who indicated they perform BSEs, 63 (86%) indicated they always evaluate gross semen motility on bright field microscopy at  $\geq 40X$  magnification, and 63 (86%) indicated they always evaluate morphology by at least one of the methods defined. However, only 29 (40%) respondents indicated that they always evaluate morphology using an

eosin-nigrosin stained slide at 1,000X magnification. Respondents who indicated they thought that morphology was most predictive of bull fertility had higher odds of indicating that they evaluated morphology as part of every BSE they performed compared to respondents who indicated semen motility was most predictive of a bull's ability to sire calves or respondents who were unsure (Table 4,  $P = 0.03$ ). We did not find significant associations for the other variables evaluated for this outcome.

Only 1% of respondents indicated they saw the diadem defect as illustrated in Figure 1 often, vs. 23% that indicated sometimes, 47% that indicated rarely, and 29% that indicated never. After aggregating variables into binary outcomes, 25% indicated sometimes/often, and 75% indicated rarely/never. The factors associated with increased odds of detecting the diadem defect sometimes/often were always using bright field microscopy at 1,000X magnification or always using phase contrast microscopy at  $\geq 400X$  (Table 5,  $P = 0.02$ ). The odds for reporting seeing the diadem defect sometimes/often were not significantly associated with using 400X bright field microscopy. We did not find significant associations between this outcome and the other variables assessed in the model.

Most respondents reported using bright field microscopy, so to test the effect of magnification with bright field microscopy on reporting seeing the diadem defect sometimes/often we removed from analysis those veterinarians evaluating semen morphology by phase contrast microscopy. In this logistic regression model, we detected a significant interaction between using magnification at 1,000X, or not, and 400X, or not, such that veterinarians who reported always using both 1,000X and 400X to examine semen morphology were more likely to report seeing the diadem defect sometimes/often than those using 400X alone ( $P = 0.048$ ). The odds for seeing the defect sometimes/often were not significantly different among veterinarians always using 1,000X and 400X, 1000X alone, or neither magnification. Figure 4 shows the results of this model, with the odds having been converted into probabilities for comparison.

Veterinarians' perceptions of how their seedstock and commercial cow-calf clients ranked different criteria for selecting a veterinarian to perform BSEs in 3 groups of 3 are listed in Tables 6 and 7 respectively, and the overall most and least important criteria for each producer category are listed in Table 8. The rankings of general services categories according to which services veterinarians feel are most important to their clients vs. which service categories veterinarians feel provide the most value to their clients are listed in Table 9.

## Discussion

The results of this study are consistent with the hypothesis that the SFT guidelines are not universally adhered to by practitioners when performing BSEs.

While 11% of veterinarians surveyed use phase contrast microscopy at  $\geq 400X$  magnification to evaluate morphology, only 40% of the respondents to this survey indicated that they follow the SFT recommendation of evaluating semen morphology using eosin-nigrosin stained slides under bright field microscopy at 1,000X magnification as part of every BSE they perform. Even more concerning, 14% of respondents who performed BSEs reported not always evaluating semen morphology by *any* of the methods listed. The finding that veterinarians have higher odds of evaluating morphology as part of every BSE if they believe it is most predictive of fertility, suggests

that veterinarians might not evaluate morphology as part of every BSE if they do not believe it is as important to fertility as motility, or are unsure of its importance. There is also the possibility that some veterinarians may view these two sperm characteristics as equally important. For this survey, it was assumed respondents who felt motility and morphology were equally important might indicate they were unsure which was more important. However, due to the structure of the question, the data from this survey do not allow for assessment of veterinarians' methods when they feel morphology and motility are equally important.

Multiple studies have demonstrated the importance of semen morphology on bull fertility.<sup>12,13,23</sup> However, it may be intuitive for practitioners to assume that semen morphological characteristics are moot if the spermatozoa are largely

non-motile. For example, a practitioner may evaluate a motility slide and determine there is no need for further evaluation due to poor motility. This approach saves time, but it could deprive the bull owner of data that may aid in formulating a prognosis for the bull in question (i.e., the type of defects present may indicate the probability of the bull's spermogram improving with time and/or use). Semen with poor motility that is otherwise free of primary and/or uncompensable defects may simply be the result of a bull accumulating large amounts of senescent sperm in the ampullae and caudal epididymides, and if this is the case, the bull is likely to improve with time and breeding.<sup>17,24</sup> In contrast, if a veterinarian examines a semen sample with poor motility and detects a high percentage of defects that arose during spermatogenesis, the prognosis may be more guarded, or at the very least may indicate a recent disruption in spermatogenesis.<sup>25</sup>

