UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

GUILHERME HENRIQUE GEBIM POLIZEL

Evaluation of sexual, tissue and morphological development genes of Nellore cattle submitted to fetal programming

Pirassununga
2021

GUILHERME HENRIQUE GEBIM POLIZEL

Evaluation of sexual, tissue and morphological development genes of Nellore cattle submitted to fetal programming

Versão Corrigida

Dissertação apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do título de Mestre em Ciências do programa de Pós-graduação em Biociência Animal.

Área de Concentração: Biologia molecular e bioinformática

Orientador: Prof. Dr. Miguel Henrique de Almeida Santana

Pirassununga

2021

Ficha Catalográfica

Ficha catalográfica elaborada pelo
Serviço de Biblioteca e Informação, FZEA/USP,
com os dados fornecidos pelo(a) autor(a)

Polizel, Guilherme Henrique Gebim

P769e Evaluation of sexual, tissue and morphological development genes of Nellore cattle submitted to fetal programming / Guilherme Henrique Gebim Polizel ; orientador Miguel Henrique de Almeida Santana. -- Pirassununga, 2021.

96 f.

Dissertação (Mestrado - Programa de Pós-Graduação em Biociência Animal) -- Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo.

1. Ciências ômicas. 2. Desenvolvimento muscular.3. Gado de corte. 4. Nutrigenética. 5. Programação fetal. I. Santana, Miguel Henrique de Almeida, orient. II. Título.

DEDICATÓRIA

Todas as minhas conquistas, que em parte estão nesta dissertação, só foram possíveis graças ao apoio de minha família, que me estimularam a sempre estudar e atingir meus objetivos com esforço e trabalho duro. Portanto, este trabalho é dedicado aos meus pais, Cibele Cristina Cardoso dos Santos Polizel e José Moacyr Polizel, e meu irmão Felipe José Gebim Polizel.

AGRADECIMENTOS

À minha família, que sempre esteve ao meu lado fornecendo todo suporte necessário ao longo da minha jornada como aluno e pesquisador. Em todos os finais de semana juntos que me aconselhavam e ajudavam a manter o foco em momentos mais difíceis desde a graduação até agora, finalizando o mestrado.

Ao meu orientador, Professor Miguel Henrique de Almeida Santana, pela oportunidade de trabalhar junto e confiar em mim como seu primeiro aluno de pós-graduação. Pela amizade, conhecimento e apoio, e frequentemente dizendo “Se vira” para minhas dúvidas e assim estimulando meu crescimento como aluno e ainda mais como pesquisador, aprendendo a contornar problemas e principalmente, resolvê-los. Na maioria das vezes ele já tinha a solução para meu problema, mas como eu aprenderia se ele sempre me fornecesse a resposta para tudo?

Aos Professores e pesquisadores Heidge Fukumasu, Aline S. M. Cesar, José B. S. Ferraz, Ricardo F. Strefezzi, Paulo Fantinato Neto e Mirele D. Poleti pela ajuda nos experimentos e disponibilização de seus equipamentos e laboratórios.

Aos meus amigos da pós-graduação, Tucano, Roberta, Arícia e Kstiga, que além de todos as festas juntos, sempre foram pessoas que pude contar para me ajudar durante esses anos em que estive no mestrado.

Ao GOPEC (Grupo de Ômicas na Pecuária) por todas as discussões e reuniões que contribuíram para a ampliação da bagagem de conhecimentos que eu tinha antes de ingressar na pós-graduação.

A todos os meus amigos que fiz desde que cheguei em Pirassununga, principalmente aos mais próximos: Dispaxo, Marpa, Marica, Tucano, Cunheta e Pinguim. E as repúblicas

Tatu Corrido e SóKanela por todas as festas e troca de experiências nesses 7 anos que estive em Pirassununga.

Aos membros da banca examinadora pelo tempo precioso no julgamento desse trabalho.

A FZEA, ao programa de Biociência Animal e a todos os professores, pesquisadores e funcionários que dele fazem parte e me concederam a oportunidade de estudar e pesquisar em uma das melhores universidades do mundo.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

A Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) por todo o suporte financeiro para a execução desses trabalhos (processo 2017/12105-2) e pela bolsa (processo 2019/02310-3).

"A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê". (Arthur Schopenhauer)

RESUMO

A produção de bovinos de corte brasileiro ocorre em grande parte no Cerrado e com animais da raça Nelore, sendo muito comum as pastagens do gênero *Brachiaria* e, neste cenário, a subnutrição de matrizes bovinas gestantes se mostra presente no país devido à maior exigência por nutrientes neste estágio fisiológico. Consequentemente, a progênie pode ser afetada em sua produtividade e desempenho ao longo da vida. Esse insulto ou estímulo nutricional pré-natal é denominado programação fetal. Dessa forma, esta dissertação teve por objetivo avaliar genes e fenótipos relacionados à precocidade sexual, o desenvolvimento corporal e a diferenciação tecidual de novilhos submetidos à programação fetal durante a fase de recria. Para isto, durante a gestação, 126 vacas da raça Nelore foram divididas em três planos nutricionais sendo que (FP, PP e NP, respectivamente): ⅓ das matrizes receberam suplementação proteico-energética por toda gestação, outro ⅓ das matrizes somente no terço final e ⅓ não receberam esse estímulo nutricional. Durante toda a fase de recria foram coletados dados fenotípicos (peso, medidas ultrassonográficas de carcaça e perímetro escrotal) e material biológico (músculo *Longissimus* e sêmen) para avaliar o efeito das diferentes estratégias de suplementação pré-natal sobre os 64 novilhos analisados. No primeiro capítulo, foi realizado a avaliação da precocidade sexual, características morfológicas e físicas do sêmen e um estudo nutrigenético para avaliar a resposta dos animais ao estímulo nutricional pré-natal. Neste capítulo foi possível observar os efeitos do fenótipo frugal sobre as características reprodutivas e que a programação fetal não exerce efeito sobre a precocidade sexual em novilhos da raça Nelore. Ainda, foi encontrado interação genótipo-ambiente e alguns genes associados com SNPs (polimorfismo de nucleotídeo único) que explicaram mais de 1% da variância genética dos animais entre os diferentes tratamentos. No capítulo 2, foi avaliado a influência da programação fetal sobre os fenótipos (espessura de gordura subcutânea e da picanha, peso, ganho médio diário, área de olho de lombo, área celular muscular e número de células musculares na área de olho de lombo) durante a fase de recria. Neste capítulo foi possível observar que a programação fetal influenciou no peso dos novilhos ao longo da fase de recria, entretanto nas idades avaliadas não mostrou efeito sobre as espessuras de gordura e avaliações histológicas. Além disso, a suplementação proteico-energética pré-natal durante toda gestação mostrou tendência à um melhor ganho médio diário e área de olho de lombo nos novilhos ao longo da fase de recria. No terceiro capítulo, o transcriptoma de 15 novilhos foi analisado para expressão gênica diferencial com o objetivo de avaliar se a programação fetal apresentou influência sobre a expressão gênica muscular dos animais; e eQTL (loci de características quantitativas de expressão) para avaliar quais genes e processos metabólicos estavam relacionados ao background genético dos animais avaliados. Neste capítulo, não foi encontrado diferenças na expressão gênica diferencial entre os tratamentos, entretanto os eQTLs encontrados controlam características relacionadas à programação fetal, o que pode estar encobrindo os possíveis efeitos da nutrição pré-natal. Em suma, esta dissertação traz grandes contribuições para o campo da programação fetal, com estudos pioneiros, contribuindo

para a descoberta de mecanismos associados à nutrição materna e os impactos sobre suas progênies.

Palavras-chave: desenvolvimento muscular; eQTLs; gado de corte; nutrigenética; suplementação pré-natal; transcriptômica.

ABSTRACT

The Brazilian beef cattle production occurs largely in the Cerrado and with Nellore animals, with *Brachiaria* pastures being very common and, in this scenario, the malnutrition of pregnant bovine dams is present in the country due to the greater demand nutrients at this physiological stage. As a result, progeny can be affected in their productivity and lifelong performance. This insult or prenatal nutritional stimulus is called fetal programming. Thus, this thesis aimed to evaluate genes and phenotypes related to sexual precocity, body development and tissue differentiation of young bulls submitted to fetal programming during the rearing phase. For this, during pregnancy, 126 Nellore cows were divided into three nutritional plans (FP, PP and NP, respectively): ⅓ of the dams received protein-energy supplementation for the entire pregnancy, another ⅓ of the cows only in the final third and ⅓ did not receive this nutritional stimulus. During the entire rearing phase, phenotypic data (weight, ultrasound measurements of the carcass and scrotal perimeter) and biological material (*Longissimus* muscle and semen) were collected to assess the effect of different prenatal supplementation strategies on the 64 analyzed bulls. In the first chapter, an assessment of sexual precocity, morphological and physical characteristics of the semen was carried out and a nutrigenetic study was carried out to assess the animals' response to prenatal nutritional stimulus. In this chapter it was possible to observe the effects of the frugal phenotype on reproductive characteristics and that the fetal programming has no effect on sexual precocity in Nellore bulls. Furthermore, genotype-environment interaction and some genes associated with SNPs (single nucleotide polymorphism) that explained more than 1% of the genetic variance of the animals between the different treatments were found. In chapter 2, the influence of fetal programming on the phenotypes (rump and backfat thickness, weight, average daily gain, ribeye area, muscle cell area and number of muscle cells in ribeye area) was evaluated during the rearing phase. In this chapter it was possible to observe that the fetal programming influenced the weight of the bulls during the rearing phase, however at the evaluated ages it did not show any effect on the fat thickness nor histological assessments. In addition, prenatal protein-energy supplementation throughout pregnancy showed a tendency towards better average daily gain and ribeye area in bulls throughout the rearing phase. In the third chapter, the transcriptome of 15 bulls was analyzed for differential gene expression in order to assess whether fetal programming had an influence on the animals' muscle gene expression; and eQTL (expression quantitative trait loci) to assess which genes and metabolic processes were related to the genetic background of the animals evaluated. In this chapter, no differences were found in the differential gene expression between treatments, however the eQTLs found control characteristics related to fetal programming, which may be masking the possible effects of prenatal nutrition. In short, this thesis brings great contributions to the field of fetal programming, with pioneering studies, contributing to the discovery of mechanisms associated with maternal nutrition and the impacts on their offspring.

Key-words: beef cattle; eQTLs; muscle development; nutrigenetics; prenatal supplementation; transcriptomics.

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

(respectively A, B, C and D) from active experiments. Networks A, B, C and D correspond respectively to the 4 processes shown in table 3. Thick cyan lines indicate the fragments of canonical pathways. The genes present in the list of significant trans-eQTL genes are marked with red circles. The legend for each item in this figure is in Additional file 1.

Additional file 1. Quick reference guide for figure 3.

LIST OF TABLES

Chapter 1

Chapter 2

Table 1. Average carcass ultrasound characteristics (BFT, RFT and REA), BW and ADG ± standard error during the entire rearing phase of Nellore young bulls submitted to fetal programming with their respective *P* values.

Chapter 3

Table 1. Ingredients and nutrients content of the matrices supplement.

Table 2. Mineral premix content of the supplement for matrices.

Table 3. Top 4 biological networks with the highest gScore by MetaCore ™ for genes from significant trans-eQTLs.

Table 4. Genes selected from the 4 main significant networks with their respective genetic variants (SNPs). These genes are present in our list of trans-eQTL genes.

Table S1. Sequencing data samples with initial filters and multimapping reads, uniquely mapped and unmapped reads rates.

Table S2. Genes from the differential expression analysis with your respectively p values between the treatments (NP, PP and FP).

Table S3. Significant muscle cis-eQTLs from young Nellore bulls regardless the prenatal treatment submitted.

Table S4. Significant muscle trans-eQTLs from young Nellore bulls regardless the prenatal treatment submitted.

LIST OF ABBREVIATIONS AND ACRONYMS

ADG – Average daily gain

ASZ1 gene – Ankyrin Repeat, SAM And Basic Leucine Zipper Domain Containing 1

ATP – Adenosine triphosphate

BCS – Body condition score

BFT – Backfat thickness

BIF – Beef Improvement Federation

BTA23 – Bos taurus 23 autosome

BTBD9 gene – BTB Domain Containing 9

BW – Body weight

CBRA – Colégio Brasileiro de Reprodução Animal

CFTR – Cystic fibrosis transmembrane conductance regulator

Cis-eQTL – Variants located within 1Mb of the associated gene

CONCEA – National Council for the Control of Animal Experimentation

CP – Crude protein

CpG – Cytosine-phosphate-guanosine

DNA – Deoxyribonucleic acid

eQTL – Expression quantitative trait loci

FDR – False discovery rate

FP – Full programming

FSH – Folicle-stimulating hormone

FTAI – Fixed-time artificial insemination

GEBVs – Genomic estimated breeding values

GO – Gene ontology

IGF-1 – Insulin-like growth fator-1

IL-6 gene – Interleukin 6

JUN gene – JUN Proto-Oncogene

LD – Linkage disequilibrium

LH – Luteinizing hormone

MCA – Muscle cell area

miR-181a gene – MicroRNA 181a-1

NCREA – Number of cells in ribeye area

NDF – Neutral detergent fiber

NF-kB – Nuclear factor kappa B

NP – Not programmed

NPM – Nucleophosmin/Nucleoplasmin family gene

PAG – Pregnancy-associated glycoproteins

piRNA – Piwi-interacting RNA

POU3F2 gene – POU Class 3 Homeobox 2

PP – Partial programming

PPARγ gene – Peroxisome proliferator-activated receptors

QTL – Quantitative trait loci

REA – Ribeye area

RFT – Rump fat thickness

RIN – RNA integrity number

SC – Scrotal circumference

SLC26A8 – Solute Carrier Family 26 Member 8

SNP – Single nucleotide polymorphism

STAT1 gene – Signal Transducer And Activator Of Transcription 1

TAT1 – Testis anion transporter

TDN – Total digestible nutrients

Trans-eQTL – Variants located farther than 1Mb from the associated gene

UGC – Ultrasound Guidelines Council

WNT – WNT gene family

WWOX gene– WW Domain Containing Oxidoreductase

SUMMARY

1. Introduction

According to *World Population Review*, approximately 225 thousand people are added to the world population per day. This population increase generates the need for new technologies and approaches in the production of food that increase its production efficiency, and so that the food supply is compatible with the demand.

The Brazilian herd consists of about 244 million cattle (USDA, 2020), with a large part of this number being on pasture, in extensive production. In this system, the animals are in large pasture areas throughout the year, and thus the climatic effects (scarcity of rain, high temperatures) have a direct influence on the productivity of the animals (SILVA; NASCIMENTO JÚNIOR, 2007). In addition, these factors interfere in the development of forage, leading to a seasonal variation in the edible portion of the pasture and directly affecting the production system of Brazilian beef cattle (PAULINO, 2004). In the case of pregnant cows, who normally remain throughout the drought period in conditions where there is a low supply of forage, it may result in a nutritional deficit in the main stages of fetal development, generating a low-performing animal (DUARTE JÚNIOR et al., 2016).

The aforementioned problem is leading to a greater scientific interest in dams and their impacts on fetal development, pathologies and productive characteristics of the progeny throughout life. Thus, clarifying mechanisms and consequences of fetal programming are essential to determine the long-term effects on growth, physiological functions, health of the offspring and the impact on productive performance and meat quality. (REHFELDT et al., 2011; MULLINIKS et al., 2013).

The term fetal programming is a concept that has been widespread since the 1990s, when the British epidemiologist David Barker began his studies on the relationship between birth weight and the risk of heart disease and diabetes (HALES; BARKER, 1992;

BARKER, 1993). Since then, other studies have been conducted in different species and with different stimuli in the pregnant female, in order to find relationships between the conditions in which the mother is subjected and the phenotype that her progeny presents. This concept can have a great impact on beef cattle, due to the consequences, positive or negative, resulting from different factors that act on pregnancy and affect the offspring.

Some authors, such as LONG et al. (2009), WU et al. (2006), Du et al. (2010) e Mulliniks et al. (2013), have already observed effects of fetal programming in beef cattle, in which maternal malnutrition has led to some consequences, such as: increase in the neonatal mortality rate, changes in body composition and growth, hormonal balance, changes in the development and functioning of organs, including respiratory, cardiovascular and intestinal systems and metabolic functions. However, according to ZAGO et al. (2019) many fetal programming studies show inconclusive results, which may be related to the different diets, breeds and gestational periods evaluated, showing the need for further studies in this field to determine the mechanisms that involve fetal programming.

In this thesis, we have elaborated three articles. The first described the impacts of fetal programming on reproductive characteristics and the effect of nutrigenetics on Nellore bulls during the rearing phase. In the second article, we discuss the effect of fetal programming on the carcass, body weight and average daily gain phenotypes of Nellore bulls during the entire rearing phase. The third article evaluated whether the different prenatal supplementation approaches had an effect on gene expression in the muscle of bulls at 15 months of age and whether the results were related to the genetic background of these animals through an eQTL analysis. With this, we brought new scientific knowledge about the effects of fetal programming in male Nellore animals and contribute with new mechanisms found for the phenotype.

1.1 References

BARKER, D. J. P. Fetal origins of coronary heart disease. **Heart**, v. 69, n. 3, p. 195–196, 1993.

DU, M. et al. Fetal programming of skeletal muscle development in ruminant animals. **Journal of animal science**, v. 88, n. 13 Suppl, 2010.

DUARTE JÚNIOR, M. et al. Suplementação de fêmeas bovinas em pastejo: aspectos nutricionais e reprodutivos. **PubVet**, v. 9, n. 7, p. 321–336, 2016.

