Final Technical Report

FCEB Project #48

Finding new sperm traits that more accurately estimate bull fertility for semen analyses

Priority Area: Reproductive efficiency – improve pregnancy in cows and heifers

Final Report

Principle Investigator: Brad Daigneault, PhD

1. Specific Aims

The overall goal of this proposal is to determine if inclusion of new bioenergetic sperm traits with current bull semen analyses can be used to more accurately predict bull fertility.

Our long-term goal is to integrate these new analyses with current breeding soundness exams to more accurately predict bull fertility rather than relying on motility and morphology. Data generated from this proposal will directly improve the beef cattle industry by strengthening the predictive value of sperm analyses to increase conception rates. Our published data demonstrate the usefulness of the Seahorse assay to identify these new mitochondrial indices of sperm. We aim to determine if the application of including bioenergetic sperm indices in semen evaluation is useful to determine differences in bull fertility that are not detected by motility and morphology.

Objectives

1. Determine if sperm from bulls of different fertility have divergent bioenergetic profiles.
2. Develop statistical models that more accurately predict bull fertility by inclusion of new mitochondrial bioenergetics traits with traditional sperm motility kinematic parameters.

2. Approach

Objective 1: Determine if sperm from bulls of different fertility have divergent bioenergetic profiles

Experimental Design & Methods -Frozen bull sperm from ten bulls ranging in fertility (-5 to +5% Estimated Relative Conception Rate (ERCR)), and with similar post-thaw motility (±5%) will be isolated by Percoll centrifugation and extended in a patent-pending to 37°C for all analyses[1]. Ejaculates will be divided and evaluated for 1) traditional motility parameters using the CASA (total motility, progressive motility, average path velocity, straight-line velocity, curvilinear velocity, amplitude of lateral head displacement, beat cross-frequency) and 2) bioenergetics using the Seahorse instrument (OCR, ATP, oxidative stress and ECAR). A minimum of 1000 spermatozoa will be analyzed at each time point for motility and 5 x 10^6 total spermatozoa for mitochondrial assays. For mitochondrial assessment, sperm suspensions (160µL) will be added to wells of a Seahorse XF^{e}24 culture plate and incubated at 37°C following our recently published protocol[1]. Soluble sensors will be added to a Seahorse sensor cartridge to directly quantify metabolic capacity. Mitochondrial toxicants (oligomycin, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), antimycin) will be serially injected into each well to obtain measurements that include basal respiration, oligomycin-induced ATP-linked respiration, FCCP-induced maximal respiration, spare capacity, proton leak, and non-mitochondrial respiration using the Seahorse XF(e)24 Extracellular Flux Analyzer (Agilent Technologies). Sperm will then be maintained at 23°C in a patent-pending medium for 24 hr at room temperature and then warmed to 37°C to repeat CASA and Seahorse measurements. All experiments will include 3 independent replicates, yielding 9 technical replicates for Seahorse and 15 independent fields for CASA. Specific measurables at 0 and 24 hr include:

1) Kinematics – CASA: TM, PM, VAP, VCL VSL, VCL, ALH, BCF (defined below)

2) Bioenergetics – Seahorse: OCR, ECAR, ATP, Basal Respiration (BR), MAX respiration, Proton Leak, and non-mitochondrial respiration

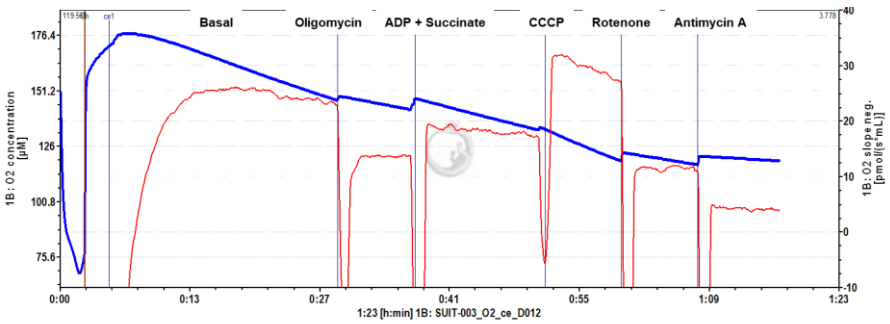
3) Mitochondrial Membrane Potential (MMP) – JC1: High, Medium, Low (previously optimized)[1]

4) Sperm morphology – Primary & Secondary defects

3. Final Report

A total of ten commercial bulls with in-house fertility indices were donated from Select Sires for these experiments. The bulls were evaluated for multiple sperm kinematics by CASA (Computer Assisted Sperm Analyses) immediately after thawing samples (T0) and then maintained at ambient temperature for analyses 24 h later (T24). Sperm samples were also subjected to novel bioenergetic analyses at respective time points using an Oroboros machine to generate multiple indices that may be related to sperm function. Initial experiments were conducted to validate the use of Oroboros for determining sperm bioenergetics. We have concluded that the Oroboros may offer superior analyses of sperm bioenergetics compared to Seahorse instrumentation because sperm remain unbound (motile) and thus measurements are more physiologically relevant (**Figure 1**).