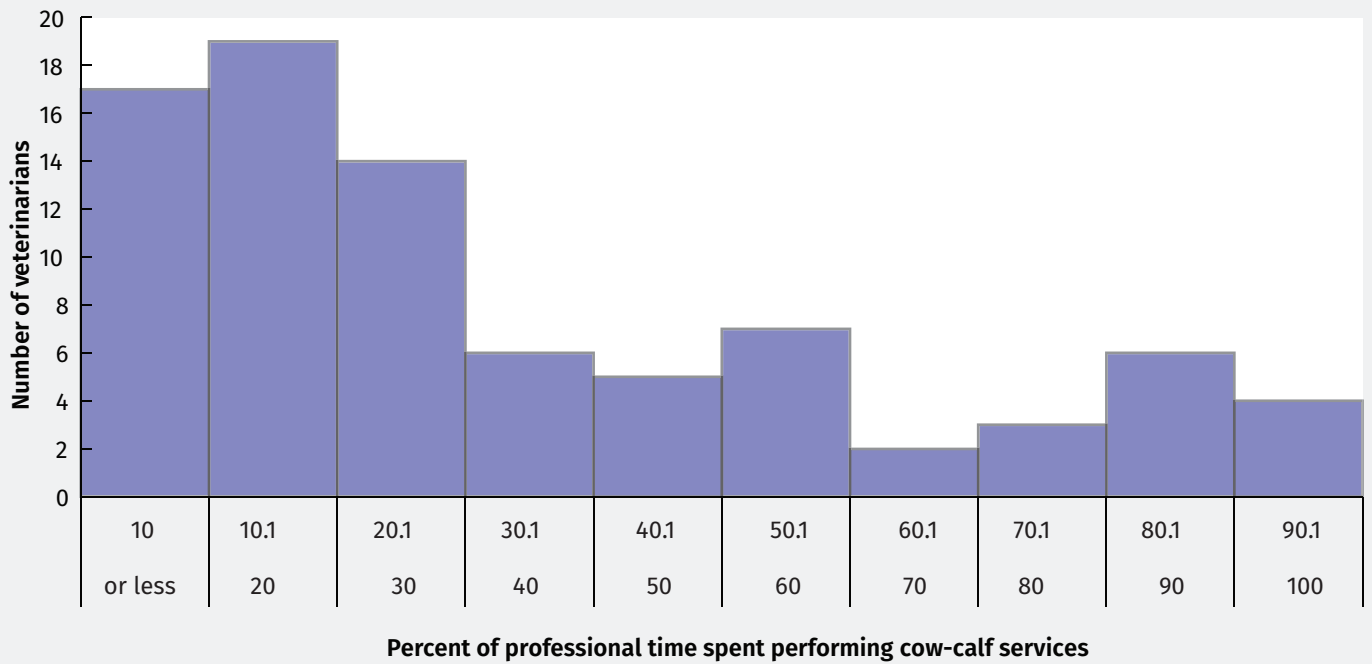
**Table 1:** Demographic data of veterinarians who responded to the survey.

Variable	Outcomes*	# of respondents/	# of respondents/ # of qualified responses
Primary state of practice	Arkansas	30/83	36%
	Louisiana	29/83	35%
	Mississippi	24/83	29%
Employment status	Associate veterinarian	18/83	22%
	Partner in a jointly owned practice	19/83	23%
	Sole owner of a practice	45/83	54%
	Relief veterinarian	1/83	1%
Gender	Male	66/83	80%
	Female	15/83	18%
	Prefer not to answer/Skipped	2/83	2%
Years in practice	0-5	11/83	13%
	6-10	10/83	12%
	11-20	13/83	16%
	21-30	23/83	28%
	31-40	18/83	22%
	41+	8/83	10%
	≤ 20†	34/83	41%
	> 20†	49/83	59%
Primary method of pregnancy diagnosis	Transrectal palpation	63/82	77%
	Transrectal ultrasonography	15/82	18%
	Blood test	4/82	5%
How often respondent estimates gestational length at pregnancy diagnosis	Always	38/82	46%
	Usually	33/82	40%
	Sometimes	9/82	11%
	Rarely	1/82	1%
	Never	1/82	1%