HALES, C. N.; BARKER, D. J. P. **Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesisDiabetologia**Springer-Verlag, , jul. 1992. Disponível em: <https://link.springer.com/article/10.1007/BF00400248>. Acesso em: 28 ago. 2020

LONG, N. M. et al. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. **Journal of Animal Science**, v. 87, n. 6, p. 1950–1959, 1 jun. 2009.

MULLINIKS, J. T. et al. Supplementation strategy during late gestation alters steer progeny health in the feedlot without affecting cow performance. **Animal Feed Science and Technology**, v. 185, n. 3–4, p. 126–132, 2013.

PAULINO, M. F. ET AL. Suplementação de bovinos em pastagens : Uma visão sistêmica. **Simpósio de produção de gado de corte**, v. 4, n. June, p. 93–139, 2004.

REHFELDT, C. et al. Advances in research on the prenatal development of skeletal muscle in animals in relation to the quality of muscle-based food. I. Regulation of myogenesis and environmental impact. **Animal**, v. 5, n. 5, p. 703–717, maio 2011.

SILVA, S.; NASCIMENTO JÚNIOR, D. Avanços na pesquisa com plantas forrageiras tropicais em pastagens : características morfofisiológicas e manejo do pastejo. **Revista Brasileira de Zootecnia**, v. 36, n. 1516–3598, p. 121–138, 2007.

USDA. **Livestock and Poultry: World Markets and Trade China Pulls Back on Meat Imports in 2021**. [s.l: s.n.]. Disponível em: <https://downloads.usda.library.cornell.edu/usda-esmis/files/73666448x/76537r01m/fx71bb52q/livestock_poultry.pdf>. Acesso em: 21 jan. 2021.

WORLD POPULATION REVIEW. **2020 World Population by Country**. Disponível em: <http://worldpopulationreview.com/>. Acesso em: 18 jan. 2021.

WU, G. et al. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. **Journal of Animal Science**, v. 84, n. 9, p. 2316–2337, 2006.

ZAGO, D.; CANOZZI, M. E. A.; BARCELLOS, J. O. J. Pregnant cow nutrition and its effects on foetal weight - A meta-analysis. **Journal of Agricultural Science**, v. 157, n. 1, p. 83–95, 2019.

2. Chapter 1: Evaluation of reproductive traits and the effect of nutrigenetics on bulls submitted to fetal programming

Guilherme Henrique Gebim Polizel[2*], Paulo Fantinato Neto[1], Raissa Braido Rangel[1], Laís Grigoletto[1], Fernando de Oliveira Bussiman[1], Roberta Cavalcante Cracco[2], Nara Pontes Garcia[1], Isabela Modolo Ruy[2], José Bento Sterman Ferraz[1], Miguel Henrique de Almeida Santana[2].

[1] Department of Veterinary Medicine, College of Animal Science and Food Engineering – USP, Av. Duque de Caxias Norte, 225, Pirassununga, SP, 13635-900, Brazil.

[2] Department of Animal Science, College of Animal Science and Food Engineering – USP, Av. Duque de Caxias Norte, 225, Pirassununga, SP, 13635-900, Brazil.

Published in Livestock Science, May 2021

DOI: https://doi.org/10.1016/j.livsci.2021.104487

Abstract

Nutritional stimuli during the gestational period in dams have long-term effects on the offspring in terms of health and production rates. This study assessed the effect of fetal programming in 126 pregnant Nellore cows on reproductive and nutrigenetic traits of the progeny during the rearing phase. For that purpose, three nutritional treatments were used in these cows during pregnancy: PP – protein-energy supplementation in the final third, FP – protein-energy supplementation during the entire pregnancy, and NP – (control) only mineral supplementation. The male progeny (64 bulls) was evaluated for scrotal circumference and seminal traits at 12, 15, and 18 months of age. In addition, we performed a genomic association (35K SNPs) for scrotal circumference at 12 and 18 months of age. Only the total sperm defects showed significant difference between groups ($P < 0.05$), regardless of age, while major and minor sperm defects and vigor showed

tendencies ($P < 0.10$). In the time analysis, only the minor sperm defects did not differ between ages, regardless of treatment ($P = 0.92$). We found genes that are associated with genetic variance at different ages and treatments (BTBD9, WNT2, ASZ1, WWOX and SLC26A8). Thus, prenatal protein-energy supplementation showed effects on the total of abnormal sperm cells between treatments, genotype-environment interaction, and some SNPs that explain more than 1% of the genetic variance on bulls during the rearing phase. These are evidences that different strategies of prenatal supplementation may have acted on epigenetic factors and may have caused changes in gene expression of animals. This contributes to the knowledge about mechanisms that involve fetal programming in beef cattle.

Keywords: Beef cattle; Maternal nutrition; Rearing phase; Scrotal circumference; SNPs.

2.1 Introduction

Rain scarcity and unfavorable environmental conditions for forage growth and development are common scenarios in beef cattle production in Brazil, especially in cows, which often face periods of low nutrient intake during pregnancy due to natural reproductive seasonality. This nutritional deficiency could hinder embryonic and fetal development, significantly affecting animal yield, increasing neonatal mortality rate, changing body composition, growth, hormonal balance, sexual precocity and modifying organ development and functions, including respiratory, cardiovascular, intestinal, and metabolic systems (WU et al., 2006; FUNSTON et al., 2010; LONG et al., 2012; DU et al., 2013; ROBINSON; CAFÉ; GREENWOOD, 2013; CUSHMAN; PERRY, 2019).

Exogenous effects could cause long-term consequences to the offspring, mainly nutritional factors in pregnant animals, since fetal life accounts for roughtly 35% to 40%

of the total life of most mammalian livestock (VONNAHME; TANNER; HILDAGO, 2018). More specifically, in beef cattle, fetal life corresponds to about 27% when slaughter occurs up to 24 months, accounting for more than 25% of total life solely nourished by the placenta.

Fetal programming is any stimulus or injury during the gestational period that has long-term effects on the progeny. Studies have related fetal programming to fertility and reproductive traits in ruminants. Differences in semen quality, testicular development, and sexual precocity were already reported (WELLER et al., 2016; COPPING et al., 2018; MCCARTY et al., 2018). However, other studies reported no influence of prenatal life (JAFARIAHANGARI et al., 2012; MOSSA et al., 2018; JOHNSON et al., 2019). The lack of knowledge of these variables is linked to the small number of males selected to become breeders (CUSHMAN; PERRY, 2019). However, reproductive traits such as scrotal circumference (SC) are of great importance because they show genetic correlations with other reproductive and weight traits (GRESSLER et al., 2000; DIAS et al., 2008), which could directly affect the productive cyclicality of the offspring.

In addition, interaction of genetics with the environment allows understanding a specific physiological or pathological state of an organism (SIMOPOULOS, 2002; SIMOPOULOS, A. P., & ORDOVÁS, 2004). The genes define susceptibility to certain conditions and environmental factors, such as the diet, determining the phenotype development (SIMOPOULOS, 2010). Nutrigenetics applied to livestock seeks to increase yield and efficiency of animals; thereby, it is essential to study the genetic factors involved in the biological mechanisms that affect gene-nutrient interactions (FONTANESI et al., 2015).

This study evaluated reproductive traits and implications of nutrigenetics during the rearing phase in Nellore bulls submitted to fetal programming.

2.2 Material and methods

2.2.1 Ethics statement

This study was approved by the Research Ethics Committee of FZEA/USP, under protocol No. 1843241117, according to the guidelines of the National Council for the Control of Animal Experimentation (CONCEA).

2.2.2 Experimental design

Initially, 126 Nellore cows were submitted to fixed-time artificial insemination (FTAI) with semen from four breeders with known genetic values. The pregnancy diagnosis was performed 30 days after FTAI. These animals were divided into three treatments: PP – Partial Programming, FP – Full Programming, and NP – Not Programmed. NP ingested only mineral supplement during the whole pregnancy (0.03% body weight). PP and FP received protein-energy supplementation daily equivalent to 0.3% of the body weight during pregnancy. PP received the supplementation only at the final third of pregnancy and the FP from the confirmation of pregnancy until delivery (see composition of supplements in Table 1 and Table 2). The NP is considered our control group, since this strategy of using only mineral supplementation is the approach commonly used in pregnant cows in extensive beef cattle production systems. The inclusion of an energy-protein supplement in the PP and FP groups are considered an additional stimulus during pregnancy. Age, body weight, and body condition scores were used as group selection criteria. All animals were allocated to pasture paddocks of *Brachiaria brizantha* cv. *Marandu*, with acces to the supplement and water *ad libitum*. The groups (NP, PP and FP) were submitted to pasture rotation in all paddocks, avoiding bias related to the quality of pastures.

After delivery, protein-energy supplementation ceased and the animals were kept together, regardless of the nutritional plan, remaining until weaning (average of 220 days old). Additionally, all animals were submitted to the same health and vaccination protocols, and nutritional managements, according to the farm routine. After weaning, the animals were separated according to sex (males and females), regardless of the treatment, and remained until the end of the rearing phase that lasted 11 months. During this period, the bulls were evaluated phenotypically at 12, 15, and 18 months of age (see sections *2.3.4* and *2.3.5*), ranging from the beginning of puberty until the end of the rearing phase.

Table 1. Ingredients and nutrients content of the matrices supplement.

Ingredients	Mineral supplement	Energetic-proteic supplement
Corn (%)	35.00	60.00
Soybean meal (%)	-	30.00
Dicalcium phosphate (%)	10.00	-
Urea 45% (%)	-	2.50
Salt (%)	30.00	5.00
Minerthal 160 MD (%)*	25.00	2.50
Total digestible nutrients (%)	26.76	67.55
Crude protein (%)	2.79	24.78
Non-protein nitrogen (%)	-	7.03
Acid detergent fiber (%)	1.25	4.76
Neutral detergent fiber (%)	4.29	11.24
Fat (%)	1.26	2.61
Calcium (g/kg)	74.11	6.20
Phosphate (g/kg)	59.38	7.24

*Mineral premix

Table 2. Mineral premix content of the supplement for matrices**.**

Minerals	Guarantee levels (25kgs)
Calcium (Ca)	200 - 230 g
Cobalt (Co)	160 mg
Copper (Cu)	2,700 mg
Sulfur (S)	60 g
Fluorine (F)	1,600 mg
Phosphor (P)	160 g
Iodine (I)	135 mg
Manganese (Mn)	2,700 mg
Selenium (Se)	80 mg
Zinc (Zn)	8,100 mg
Sodium monensin	4,000 mg

2.2.3 Dams evaluation

We evaluated all 126 cows for rump fat thickness (RFT) and weight before the breeding season and pre-delivery. Carcass ultrasound was performed by a certified technician from the Ultrasound Guidelines Council (UGC), according to the methodology in the Beef Improvement Federation (BIF) (BIF, 1996). The RFT was measured at the intersection of *Biceps femoris* and *Gluteus medius* between the ileum and the ischium.

We used an Aloka SSD-500 ultrasound device equipped with a 17.2 cm linear transducer at 3.5 MHz frequency (Aloka Co. Ltd., Wallingford, CT, USA). To optimize the transducer contact with the animal skin, vegetable oil was used as a conductor of ultrasonic waves (SANTANA et al., 2015). The Lince® software (M&S Consultoria Agropecuária Ltda., Pirassununga, São Paulo State, Brazil) was used to analyze the images. Additionally, the individual weight of each animal was obtained (Coimma Scales, Dracena, São Paulo State, Brazil).

2.2.4 Andrological assessment

At 12, 15, and 18 months of age, 64 bulls were evaluated for testicular symmetry and integrity, spermatic cord, and epididymis by palpation. The scrotal circumference (SC) was measured with a millimeter tape at the largest testicular circumference. After measuring the SC, each animal was submitted to testicular ultrasound to assess the integrity of the structures (Mindray DP 2200 VET – Mindray Bio-Medical Electronics Co., Ltd.; Shenzhen, Guangdong, China). Then, rectal palpation was performed to assess possible abnormalities of the accessory glands (seminal vesicle, ampoule of vas deferens and prostate). Semen was collected by an electro-ejaculator preceded by hair saving and cleaning of the foreskin with sterile saline solution (NaCl 0.9).

2.2.5 Analysis and seminal parameters

In pen management, the presence or absence of sperm and the ejaculate physical characteristics were evaluated using an optical microscope (Nikon Eclipse E-200, Nikon Corporation; Minato, Tokyo, Japan) at 100x and 400x magnification. For the collection, the tubes remained in water bath at 35 °C to avoid thermal shock of the semen. The collection tubes were composed by a funnel, thermal insulating material around the tube, and a cable to facilitate the collection. After collection, a drop of the ejaculate was placed on the slide to evaluate mass movement. A second drop was placed between the slide and cover slip to assess motility and vigor. Semen samples were diluted in formaldehyde saline solution for further sperm analysis of morphology (HANCOCK, 1957). Sperm morphology evaluation was conducted by counting 200 cells in the wet chamber technique under differential interference contrast microscopy (DIC - Nikon Eclipse Ni-U 80i, Nikon Corporation; Minato, Tokyo, Japan) at 1000x magnification. The proportion of abnormal cells was classified into major and minor defect proportions, as described by

Blom (1973). The characteristics of each animal were registered on individual forms and all procedures were performed based on recommendations of CBRA (2013).

2.2.6 Nutrigenetic evaluation

To study the nutrigenetic effects on fetal programming, a genomic association was performed using a single nucleotide polymorphism (SNP) panel (35K) for the SC characteristics at 12 and 18 months, searching for SNPs and genome regions associated to phenotypes. The analyses were conducted using the BLUPF90 software (MISZTAL, 2002). The software PREGSF90 (AGUILAR et al., 2014) was used as an interface for the genomic module to process the genomic information and POSTGSF90 (AGUILAR et al., 2014) was used to back solve the genomic estimated breeding values (GEBVs) for each trait (used as pseudophenotypes in the analyses) to estimate substitution allele effects. This methodology was applied by inserting "weight" to each iteration (in total three iterations to be performed) to the SNPs as described by WANG et al. (2014). The Pearson's correlation between genetic values was performed for each treatment to analyze the genotype-environment interaction (ROBERTSON, 1959).

2.2.7 Statistical analysis

The effects of treatments (NP, PP, and FP) and time on phenotypes were evaluated by the analysis of variance and the means were compared by the Tukey-Kramer test, with contrasts considered significant when $P < 0.05$ and tendency when $P < 0.10$. All procedures were performed using the MIXED procedure of the statistical package SAS University Edition (SAS/STAT®, SAS Institute Inc, NC, USA). The data were corrected for the age of animals, and the age of matrices and paternity were used in the linear model. In addition, the residuals were tested for normality (Shapiro-Wilk test) and the measurements that did not follow normality were transformed using log10 or arc sine,

evaluating which transformation met the normal distribution requirements. For the seminal traits of 12 months, only a precocity frequency analysis was performed using the Prisma® software using the Chi-Square test (X^2), due to the low prevalence of these traits in the collection. Nutrigenetics was evaluated by including the three treatments as a fixed effect in the model, after a significance test. The weighted single-step methodology (WssGBLUP; WANG et al., 2012) was applied using the animal model, including treatments as a fixed effect, the age of the matrix was used as a covariate, and the additive and residual effects were considered random effects. The statistical model used is represented by the following equation:

$$Y = X\beta+Z\alpha+\varepsilon$$

Where: Y is the vector of phenotypic observations for each trait, β is the vector of solutions for fixed effects, α is the vector of predictions for the random additive genetic effect, ε is the vector for random residual effect and, X and Z are the corresponding incidence matrices.

The same statistical models described to estimate genetic values were used to identify the genomic windows associated to the traits. After the analysis, genomic regions with 1 Mb surrounding markers that explained at least 1% of the additive genetic variance were constructed and, from these regions, a gene search (chromosome number and genomic coordinates as inputs) was conducted using the tool *Ensembl Biomart* (reference genome ARS-UCD1.2; ZERBINO et al., 2018), generating the list of candidate genes. Additionally, for each candidate gene, its function was prospected by a literature search and when there was no function related to the traits studied, these genes were considered a false positive signal.

2.3 Results

2.3.1 Weight and rump fat thickness in dams

The weight and RFT of cows at FTAI were similar between the treatments, reinforcing the phenotypic level in the animals inseminated in this study. The pre-delivery evaluation showed a significant difference in these traits between the groups ($P < 0.01$), in which the NP and FP treatments presented a difference between weight, while FP showed a difference from the others in terms of RFT.

2.3.2 Precocity frequency at 12 months

Regardless of the treatment, the animals showed a high rate of sperm absence in the ejaculate (azoospermy) and low proportion of bulls with sperm motility at 12 months of age. In addition, there was no difference between treatments for the frequency of animals by trait ($P = 0.43$) (Figure 1). This analysis was carried out exclusively for this age, due to the low rate of pubertal animals, evaluating the difference in precocity between animals in the treatments at 12 months of age.

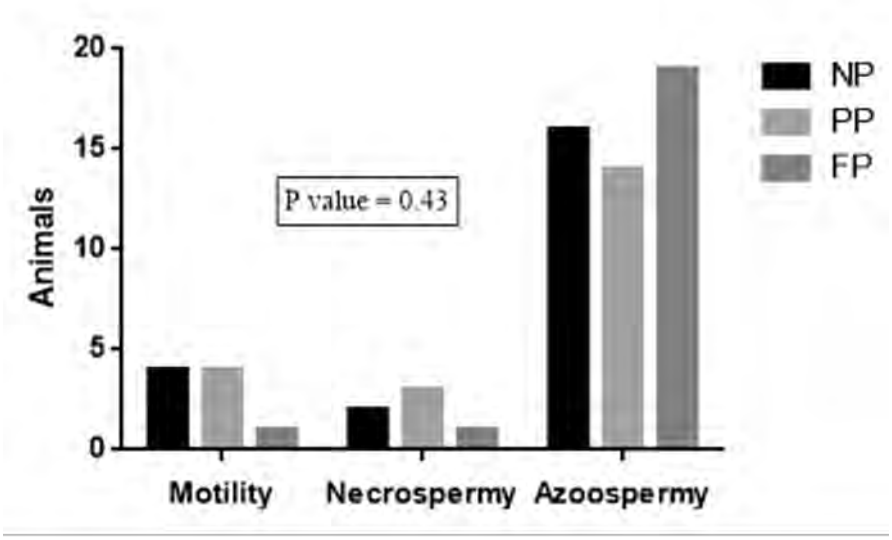

Figure 1. Precocity analysis of animals at 12 months of age between treatments of fetal programming (NP, PP, and FP), showing numerically differences between treatments; however, the *P* value (0.43) shows that this difference it is not statistically significant.

