

**Final Technical Report**  
**FCEB Project #9**

## Final Report to the Florida Cattle Enhancement Board

### EFFECT OF OVER CONDITIONED BULLS ON FERTILITY

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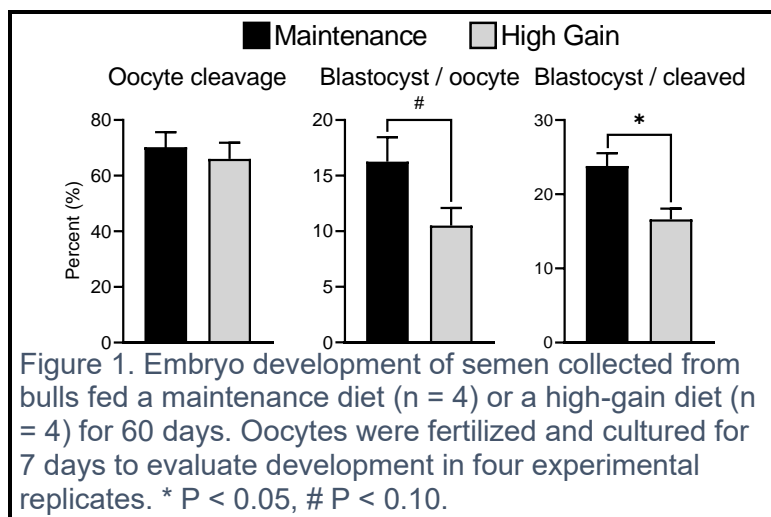
Co-principal investigators: Nicolas DiLorenzo, Dept of Animal Sciences, University of Florida. Pedro Fontes, Dept of Animal and Dairy Science, University of Georgia

Program Area Priority: This project addresses the Florida Cattle Enhancement Board priorities of Reproduction Efficiency, specifically “Improving pregnancy attainment of cows and heifers” and “Understand early embryonic and in utero losses.”

#### SPECIFIC AIMS

Most reproduction in Florida beef cattle is facilitated through live cover using bulls. Therefore, the fertility of these bulls is of paramount importance to the economic viability to the Florida beef industry. Currently, the only estimate of a young bull’s fertility is a breeding soundness exam that does not provide an accurate measure of fertility for that animal. In the beef industry, young bulls are often fed to achieve high rates of body weight gain and fat deposition to increase marketability at sale. However, the consequence of this early over conditioning on fertility is yet to be determined. We have shown that semen collected from mature bulls fed a high-gain diet (designed to induce a 4 lb average daily gain) for 60 days have a reduced ability to produce blastocyst stage embryos after in vitro fertilization compared to mature bulls fed a maintenance diet (designed to maintain body weight; Fig. 1). Interestingly, semen parameters (motility and morphology) were not different when evaluated using conventional breeding soundness examination techniques. This suggests that over conditioned mature bulls have compromised fertility and current methods utilized to estimate bull fertility utilized in the beef industry fail to identify this decrease in fertility. Noteworthy, this study was performed to gain an insight into the impact of highly anabolic diets on bull fertility. The question remains, what is the impact of over conditioning young, growing bulls on fertility? We hypothesize that over

conditioning of young bulls will compromise fertility. We aim to 1) correlate semen parameters of young bulls from the University of Florida’s NFREC Bull Test Program and University of Georgia’s Bull Evaluation Program with growth and fatness; and 2) evaluate the fertility of young bulls grown with either a moderate or high gain diet using in vitro fertilization. The young bulls used in these



studies have already been developed at the University of Georgia under the supervision of Dr Pedro Fontes. These studies will allow us to better understand the importance of bull nutrition on subsequent fertility, potentially changing the way we think about raising bulls. The idea that the diet of young bulls can impact future fertility is of particularly interest to the Florida beef industry, where young bulls are often fed to achieve high rates of body weight gain and fat deposition for maximal profit at sale. This project addresses the Florida Cattle Enhancement Board priorities of “Improving pregnancy attainment of cows and heifers” and “Understanding early embryonic and in utero losses.”

## **APPROACH**

The studies outlined here will demonstrate the impact of excessive fat deposition and accelerated growth on subsequent fertility in young bulls.

Aim 1: Correlate semen parameters of young bulls at the University of Florida NFREC bull test with growth and fatness. Approximately 100 young bulls finish the 112-day University of Florida NFREC Florida Bull Test each year. Bulls consist of various breeds and arrive at the facility weighing approximately 870 lbs. At the completion of the test, each bull has average daily gain and weight per day of age calculated, in addition to a breeding soundness exam. We will take advantage of this unique population of bulls to correlate detailed semen characteristics with growth and fatness of bulls. In addition to average daily gain and weight per day of age for each bull, we will also measure subcutaneous fat, rump fat, intramuscular fat, and ribeye area on day 0 and day 112 of the test using carcass ultrasound. Semen collected from each bull at day 112 of the test will be evaluated by detailed semen analysis that includes computer-assisted semen analysis (CASA), sperm DNA integrity, oxidative damage, and mitochondrial membrane potential. These tests are far beyond the parameters normally evaluated during a breeding soundness exam. Collectively, these data will allow us to correlate various growth and fatness parameters of young bulls with a detailed assessment of semen, providing a valuable novel insight into the effects of growth and fatness on sperm quality.