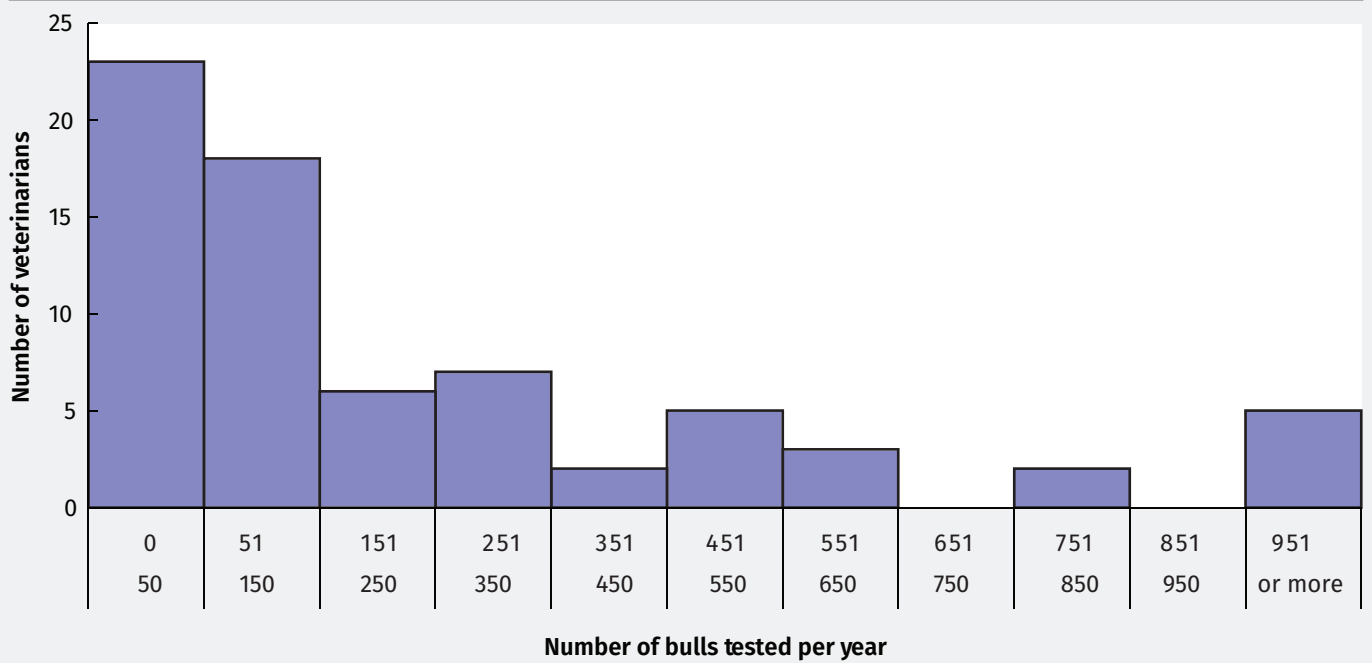
\* Respondents were asked to only indicate one outcome

† Aggregated outcomes

**Figure 2:** Percentage of time responding veterinarians spent in cow-calf practice.



**Figure 3:** Distribution of responding veterinarians by number of bulls tested per year.



**Table 2:** Physical exam and semen criteria that responding veterinarians evaluated as part of every BSE they perform.

Variable	Criteria*	# of respondents/ # of qualified responses
Physical exam criteria evaluated as part of every BSE	Palpate testicles/epididymis	69/73 95%
	Measure scrotal circumference	68/73 93%
	Assess body condition score	64/73 88%
	Assess full length of penis	62/73 85%
	Assess conformation	60/73 82%
	Assess eyes	59/73 81%
	Palpate accessory sex glands	50/73 68%
	Palpate inguinal rings	19/73 26%
	Take rectal temperature	2/73 3%
Heart & lung auscultation	1/73 1%	
Semen criteria evaluated as part of every BSE	Gross motility on bright field microscopy at $\geq 40X$	63/73 86%
	Morphology by any method <sup>†</sup>	63/73 86%
	Subjectively assess sperm concentration	48/73 66%
	Morphology with eosin-nigrosin stain at 400X	41/73 56%
	Individual motility on bright field microscopy at $\geq 100X$	35/73 48%
	Morphology with eosin-nigrosin stain at 1,000X	29/73 40%
	Gross motility without magnification	27/73 37%
	Individual motility on phase contrast at $\geq 100X$	9/73 12%
	Morphology with phase contrast at $\geq 400X$	8/73 11%
Quantitatively measure sperm concentration	5/73 7%	

\* Respondents were asked to indicate all criteria that applied

<sup>†</sup> Aggregated criteria

An alternative scenario would be when a veterinarian examines a motility slide, determines the bull is fertile due to excellent motility, and foregoes preparation and/or examination of a morphology slide. Failure to examine a morphology slide on bulls with normal motility is a more egregious omission, because bulls with normal motility may have high percentages of sperm defects that may not only result in sub-fertile breedings from that bull, but could theoretically impact the fertility of other bulls in the pasture if ovulated oocytes are blocked to their sperm by defective sperm from the sub-fertile bull.<sup>17,20-22,26,27</sup>