2.3.3 Scrotal circumference

Scrotal circumference (SC) showed no difference between treatments for any age nor when analyzed together for repeated measurements over time; however, all treatments showed differences between different ages ($P < 0.01$; Table 3).

2.3.4 Physical characteristics of sperm

There was no difference between prenatal treatments for semen physical characteristics (motility, vigor, and mass movement). However, motility tended to increase in PP compared to FP ($P < 0.10$). In addition, motility tended to increase ($P < 0.10$) with the advancement of age in FP, which did not occur in other treatments. For sperm vigor, NP showed a tendency to increase at 15 months of age ($P < 0.10$) only in relation to the FP group, which was not repeated at 18 months. FP and PP showed an increase in mass movement over time ($P < 0.05$), which was not demonstrated by NP (see results in Table 3).

Table 3. Reproductive characteristics of Nellore bulls submitted to fetal programming**.**

Traits	Age	NP	PP	FP	*P* value[1]	*P* value[3]
Motility (%)	15 months	26.33 ± 8.09	33.59 ± 7.32	11.68 ± 5.41	0.12	0.07
	18 months	49.22 ± 6.16	48.94 ± 6.29	37.44 ± 7.05	0.49	
	P value[2]	0.21	0.51	0.08		
	P value[4]		< 0.01			
Vigor (0-5)	15 months	1.98 ± 0.45	2.33 ± 0.34	1.01 ± 0.38	0.08	0.12
	18 months	2.90 ± 0.30	2.84 ± 0.29	2.59 ± 0.42	0.89	
	P value[2]	0.55	0.88	0.02		
	P value[4]		< 0.01			
Mass movement (0-5)	15 months	1.27 ± 0.42	1.02 ± 0.27	0.32 ± 0.19	0.11	0.44
	18 months	2.24 ± 0.32	2.72 ± 0.40	2.40 ± 0.45	0.67	
	P value[2]	0.47	0.01	<0.01		
	P value[4]		< 0.01			
Major sperm defects (%)	15 months	26.77 ± 5.61	17.34 ± 3.16	32.17 ± 6.50	0.13	0.09
	18 months	2.68 ± 0.53	5.02 ± 0.94	5.55 ± 1.50	0.14	
	P value[2]	< 0.01	0.09	< 0.01		
	P value[4]		< 0.01			
Minor sperm defects (%)	15 months	6.31 ± 1.53	9.38 ± 2.16	10.97 ± 2.18	0.25	0.06
	18 months	5.56 ± 0.83	8.23 ± 2.16	12.77 ± 4.23	0.18	
	P value[2]	0.99	0.99	0.99		
	P value[4]		0.92			
Total of abnormal sperm cells (%)	15 months	33.09 ± 6.08	26.73 ± 3.62	43.14 ± 6.11	0.1	0.02
	18 months	8.24 ± 1.15	13.24 ± 2.10	18.32 ± 4.77	0.09	
	P value[2]	< 0.01	0.16	< 0.01		
	P value[4]		< 0.01			
Scrotal circumference (cm)	12 months	22.24 ± 0.55[a]	22.59 ± 0.37[a]	21.7 ± 0.37[a]	0.37	0.36
	15 months	27.57 ± 0.52[b]	28.27 ± 0.63[b]	28.49 ± 0.45[b]	0.44	
	18 months	29.45 ± 0.55[c]	29.57 ± 0.57[b]	30.78 ± 0.49[c]	0.36	
	P value[2]	< 0.01	< 0.01	< 0.01		
	P value[4]		< 0.01			

The data are expressed as means of the characteristics ± standard error of the mean. The *P* value is evaluated for ages and treatments separately and for repeated measures over time. It was considered significant when $P < 0.05$ and with tendency when $0.10 > P > 0.05$.

[1] *P* value between groups in the same age

[2] *P* value between ages in the same group

[3] *P* value between groups regardless of age

[4] *P* value between ages regardless of group

2.3.5 Sperm morphological evaluation

Reproductive traits for the different groups during the experimental period are shown in Table 3. FP had a greater number of abnormal sperm ($P < 0.05$) than NP and PP and at 18 months. NP showed a tendency ($P < 0.10$) of less abnormality when compared to the FP. PP showed a tendency for a smaller percentage of major defects than FP, while NP tended to have a smaller proportion of minor defects than FP ($P < 0.10$). NP and FP significantly reduced the proportion of total and major defects with increasing age, while PP did not show any difference for the proportion of these defects between collections at 15 and 18 months (see results in Table 3).

2.3.6 Repeated measures over time

All traits showed a significant difference in time, except for minor defects, demonstrating that animals developed and decreased the proportion of abnormal sperm cells and presented better results for the sperm physical characteristics (motility, vigor, and mass movement) and SC, regardless of the treatment (Table 3).

2.3.7 Pearson's correlation

The correlation estimates between GEBVs in different environments ranged from 0.36 to 0.54 at both ages evaluated. The lowest correlation was found between FP and NP at 18 months (0.36; *P* value < 0.05). The strongest correlations were found between PP and NP at 18 (0.54, *P* value < 0.01) and between NP and FP at 12 months (0.54, *P* value < 0.01). Whereas for the treatment groups, at 12 months, the weakest correlation occurred between NP and PP (0.37, $P < 0.05$). At 18 months, the weakest correlation occurred between NP and FP at 0.36 ($P < 0.01$) (Table 4).

Table 4. Correlation between genetic values for scrotal circumference at 12 and 18 months in different treatments.

SC12			SC18		
	PP	FP		PP	FP
NP	0.37*	0.54**	NP	0.54**	0.36*
PP		0.48**	PP		0.49**

* *P* value <0.05

** *P* value <0.01

2.3.8 Genomic windows

Among the genes found, the following showed functions related to reproductive traits and fetal programming: BTBD9, whose genomic window explaind 1.48% of the genetic variance of NP treatment at 12 months; WNT2 and ASZ1, both located in the same genomic window that explained 2.83% of the genetic variance of PP treatment at 18 months and 3.11% at 12 months of FP treatment; WWOX, whose genomic window explaind 1.52% of the genetic variance of PP treatment at 18 months, and 1.19% of FP treatment at 12 months; and SLC26A8, which presents the same genomic window of MAPK14, explaining 5.11% of genetic variance at 18 months of PP treatment and 6.21% of FP treatment at 12 months (see genes and genetic windows with their explained genetic variance in Tables S1, S2, S3, S4, S5 and S6 in the supplementary material).

2.4 Discussion

This study is innovative and the data will be used to create a more robust database. To date, few studies related fetal programming to male reproductive traits, highlighting the significant contribution of this study to the knowledge of fetal programming.

Based on our results, prenatal supplementation in dams compensated for the drought period and it induced the desired effect by minimizing malnutrition during pregnancy and proved the occurrence of fetal programming.

According to Wolf et al. (1965), age at puberty is defined when the first ejaculate occurs with 10% motility and 50 million total sperm. In our study, only 9.4% of the animals had appropriate seminal characteristics to be considered pubertal (Figure 1). However, the rest (90.6%) did not yet reach pubertal development at 12 months of age, corroborating Freneau et al. (2006), who reported an average of 14.8 months for age at puberty in Nellore animals on pasture with mineral supplementation. The results of sperm morphology were evaluated only when bulls were 15 and 18 months old, when a large proportion of animals presented sperm, reducing the number of missing data. In addition, sexual precocity is closely related to a transient LH peak, apparently mediated by an increase in IGF-I concentration. Nevertheless, interaction between physiological and nutritional mechanisms that regulate the reproductive development in males is not yet clear. (BOLLWEIN; JANETT; KASKE, 2016). At 12 months old, the animals studied were still way below their reproductive potential. Possibly, most animals had not reached the transient LH peak, allowing to conclude that fetal programming has no effect on reproductive performance at this age.

According to the results in Table 3, animals showed greater SC due to testicular development as they age. These findings are consistent with studies on ruminants that reported no effects of fetal programming on SC (RAE et al., 2002; JAFARIAHANGARI et al., 2012; MOSSA et al., 2018), although research relating reproductive traits in males to the prenatal environment is still scarce. One factor that hinders the effect of maternal nutrition on SC is the high heritability of the trait for bulls from 12 to 24 months of age (~ 0.5) (KASTELIC; THUNDATHIL, 2017), an accurate puberty predictor, more than body weight or age (KNIGHTS et al., 1984; SMITH; BRINKS; RICHARDSON, 1989; BOLIGON; BALDI; DE ALBUQUERQUE, 2011).

To achieve puberty, an ejaculate with at least 70% of normal sperm is required (WOLF; ALMQUIST; HALE, 1965); thus, all groups (NP, PP, and FP) achieved puberty at 18 months. However, this does not mean that the treatments were homogeneous in terms of sperm morphology at this age (Table 3). According to Sullivan et al. (2010), bulls from dams that received high levels of protein and energy showed lower FSH concentrations, lower testicular weight, and a tendency towards a smaller diameter of seminiferous tubules. As FSH stimulates sperm capacity (MCLACHLAN et al., 1995), it is related to seminal traits. These results corroborate our findings in which the group that received supplementation throughout pregnancy (FP) demonstrated a higher proportion of total defects and a tendency of lower other morphological characteristics, vigor, and sperm motility. Another study reported that over-nutrition of pregnant sheep reduces prepubertal testosterone concentrations, testicular volume, and delays puberty age (DA SILVA et al., 2001). On the other hand, some studies have shown that malnutrition compromises reproductive traits (KOTSAMPASI et al., 2009; TOLEDO et al., 2011; COPPING et al., 2018). According to Evans et al. (2016), different nutritional stimuli may be associated to similar and/or opposite effects and the species studied, thus, requiring further investigation.

The thrifty phenotype hypothesis is one of the theories that explains the results where the NP group showed a tendency or even a higher reproductive phenotypic in relation to FP (HALES; BARKER, 1992), which was later reviewed and complemented by Gluckman and Hanson (2005). The authors propose that any change in the intrauterine environment, related to the different levels of prenatal supplementation of the cow in our study, leads to adaptations of the fetus to maximize its chances of survival in the maternal environment. Thereby, animals from NP may have undergone these changes, which possibly persisted during postnatal life in our study, meaning that these animlas were

more prepared for stressful situations and may have achieved puberty earlier, reflecting a lower phenotypic potential due to lower physiological demands.

Additionally, the only trait that did not improve with age was minor defects (P = 0.92), possibly because most defects found were related to cytoplasmatic droplets. As the bulls became older, maturational changes led to the translocation of proximal droplets to the sperm annular region. This process leads to the replacement of defects, and a defect that was previously classified as major, ends up as a minor. Finally, when the animals reach complete sexual development, they no longer present this type of morphological abnormality (THUNDATHIL et al., 2001). The phenotypic divergences found in studies on fetal programming may be related to the different diet, breed, and gestational period evaluated (ZAGO; CANOZZI; BARCELLOS, 2019).

SNP markers with high effects, which explain 1% or more of the genetic variance, did not overlap in different environments (treatments of fetal programming). Overlapping genomic windows have different effects; therefore, they explain different percentages of the genetic variance, which is also indicative of the genotype by environment interaction. The Pearson's correlation between GEBVs across different environments approximates the genetic correlation among different traits (i.e. a trait differently expressed by environment effects). When this estimate is high, the GEBVs tend to be close for each environment and as a result, the SNP effects are also approximately close. In our analysis, as the correlations were all below 0.8, differences between allele substitution effects could be related to environment forces changing genotypic expression. To further examine the change in SNP effects between environments in detail, the main genes associated to these regions and their actions were identified.

The gene BTBD9 is associated to a quantitative trait loci (QTL) the Bos Taurus 23 autosome (BTA23) that affects birth rate, increasing the number of stillbirths, due to

a deletion-type mutation between 12.28 and 12.81 Mbp in BTA23 (SAHANA et al., 2016). This gene was found in the NP group, but not in the PP and FP groups, meaning that fetal programming could possibly contribute to the lower occurrence of abnormalities in male fertility. WNT2 is a gene that encodes signaling proteins involved in the regulation of cell fate and organization during embryogenesis, related to placental vascularization and pregnancy health, as well as in the concentration of PAG (pregnancy-associated glycoproteins) in maternal blood and milk. These glycoproteins potentially influence not only placental development and maintenance of pregnancy, but also embryo survival, proteolytic activity, and immune modulation (SANTOS et al., 2018). The same authors demonstrated that 43 QTLs in this region affected the number of stillbirths, the conception rate, and the interval from the first to the last inseminations, indicating a positive correlation between fertility and the maternal PAG effect. In addition, low birth weight in humans has also been related to changes in the placenta due to methylations in the WNT2 gene (FERREIRA et al., 2011).

The WWOX gene is associated with steroid metabolism and represents great expression in the testicular tissue of animals (highly conserved among species), mainly in Leydig cells, which are responsible for testosterone production. This gene is also highly expressed in the pituitary gland, which produces LH and FSH, regulators of the functions of Leydig and Sertoli cells, respectively (NUNEZ; LUDES-MEYERS; ALDAZ, 2006). Ludes-Meyers et al. (2007) demonstrated that the partial interruption of WWOX expression leads to defects in testicles of mice. These hypomorphic animals showed atrophy of seminiferous tubules associated to the WWOX gene, linked to Leydig cell hyperplasia, resulting in premature testicular degeneration and fertility reduction. Therefore, WWOX plays an essential role in various stages of testicular function and differentiation.

The ASZ1 gene encodes the protein known as GASZ, specific for germ cells, which plays a central role in spermatogenesis. Orthological genes encoding GASZ have been identified in rats, cows, baboons, chimpanzees, and humans. The phylogenetic analyses reveal that GASZ proteins are highly conserved in these species (YAN et al., 2002). Nuages are cytoplasmic electrodense structures in germ cells and there is a specific type of nuage (pi-body), also called intermitochondrial cement, which houses sperm mitochondria and spermatocytes (CHUMA et al., 2009). Many germ cell-specific proteins are associated to this type of nuage, including GASZ. Loss of function of these proteins usually leads to the interruption of the nuage formation, defective piRNA biosynthesis, unregulated transposon expression, and male infertility (MA et al., 2009).

The gene SLC26A8 is responsible for encoding the TAT1 protein (testis anion transporter), which mediates the transport of chloride, sulfate, and oxalate, specifically expressed in male germ cells and mature sperm (TOURÉ et al., 2001; LOHI et al., 2002). This transporter is part of a complex of proteins in the annulus, a structure that connects the intermediate piece and the final piece of mammalian sperm. (TOURÉ et al., 2001). In conjunction with CFTR (cystic fibrosis transmembrane conductance regulator), the TAT1 protein appears to help in regulating the Cl- / HCO3- flows needed for sperm motility and training (RODE et al., 2012). Touré et al. (2007) generated mice with a targeted interruption of the TAT1 gene to investigate the function of TAT1. The mice were sterile due to the lack of sperm motility and the reduced potential for fertilization. The structural analyses revealed defects in flagellar differentiation leading to abnormal annulus, disorganization of the junction of the final intermediate piece, hook curvature of the sperm tail with rupture of axial structures, and abnormal assembly of the mitochondrial sheath. Although the ATP levels were normal, consumption was greatly reduced. These results indicate that TAT1 is a critical component of the spermatozoid annulus, essential

for proper tail differentiation and motility and, therefore, has a great effect on male fertility. The absence of annulus is also associated to defects in tail differentiation and asthenozoospermia in humans (TOURE et al., 2011).

The genes WWOX, ASZ1, and SLC26A8 are important for sexual development and male fertility, which explain high percentages of genetic variability. In our study, these genes were found only in the PP and FP groups, indicating that fetal programming could benefit the sexual development of the offspring. The Pearson's correlation below 0.8 demonstrated a difference between the genetic values of animals of distinct treatments, which explained different responses to the environments, highlighting the effect of nutrigenetics in our study.

As stated by Robertson (1959), the genetic correlation between a trait measured in different environments with a value lower than 0.8 is an indicator of genotype-environment interaction. Therefore, the low correlations found between the genetic values for SC of the different treatments suggest that fetal programming (maternal environment) has an effect on the genotype of animals (Table 4).

In addition, the correlations and genomic association found in our study evidenced that fetal programming had an effect on the gene expression of animals. This could be explained by epigenetics, resulting in rapid changes in response to environmental adaptations, which are related to different levels of prenatal supplementation in our study. Epigenetic changes involve two main processes, methylation of DNA at cytosines in cytosine-guanosine (CpG) pairs and several changes in proteins that involve DNA (histones), such as methylation or acetylation. These processes determine whether or not a specific gene is available for transcription (REYNOLDS; CATON, 2012; REYNOLDS; WARD; CATON, 2017) and, in the case of low availability of nutrients during pregnancy, favor the appearance of the thrifty phenotype (HALES; BARKER, 2001; WELLS, 2007;

VAAG et al., 2012) and these changes may persist for generations (RAVELLI et al., 1998).

2.5 Conclusion

Prenatal nutritional stimulus influences the total of abnormal sperm cells during the rearing phase and shows evidence that fetal programming may act on epigenetic factors and cause changes in gene expression of animals. Therefore, our results bring opportunities for further studies to improve the knowledge about factors that comprise the physiology of fetal programming.