Aim 2: Evaluate the fertility of young bulls grown with either a moderate or high gain diet using in vitro fertilization. Forty-four half sibling bulls have been developed at the University of Georgia’s Eatonton Beef Research Unit. At the age of 13 months these bulls were blocked body weight and subcutaneous fat before being randomly assigned to either a moderate gain (2.5 lb of average daily gain) or high gain (4.0 lb of average daily gain) diet for 120 days (17 months of age). Bulls fed the moderate gain diet achieved an average daily gain of 2.7 lb/day, while bulls fed the high gain diet achieved an average daily gain of 4.4 lb/day. At the end of the feeding period all bulls were evaluated using a standard breeding soundness exam, semen was collected, extended and frozen. This semen will be used to evaluate bull fertility using in vitro fertilization. Oocytes will be purchased from Simplot and shipped overnight to the University of Florida in oocyte maturation medium. Groups of 50 oocytes will be subjected to in vitro fertilization using semen from each of the 44 collected bulls. Following fertilization, oocytes will be cultured for a total of 7.5 days in commercial embryo culture medium (IVF Biosciences). The rate

of fertilization will be estimated by oocyte cleavage at day 3, and the stage and quality of embryos will be evaluated at day 7.5, providing information on blastocysts development, embryo arrest and embryo death. Each bull will be used in the in vitro fertilization system a total of three times to evaluate the bull's capacity to develop high quality, blastocyst stage embryos. Resultant blastocyst stage embryos will be preserved for future analysis using immunohistochemistry and gene expression to evaluate embryo quality. This approach will allow us to investigate the impact of over conditioning young bulls in unprecedented detail, helping Florida cattlemen unravel the ongoing industry debate regarding the ideal nutritional program for bull development.

## **RESULTS**

The project was initiated in Spring 2024. A total of 29 bulls (n = 15 moderate gain; n = 14 high gain) were used for in vitro production of embryos. A total of 3,458 oocytes have been subjected to fertilization over six independent experimental replicates with each bull being used three times. These experiments have yielded in excess of 800 blastocyst stage embryos (36.0% blastocyst rate). While the experimentation to generate embryos has concluded we are still analyzing the data obtained from these intensive experiments; however, the moderate gain bulls have an average blastocyst development rate of 37.7% and the high gain bulls have an average blastocyst development rate of 34.2% which suggests that the developmental potential of high gain bulls is lower than moderate gain bulls. In addition to the main developmental outcomes of blastocyst development, we are also quantifying specific cell biology milestones of blastocysts to determine cell number, differentiation, and apoptosis.

In addition to the embryos created above for the specific award, we expanded our experimentation to evaluate the morphokinetics of embryo development by using time lapse embryo culture to carefully quantify the developmental milestones of embryo development of individual bulls. These studies have been completed and data analysis is ongoing due to the large amount of acquired information. In addition, we have also transferred blastocyst stage embryos into recipients' cows that were then flushed at day 15 of development to assess embryo elongation. To date, a total of 96 embryos have been transferred to 40 recipients to evaluate the effect of bull nutrition on embryo elongation. These studies are in addition to those outlined in the initial award and are continuing based on the positive results acquired in the original aims of the proposal.

The plan to evaluate bulls at the NFREC bull test had to be changed due to the time of funding release (the test had already been completed). However, we will be able to conduct the proposed evaluation at the Georgia bull test instead and this has now been planned.

**PLEASE REMIT TO:**

UNIVERSITY OF FLORIDA BOARD OF TRUSTEES  
 Contracts & Grants  
 PO Box 931297  
 Atlanta, GA 31193-1297

Invoice Date: 08/15/2024  
 Invoice Period: 05/01/2024 - 07/31/2024  
 Principal Investigator: Bromfield, John James  
 Award Begin Date: 10/30/2023  
 Award End Date: 07/31/2024

**SPONSOR:**

FL CATTLE ENHANCEMENT BOARD  
 P.O. Box 421929  
 Kissimmee FL 34742-1929  
 United States

UF FEIN: 59-6002052

Sponsor Award ID: 9  
 Award Title: Effect of Over Conditioned Bulls on Fertility  
 Award Amount: \$54,622.00

<b>Invoice #</b>	I000130496
<b>UF Award #</b>	AWD15788
<b>Primary Project #</b>	P0324556
<b>Primary Department:</b>	60090000
<b>Current Invoice Amount:</b>	\$4,676.14

Description	Current	Cumulative
Personnel - Salary	(\$15.41)	\$13,067.95
Personnel - Fringe Benefits	(\$1.83)	\$1,541.95
Other Expenses	\$4,192.20	\$30,045.00
Direct Cost	\$4,174.96	\$44,654.90
Facilities and Administrative Costs	\$501.18	\$5,358.82
<b>Total</b>	\$4,676.14	<b>\$50,013.72</b>

For billing questions, please call 352.392.1235  
 Torres, Kannika S [kannika@ufl.edu](mailto: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*

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 Certifying Official

Payment History	
Cumulative Invoices:	\$50,013.72
Payments Received:	\$45,337.58
Outstanding Balance:	\$4,676.14
Note: Outstanding balance includes current invoice amount	

FOR UF USE ONLY			Additional Projects: N	
Project ID	Deptid	Department Name	Current	Cumulative