Nuclear vacuoles are an example of a defect that can impact bull fertility in a manner that other bulls cannot compensate for. The head shape of spermatozoa with vacuoles is often normal. As a result, they are not filtered out at the cervix or uterotubal junction the same as other defective spermatozoa.<sup>23</sup> This trait can enable vacuole containing spermatozoa to compete with normal spermatozoa for attachment to the zona pellucida and induce the block to polyspermy. However, multiple studies have shown that nuclear vacuole containing sperm can be less likely to produce a viable embryo, even when they successfully penetrate the zona pellucida.<sup>20-22,26,27</sup> The ability to penetrate the zona pellucida and induce the block to polyspermy, coupled with a decreased ability to produce a viable embryo, makes this type of defect uncompensable. For this reason, the investigators chose to assess the frequency that

veterinarians detect nuclear vacuoles arranged in the diadem defect. While the diadem defect is not the only way vacuoles will present in spermatozoa, it is a conspicuous manifestation of this defect, so it stands to reason that if a veterinarian fails to detect the diadem defect, more subtle presentations of the nuclear vacuoles may go undetected.

It is not surprising that veterinarians who always use bright field microscopy at 1,000X magnification and/or phase contrast microscopy at  $\geq 400X$  magnification had higher odds of reporting more frequent detection of the diadem defect. However, the finding that veterinarians who always examine stained slides only on bright field microscopy at both 400X and 1,000X had significantly higher odds of reporting more frequent detection of the diadem defect compared to veterinarians who always examine morphology at 400X, was unexpected. This finding suggests that it is not magnification alone that explains seeing the diadem defect more often. A possible explanation may be that, in addition to using the right magnification, taking the time to do a thorough examination also enables veterinarians to detect these types of defects. Notwithstanding, we cannot say for sure why we observed this last result. It also bears noting that some veterinarians indicated they did not always evaluate morphology on an eosin-nigrosin stained slide either at 1,000X or 400X magnification, but still indicated they saw the diadem defect sometimes/often. These veterinarians may not always evaluate morphology but

**Table 3:** Practices of responding veterinarians related to their record keeping, reading stained slides, and how often they perform *Tritrichomonas* testing when they perform BSEs.

Variable	Outcomes	# of respondents/ # of qualified responses	Percent
Record keeping practices*	Use SFT paper form	36/73	49%
	Use a different paper form	35/73	48%
	Use SFT digital form	4/73	5%
	Use a different digital form	4/73	5%
	Keep stained slides as part of record	5/73	7%
	Record digital images of slides	0/73	0%
Read stained slides†	Chute side	58/73	79%
	At a later time	11/73	15%
	Don't read stained slides	4/73	5%
Tests for <i>Tritrichomonas</i> at BSE†	> 80% of the time	12/73	16%
	61-80% of the time	8/73	11%
	41-60% of the time	9/73	12%
	21-40% of the time	18/73	25%
	≤ 20% of the time	26/73	36%

\* Respondents were asked to indicate all criteria that applied

† Respondents were asked to only indicate one outcome

**Table 4:** Logistic regression model for relationship between evaluating morphology by any method as part of every BSE and opinions regarding which aspect of the BSE is most predictive of fertility. Respondents who indicated they believed percent morphologically normal sperm was most predictive of a bull's ability to sire calves (instead of indicating percent motility was most predictive or were unsure) had 11.2 times the odds of indicating that they evaluated morphology as part of every BSE they performed.

Variable	Evaluates morphology as part of every BSE	Does not evaluate morphology as part of every BSE	Estimate	Standard error	OR	95% C.I.	P-value
Respondent believes morphology is most predictive of fertility	35	1	2.42	1.08	11.2	1.3-94.1	0.03
Respondent believes motility is most predictive of fertility or is unsure	28	9		Ref.			
Intercept			1.14	0.38			

\* Values in column indicate number of respondents in each category

† Other variables assessed include years of practice, gender, number of bulls tested per year, and percentage of time spent in cow-calf practice