2.6 References

AGUILAR, I. et al. **PREGSF90 – POSTGSF90: Computational Tools for the Implementation of Single-step Genomic Selection and Genome-wide Association with Ungenotyped Individuals in BLUPF90 Programs10th World Congress of Genetics Applied to Livestock Production**, 2014. . Disponível em: <http://nce.ads.uga.edu/wiki/doku.php].>. Acesso em: 18 maio. 2021.

BLOM; E. The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. **Nord. Vet. Med.**, v. 25, p. 383–391, 1973. Disponível em: <https://ci.nii.ac.jp/naid/10024782118>. Acesso em: 11 set. 2020.

BOLIGON, A. A.; BALDI, F.; DE ALBUQUERQUE, L. G. Genetic parameters and relationships between growth traits and scrotal circumference measured at different ages in Nellore cattle. **Genetics and Molecular Biology**, v. 34, n. 2, p. 225–230, 2011. Disponível em: <www.sbg.org.br>. Acesso em: 26 ago. 2020.

BOLLWEIN, H.; JANETT, F.; KASKE, M. **Impact of nutritional programming on the growth, health, and sexual development of bull calvesDomestic Animal Endocrinology**Elsevier Inc., , 1 jul. 2016. .

CBRA. Manual para exame andrológico e avaliação de sêmen animal. Belo Horizonte, CBRA, 1998. 2013.

CHUMA, S. et al. **Ultrastructural characterization of spermatogenesis and its evolutionary conservation in the germline: Germinal granules in mammalsMolecular and Cellular Endocrinology**Elsevier, , 10 jul. 2009. .

COPPING, K. J. et al. Peri-conception and first trimester diet modifies reproductive development in bulls. **Reproduction, Fertility and Development**, v. 30, n. 5, p. 703, 9

maio 2018. Disponível em: <http://www.publish.csiro.au/?paper=RD17102>. Acesso em: 13 ago. 2020.

CUSHMAN, R. A.; PERRY, G. A. **Developmental Programming of Fertility in LivestockVeterinary Clinics of North America - Food Animal Practice**W.B. Saunders, , 1 jul. 2019. . Disponível em: <https://doi.org/10.1016/j.cvfa.2019.02.003>. Acesso em: 13 ago. 2020.

DA SILVA, P. et al. Influence of placentally mediated fetal growth restriction on the onset of puberty in male and female lambs. **Reproduction**, v. 122, n. 3, p. 375–383, 1 set. 2001. Disponível em: <https://rep.bioscientifica.com/view/journals/rep/122/3/375.xml>. Acesso em: 27 ago. 2020.

DIAS, J. C. et al. Correlações genéticas e fenotípicas entre características reprodutivas e produtivas de touros da raça Nelore. **Pesquisa Agropecuaria Brasileira**, v. 43, n. 1, p. 53–59, 2008.

DU, M. et al. Meat science and muscle Biology Symposium: Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **Journal of Animal Science**, v. 91, n. 3, p. 1419–1427, mar. 2013. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/23100595/>. Acesso em: 12 ago. 2020.

EVANS, N. P.; BELLINGHAM, M.; ROBINSON, J. E. **Prenatal programming of neuroendocrine reproductive functionTheriogenology**Elsevier Inc., , 1 jul. 2016. .

FERREIRA, J. C. et al. WNT2 promoter methylation in human placenta is associated with low birthweight percentile in the neonate. **Epigenetics**, v. 6, n. 4, p. 440–449, 2011. Disponível em: <https://doi.org/10.4161/epi.6.4.14554>. Acesso em: 10 set. 2020.

FONTANESI, L. et al. Next Generation Semiconductor Based-Sequencing of a Nutrigenetics Target Gene (GPR120) and Association with Growth Rate in Italian Large White Pigs. **Animal Biotechnology**, v. 26, n. 2, p. 92–97, 13 abr. 2015.

FRENEAU, G. E. et al. Puberdade em touros Nelore criados em pasto no Brasil: Características corporais, testiculares e seminais e de índice de capacidade andrológica por pontos. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 58, n. 6, p. 1107–1115, 2006.

FUNSTON, R. N. et al. Winter grazing system and supplementation of beef cows during late gestation influence heifer progeny. **Journal of Animal Science**, v. 88, n. 12, p. 4094–4101, dez. 2010. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/20709872/>. Acesso em: 12 ago. 2020.

GLUCKMAN, P.; HANSON, M. **The Fetal Matrix: Evolution, Development and Disease**. [s.l: s.n.]

GRESSLER, S. L. et al. Estudo das Associações Genéticas entre Perímetro Escrotal e

Características Reprodutivas de Fêmeas Nelore. **Revista Brasileira de Zootecnia**, v. 29, n. 2, p. 427–437, mar. 2000.

HALES, C. N.; BARKER, D. J. P. **Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesisDiabetologia**Springer-Verlag, , jul. 1992. . Disponível em: <https://link.springer.com/article/10.1007/BF00400248>. Acesso em: 28 ago. 2020.

HALES, C. N.; BARKER, D. J. P. The thrifty phenotype hypothesis. **British Medical Bulletin**, v. 60, n. 1, p. 5–20, 1 nov. 2001. Disponível em: <https://academic.oup.com/bmb/article/60/1/5/322752>. Acesso em: 25 set. 2020.

HANCOCK, J. L. The morphology of boar spermatozoa. **Journal. Royal Microscopical Society (Great Britain)**, v. 76, n. 3, p. 84–97, out. 1957.

JAFARIAHANGARI, Y. et al. The effect of pre-natal maternal environment on live weight, reproductive and semen characteristics in ram lambs. **Small Ruminant Research**, 2012.

JOHNSON, C. et al. Impacts of residual feed intake and pre-natal diet on reproductive potential of bulls. **Animal Production Science**, v. 59, n. 10, p. 1827, 19 set. 2019. Disponível em: <http://www.publish.csiro.au/?paper=AN18301>. Acesso em: 24 ago. 2020.

KASTELIC, J. P.; THUNDATHIL, J. C. **Predicting and Promoting Fertility in Beef BullsProceedings, Applied Reproductive Strategies in Beef Cattle**. [s.l: s.n.].

KNIGHTS, S. A. et al. Estimates of Heritabilities and of Genetic and Phenotypic Correlations among Growth and Reproductive Traits in Yearling Angus Bulls1. **Journal of Animal Science**, v. 58, n. 4, p. 887–893, 1 abr. 1984. Disponível em: <https://academic.oup.com/jas/article/58/4/887-893/4658307>. Acesso em: 26 ago. 2020.

KOTSAMPASI, B. et al. Reduced Sertoli cell number and altered pituitary responsiveness in male lambs undernourished in utero. **Animal Reproduction Science**, v. 114, n. 1–3, p. 135–147, 1 ago. 2009.

LOHI, H. et al. Functional characterization of three novel tissue-specific anion exchangers SLC26A7, -A8, and -A9. **Journal of Biological Chemistry**, v. 277, n. 16, p. 14246–14254, 19 abr. 2002. Disponível em: <http://www.jbc.org/>. Acesso em: 10 set. 2020.

LONG, N. M. et al. Effects of early- to mid-gestational undernutrition with or without protein supplementation on offspring growth, carcass characteristics, and adipocyte size in beef cattle. **Journal of Animal Science**, v. 90, n. 1, p. 197–206, jan. 2012.

LUDES-MEYERS, J. H. et al. *WWOX* hypomorphic mice display a higher incidence of B-cell lymphomas and develop testicular atrophy. **Genes, Chromosomes and Cancer**, v. 46, n. 12, p. 1129–1136, 1 dez. 2007. Disponível em:

<http://doi.wiley.com/10.1002/gcc.20497>. Acesso em: 10 set. 2020.

MA, L. et al. GASZ Is Essential for Male Meiosis and Suppression of Retrotransposon Expression in the Male Germline. **PLoS Genetics**, v. 5, n. 9, p. e1000635, 4 set. 2009. Disponível em: <https://dx.plos.org/10.1371/journal.pgen.1000635>. Acesso em: 10 set. 2020.

MCCARTY, K. J. et al. Effect of chronic melatonin supplementation during mid to late gestation on maternal uterine artery blood flow and subsequent development of male offspring in beef cattle. **Journal of Animal Science**, v. 96, n. 12, p. 5100–5111, 3 dez. 2018. Disponível em: <https://academic.oup.com/jas/article/96/12/5100/5093175>. Acesso em: 13 ago. 2020.

MCLACHLAN, R. I. et al. **Hormonal control of spermatogenesisTrends in Endocrinology and Metabolism**Elsevier Current Trends, , 1 abr. 1995. .

MISZTAL, I. **BLUPF90 and related programs Challenges for genetic evaluation**. [s.l: s.n.]. Disponível em: <https://www.researchgate.net/publication/262224129>. Acesso em: 28 mar. 2021.

MOSSA, F. et al. Testicular development in male lambs prenatally exposed to a high-starch diet. **Molecular Reproduction and Development**, v. 85, n. 5, p. 406–416, maio 2018. Disponível em: <http://doi.wiley.com/10.1002/mrd.22974>. Acesso em: 23 dez. 2019.

NUNEZ, M. I.; LUDES-MEYERS, J.; ALDAZ, C. M. WWOX protein expression in normal human tissues. **Journal of Molecular Histology**, v. 37, n. 3–4, p. 115–125, 2006. Disponível em: <https://link.springer.com/content/pdf/10.1007/s10735-006-9046-5.pdf>. Acesso em: 10 set. 2020.

RAE, M. T. et al. The effects of undernutrition, in utero, on reproductive function in adult male and female sheep. **Animal Reproduction Science**, v. 72, n. 1–2, p. 63–71, 15 jul. 2002.

RAVELLI, A. C. J. et al. Glucose tolerance in adults after prenatal exposure to famine. **Lancet**, v. 351, n. 9097, p. 173–177, 17 jan. 1998.

REYNOLDS, L. P.; CATON, J. S. **Role of the pre- and post-natal environment in developmental programming of health and productivityMolecular and Cellular Endocrinology**Elsevier, , 6 maio 2012. .

REYNOLDS, L. P.; WARD, A. K.; CATON, J. S. Epigenetics and developmental programming in ruminants long-term impacts on growth and development. In: **Biology of Domestic Animals**. [s.l: s.n.]p. 85–121.

ROBERTSON, A. Experimental design on the measurement of heritabilities and genetic correlations. **biometrical genetics**, p. 186, 1959.

ROBINSON, D. L.; CAFÉ, L. M.; GREENWOOD, P. L. Meat Science and muscle Biology Symposium: Developmental programming in cattle: Consequences for growth, efficiency, carcass, muscle, and beef quality characteristics. **Journal of Animal Science**, v. 91, n. 3, p. 1428–1442, mar. 2013. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/23230118/>. Acesso em: 12 ago. 2020.

RODE, B. et al. The testis anion transporter TAT1 (SLC26A8) physically and functionally interacts with the cystic fibrosis transmembrane conductance regulator channel: A potential role during sperm capacitation. **Human Molecular Genetics**, v. 21, n. 6, p. 1287–1298, 15 mar. 2012. Disponível em: <https://academic.oup.com/hmg/article/21/6/1287/558584>. Acesso em: 10 set. 2020.

SAHANA, G. et al. A 0.5-Mbp deletion on bovine chromosome 23 is a strong candidate for stillbirth in Nordic Red cattle. **Genetics Selection Evolution**, v. 48, n. 1, p. 1–12, 18 abr. 2016. Disponível em: <https://link.springer.com/articles/10.1186/s12711-016-0215-z>. Acesso em: 10 set. 2020.

SANTANA, M. H. A. et al. A genomewide association mapping study using ultrasound-scanned information identifies potential genomic regions and candidate genes affecting carcass traits in Nellore cattle. **Journal of Animal Breeding and Genetics**, v. 132, n. 6, p. 420–427, 2015.

SANTOS, D. J. A. et al. Genetic and nongenetic profiling of milk pregnancy-associated glycoproteins in Holstein cattle. **Journal of Dairy Science**, v. 101, n. 11, p. 9987–10000, 1 nov. 2018.

SIMOPOULOS, A. P., & ORDOVÁS, J. M. Nutrigenetics and Nutrigenomics. **Karger Medical and Scientific Publishers**, v. 93, 2004. Disponível em: <https://books.google.com.br/books?hl=pt-BR&lr=&id=9-c1Snwe-IcC&oi=fnd&pg=PR5&dq=+Simopoulos+AP,+Ordovas+JM,+eds.+2004.+Nutrigenetics+and+nutrigenomics.+World+Rev.+Nutr.+Diet.+Vol.+93&ots=GE7p4Q63O9&sig=NWWop2IdMNgNJ0qFVx18e9SX0PQ#v=onepage&q=Simopoulos AP>. Acesso em: 12 nov. 2020.

SIMOPOULOS, A. P. Genetic variation and dietary response: Nutrigenetics/nutrigenomics. **Asia Pacific Journal of Clinical Nutrition**, v. 11, n. SUPPL. 6, p. S117–S128, out. 2002. Disponível em: <http://doi.wiley.com/10.1046/j.1440-6047.11.s6.3.x>. Acesso em: 12 nov. 2020.

SIMOPOULOS, A. P. **Nutrigenetics/nutrigenomicsAnnual Review of Public Health** Annual Reviews , , 21 abr. 2010. . Disponível em: <www.annualreviews.org>. Acesso em: 12 nov. 2020.

SMITH, B. A.; BRINKS, J. S.; RICHARDSON, G. V. Estimation of Genetic Parameters among Breeding Soundness Examination Components and Growth Traits in Yearling Bulls. **Journal of Animal Science**, v. 67, n. 11, p. 2892–2896, 1 nov. 1989. Disponível em: <https://academic.oup.com/jas/article/67/11/2892-2896/4697175>. Acesso em: 26

ago. 2020.

SULLIVAN, T. M. et al. Dietary manipulation of Bos indicus × heifers during gestation affects the prepubertal reproductive development of their bull calves. **Animal Reproduction Science**, v. 118, n. 2–4, p. 131–139, 1 abr. 2010.

THUNDATHIL, J. et al. The use of in vitro fertilization techniques to investigate the fertilizing ability of bovine sperm with proximal cytoplasmic droplets. **Animal Reproduction Science**, v. 65, n. 3–4, p. 181–192, 30 mar. 2001.

TOLEDO, F. C. et al. In utero protein restriction causes growth delay and alters sperm parameters in adult male rats. **Reproductive Biology and Endocrinology**, v. 9, n. 1, p. 94, 24 jun. 2011. Disponível em: <http://rbej.biomedcentral.com/articles/10.1186/1477-7827-9-94>. Acesso em: 28 ago. 2020.

TOURE, A. et al. Septins at the annulus of mammalian sperm. In: Biological Chemistry, 8–9, **Anais**...De Gruyter, 1 ago. 2011. Disponível em: <https://www.degruyter.com/view/journals/bchm/392/8-9/article-p799.xml>. Acesso em: 10 set. 2020.

TOURÉ, A. et al. Tat1, a Novel Sulfate Transporter Specifically Expressed in Human Male Germ Cells and Potentially Linked to RhoGTPase Signaling. **Journal of Biological Chemistry**, v. 276, n. 23, p. 20309–20315, 8 jun. 2001. Disponível em: <http://www.jbc.org/>. Acesso em: 10 set. 2020.

TOURÉ, A. et al. The Testis Anion Transporter 1 (Slc26a8) is required for sperm terminal differentiation and male fertility in the mouse. **Human Molecular Genetics**, v. 16, n. 15, p. 1783–1793, 1 ago. 2007. Disponível em: <https://academic.oup.com/hmg/article/16/15/1783/576972>. Acesso em: 10 set. 2020.

VAAG, A. A. et al. The thrifty phenotype hypothesis revisited. **Diabetologia**, v. 55, n. 8, p. 2085–2088, 2012. Disponível em: <https://link.springer.com/content/pdf/10.1007/s00125-012-2589-y.pdf>. Acesso em: 25 set. 2020.

VONNAHME, K. A.; TANNER, A. R.; HILDAGO, M. A. V. Effect of maternal diet on placental development, uteroplacental blood flow, and offspring development in beef cattle. **Animal Reproduction**, v. 15, p. 912–922, 2018.

WANG, H. et al. Genome-wide association mapping including phenotypes from relatives without genotypes. **Genetics Research**, v. 94, n. 2, p. 73–83, abr. 2012. Disponível em: <https://doi.org/10.1017/S0016672312000274>. Acesso em: 18 maio. 2021.

WANG, H. et al. Genome-wide association mapping including phenotypes from relatives without genotypes in a single-step (ssGWAS) for 6-week body weight in broiler chickens. **Frontiers in Genetics**, v. 5, n. MAY, p. 134, 20 maio 2014. Disponível em: <http://journal.frontiersin.org/article/10.3389/fgene.2014.00134/abstract>. Acesso em:

10 set. 2020.

WELLER, M. M. D. C. A. et al. Effect of maternal nutrition and days of gestation on pituitary gland and gonadal gene expression in cattle. **Journal of Dairy Science**, v. 99, n. 4, p. 3056–3071, 1 abr. 2016.

WELLS, J. C. K. **The thrifty phenotype as an adaptive maternal effectBiological Reviews**John Wiley & Sons, Ltd, , 1 fev. 2007. . Disponível em: <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-185X.2006.00007.x>. Acesso em: 25 set. 2020.

WOLF, F. R.; ALMQUIST, J. O.; HALE, E. B. Prepuberal Behavior and Puberal Characteristics of Beef Bulls on High Nutrient Allowance. **Journal of Animal Science**, v. 24, n. 3, p. 761–765, 1 ago. 1965. Disponível em: <https://academic.oup.com/jas/article/24/3/761-765/4701309>. Acesso em: 20 dez. 2019.