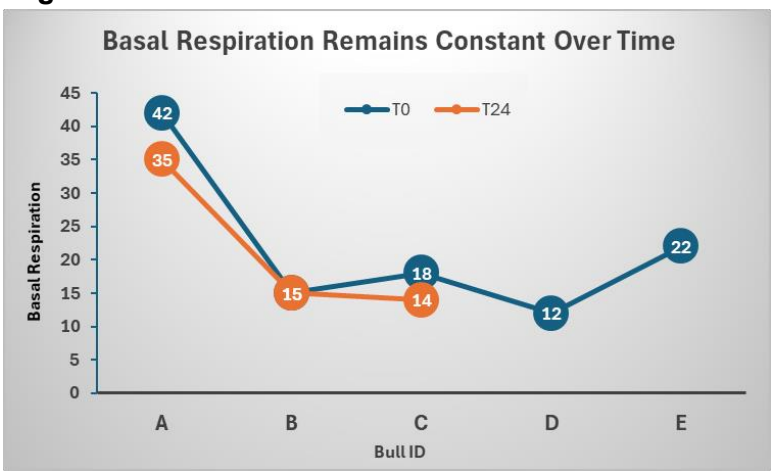
Figure 1. Below are outputs for Oroboros, demonstrating feasibility of using 2 million total cells, which may be useful for sexed sperm. Note that the data below are using 50% less sperm than above. These data demonstrate that Oroboros may be used to measure sperm bioenergetic indices.



Chemical	Function	Mitochondrial respiration
No chemical	Basal respiration	→
Oligomycin	ATP synthase inhibidor	↓
ADP + succinate	Mitochondrial stimulation	→ or ↑
CCCP	Mitochondrial uncoupler	↑
Rotenone	Complex I inhibitor	↓
Antimycin A	Complex III inhibitor	↓

We aimed to determine if the basal respiration of sperm changed after 24 h to determine the feasibility of using these data as early measurements of sperm function that may be useful to include in a fertility model. As seen in Figure 2, basal respiration of sperm after 24 h does not change noticeably, suggesting that sperm motility measurements captured for semen analyses may not be tightly correlated to basal respiration.