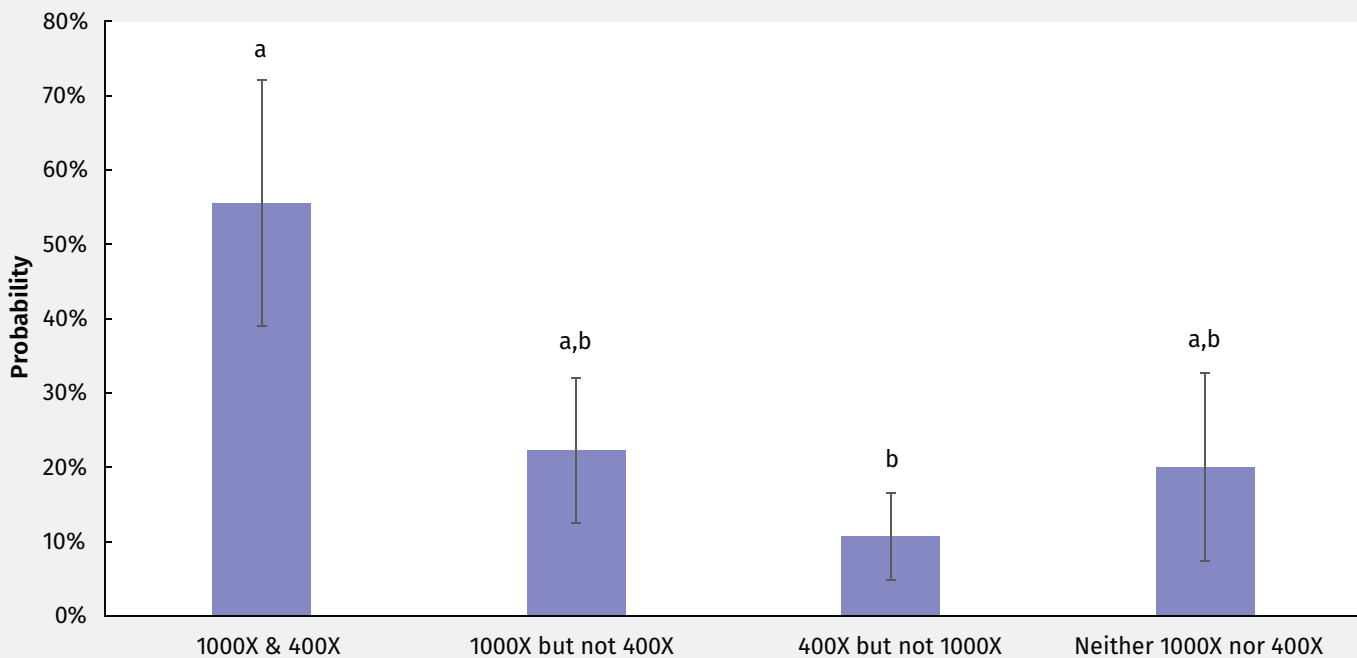
**Table 5:** Multivariable logistic regression model for relationships between microscopy methods/magnification and whether veterinarians indicated they detect the diadem defect. The model evaluates the odds that the respondent reported observing the diadem defect sometimes/often rather than rarely/never. Accounting for respondents who indicated they always use phase contrast microscopy, respondents who always use bright field microscopy at 1000X magnification had 4.1 times the odds of indicating they see the diadem defect sometimes/often. Accounting for respondents who indicated they always used bright field microscopy at 1000X, respondents who always use phase contrast microscopy  $\geq 400X$  had 5.4 times the odds of indicating they see the diadem defect sometimes/often.

Microscopic method always used		Detect diadem defect sometimes/often*	Detect diadem defect rarely/never*	Estimate	Standard error	OR	95% C.I.	P - value
Bright field microscopy at 1,000X	Yes	11	18	1.40	0.61	4.1	1.2-13.3	0.02
	No	7	37					
Phase contrast at $\leq 400X$	Yes	4	4	1.68	0.84	5.4	1.1-27.7	0.04
	No	14	51					
Intercept				-2.01	0.49			

\* Values in column indicate number of respondents in each category

† Other variables assessed include years of practice, gender, number of bulls tested per year, percentage of time spent in cow-calf practice, and whether respondents evaluated morphology by any method as part of every BSE they performed

**Figure 4:** Probability of respondents indicating they see the diadem defect sometimes/often categorized by magnification(s) always used on bright field microscopy to evaluate semen morphology on eosin-nigrosin stained slides under bright field microscopy. Veterinarians who indicated they always use phase contrast at  $\geq 400X$  magnification were excluded from the analysis. Error bars indicate 1 standard error unit from the mean.





**Table 6:** Veterinary perceptions of which criteria their seedstock cow-calf clients consider most important in choosing a veterinarian to perform BSEs.