WU, G. et al. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. **Journal of Animal Science**, v. 84, n. 9, p. 2316–2337, 2006.

YAN, W. et al. Identification of Gasz, an Evolutionarily Conserved Gene Expressed Exclusively in Germ Cells and Encoding a Protein with Four Ankyrin Repeats, a Sterile-α Motif, and a Basic Leucine Zipper. **Molecular Endocrinology**, v. 16, n. 6, p. 1168–1184, 1 jun. 2002. Disponível em: <https://academic.oup.com/mend/article/16/6/1168/2741548>. Acesso em: 10 set. 2020.

ZAGO, D.; CANOZZI, M. E. A.; BARCELLOS, J. O. J. Pregnant cow nutrition and its effects on foetal weight - A meta-analysis. **Journal of Agricultural Science**, v. 157, n. 1, p. 83–95, 2019.

ZERBINO, D. R. et al. Ensembl 2018. **Nucleic Acids Research**, v. 46, n. D1, p. D754–D761, 1 jan. 2018. Disponível em: <https://academic.oup.com/nar/article/46/D1/D754/4634002>. Acesso em: 10 set. 2020.

3. Chapter 2: Effects of fetal programming on performance and tissue development of beef cattle offspring in rearing phase

Guilherme Henrique Gebim Polizel[1*], Ricardo de Francisco Strefezzi[2], Roberta Cavalcante Cracco[1], Arícia Christofaro Fernandes[1], Cassiano Bordignon Zuca[1], Henrique Hespanhol Castellar[1], Geovana Camila Baldin[1], Miguel Henrique de Almeida Santana[1].

[1] Department of Animal Science, College of Animal Science and Food Engineering – USP, Av. Duque de Caxias Norte, 225, Pirassununga, SP, 13635-900, Brazil.

[2] Department of Veterinary Medicine, College of Animal Science and Food Engineering – USP, Av. Duque de Caxias Norte, 225, Pirassununga, SP, 13635-900, Brazil.

Abstract

This study evaluated the effects of fetal programming on body weight (BW), average daily gain (ADG), rump fat thickness (RFT), backfat thickness (BFT), ribeye area (REA), muscle cell area (MCA) and number of cells in REA (NCREA) of young Nellore bulls during the rearing period. After pregnancy confirmation, 126 Nellore cows were separated into three prenatal nutritional treatments (NP – (control) only mineral supplementation, PP – protein-energy supplementation in the final third, and FP – protein-energy supplementation during the entire pregnancy). Upon calving, 63 males were evaluated during the entire rearing phase and for histological assessments 7 animals per treatments were randomly selected. All phenotypes were subjected to an analysis of variance (ANOVA). The different prenatal stimuli had no effect on BFT, RFT, MCA and NCREA ($P>0.05$); however, prenatal nutrition influenced BW of the animals during the rearing phase ($P<0.01$) and showed a tendency on ADG ($P=0.09$) and REA ($P=0.08$). The results of this study showed that prenatal nutrition in Zebu cattle has an effect on the

performance during the rearing phase, due to muscle development (REA) and probably also due to the increase in other organ mass.

Keywords: Body weight, Carcass traits, Maternal nutrition, Muscle development, Nellore cattle

3.1 Introduction

Beef cattle from tropical regions is characterized by an extensive production model and animals are susceptible to the effects of climatic variation, forage quality, and food availability (SILVA; NASCIMENTO JÚNIOR, 2007). These edaphoclimatic variations can affect the pregnant cows, during most of their pregnancy.

Fetal programming is a concept that has been widely addressed in the literature, given the vast range of factors that influence the progeny. Nutritional input during gestation of cows affects the vascular and placental development (BELKACEMI et al., 2010). The placenta works as an intermediate mechanism between the fetus and mother and is responsible for fetal development and nutrition, hormone production, and immune barrier (CASTRO-RODRÍGUEZ et al., 2020). In this scenario, changes in the maternal environment can decrease uterus-placental blood flow and affect the progeny (DICKINSON et al., 2016). Thus, nutrition is the environmental factor with the greatest influence on the fetus, possibly affecting the offspring throughout its life (WU et al., 2004) and influencing important productive indices for that animal category in beef cattle.

The development and growth of skeletal muscle are processes controlled by undifferentiated mesenchymal cells in the embryonic phase, with the maternal nutritional supply playing a key role in these processes (JENNINGS et al., 2016). Undifferentiated mesenchymal cells at this stage of development have the function of creating myocytes, adipocytes and fibroblasts (DU et al., 2013). These cells, depending on the nutritional

status of the dam and the gestational period, may show hypertrophy or hyperplasia (BONNET et al., 2010; DU et al., 2010; UNDERWOOD et al., 2010). Hyperplasia is related to the increase in the number of cells, and this process occurs only during pregnancy, that is, the number of cells that the calf presents at birth will be the same when slaughtered (PICARD et al., 2002). Thus, muscle development during the fetal stage is decisive for the number of muscle fibers and muscle mass, improving carcass quality (ZHU et al., 2004).

This study assessed the phenotypic effects (fat thickness, body weight, average daily gain, ribeye area, muscle cell area and number of cells in ribeye area) caused by different fetal programming strategies in beef cattle during the rearing period.

3.2 Material and methods

3.2.1 Ethics statement

The Research Ethics Committee of College of Animal Science and Food Engineering from São Paulo University approved this study, under protocol No. 1843241117, according to the guidelines of the National Council for the Control of Animal Experimentation (CONCEA).

3.2.2 Experimental design

A group of 126 Nellore was fixed-time artificially inseminated (FTAI) with semen from four breeders with known genetic values and, after confirmation of pregnancy, the cows were divided into three treatments: NP – Not programmed, PP – Partial Programming and FP – Full Programmed. NP cows ingested only mineral supplement during the entire pregnancy (0.03% of BW). PP and FP received protein-energy supplementation equivalent to 0.3% of the average BW per day during pregnancy. The PP group received supplementation only in the final third of pregnancy. The FP group

received the supplementation from the confirmation of pregnancy (30 days) until delivery. The mineral supplement contained 26.76% of total digestible nutrients (TDN), 2.79% of crude protein (CP), 4.29% of neutral detergent fiber (NDF) and 1.26% of fat. The protein-energy supplement contained 67.55% (TDN), 24.78% (CP), 11.24% (NDF) and 2.61% (fat; more details about compounds and ingredients of the supplements were described by Polizel et al. (2021)). During pregnancy, pastures where the different treatments remained (all paddocks with *Brachiaria brizantha* cv. Marandu) were evaluated and presented similar nutritional values: NP (TDN = 63.07% ± 1.45%, CP = 7.38% ± 1.72% and NDF = 59.03% ± 3.68%), PP (TDN = 64.1% ± 2.34%, CP = 7.82 ± 2.29% and NDF = 61.43% ± 5.05) and FP (TDN = 61.43% ± 2.13%, CP = 7.40% ± 2.30% and NDF = 58.49% ± 4.11%).

Cow age, body condition score (BCS), and BW were used to block the dams in each treatment. The effects of the treatments on the dams were briefly described by Polizel et al. (2021).

After calving, all animals were submitted to the same environmental conditions (sanitary and nutritional) and protein-energy supplementation ceased. The animals (dams and calves) were separated according to the month of birth, regardless of the nutritional plan, where they remained until weaning (210 days ± 28 days). After weaning, the animals were separated according to sex (males and females), regardless of the treatment and remained until the end of the rearing phase (11 months; 540 days ± 28 days).

3.2.3 Evaluation of carcass ultrasonography traits

We performed carcass ultrasonography at 210, 365, 450, and 540 days of age on average in 63 male animals. Rump fat thickness (RFT) at the intersection of *Biceps femoris* and *Gluteus medius* between the ileum and ischium, backfat thickness (BFT) between the 12th and 13th ribs and ribeye area (REA) in *Longissimus* muscle also between

the 12th and 13th ribs were measured by a certified technician from the Ultrasound Guidelines Council (UGC), according to the methodology of Beef Improvement Federation (BIF). We used the Aloka SSD-500 ultrasound equipped with a 17.2 cm linear transducer at a frequency of 3.5 MHz (Aloka Co. Ltd., Wallingford, CT, USA) in the ultrasound. Vegetable oil was used as a conductor of ultrasonic waves to optimize the means of transducer contact with the skin of the animals (BONIN et al., 2015). In addition, a silicone acoustic guide was used to adapt the transducer to the curvature of the dorsal-lumbar region of animals for the BFT and REA measurements. After collection, the images were stored for further analysis. The Lince® software (M&S Consultoria Agropecuária Ltda., Pirassununga, São Paulo, Brazil) was used to read and generate the specific measurements of each trait.

3.2.4 Evaluation of body weight and average daily gain

The BW of 63 young bulls were measured individually at 210, 255, 318, 365, 450, and 540 days of age on average using a Coimma® electronic scale. The ADG was estimated as the slope of a linear regression of weights at age (210 to 540 days).

3.2.5 Sampling and morphometric evaluation by histochemical and image analyses

Seven male animals were randomly selected per treatment, resulting in a group of 21 animals submitted to biopsy of the *Longissimus* muscle (between 9th and 10th ribs) at 210 and 450 days of age. The region was cleaned with water and detergent to perform the trichotomy and a 10% povidone-iodine solution. Biopsy was started ten minutes after local anesthesia was applied (Lidocaine 2%) and 2 cm^3 samples were collected and the incision sutured. The animals were monitored and treated with antibiotics and anti-inflammatory drugs. The samples were fixed in fresh 10% buffered formalin solution for 48 hours, followed by routine histological processing (PROPHET et al., 1992). Three-µm

transverse histological sections were obtained and stained with Hematoxylin and eosin for image analysis.

Muscle cell area (MCA) was evaluated with an optical microscope (DM500, Leica®) coupled to a high-definition digital camera (ICC50 HD, Leica®). Morphometry was performed using an image analysis software (Image Pro Plus 4.5; Media Cybernetics, Silver Spring, USA). Five high-power fields (HPF, 40x objective) were randomly selected and the images were saved as ".jpg" files. All muscle cells in each image, presenting sharp cytoplasmic limits, were manually contoured by tracing their margins with the aid of a mouse. MCA was calculated as the average for all cells that were measured per animal.

To estimate the number of cells in the ribeye area (NCREA) at 450 days of age, REA was adjusted from cm^2 to μm^2 and the following formula was used for each animal:

$$Number\ of\ cells\ in\ REA = \frac{REA\ (450\ days)}{Average\ of\ cell\ area\ (450\ days)}$$

3.2.6 Statistical analysis

The data were tested for normality (Shapiro Wilk) and subjected to an analysis of variance (ANOVA) in the MIXED procedure of the SAS® OnDemand for Academics software. In addition, BW, RFT, BFT, REA and MCA assessments were analyzed individually at each age and over time, considering repeated measures over time. The sires and the ages of dams were used as fixed effects. Animal age was used as covariate in the analysis and the differences between treatments were considered significant when P value < 0.05 and tendency when $0.05 < P < 0.10$ by the Tukey Kramer test.

3.3 Results

3.3.1 Carcass ultrasound traits

For the fat thickness (backfat and rump fat), there was no significant difference between treatments in each period (*P* value > 0.05) and throughout the rearing phase, regardless of the animal age (*P* value > 0.05; Table 1). Ribeye area did not show differences between treatments in individual assessments (*P* value > 0.05); however, it showed a tendency to be higher in FP treatment than in NP (*P* value = 0.08; Table 1) throughout the rearing phase.

3.3.2 Body weight and average daily gain

Assessments of animal BW throughout the rearing phase showed that bulls in the FP treatment were superior to the NP treatment (*P* value < 0.01); however, animals in the PP group did not show differences. In the individual weightings, BW did not show any difference (*P* value > 0.05) between treatments and ADG tended to be higher in the FP group than in the PP group (*P* value = 0.09; Table 1) during the rearing period.

Table 1. Average carcass ultrasound characteristics (BFT, RFT and REA), BW and ADG ± standard error during the entire rearing phase of Nellore young bulls submitted to fetal programming with their respective *P* values.

Traits	Age	NP	PP	FP	P value
Backfat thickness (mm)	210 days	1.67 ± 0.30	1.58 ± 0.31	1.71 ± 0.28	0.99
	365 days	0.66 ± 0.21	0.39 ± 0.17	0.43 ± 0.18	0.45
	450 days	0.62 ± 0.22	0.49 ± 0.20	0.53 ± 0.18	0.93
	540 days	1.52 ± 0.27	1.79 ± 0.33	1.35 ± 0.28	0.78
	210 to 540 days	1.12 ± 0.13	1.06 ± 0.14	1.00 ± 0.13	0.66
Rump fat thickness (mm)	210 days	2.35 ± 0.38	2.80 ± 0.33	2.42 ± 0.32	0.51
	365 days	1.05 ± 0.22	0.80 ± 0.28	0.99 ± 0.25	0.77
	450 days	1.48 ± 0.28	1.52 ± 0.29	1.83 ± 0.28	0.77
	540 days	2.97 ± 0.31	3.12 ± 0.38	3.25 ± 0.40	0.86
	210 to 540 days	1.96 ± 0.17	2.06 ± 0.19	2.12 ± 0.18	0.98
Ribeye area (cm^2)	210 days	45,78 ± 1,91	47,86 ± 1,90	47,81 ± 2,09	0.66
	365 days	58,28 ± 1,63	58,46 ± 1,99	60,42 ± 2,19	0.50
	450 days	67,42 ± 1,53	68,32 ± 1,86	69,51 ± 2,27	0.56
	540 days	76,81 ± 1,80	79,18 ± 2,10	80,35 ± 2,86	0.43
	210 to 540 days	62.07 ± 1.49	63.45 ± 1.62	64.52 ± 1.75	0.08
Body Weight (kg)	210 days	219.56 ± 5.85	231.20 ± 5.34	231.70 ± 4.91	0.15
	255 days	253.70 ± 5.36	262.10 ± 5.76	264.02 ± 5.19	0.33
	318 days	269. 59 ± 5.38	275.01 ± 6.20	280.20 ± 5.72	0.42
	365 days	293.44 ± 5.30	296.34 ± 6.29	301.51 ± 5.80	0.55
	450 days	370.22 ± 5.73	370.64 ± 6.37	382.93 ± 5.87	0.20
	540 days	430.23 ± 5.02	429.38 ± 6.47	439.61 ± 4.65	0.17
	210 to 540 days	306.12 ± 6.67[a]	310.77 ± 6.70[ab]	316.66 ± 6.78[b]	<0.01
Average daily gain (kg/day)	210 to 540 days	0.58 ± 0.01	0.55 ± 0.02	0.58 ± 0.01	0.09

The last line of each trait represents the repeated measures over time with their P value, respectively.

3.3.3 Evaluation of muscle cell area (MCA) and number of cells in ribeye area (NCREA)

There were no differences in MCA between prenatal nutritional treatments in individual assessments (210 days and 450 days of age; *P* value > 0.05; Figure 1) nor in the evaluation performed over time (*P* value = 0.86; Figure 1). This result was repeated

in regard to the NCREA at 450 days of age, in which the different prenatal supplementation strategies had no effect (*P* value = 0.75; Figure 1).

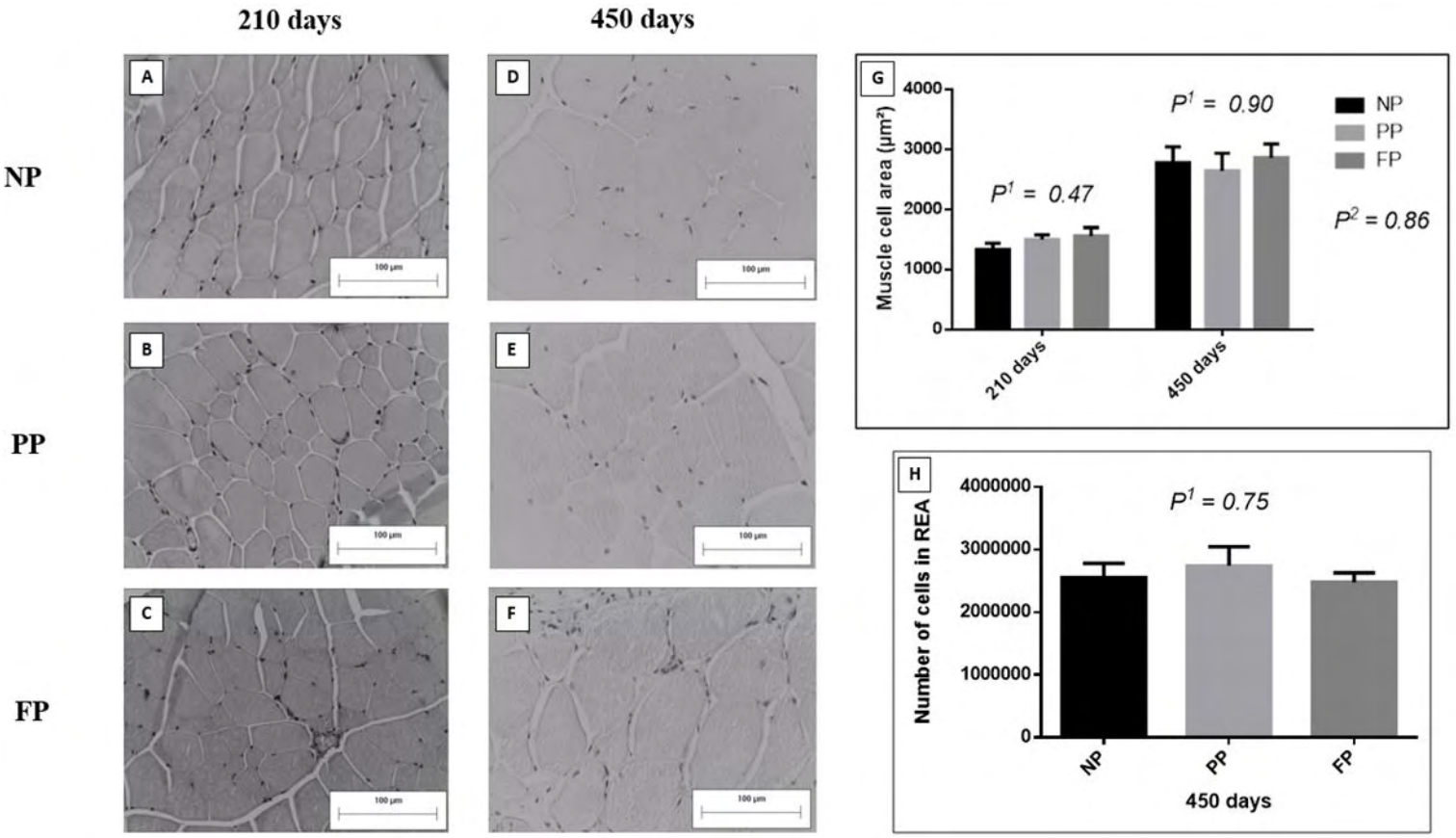

Figure 1. Muscle cell area (MCA; µm^2) at 210 days and 450 days of age and number of cells in ribeye area (NCREA) at 450 days of age of the skeletal muscle of the offspring resulting from different treatments. NP (A, D), PP (B, E) and FP (C, F) are represented in histological images from the skeletal muscle of the offspring at different ages (210 days and 450 days). Bars represent means ± standard error of MCA (G) and NCREA (H). P^1 corresponds to *P* value between treatments for each age and P^2 corresponds to *P* value between treatments regardless of age for each evaluated characteristic.