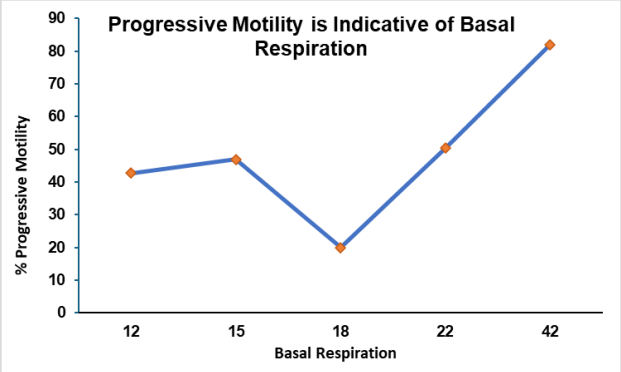
Figure 2

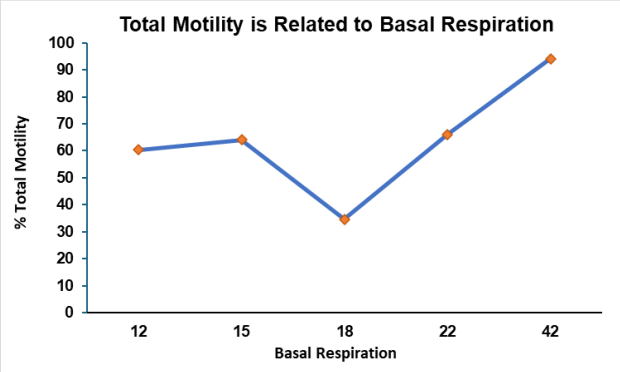


Key Industry Important Findings

1. Basal Respiration is related to sperm motility

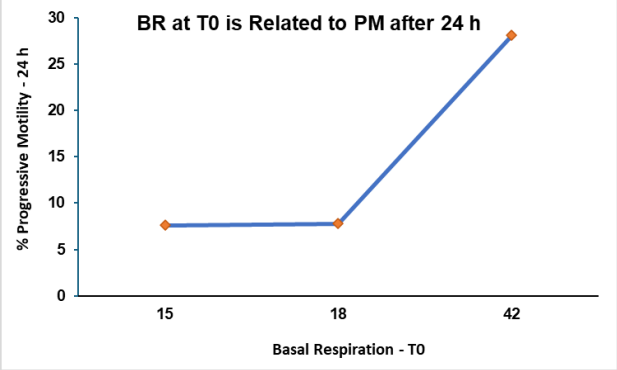
Our laboratory has determined that low basal respiration of sperm may be correlated with lower progressive and total motility. These findings are important because the data suggest that cattle inseminated with sperm of lower basal respiration may not be suitable for protocols that include increased insemination to ovulation intervals because sperm may not have the mitochondrial capacity to reach the site of fertilization.

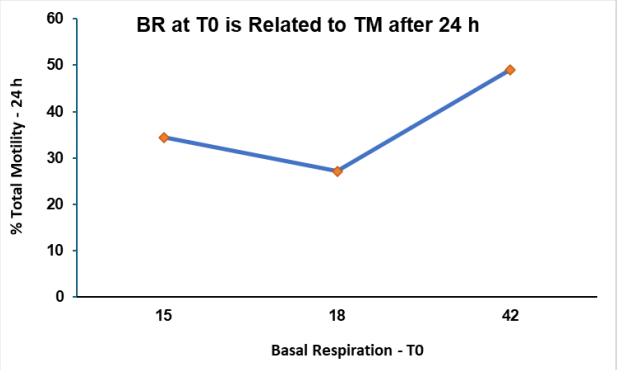




2. Basal Respiration of sperm at T0 is correlated to total and progressive motility after 24 h

By measuring the basal respiration of frozen-thawed sperm immediately after thawing we have preliminary data to suggest that these numbers can be used to estimate the motility of samples after 24 h. This finding is important because it suggests that perhaps this novel metric of sperm function may be more useful than traditional motility measurements that are used to assess sperm quality prior to insemination.





Summary and Conclusion

The project provides preliminary evidence that measuring the bioenergetic capacity of frozen-thawed bull sperm provides novel indices that may be utilized as additional or superior measurements of sperm assessment. Additional bulls are currently being added to those presented within this report. We have recently obtained the fertility indices of these bulls and when the data are collected, will continue with the statistical analyses outlined above to determine the utility of adding these novel indices into BSE exams. We

anticipate that these experiments will provide industry and veterinary professionals with additional tools to select superior sperm for optimized artificial insemination protocols that may improve current semen selection techniques.

*We would like to thank the FCEB for supporting this project and anticipate an impactful publication upon further analyses of the current work.

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Invoice Period:	05/01/2024 - 07/31/2024
Principal Investigator:	Daigneault,Bradford William
Award Begin Date:	10/30/2023
Award End Date:	07/31/2024
UF FEIN:	59-6002052

Sponsor Award ID:	48
Award Title:	Finding new sperm traits that more accurately estimate bull fertility for semen analyses
Award Amount:	$39,507.00

Invoice #	I000130492
UF Award #	AWD15822
Primary Project #	P0325166
Primary Department:	60090000
Current Invoice Amount:	$30,233.71

Description	Current	Cumulative
Personnel - Salary	$18,647.47	$24,336.86
Personnel - Fringe Benefits	$2,191.03	$2,862.34
Materials and Supplies	$4,296.26	$5,632.96
Other Expenses	$939.84	$1,859.58
Direct Cost	$26,994.34	$34,691.74
Facilities and Administrative Costs	$3,239.37	$4,163.07
Total	$30,233.71	$38,854.81

For billing questions, please call 352.392.1235
Torres,Kannika S kannika@ufl.edu
Please reference the UF Award Number and Invoice Number in all correspondence

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Kannika Torres

Certifying Official

Payment History	
Cumulative Invoices:	$38,854.81
Payments Received:	$8,621.10
Outstanding Balance:	$30,233.71
Note: Outstanding balance includes current invoice amount	

FOR UF USE ONLY Additional Projects: N

Project ID	Deptid	Department Name	Current	Cumulative
P0325166	60090000	AG-ANIMAL SCIENCES	$30,233.71	$38,854.81