Group	Criteria*	Most important	Least important
1	The ability of the veterinarian to accurately predict a bull's fertility/calf-output	69/83 (83%)	3/83 (4%)
	How fast the veterinarian can test the bulls (i.e., how many they can get through in an hour)	13/83 (16%)	39/83 (47%)
	How accurately the veterinarian can measure scrotal circumference	1/83 (1%)	41/83 (49%)
2	The quality of the working relationship with the veterinarian	54/83 (65%)	14/83 (17%)
	The pass/fail rate of the veterinarian	20/83 (24%)	48/83 (58%)
	How fast the veterinarian can provide test results (i.e., at the time of exam vs. the next day)	9/83 (11%)	21/83 (25%)
3	The cost of the test	18/83 (22%)	24/83 (29%)
	The availability of the veterinarian	60/83 (72%)	2/83 (2%)
	How close the veterinarian is to your location	5/83 (6%)	57/83 (69%)

\* Respondents were able to only give one ranking per criteria

**Table 7:** Veterinary perceptions of which criteria their commercial cow-calf clients consider most important in choosing a veterinarian to perform BSEs.

Group	Criteria*	Most important	Least important
1	The ability of the veterinarian to accurately predict a bull's fertility/calf-output	66/83 (80%)	2/83 (2%)
	How fast the veterinarian can test the bulls (i.e., how many they can get through in an hour)	16/83 (19%)	24/83 (29%)
	How accurately the veterinarian can measure scrotal circumference	1/83 (1%)	57/83 (69%)
2	The quality of the working relationship with the veterinarian	46/83 (55%)	19/83 (23%)
	The pass/fail rate of the veterinarian	10/83 (12%)	48/83 (58%)
	How fast the veterinarian can provide test results (i.e., at the time of exam vs. the next day)	27/83 (33%)	16/83 (19%)
3	The cost of the test	29/83 (35%)	17/83 (20%)
	The availability of the veterinarian	52/83 (63%)	4/83 (5%)
	How close the veterinarian is to your location	2/83 (2%)	62/83 (75%)

\* Respondents were able to only give one ranking per criteria

**Table 8:** Veterinary perceptions of the criteria commercial and seedstock cow-calf clients consider overall most and least important when choosing a veterinarian to perform BSEs.

Producer type	Criteria*	Most important	Least important
Seedstock	The ability of the veterinarian to accurately predict a bull's fertility/calf-output	36/83 (43%)	2/83 (2%)
	How fast the veterinarian can test the bulls (i.e., how many they can get through in an hour)	1/83 (1%)	16/83 (19%)
	How accurately the veterinarian can measure scrotal circumference	---	13/83 (16%)
	The quality of the working relationship with the veterinarian	27/83 (33%)	3/83 (4%)
	The pass/fail rate of the veterinarian	3/83 (4%)	11/83 (13%)
	How fast the veterinarian can provide test results (i.e., at the time of exam vs. the next day)	---	4/83 (5%)
	The cost of the test	5/83 (6%)	9/83 (11%)
	The availability of the veterinarian	11/83 (13%)	---
Commercial	How close the veterinarian is to your location	---	25/83 (30%)
	The ability of the veterinarian to accurately predict a bull's fertility/calf-output	37/83 (45%)	1/83 (1%)
	How fast the veterinarian can test the bulls (i.e., how many they can get through in an hour)	4/83 (5%)	9/83 (11%)
	How accurately the veterinarian can measure scrotal circumference	---	27/83 (33%)
	The quality of the working relationship with the veterinarian	17/83 (20%)	1/83 (1%)
	The pass/fail rate of the veterinarian	1/83 (1%)	14/83 (17%)
	How fast the veterinarian can provide test results (i.e., at the time of exam vs. the next day)	4/83 (5%)	4/83 (5%)
	The cost of the test	10/83 (12%)	7/83 (8%)
The availability of the veterinarian	9/83 (11%)	1/83 (1%)	
How close the veterinarian is to your location	1/83 (1%)	19/83 (23%)	

\* Respondents were able to only give one ranking per criteria

**Table 9:** Rankings of general service categories by which categories veterinarians perceive their clients value most compared to which categories veterinarians believe provide the most value to their client.

Ranking criteria	Service category*	Most important	Least important
Service categories that veterinarians feel are most important to their clients	Access to emergency services	38/83 (46%)	10/83 (12%)
	Access to prescription drugs	31/83 (37%)	20/83 (24%)
	Access to preventive herd health services	14/83 (17%)	53/83 (64%)
Service categories that veterinarians feel provide the most value	Access to emergency services	22/83 (27%)	17/83 (20%)
	Access to prescription drugs	2/83 (2%)	56/83 (67%)
	Access to preventive herd health services	59/83 (71%)	10/83 (12%)

\* Respondents were able to only give one ranking per service category

may still evaluate morphology often enough to see this defect at least occasionally. These findings from this survey of veterinarians suggest that some BSE methods may reduce the likelihood of detecting the diadem defect.

