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Assessment of Novel Semen Evaluation Technologies and Breed Comparisons in Yearling Beef Bulls

A. R. Hartman
Kansas State University, arhartma@k-state.edu

I. E. Batey
Kansas State University, iebatey@k-state.edu

D. M. Grieger
Kansas State University, dgrieger@ksu.edu

See next page for additional authors

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Authors

A. R. Hartman, I. E. Batey, D. M. Grieger, and K. E. Fike

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Abstract

The objective of this study was to evaluate correlations of sperm quality assessments and breed comparisons as observed during yearling beef bull breeding soundness exams (BSE). Ejaculates were collected via electroejaculation from yearling Charolais (n = 23) and Angus (n = 23) bulls as part of BSE. One veterinarian conducted BSE, and one technician conducted sperm quality assessments. Additional sperm motility analysis was conducted with the iSperm. Ejaculates meeting minimum thresholds for passing a BSE were subjected to flow cytometry. Pearson's correlation coefficients were determined, and breed comparisons were made using GLIMMIX in SAS. The iSperm analyzer gross and progressive motilities were correlated ($r = 0.30$; 0.38; $P < 0.001$) with the progressive motility assessed by the technician. Neither iSperm ($P = 0.26$) nor visual assessment ($P = 0.66$) of sperm motility differed between breeds. Bull breed did not influence total percentage of viable cells ($P = 0.83$), percentage of viable cells with intact acrosomes ($P = 0.83$), or percentage of live sperm cells with positive reactive oxygen species (ROS) status ($P = 0.92$). Sperm from Charolais bulls (31.1% ± 3.35) tended ($P = 0.10$) to have greater percentage of positive mitochondrial energy potential as compared with Angus bulls (17.6% ± 3.35). Percentage of live spermatozoa with negative ROS status was moderately correlated with the percentage of spermatozoa exhibiting secondary abnormalities ($r = 0.33$; $P = 0.02$). Percentage of live spermatozoa with disrupted acrosomes was strongly correlated ($r = 0.66$; $P < 0.001$) with percentage of live spermatozoa with negative ROS. Percentage of live spermatozoa with positive ROS status was correlated ($r = 0.58$; $P < 0.001$) with percentage of spermatozoa with active mitochondrial membranes. Sperm motility data assessed by the technician and iSperm data are positively correlated, offering producers an on-farm evaluation tool. Though the bull breed had little influence on sperm quality assessments, negative ROS status appears to impair sperm health and function.

Introduction

There is currently no definitive test that evaluates a bull's fertility. Current semen evaluation techniques include evaluation of motility and morphology, and although these are insightful tools for bull fertility, they are not definitive fertility tests and are highly subjective. In recent years many new fertility markers have been identified that provide an objective analysis of spermatozoa and an insight into identifying sub-fertile bulls. The use of flow cytometry is a method of analysis that works through excitation and

emissions spectra and aids in the detection of fertility markers. Specific colors bind to sperm based on the functional status of the individual cells. In recent years development of assays that target components known to be essential to bull fertility has advanced our knowledge of sperm functional statuses (Bucher et al., 2019). Reactive oxygen species are endogenous, highly reactive, oxygen- and nitrogen-bearing molecules that can be found throughout the body (Krumova and Gonzalo, 2016). In semen, ROS affects spermatozoa characteristics, including mitochondrial membrane potential, acrosomal integrity, and structural abnormalities that can influence spermatozoa function. In bulls, the presence of ROS in semen has been shown to have a direct impact on the function of spermatozoa as well as a relationship to bull fertility (Kumaresan et al., 2017; Leite et al., 2022).

Use of on-farm technologies for semen evaluation in cattle is limited; however, a product called the iSperm offers hope that these technologies may become more accessible. The iSperm works through the camera on an iPad Mini and is a relatively easy-to-use, affordable, and portable semen analysis device. This device has been validated in equines and canines but not in the bovine (Moraes et al., 2019; Dini et al., 2019; Domain et al., 2022).