3.4 Discussion

Maresca et al. (2019) evaluated whether the different levels of protein added to the diet of dams affected meat quality and carcass characteristics. The authors found no influence of prenatal diet on fat thickness of the animals evaluated during rearing phase. Coutinho et al. (2015) reported that most Nellore animals raised in an extensive production system up to 20 months of age did not develop subcutaneous fat, which hinders the identification of differences between treatments at the ages evaluated. In addition, our results show that subcutaneous fat thickness is not a measure with linear growth when the animals are grazing. This is because the rearing phase lasts approximately 11 months; thus, animals are affected by variations in supply and quality of pasture throughout the year, which decrease energy reserve of animals (BFT and RFT).

Bell and Greenwood (2016) and Robinson et al. (2013) showed that intrauterine growth retardation induced by nutritional variations during pregnancy can compromise progeny growth and require more time to reach market weight. Greenwood and Café (2007) concluded that maternal restriction in early periods of pregnancy affects growth during fetal life, implying in lower BW and ADG in progenies during the rearing and finishing phases from of that underwent nutritional restriction.

Animals in the FP group showed higher BW and tendency in ADG, due to the good nutritional supply throughout the gestational period, however this higher BW is not due to MCA and NCREA, which did not show differences between treatments. REA showed tendency to be greater in the FP group than in NP group, nevertheless this result was not supported by the other muscle assessments. So based only in our histological results, the prenatal treatments did not affect the hyperplasia rate of muscle cells during primary myogenesis nor the hypertrophic rate of muscle cell, as reported by Du et al. (2010). REA is used as an indicator of muscularity, edible carcass portion and yield of

commercial value cuts (BERGEN et al., 1996; BIANCHINI et al., 2007; LOPES et al., 2012). This variable may be demonstrating that these characteristics had an impact during pregnancy, with an increase in hyperplasia and/or hypertrophy, even though it is not possible to confirm this result at the cellular level by histological assessments. In addition, different types of skeletal muscles have different effects as a result of fetal programming, varying according to muscle function and/or fiber composition (PARADIS et al., 2017), impacting differently on the carcass. Thus, the differences showed here in regard to BW are related to muscle development (REA) and has no relationship with subcutaneous fat (RFT and BFT).

Another hypothesis is that FP may be developing other tissues and organs more efficiently, explaining the higher BW. Maternal nutrient restriction in ruminants leads to placental insufficiency in meeting fetal nutritional needs and may result in smaller bones and a greater proportion of bone to muscle and adipose tissue (BELL; GREENWOOD, 2016; HOFFMAN et al., 2017). Although bones are not the tissue of greatest interest in livestock, they have priority in nutrient use over soft carcass tissues (HAMMOND, 1944) and play essential roles in the development of skeletal muscle, also impacting the meat production chain (BELL; GREENWOOD, 2016).

It has been reported by several authors that the visceral organ mass may be affected by prenatal nutrition. Meyer et al. (2010) and Duarte et al. (2013) demonstrated that prenatal diet interferes with the gastrointestinal tract, affecting the reticular mass, small intestine mass, and the vascular and cell development of the intestine. Long et al. (2010) showed that steers whose dams were fed with low-nutrition diet had lower lung and tracheal weights, however in other organs no differences were found in this study. Long et al. (2021) reported a decrease in fetal pancreatic mass in fetuses from cows that suffered food restriction during pregnancy and associated endocrine effects.

In summary, maternal nutritional supply can impact several tissues and organs, and thus lead to greater body weight, which may be occurring with the FP treatment in our study compared to the NP. However, responses to different prenatal stimuli vary by breed, supplementation level, period, nutritional differences between the groups that will be evaluated in each experiment and evaluated phenotype, which may explain the range of responses found in the literature regarding different phenotypes in ruminants.

3.5 Conclusion

Fetal programming in Nellore cattle improves BW during the rearing phase of animals and shows tendency in ADG and REA; however, different prenatal supplementation strategies do not affect fat thickness nor histological muscle cell assessments. This study showed that there was impact of prenatal nutrition on muscle (REA) in rearing phase, and the greater BW shown is probably too related to increased mass in other organs.

3.6 References

BELKACEMI, L. et al. Maternal Undernutrition Influences Placental-Fetal Development. **Biology of Reproduction**, v. 83, n. 3, p. 325–331, 1 set. 2010. Disponível em: <https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod.110.084517>. Acesso em: 13 abr. 2021.

BELL, A. W.; GREENWOOD, P. L. **Prenatal origins of postnatal variation in growth, development and productivity of ruminantsAnimal Production Science**CSIRO, , 18 jul. 2016. . Disponível em: <https://www.publish.csiro.au/an/AN15408>. Acesso em: 14 abr. 2021.

BERGEN, R. D. et al. Prediction of lean yield in yearling bulls using real-time ultrasound. **Canadian Journal of Animal Science**, v. 76, n. 3, p. 305–311, 1996. Disponível em: <https://cdnsciencepub.com/doi/abs/10.4141/cjas96-046>. Acesso em: 9 jun. 2021.

BIANCHINI, W. et al. Effect of genetic group on carcass traits and fresh and aged beef tenderness from young cattle. **Revista Brasileira de Zootecnia**, v. 36, n. 6 SUPPL., p. 2109–2117, 2007. Disponível em: <www.sbz.org.br>. Acesso em: 9 jun. 2021.

BONIN, M. N. et al. Visual body-scores selection and its influence on body size and ultrasound carcass traits in Nellore cattle. **Journal of Animal Science**, v. 93, n. 12, p. 5597–5606, 1 dez. 2015. Disponível em: <https://academic.oup.com/jas/article/93/12/5597/4717807>. Acesso em: 16 abr. 2021.

BONNET, M. et al. Ontogenesis of muscle and adipose tissues and their interactions in ruminants and other species. **Animal**, v. 4, n. 7, p. 1093–1109, 1 jan. 2010.

CASTRO-RODRÍGUEZ, D. C. et al. **Maternal interventions to prevent adverse fetal programming outcomes due to maternal malnutrition: Evidence in animal modelsPlacenta**W.B. Saunders Ltd, , 1 dez. 2020. .

COUTINHO, C. et al. Growth curves of carcass traits obtained by ultrasonography in three lines of Nellore cattle selected for body weight. **Genetics and Molecular Research**, v. 14, n. 4, p. 14076–14087, 2015. Disponível em: <http://dx.doi.org/10.4238/2015.October.29.27>. Acesso em: 16 abr. 2021.

DICKINSON, H. et al. **A review of fundamental principles for animal models of DOHaD research: An Australian perspectiveJournal of Developmental Origins of Health and Disease**, 2016. .

DU, M. et al. Fetal programming of skeletal muscle development in ruminant animals. **Journal of animal science**, v. 88, n. 13 Suppl, 2010.

DU, M. et al. Meat science and muscle Biology Symposium: Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **Journal of Animal Science**, v. 91, n. 3, p. 1419–1427, mar. 2013. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/23100595/>. Acesso em: 12 ago. 2020.

DUARTE, M. S. et al. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. **Livestock Science**, v. 153, n. 1–3, p. 60–65, 1 maio 2013.

GREENWOOD, P. L.; CAFE, L. M. Prenatal and pre-weaning growth and nutrition of cattle: Long-term consequences for beef production. **Animal**, v. 1, n. 9, p. 1283–1296, 2007.

HAMMOND, J. Physiological factors affecting birth weight. **Proceedings of the Nutrition Society**, v. 2, n. 1–2, p. 8–14, 1944. Disponível em: <https://scholar.google.com.br/scholar?hl=pt-BR&as_sdt=0%2C5&q=Physiological+factors+affecting+birth+weight&btnG=>. Acesso em: 4 jun. 2021.

HOFFMAN, M. L. et al. Physiology and endocrinology symposium: The effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism. **Journal of Animal Science**, v. 95, n. 5, p. 2222–2232, 1 maio 2017.

JENNINGS, T. D. et al. The influence of maternal nutrition on expression of genes responsible for adipogenesis and myogenesis in the bovine fetus. **Animal**, v. 10, n. 10, p. 1697–1705, 1 out. 2016.

LONG, J. M. et al. Maternal nutrient restriction alters endocrine pancreas development in fetal heifers. **Domestic Animal Endocrinology**, v. 74, p. 106580, 1 jan. 2021.

LONG, N. M. et al. Effects of nutrient restriction of bovine dams during early gestation on postnatal growth, carcass and organ characteristics, and gene expression in adipose tissue and muscle. **Journal of Animal Science**, v. 88, n. 10, p. 3251–3261, 1 out. 2010. Disponível em: <https://academic.oup.com/jas/article/88/10/3251/4764175>. Acesso em: 27 dez. 2019.

LOPES, L. S. et al. Características de carcaça e cortes comerciais de tourinhos Red Norte e Nelore terminados em confinamento. **Revista Brasileira de Zootecnia**, v. 41, n. 4, p. 970–977, 2012. Disponível em: <www.sbz.org.br>. Acesso em: 9 jun. 2021.

MARESCA, S. et al. The influence of protein restriction during mid- to late gestation on beef offspring growth, carcass characteristic and meat quality. **Meat Science**, v. 153, n. March, p. 103–108, 2019. Disponível em: <https://doi.org/10.1016/j.meatsci.2019.03.014>.

MEYER, A. M. et al. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. **Journal of Animal Science**, v. 88, n. 7, p. 2410–2424, 1 jul. 2010. Disponível em: <https://academic.oup.com/jas/article/88/7/2410/4745653>. Acesso em: 4 jun. 2021.

PARADIS, F. et al. Maternal nutrient restriction in mid-to-late gestation influences fetal mRNA expression in muscle tissues in beef cattle. **BMC Genomics**, v. 18, n. 1, p. 632, 18 ago. 2017. Disponível em: <http://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-017-4051-5>. Acesso em: 27 jan. 2021.

PICARD, B. et al. **Muscle fibre ontogenesis in farm animal speciesReproduction Nutrition Development**EDP Sciences, , 1 set. 2002. . Disponível em: <http://dx.doi.org/10.1051/rnd:2002035>. Acesso em: 24 maio. 2021.

POLIZEL, G. H. G. et al. Evaluation of reproductive traits and the effect of nutrigenetics on bulls submitted to fetal programming. **Livestock Science**, v. 247, p. 104487, 1 maio 2021. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1871141321000950>. Acesso em: 7 abr. 2021.

PROPHET, E. et al. **Laboratory methods in histotechnology**. [s.l: s.n.]

ROBINSON, D. L.; CAFÉ, L. M.; GREENWOOD, P. L. Meat Science and muscle Biology Symposium: Developmental programming in cattle: Consequences for growth, efficiency, carcass, muscle, and beef quality characteristics. **Journal of Animal Science**, v. 91, n. 3, p. 1428–1442, mar. 2013. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/23230118/>. Acesso em: 12 ago. 2020.

SILVA, S.; NASCIMENTO JÚNIOR, D. Avanços na pesquisa com plantas forrageiras tropicais em pastagens : características morfofisiológicas e manejo do pastejo. **Revista Brasileira de Zootecnia**, v. 36, n. 1516–3598, p. 121–138, 2007.

UNDERWOOD, K. R. et al. Nutrition during mid to late gestation affects growth, adipose tissue deposition, and tenderness in cross-bred beef steers. **Meat Science**, v. 86, n. 3, p. 588–593, nov. 2010.

WU, G. et al. **Maternal nutrition and fetal development Journal of Nutrition** American Institute of Nutrition, , 1 set. 2004. . Disponível em: <https://academic.oup.com/jn/article/134/9/2169/4688801>. Acesso em: 13 abr. 2021.

ZHU, M.-J. et al. Effect of Maternal Nutrient Restriction in Sheep on the Development of Fetal Skeletal Muscle1. **Biology of Reproduction**, v. 71, n. 6, p. 1968–1973, 2004.

4. Chapter 3: Identification of eQTLs and differential gene expression associated with fetal programming in beef cattle

Guilherme Henrique Gebim Polizel[1*], Aline Silva Mello Cesar[2], Roberta Cavalcante Cracco[1], Arícia Christofaro Fernandes[1], Gustavo Morandini Reginato[3], Pedro Luiz Porfirio Xavier[3], Isabela Mortari[1], Édison Furlan[1], Heidge Fukumasu[3], Miguel Henrique de Almeida Santana[1].

[1] Department of Animal Science, College of Animal Science and Food Engineering – USP, Av. Duque de Caxias Norte, 225, Pirassununga, SP, 13635-900, Brazil.

[2] Departament of Agri-food Industry, Food and Nutrition, Luiz de Queiroz College of Agriculture – USP, Av. Pádua Dias, 11, Piracicaba, SP, 13418-900, Brazil.

[3] Department of Veterinary Medicine, College of Animal Science and Food Engineering – USP, Av. Duque de Caxias Norte, 225, Pirassununga, SP, 13635-900, Brazil.

Abstract

This study assessed differential gene expression and identified expression quantitative trait loci (eQTLs) from samples of *Longissimus lumborum* muscle from bulls at 15 months of age submitted to fetal programming. Upon confirmation of pregnancy, 126 dams were separated into three prenatal nutritional treatments. At birth, 63 males were genotyped with GGP LD BeadChip and the skeletal muscle of 15 bulls was sequenced (RNA-seq) at 15 months of age. The EdgeR package **(**based on negative binomial distributions) was used for differential gene expression and the Matrix eQTL package (association between each gene and each genetic variation) was used for the eQTLs analysis (R statistical). The functional enrichment analysis was performed using the MetaCore® software. No genes differentially expressed were found between treatments (FDR>0.05); nevertheless, we found 610,467 eQTLs (894 cis-eQTLs and 609,573 trans-

eQTLs; FDR<0.05). The functional enrichment analysis allowed to identified terms from Gene Ontology (GO terms) related to genes associated to trans-eQTLs (FDR<0.05) as well as metabolic pathways (>gScore). Most biological pathways and genes found had been previously associated to fetal programming. Thus, the eQTLs found here may be possibly masking the effects of prenatal nutrition on skeletal muscle of the animals evaluated.

Keywords: Nellore, Prenatal nutrition, SNPs, Trans-eQTLs, WNT family genes

4.1 Introduction

Fetal programming occurs at a critical period in the embryonic and fetal stages of mammals, changing from cell differentiation to development of organs and tissues. Nutritional stimuli have a direct effect on this process and are some of the most relevant extrinsic factors in gene-environment interaction, influencing the intrauterine development of offspring and thus generating long-term consequences (TOCA; TONIETTI; VECCHIARELLI, 2015).

The fetus or embryo interactions with uterine environment create conditions to prepare offspring for postnatal life, because the extra-uterine environment usually corresponds to the similar conditions the progenies are submitted to during pregnancy (GLUCKMAN et al., 2005). This process of intrauterine adaptation by fetus is called developmental plasticity (WEST-EBERHARD, 2005). In the event of incompatibility between the environments of these different life stages, development plasticity can change metabolic parameters and generate incompatible phenotypes (BATESON et al., 2004).

Recent studies have assessed the prenatal nutritional effects on progeny of different species (AYSONDU; OZYUREK, 2020; RAMÍREZ et al., 2020; FRANCO et

al., 2021). In addition, fetal programming may have a major impact on fertility, health, growth, body composition, meat quality, and carcass yield in cattle (LONG et al., 2009; MULLINIKS et al., 2013; GREENWOOD; BELL, 2019; RAMÍREZ et al., 2020), significantly affecting profitability of meat production chain. (FUNSTON; LARSON; VONNAHME, 2010). In this context, skeletal muscle becomes the most important tissue in beef cattle.

Most traits of livestock are considered complex or quantitative (CLARK; WHITELAW, 2003), as they are controlled by a combination of genetics, epigenetics, and environment (GALLAGHER; CHEN-PLOTKIN, 2018). Thus, these multiple factors (WOOD; ESKO; YANG, 2014) hinder the understanding of biological mechanisms involved in development and performance of organisms.