This is concerning because nuclear vacuoles can be relatively common. A study of 46 beef bulls at an artificial insemination center in Canada showed that all ejaculates had some spermatozoa with vacuoles, and 13% had  $\geq 20\%$  of spermatozoa with vacuoles.<sup>28</sup> Another study of 411 bulls reported that 43.1% had no vacuoles, and 6.8% had 10-70% vacuoles. Because veterinarians reporting their frequency of detecting the diadem defect is subjective and prone to respondent bias, and this data set only represents veterinarians in a specific geographical region, further research is warranted to determine the extent to which veterinarians are failing to detect these types of defects, how this affects the accuracy of the BSE, and how the fertility of multi-sire herds is impacted when they are bred by bulls that are not adequately screened for these defects. Furthermore, because this study did not specifically ask veterinarians why they do, or do not, perform certain components of the BSE, the omission of these components is also an area that remains to be studied further.

At least one other author has posited that veterinary compliance with established BSE standards may not be universal, and those observations were from a region outside of the geographical area for this study.<sup>16,17</sup> Nevertheless, because our data were gathered from a limited number of respondents in a specific geographical region, caution should be used in extrapolating these results to other regions. The limited number of respondents also may have reduced the power of this study to detect significant associations between our outcome variables and the other explanatory variables evaluated during the first step of manual forward selection.

## Conclusions

This survey demonstrated that compliance with the SFT established guidelines for performing BSEs is not universal in the regions surveyed, and that veterinarians' perceptions regarding the predictive value of sperm morphology vs. motility influence the way they perform these evaluations. Furthermore, the method of microscopic examination may affect the probability to detect nuclear vacuoles.

## Acknowledgements

This study was a contribution of the Beef Cattle Population Health and Reproduction Program at Mississippi State University. The authors of this study would like to acknowledge and thank the Mississippi Board of Veterinary Medicine, the Louisiana Board of Veterinary Medicine and the Arkansas State Board of Veterinary Medical Examiners for their cooperation in performing this survey as well as our colleagues in these jurisdictions who took the time to respond candidly and openly to our survey. We would also like to thank Dr. Tony Parsons and Dr. Josh Harper of Blackfoot, Idaho, and Dr. Wayne Ayers of Wilder, Idaho, for taking the time to review the questions on the survey and for their contributions to the format and content. We would also like to thank Dr. Robert Wills for consulting on the study design and data analysis of this study.

## Funding

The funding for this research was provided by a House Officer Grant of Mississippi State University's College of Veterinary Medicine.

## Endnotes

<sup>a</sup> Sympa, Mailing List Management Software, [www.sympa.community/sympa](http://www.sympa.community/sympa)

<sup>b</sup> Qualtrics, Provo, UT

<sup>c</sup> Microsoft Excel, Microsoft Corporation, Redmond, WA

<sup>d</sup> PROC LOGISTIC, SAS for Windows v9.4, SAS Institute, Inc., Cary, NC

## Conflicts of interest

The authors of this study have no conflicts of interest to report.

## References

1. Chenoweth PJ, Hopkins FM, Spitzer JC, Larsen RE. New guidelines for the evaluation of bulls for breeding soundness. *Proc Am Assoc Bov Pract*, Sept 16-10 1993. 1994;26:105-107.
2. Koziol JH, Armstrong CL. *Society for Theriogenology Manual for Breeding Soundness Examination of Bulls 2nd Edition*. Society for Theriogenology; 2018.
3. Carroll EJ, Ball L, Scott JA. Breeding soundness in bulls—a summary of 10,940 examinations. *J Am Vet Med Assoc* 1963;142(10):1105-1111.
4. Bartlett DE. Society for Theriogenology. History and term of theriogenology. Accessed August 29, 2023. <https://www.therio.org/page/HistoryTheriogenolog>
5. Kastelic J, Thundathil J. Breeding soundness evaluation and semen analysis for predicting bull fertility. *Reprod Domest Anim* 2008;43:368-373. <https://doi:10.1111/j.1439-0531.2008.01186.x>
6. Barth AD. Personal communication. September 7, 2023.
7. Kastelic JP. Personal Communication. September 11, 2023.
8. Koziol JH. Personal Communication. September 17, 2023.
9. Bull breeding soundness evaluation guidelines established by Society for Theriogenology. Published online 2022. [www.therio.org](http://www.therio.org)
10. Chenoweth PJ. Bull breeding soundness, semen evaluation and cattle productivity. *Anim Reprod Sci* 2016;169:32-36. <https://doi:10.1016/j.anireprosci.2016.03.001>
11. Menegassi SRO, Barcellos JOJ, Lampert V do N, Borges JBS, Peripolli V. Bioeconomic impact of bull breeding soundness examination in cow-calf systems. *Rev Bras Zootec* 2011;40(2):441-447. <https://doi:10.1590/S1516-35982011000200028>
12. Wiltbank JN, Parish NR. Pregnancy rate in cows and heifers bred to bulls selected for semen quality. *Theriogenology* 1986;25(6):779-783. [https://doi:10.1016/0093-691X\(86\)90093-2](https://doi:10.1016/0093-691X(86)90093-2)
13. Fitzpatrick LA, Fordyce G, McGowan MR, et al. Bull selection and use in northern Australia Part 2. Semen traits. *Anim Reprod Sci* 2002;71:39-49. [https://doi:10.1016/S0378-4320\(02\)00024-6](https://doi:10.1016/S0378-4320(02)00024-6)
14. Coulter GH, Kozub GC. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. *J Anim Sci* 1989;67(7):1757-1766. <https://doi:10.2527/jas1989.6771757x>