Bull breed influences bull fertility. Barth and Waldner (2002) found that Angus bulls were more likely to pass a BSE than Charolais bulls, and Brito et al. (2002) found differences in motility and ejaculate concentrations among *Bos taurus* bulls when compared to *Bos indicus* but proposed no suggested explanation for these differences. Others have shown that breed influences motility, morphology, concentration, and volume of ejaculates (Hartman, 2021). As the need for a better understanding of bull fertility grows, the influence of breed has been largely understudied.

Experimental Procedures

Ejaculates were collected via electroejaculation on one of three consecutive days from Angus and Charolais yearling bulls (403 ± 11 days of age; n = 46) as part of a BSE. One veterinarian conducted all BSE, and ejaculates were evaluated by one technician. Ejaculates were diluted in BoviFree to accomplish a 1:5 dilution based on manufacturer recommendations, and an additional sperm motility and concentration analysis was conducted with the iSperm analyzer. Ejaculates meeting minimum thresholds for passing a BSE were diluted to 70 million cells/mL using BoviFree and sent overnight for flow cytometry evaluation. Flow cytometry assays included acrosome and cell membrane integrity, mitochondrial energy potential, and oxidation status.

Data for analysis comparisons were assessed using Pearson's correlation coefficients in SAS. The GLIMMIX procedure of SAS with bull as experimental unit, bull breed as the main effect, and collection date as a random variable was used to assess potential differences in sperm quality variables between breeds.

Results and Discussion

Percentage of live spermatozoa with positive ROS status was correlated ($r = 0.53$; $P < 0.001$) with percentage progressive motility (Table 1). Percentage of live spermatozoa with negative ROS status was moderately correlated with percentage spermatozoa exhibiting secondary abnormalities ($r = 0.33$; $P = 0.02$) and tended to be lowly correlated ($r = 0.28$; $P = 0.06$) with percentage spermatozoa exhibiting primary abnor-

malities. Percentage of live spermatozoa that had disrupted acrosomes was strongly correlated ($r = 0.66$; $P < 0.001$) with percentage live spermatozoa with negative ROS and moderately negatively correlated ($r = -0.31$; $P = 0.04$) with percentage live spermatozoa with positive ROS. These results for the relationship between ROS and acrosome integrity are similar to those observed by Kumaresan et al. (2017). The percentage of live spermatozoa with positive ROS status was correlated ($r = 0.58$; $P < 0.001$) with the percentage of spermatozoa with active mitochondrial membranes. Leite et al. (2022) found that when there were increased levels of ROS and impaired mitochondrial membranes, this often resulted in lower fertility in bulls, which supports our findings of this relationship. Live spermatozoa with positive ROS were strongly correlated ($P < 0.001$) with live spermatozoa ($r = 0.94$) and live spermatozoa with intact acrosomes ($r = 0.92$). Bucher et al. (2019) found that evaluation of the viability, acrosomal status, and mitochondrial function of cryopreserved bovine sperm could be predictive of sperm functional status. Thus, our data confirm previous research showing detrimental effects of ROS on spermatozoa function.

Both gross and progressive motilities were significantly correlated ($r = 0.30$; 0.38; $P < 0.001$) to the technician's assessment of progressive motility. These results are similar to previous research comparing assessments by technicians to the iSperm when evaluating stallion semen (Moraes et al., 2019; Dini et al., 2019). Our results are comparable to previous results when validating the iSperm for canine use (Domain et al., 2022).

Neither iSperm ($P = 0.26$) nor visual assessment ($P = 0.66$) of sperm motility differed among breeds (Table 2). Bull breed did not influence the total percentage of viable cells ($P = 0.83$) or viable cells with intact acrosomes ($P = 0.83$). When evaluating oxidation status by measuring reactive oxygen species, the bull breed did not influence ($P = 0.92$) the percentage of live sperm cells with positive reactive oxygen species status. There was a tendency ($P = 0.10$) for a greater percentage of sperm from Charolais bulls (31.1% ± 3.35) to have positive mitochondrial energy potential as compared with Angus bulls (17.6% ± 3.35). These results differ from those found by Barth and Waldner (2002), who saw differences in motility in relation to BSE between Angus and Charolais bulls. In our study, the bull breed appears to have little influence on sperm quality assessments among yearling bulls meeting the threshold requirements for passing BSE.