The analysis of expression quantitative trait loci (eQTL) evaluates the association of the single-nucleotide polymorphisms (SNPs) with the expression level of the different genes expressed in a given type of cell or tissue (DEELEN et al., 2015; LITTLEJOHN et al., 2016). This analysis allow a better understanding of genetic variability on pathways and functions of the genes involved (CARMELO; KADARMIDEEN, 2020). In addition, this data integration at different molecular levels may reveal mechanisms not yet established in the literature (ROHART et al., 2017). Therefore, this study assessed the differential gene expression and genetic variations associated with levels of gene expression (eQTLs) of Longissimus lumborum muscle from young bulls with 15 months of age submitted to fetal programming.

4.2 Material and methods

4.2.1 Ethics statement

This study was approved by the Research Ethics Committee of FZEA/USP, under protocol No. 1843241117, according to the guidelines of the National Council for the Control of Animal Experimentation (CONCEA).

4.2.2 Experimental design

In this study, Nellore dams (n = 126) was fixed-time artificially inseminated (FTAI) with semen from four different animals with known genetic values. All cows were allocated to pasture paddocks of *Brachiaria brizantha* cv. Marandu, with access to supplement and water *ad libitum*. Upon confirmation of pregnancy, the cows were divided into three nutritional treatments: (1) Not Programmed Diet (NP; control): basal diet with only mineral supplementation throughout pregnancy, equivalent to 0.03% of body weight per day; (2) Partial Programmed Diet (PP): basal diet with protein-energy supplementation equivalent to 0.3% of body weight per day during the final third of pregnancy; (3) Full Programmed Diet (FP): basal diet with protein-energy supplementation equivalent to 0.3% of body weight per day during whole pregnancy from the confirmation of pregnancy (see diets and components in Tables 1 and 2). During the gestational period, rotation of pasture paddocks was used in all groups to prevent any interference of pasture quality on the treatments. The pastures used in the different treatments showed similar nutritional values: NP – TDN (total digestible nutrients) = 63.07% ± 1.45% and CP (crude protein) = 7.38% ± 1.73%; PP – TDN = 64.1% ± 2.34% and CP = 7.82 ± 2.29%; FP – TDN = 61.43% ± 2.13% and CP = 7.40% ± 2.30%. The treatments were divided according to age, body weight, and body condition score.

Table 1. Ingredients and nutrients content of the matrices supplement.

Ingredients	Mineral supplement	Energetic-proteic supplement
Corn (%)	35.00	60.00
Soybean meal (%)	-	30.00
Dicalcium phosphate (%)	10.00	-
Urea 45% (%)	-	2.50
Salt (%)	30.00	5.00
Minerthal 160 MD (%)*	25.00	2.50
Total digestible nutrients (%)	26.76	67.55
Crude protein (%)	2.79	24.78
Non-protein nitrogen (%)	-	7.03
Acid detergent fiber (%)	1.25	4.76
Neutral detergent fiber (%)	4.29	11.24
Fat (%)	1.26	2.61
Calcium (g/kg)	74.11	6.20
Phosphate (g/kg)	59.38	7.24

*Mineral premix

Table 2. Mineral premix content of the supplement for matrices**.**

Minerals	Guarantee levels (25kgs)
Calcium (Ca)	200 - 230 g
Cobalt (Co)	160 mg
Copper (Cu)	2,700 mg
Sulfur (S)	60 g
Fluorine (F)	1,600 mg
Phosphor (P)	160 g
Iodine (I)	135 mg
Manganese (Mn)	2,700 mg
Selenium (Se)	80 mg
Zinc (Zn)	8,100 mg
Sodium monensin	4,000 mg

Upon birth, cow-calf pairs were allocated together, regardless of the treatment, to facilitate the management of calves, which were subjected to the same environmental conditions (vaccination, management and nutrition) according to the farm routine. Calves were weaned at an average age of 220 ± 28 days and then separated according to sex (males and females) for the rearing phase that lasted 11 months.

At 15 months of age, 48 animals were randomly selected for biopsy of *Longissimus lumborum* muscle (between the 9th and 10th ribs). The muscle samples were collected during their biopsy, immediately frozen in liquid nitrogen, and then stored in an ultra-freezer (-80°C) until RNA extraction. Of the 48 animals, 15 male animals were selected (5 per treatment) and all offspring was from the same sire (CFM Minério; genealogy widely used in Brazil).

4.2.3 RNA extraction and sequencing

The RNA was extracted using TRIzol reagent (Life Technologies, Carlsbad, CA, USA), according to the manufacturer instructions. The total RNA was extracted from 100 mg of muscle tissue, quantified by the DS-11 spectrophotometer (Denovix, Wilmington, DE, USA), and evaluated for integrity by Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) at the end of the process. The average RIN (RNA integrity number) of the samples was 7.3.

We used 0.1-1 µg of RNA to prepare the library, according to the protocol established in the TruSeq Stranded mRNA Reference Guide (Illumina, San Diego, CA, USA). The libraries were quantified by quantitative PCR using the KAPA Library Quantification kit (KAPA Biosystems, Foster City, CA, USA) and the average library size was assessed by Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). A single flow-cell sequencing lane for the 15 samples, using the TruSeq PE Cluster kit v3-cBot-HS (Illumina, San Diego, CA, USA), was clustered and sequenced using the HiSeq2500 equipment (Illumina, San Diego, CA, USA) with the TruSeq Stranded mRNA kit, according to the manufacturer instructions. The sequencing analysis was carried out by the company NGS Soluções Genômicas, Piracicaba, São Paulo, Brazil.

4.2.4 Differential expression analysis

The analysis of differential gene expression was performed to identify genes expressed differentially in the skeletal muscle of animals submitted to different prenatal nutritional stimuli. Initially, the data generated was filtered, removing sequences of adapters and reads of low complexity by the program SeqyClean version 1.9.10 (ZHBANNIKOV et al., 2017). The quality control was carried out using the FASTQC version 0.11.9 program (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). STAR version 02021 software (DOBIN et al., 2013) was used to map reads against the reference genome *Bos taurus* ARS-UCD1.2.95 (available at: ftp://ftp.ensembl.org/pub/release-102/fasta/bos_taurus/).

The differential expression analysis was performed in the R (version 4.0.2) statistical environment by the EdgeR package, which has the levels of gene expression based on negative binomial distributions (ROBINSON; MCCARTHY; SMYTH, 2010). The statistical analysis was performed after removing genes with 0 count, with low expression (less than one read per sample on average), and that did not show more than 10 counts in at least three samples.

4.2.5 Genotyping data

Bulbs of hairs of the animal tail were the biological material for DNA extraction, carried out by the automated robot MICRO LAB ID STARlet® (Hamilton) using the NucleoSpin® 96 extraction kit (MachereyNagel). All animals involved in the experiment were genotyped (sires, dams and offsprings). Genotyping was performed according to the standard assay Infinium Assay II for the HiScanSQ® platform (Illumina, USA) with a genotyping panel specific for GeneSeek Genomic Profiler Bos Indicus GGP Nellore LD BeadChip (35,339 markers). All genotypes were imputed to a panel containing 735,965

markers using the FIMPUTE 2.2 software (SARGOLZAEI; CHESNAIS; SCHENKEL, 2014). The reference population consisted of 2,502 bulls (most representative Nellore bulls) genotyped with the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA, USA) containing 777,962 markers. Prior to imputation, the samples were edited for rate (< 90%) and minor allele frequency (MAF ≤ 5%) and for genotyped and reference populations. SNPs not assigned to any chromosome and assigned to sex chromosomes were removed from the data set. These data are already available for most recent reference genomes (ARS-UCD1.2).

4.2.6 Identification of eQTL, enrichment analysis, and biological networks

The matrix eQTL R package (SHABALIN, 2012) was used to identify associations between the genetic variants and the gene expression (XIA et al., 2017) of 15 animals, regardless of the treatment during pregnancy. The age of dams was included as a covariate in the model. Cis-eQTLs were considered SNPs associated to the variation in gene expression at distances up to 1 Mb (local variants) and trans-eQTLs at distances greater than 1 Mb from the gene (distant variants). The matrix eQTL tests the association between each gene and each SNP assuming the genotype as an additive effect, performing individual tests for each gene-marker pair and correcting for false discovery rate (FDR < 0.05; (BENJAMINI; HOCHBERG, 1995)). The list of significant genes in the eQTL analysis was used in MetaCore software (Clarivate Analytics, Boston, MA; https://portal.genego.com/) for functional enrichment of cis-eQTL and trans-eQTLs simultaneously and for assembling networks of biological processes (GO Processes) with the highest gScore. The processes of genes ontology (GO) were considered significant with FDR ≤ 0.05 and biological networks with a p value ≤ 0.05. This analysis assesses whether the result of differential gene expression is related to the genetic background of the animals evaluated.

4.3 Results

4.3.1 Sequencing data and differential gene expression

After initial filtering of reads, 90.77% of the database remained, generating an average rate of 88.3% of uniquely mapped reads with an average of unmapped reads of 9.33% and an average of multiple mapped reads of 2.37% (Table S1). The second stage of data filtering displayed 27,233 genes initially in the database with 13,738 genes expressed in *Longissimus lumborum* muscle of the animals evaluated and the filtered genes were submitted to the analysis of differential gene expression. This evaluation did not result in a significant difference (adjusted p value > 0.05) in the levels of gene expression between treatments (NP, PP, and FP) in Nellore bulls at 15 months of age. (Figure 1; Table S2).

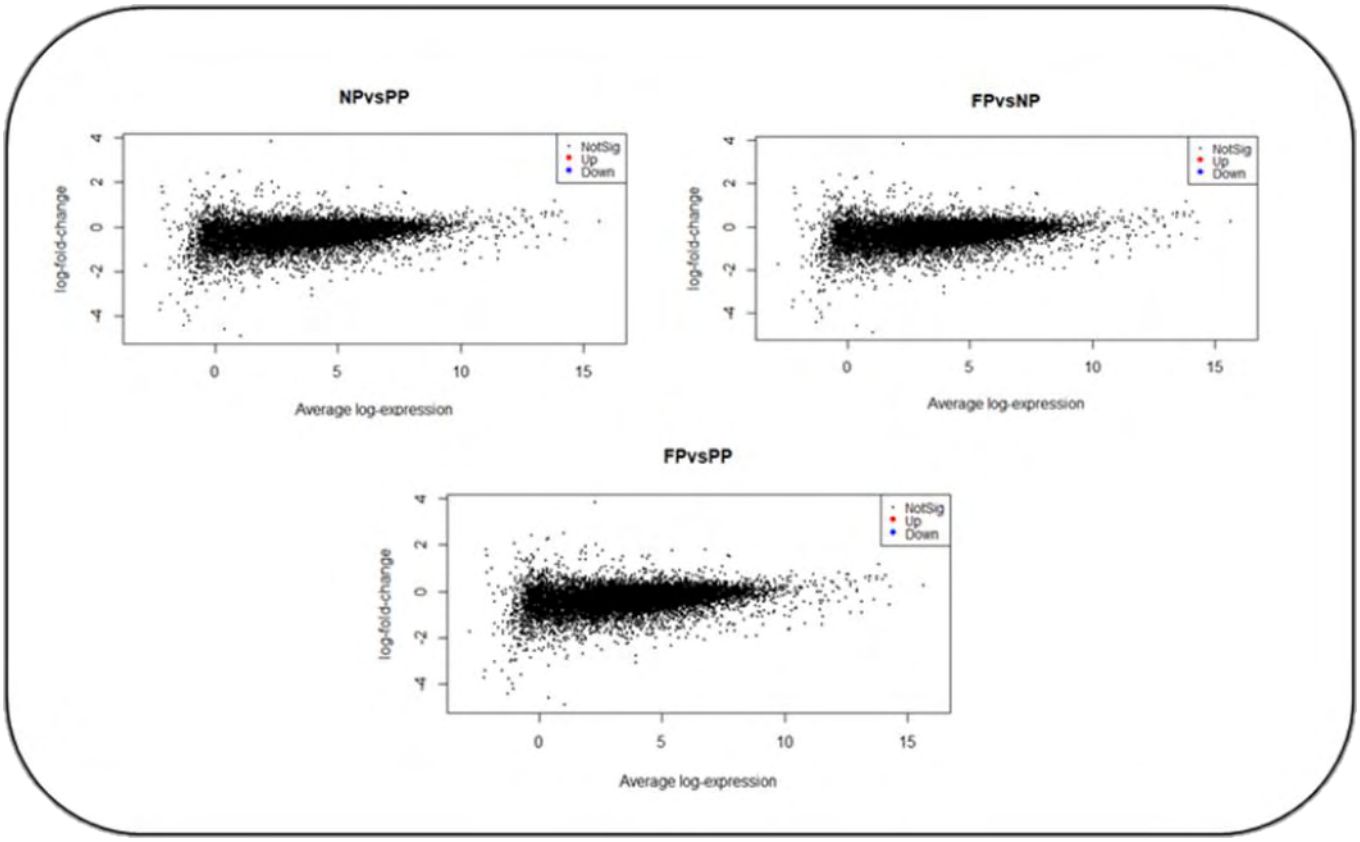

Figure 1. This figure shows the contrast graphs between the three treatments, plotting log-fold-changes of linear model against the average log-expression (log-CPM), thus

allowing the visualization of all genes graphically. In none of the contrasts was there any differential gene expression (adjusted p value > 0.05).

4.3.2 Identification of eQTLs and functional enrichment analysis

We found 610,467 significant eQTLs (FDR < 0.05) of the 15 *Longissimus lumborum* samples evaluated, with 894 cis-eQTLs (variants located within 1Mb of the associated gene; Table S3) and 609,573 trans-eQTLs (variants located farther than 1Mb from the associated gene; Table S4). The analysis showed 122,142 SNPs influencing the expression of 6,560 genes. Different SNPs were associated to the same genes, which may indicate linkage disequilibrium (LD) of some genetic variants or multiple SNPs associated with the same gene. The significant genes from eQTLs were enriched (biological processes GO category) and demonstrated no significant biological process FDR > 0.05) for cis-eQTL; however, several biological processes were found for trans-eQTL (Figure 2).

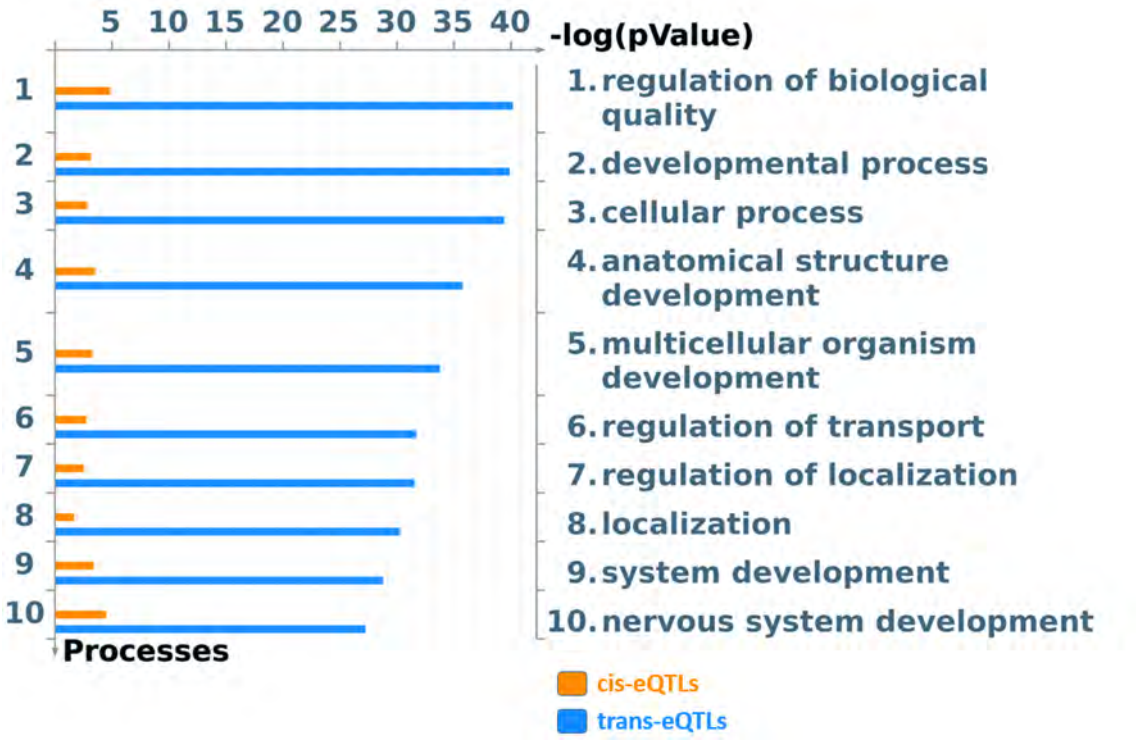

Figure 2. The top 10 significantly enriched processes from cis-eQTLs (FDR > 0.05; not significant) and trans-eQTL (FDR < 0.05; significant) genes.

The biological quality regulation for cis-eQTL presented the lowest FDR value (2.338e-3). The top 10 processes for trans-eQTLs demonstrated high significance: regulation of biological quality (FDR= 7.911e-37), developmental process (FDR=7.911e-37), cellular process (FDR=1.539e-36), anatomical structure development (FDR=6.368e-33), multicellular organism development (FDR=5.520e-31), transport regulation (FDR=5.700e-29), location regulation (FDR=6.753e-29), location (FDR=1.226e-27), system development (FDR=3.804e-26), and nervous system development (FDR=1.131e-24).

4.3.3 Trans-eQTL biological networking

We selected four networks of biological processes with the highest gScore values and with p value < 0.01 from the results of functional enrichment (Table 3).

Table 3. Top 4 biological networks with the highest gScore by MetaCore™ for genes from significant trans-eQTLs.