15. Farin PW, Chenceth PJ, Tomky DF, Ball L, Pexton JE. Breeding soundness, libido and performance of beef bulls mated to estrus synchronized females. *Theriogenology* 1989;32(5):717-725.
16. Barth AD. Review: The use of bull breeding soundness evaluation to identify subfertile and infertile bulls. *Animal* 2018;12:s158-s164. <https://doi.org/10.1017/S1751731118000538>
17. Barth AO. Case-based studies of infertility in bulls. *Proc Am Assoc Bov Pract*, 2012;45:50-59. <https://doi.org/10.21423/aabpro20123871>
18. Hopper RM. *Bovine Reproduction*. 2nd ed. Wiley Blackwell; 2021.
19. Hopper RM. *Bovine Reproduction*. 1st ed. Wiley Blackwell; 2015.
20. Miller DM, Hrudka F, Cates WF, Mapletoft RJ. Infertility in a bull with a nuclear sperm defect: A case report. *Theriogenology* 1982;17(6):611-621. [https://doi.org/10.1016/0093-691X\(82\)90059-0](https://doi.org/10.1016/0093-691X(82)90059-0)
21. Fernandes CE, Dode MAN, Pereira D, Silva AEDF. Effects of scrotal insulation in Nelore bulls (*Bos taurus indicus*) on seminal quality and its relationship with in vitro fertilizing ability. *Theriogenology* 2008;70(9):1560-1568. <https://doi.org/10.1016/j.theriogenology.2008.07.005>
22. Saacke RG, Bame J, Vogler CJ, Nadir S, Mullins J. Association of sperm nuclear vacuoles (craters) with failure of sperm to sustain embryonic development after fertilization in cattle. *J Anim Sci* 1992;70(Suppl. 1:256 abst.):256.
23. Saacke RG. Sperm morphology: Its relevance to compensable and uncompensable traits in semen. *Theriogenology* 2008;70(3):473-478. <https://doi.org/10.1016/j.theriogenology.2008.04.012>
24. Barth AD. Sperm accumulation in the ampullae and cauda epididymides of bulls. *Anim Reprod Sci* 2007;102(3-4):238-246. <https://doi.org/10.1016/j.anireprosci.2006.11.005>
25. Barth AD, Bowman PA. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. *Can Vet J* 1994;35:10.
26. Pilip R, Del Campo MR, Barth AD, Mapletoft RJ. In vitro fertilizing characteristics of bovine spermatozoa with multiple nuclear vacuoles: A case study. *Theriogenology* 1996;46(1):1-12. [https://doi.org/10.1016/0093-691X\(96\)00136-7](https://doi.org/10.1016/0093-691X(96)00136-7)
27. Thundathil J, Palasz AT, Barth AD, Mapletoft RJ. Fertilization characteristics and in vitro embryo production with bovine sperm containing multiple nuclear vacuoles. *Mol Reprod Dev* 1998;50(3):328-333. [https://doi.org/10.1002/\(SICI\)1098-2795\(199807\)50:3<328::AID-MRD9>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1098-2795(199807)50:3<328::AID-MRD9>3.0.CO;2-L)
28. Coulter GH, Oko RJ, Costerton JW. Incidence and ultrastructure of "crater" defect of bovine spermatozoa. *Theriogenology* 1978;9(2):165-173. [https://doi.org/10.1016/0093-691X\(78\)90147-4](https://doi.org/10.1016/0093-691X(78)90147-4)