Implications

Technician and iSperm assessment of sperm motility data are positively correlated, offering producers an on-farm evaluation tool. Though the bull breed has little influence on sperm quality assessments, negative ROS status in sperm appears to impair sperm health and function.

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We greatly appreciate Fink Beef Genetics for providing the bulls for this project, Sharon Tucker for performing the semen evaluation, Blue Rapids Veterinary for performing the BSE, and the crew at Fort Keogh Livestock & Range Research Laboratory for performing the flow cytometry analysis.

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Table 1. Pearson's correlation coefficients of sperm attributes from ejaculates collected following breeding soundness exams

Item	% Live negative ROS spermatozoa[1]	% Live positive ROS spermatozoa[2]
	r (*P*-value)	
% Primary abnormalities[3]	0.28 (0.06)	-0.15 (0.33)
% Secondary abnormalities[4]	0.33 (0.02)	-0.23 (0.12)
% Progressive motility[5]	-0.27 (0.10)	0.53 (<0.001)
% Live with intact acrosome[6]	-0.16 (0.29)	0.92 (<0.001)
% Live with disrupted acrosome[7]	0.66 (<0.001)	-0.31 (0.04)
% Live[8]	-0.19 (0.22)	0.94 (<0.001)
% Polarized[9]	0.03 (0.84)	0.58 (<0.001)

[1]Percentages of spermatozoa from ejaculate with an intact cell membrane that have negative reactive oxygen species (ROS).
[2]Percentages of spermatozoa from ejaculate with an intact cell membrane that have positive ROS.
[3]Percentage of spermatozoa from ejaculate exhibiting primary abnormalities.
[4]Percentage of spermatozoa from ejaculate exhibiting secondary abnormalities.
[5]Percentages of spermatozoa from ejaculate that are progressively motile.
[6]Percentage of spermatozoa from ejaculate with an intact cell membrane and acrosome.
[7]Percentage of spermatozoa from ejaculate with an intact cell membrane and disrupted acrosome.
[8]Percentage of spermatozoa from ejaculate with an intact cell membrane.
[9]Percentage of spermatozoa from ejaculate with polarized mitochondrial membranes.

Table 2. Sperm quality assessments using visual analysis and flow cytometry on ejaculates from Angus and Charolais breeds of yearling bulls meeting BSE threshold requirements

	Least squares mean ± Standard error of mean		
Factor	Angus n = 23	Charolais n = 23	*P*-value of factor
Bull age, days	402.9 ± 2.36	403.3 ± 2.36	0.90
Semen characteristic			
Technician progressive motility,[1] %	43.7% ± 1.69	47.39 ± 1.69	0.26
iSperm progressive motility,[2] %	50.1 ± 2.26	47.8 ± 3.18	0.66
iSperm gross motility,[2] %	71.6 ± 2.78	70.5 ± 2.78	0.82
Cells live and viable,[3] %	42.3 ± 3.95	43.6 ± 3.95	0.83
Cells live with intact acrosome,[4] %	41.5 ± 3.40	42.6 ± 3.40	0.83
Cells viable with positive reactive oxygen species,[5] %	29.1 ± 3.52	28.5 ± 3.52	0.92
Active mitochondrial potential,[6] %	17.6 ± 3.35	31.1 ± 3.35	0.10

[1]Ejaculate gross motility was analyzed by a single veterinarian as a part of a breeding soundness exam.
[2]Progressive and gross motility of each ejaculate were analyzed using the iSperm software and manufacturer recommendations.
[3]Percentages of live and viable cells were determined by flow cytometry using the Invitrogen Live/Dead sperm viability kit.
[4]Percentages of live cells with intact acrosomes and sperm membrane integrity were determined by flow cytometry using the IMV Technologies acrosome and sperm membrane integrity assay.
[5]Percentages of viable cells with a positive reactive oxygen species were determined by flow cytometry using the IMV Technologies Easy Kit 3: Oxidation molecule D assay.
[6]Percentages of spermatozoa with active mitochondrial potential were determined by flow cytometry using IMV Technologies Easy Kit 2: Mitochondrial activity assay.