Network	GO processes	Total nodes	Pathways	p-Value	gScore
Beta-catenin, TCF7L2 (TCF4), Axin, Tcf(Lef), GSK3 beta	regulation of cell population proliferation (91.8%; 3.605e-39), canonical Wnt signaling pathway (40.8%; 8.546e-33), tissue development (81.6%; 1.960e-29), regulation of Wnt signaling pathway (51.0%; 7.401e-29), regulation of cell differentiation (81.6%; 8.255e-29).	52	41	2.740E-70	90.06
Shc, c-Raf-1, SOS, ERK1/2, PKC-alpha	positive regulation of multicellular organismal process (77.6%; 1.569e-26), regulation of cell motility (63.3%; 1.634e-26), regulation of MAPK cascade (59.2%; 2.004e-26), regulation of protein phosphorylation (71.4%; 2.448e-26), regulation of locomotion (63.3%; 7.363e-26).	56	9	1.180E-60	51.28
TRAF6, NF-kB, TAK1(MAP3K7), MyD88, TAB2	cellular response to interleukin-1 (66.7%; 3.124e-49), response to interleukin-1 (68.8%; 3.709e-49), positive regulation of NF-kappaB transcription factor activity (58.3%; 1.074e-44), cellular response to cytokine stimulus (87.5%; 6.885e-44), response to cytokine (89.6%; 1.526e-43).	50	12	3.000E-46	44.95
GATA-1, FAK1, Collagen IV, ERK2 (MAPK1), alpha-2/beta-1 integrin	positive regulation of wound healing (16.3%; 5.437e-11), developmental process (81.6%; 7.330e-11), positive regulation of response to wounding (16.3%; 2.457e-10), germ cell migration (10.2%; 1.639e-09), anatomical structure development (75.5%; 2.095e-09).	50	3	2.290E-52	36.23

These networks are created in real time and are exclusive to the uploaded data. The gene content of the uploaded files is used as the input list for generation of biological networks using Analyze network algorithm with default settings. This is a variant of the shortest paths algorithm with main parameters of relative enrichment with the uploaded data, and relative saturation of networks with canonical pathways. These networks are built on the fly and unique for the uploaded data. In this workflow the networks are prioritized based on the number of fragments of canonical pathways on the network.

The top four networks account for several biological processes (regulation of Wnt signaling pathway, cell differentiation regulation, regulation of cell population proliferation, positive regulation of multicellular organismal process, anatomical structure development, cellular response to interleukin-1, developmental process, protein phosphorylation), which are closely related to the effects triggered by fetal programming (Figure 3).

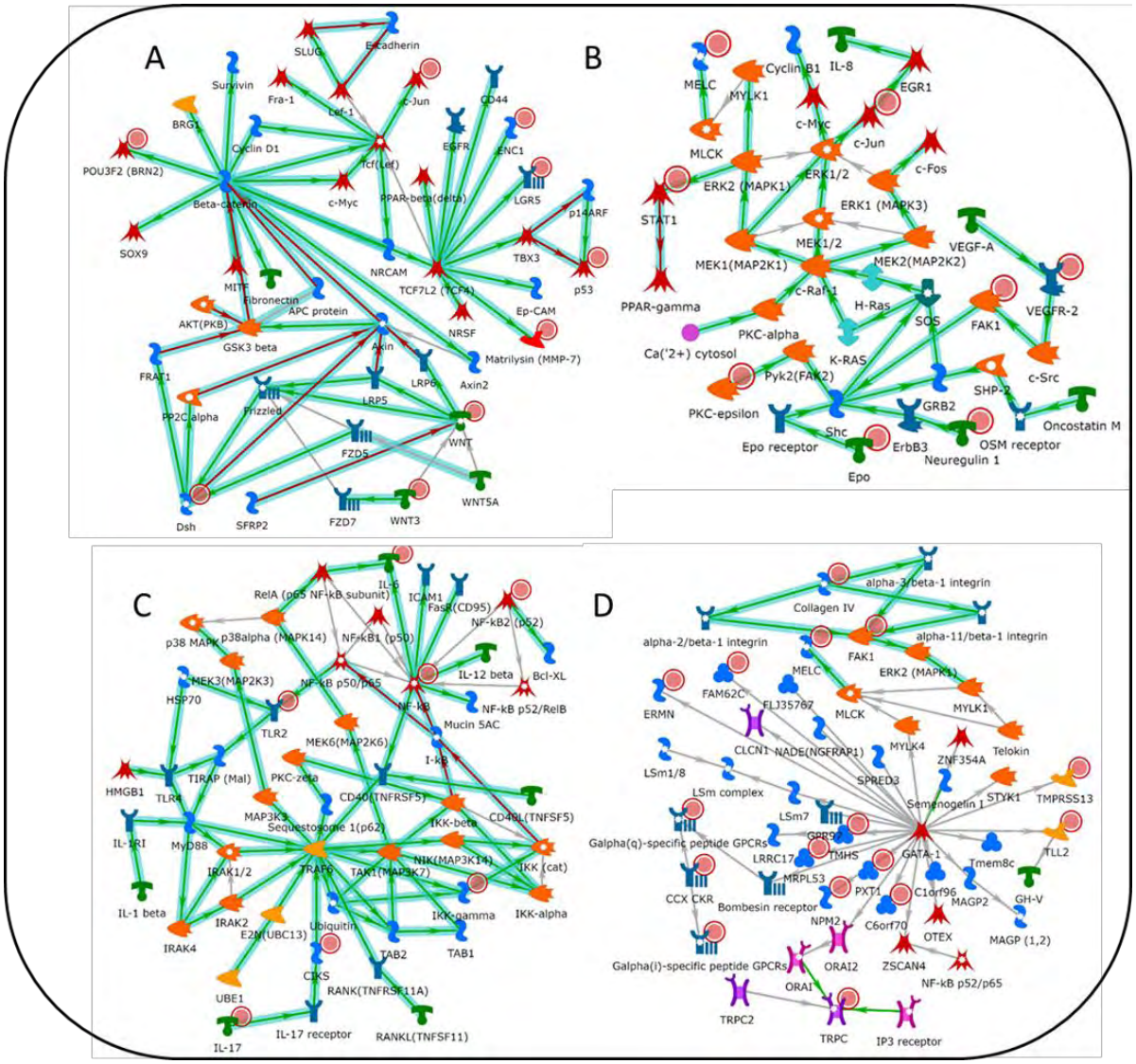

Figure 3. All networks are drawn from scratch by GeneGo annotators and manually curated and edited. The top 4 scored (by the number of pathways and gScore) network (respectively A, B, C and D) from active experiments. Networks A, B, C and D correspond respectively to the 4 processes shown in table 3. Thick cyan lines indicate the

fragments of canonical pathways. The genes present in the list of significant trans-eQTL genes are marked with red circles. The legend for each item in this figure is in Additional file 1.

4.3.4 Genes and SNPs

We selected four transcription factors (JUN, STAT1, NF-kB2, POU3F2), two genes (IL-6, NPM2), and a family of genes (WNT1, WNT3, WNT-3a, WNT-5b, WNT-7a, WNT -7b, WNT-9b) from the biological networks on our list of significant genes for trans-eQTLs with their respective SNPs (Table 4). These genes and transcription factors were selected based on their functions described in the literature and potential for relationship with fetal programming. Based on the results of functional enrichment and the associated GO terms, the selected genes and transcription factors are related to processes, such as intrauterine development, cell differentiation, regulation of gene expression, and immunological processes.

Table 4. Genes selected from the 4 main significant networks with their respective genetic variants (SNPs). These genes are present in our list of trans-eQTL genes.

Genes/Transcript Factors	SNPs
WNT gene family	162 genetic variants are correlated (view in table S4)
NPM2	BOVINEHD0200018117, BOVINEHD0200019309, BOVINEHD0200026945, BOVINEHD0300027944, BOVINEHD0900028725, BOVINEHD1100025688, BOVINEHD1400010230, BOVINEHD1400024462, BOVINEHD1400010312, BOVINEHD1400010348, BOVINEHD1700016553, BOVINEHD1800019633, BOVINEHD1900007688, BOVINEHD1900008702, BOVINEHD2000000967, BOVINEHD2000000975, BOVINEHD2000000994, BOVINEHD2000002621, BOVINEHD2100002799, BOVINEHD2400004171, BOVINEHD0700024809, BOVINEHD1300010724
IL-6	BOVINEHD0200009350, BOVINEHD0200009353, BOVINEHD1300020973, BOVINEHD1300020974, BOVINEHD1300024336, BOVINEHD1300024345
POU3F2	BOVINEHD0300031114, BOVINEHD0900008970, BOVINEHD0900016348, BOVINEHD0700024392, BOVINEHD2300008493, BOVINEHD2300008507, BOVINEHD2800008404, BOVINEHD2800008415)
JUN	BOVINEHD0300032161
STAT1	BOVINEHD0700003346, BOVINEHD0700003363
NF-kB2	BOVINEHD0900022675, BOVINEHD0900022676

4.4 Discussion

To the best of our knowledge and based on a literature search, this is the first study to evaluate eQTLs in Nellore cattle submitted to fetal programming. The pioneering nature of this study encourages further studies with different tissues and productive phases of Nellore animals from different prenatal stimuli.

The Nellore breed accounts for roughly 80% of the total beef cattle herd in Brazil (ACNB, 2021). Nellore was introduced around the 1960s with descendants of six main bulls (MAGNABOSCO; CORDEIRO; TROVO, 1997) and, since the 1950s, breeding programs have been established to optimize the adaptability and performance of these animals to different Brazilian biomes (CARVALHEIRO, 2014; ALBUQUERQUE et al.,

2017). Artificial insemination has been widely used in Brazil (FOOTE, 2002) and most semen used in animal rearing comes from few breeders (MUDADU et al., 2016). All these factors may influence genetic diversity of Nellore herd in Brazil (MARCONDES et al., 2007; MUDADU et al., 2016; GIONBELLI et al., 2018). In addition, the adaptation process of animals, due to climatically challenges, has fostered the identification of new genetic variants (SNPs) mainly related to reproductive processes, heat tolerance, and resistance to diseases (FERNANDES JÚNIOR et al., 2020). Therefore, even after decades since its introduction to Brazil, Nellore breed has an excellent capacity to adapt to low supply and quality of pastures, rain scarcity, and high temperatures.

Our results show that the daily addition of protein-energy supplement corresponding to 0.3% of the body weight of mothers did not demonstrate genes differentially expressed in offspring skeletal muscle (FDR > 0.05) between the treatments FP (entire pregnancy), PP (only the final third of the pregnancy), and NP (absence of protein-energy supplementation during the entire pregnancy). Possibly, this supplementation level in Nellore dams cannot influence gene expression significantly in their progenies at 15 months of age.

We hypothesize that some effects of fetal programming on the life of mammals described in the literature (DU et al., 2010; MULLINIKS et al., 2013; BOLLWEIN; JANETT; KASKE, 2016; COSTA et al., 2019; HUBER et al., 2020; LONG et al., 2021) may be covered by eQTLs related to features influenced by maternal nutrition during the gestational period. Thus, if genetic variants control the response to a specific stimulus in a specific group or breed, regardless of the stimulus submitted, the control of post-stimulus performance is carried out by the genetic variant, minimizing the environmental effect. Our theory is that fetal programming in Nellore animals is hampered due to the adaptive process that the breed has constantly undergone since its implantation to country,

such as resistance to drought and low food supply (SAMPAIO et al., 2010), also observed during the gestation of these animals. This may have developed transgenerational eQTLs that control embryonic and fetal development, cell differentiation, organ and tissue development, and immune response.

Studies (GICQUEL; EL-OSTA; LE BOUC, 2008; WADHWA et al., 2012; CHEN et al., 2014; O'DONNELL et al., 2017; QIU et al., 2017) show that the genetic background can affect variation in the resilience levels to prenatal stressful effects on the offspring. Teh et al. (2014) showed that genetic variations in ethnic groups in Singapore may influence epigenetic changes, concluding that 25% of variability in specific methylated regions in children is explained by genetic differences. The same authors found a correlation between genotype, different stimuli in the uterine environment, and variation of methylated regions, contributing to the theory formulated in this study for Nellore animals with different nutritional plans during pregnancy.

Regulation of canonical WNT signaling pathway involves several embryological processes, proliferation, migration, differentiation, death, and function of several cell types, participating generally in the development and homeostasis of the organism (NOVAKOFSKI, 2004; JOHNSON; RAJAMANNAN, 2006). Studies show that blocking the β-catenin pathway reduces the total number of muscle cells (PAN et al., 2005; YAMANOUCHI et al., 2007). The positive regulation of this pathway is related to the promotion of myogenesis and, conversely, the negative regulation with adipogenesis (SHANG et al., 2007). WNT-3a and WNT-7a are directly involved in the process of forming cartilaginous tissue and regulating chondrogenesis (RUDNICKI; BROWN, 1997; KENGAKU et al., 1998; CHURCH et al., 2002). Therefore, the JUN gene acts in conjunction with WNT-3a and generates a cascade of reactions that can lead to chondrogenesis inhibition and chondrocyte de-differentiation (HWANG et al., 2005). The

role of POU3F2 in conjunction with WNT signalling is related to the development of melanocytes and melanoma (DELMAS et al., 2007; LARUE; DELMAS, 2009) and also plays a key role in neural development (SALINAS; ZOU, 2008). Additionally, the canonical WNT signaling pathway showed different patterns of coexpression in the skeletal muscle of Brangus-Angus crossbred calves subjected to different prenatal nutritional stimuli (LIU et al., 2020).

STAT1 has several functions in biological processes, namely proliferation, cell differentiation, promotion of apoptotic cell death (KIM; LEE, 2007), muscle regeneration, myogenesis (SUN et al., 2007; GAO et al., 2012), and repression of PPARγ2 transcription in adipocytes (HOGAN; STEPHENS, 2001). In addition, the interaction of this transcription factor with insulin resistance, characteristic of type 2 diabetes mellitus, was recently discovered. (COX et al., 2020). The latter role is extremely important for fetal programming, since several species have demonstrated a relationship between low fetal growth and risk of developing type 2 diabetes mellitus (JONES; OZANNE, 2009).

The IL-6 gene produces proteins of the class cytokines; however, when expressed and released into the muscle, these proteins are called myokines and exert endocrine, paracrine, immunological, and metabolic functions in the organ (PEDERSEN et al., 2007). During muscle contraction, significant amounts of IL-6 are released into the circulation, increasing its concentration in the blood plasma (STEENSBERG et al., 2000). This process is probably related to the low muscle glycogen stock and therefore needs blood glucose to maintain energy levels (GLEESON, 2000). This gene and the transcription factor NF-kB are also related to function loss and muscle mass (THOMA; LIGHTFOOT, 2018). Studies show that NF-kappa-beta is also related to myogenesis during fetal skeletal muscle development (ARDITE et al., 2004; LANGEN et al., 2004;

WANG et al., 2007; BAKKAR et al., 2008) and adipogenesis (BERG et al., 2004; HEMMRICH et al., 2007; REYNA et al., 2008).

NPM-2 is a gene with a maternal effect related to embryological development, participating in processes, such as chromatin and nucleolar organization (BURNS et al., 2003). This gene expression is temporarily regulated during embryogenesis by miR-181a (LINGENFELTER et al., 2011). The family of nucleoplasmines/nucleophosmin (NPM1, NPM2, and NPM3) has as main functions in cellular processes, such as genome stability, ribosomal biogenesis, DNA duplication, and transcription regulation (FREHLICK; EIRÍN-LÓPEZ; AUSIÓ, 2007). These biological processes are essential for responses of an individual to a stimulus or an insult during pregnancy, mainly because it is related to epigenetic mechanisms.

In summary, the genes discussed present functional and biological issues closely related to the effects of fetal programming described in the literature. These genes are the result of significant eQTLs from Longissimus lumborum muscle of the animals evaluated, regardless of the treatment received during pregnancy. The results show that the eQTLs found are possibly responsible for suppressing effects from different prenatal nutritional stimuli, as highlighted in our theory. Further studies are needed to assess resilience of the Nellore cattle to epigenetic effects during pregnancy to properly determine the effective prenatal stimulation level that induces these changes.

4.5 Conclusion

The protein-energy supplement during the final third and throughout pregnancy does not affect muscular gene expression of Nellore bulls at 15 months of age. However, the population evaluated showed significant eQTLs in the *Longissimus lumborum* muscle that control features possibly influenced by fetal programming, highlighting new adaptation mechanisms of Nellore breed not yet described in the literature. Thus, the

genetic background of these animals may possibly present greater tolerance to changes in the epigenome and may be masking part of the fetal programming effects.

4.6 References

ACNB. **Associação dos criadores de Nelore do Brasil/A Raça - Histórico**. Disponível em: <http://www.nelore.org.br/Raca/Historico>. Acesso em: 18 fev. 2021.

ALBUQUERQUE, L. et al. Beef cattle genomic selection in tropical environments. In: Proc. Assoc. Advmt. Anim. Breed. Genet, **Anais**...2017. Disponível em: <https://www.researchgate.net/publication/323029639>. Acesso em: 18 fev. 2021.

ARDITE, E. et al. Glutathione depletion impairs myogenic differentiation of murine skeletal muscle C2C12 cells through sustained NF-κB activation. **American Journal of Pathology**, v. 165, n. 3, p. 719–728, 1 set. 2004.

AYSONDU, M. H.; OZYUREK, S. **Influences of Maternal Undernutrition on Placental Development and Birth Weight in SheepLarge Animal Review**. [s.l: s.n.]. Disponível em: <https://www.largeanimalreview.com/index.php/lar/article/view/168>. Acesso em: 27 jan. 2021.

BAKKAR, N. et al. IKK/NF-κB regulates skeletal myogenesis via a signaling switch to inhibit differentiation and promote mitochondrial biogenesis. **Journal of Cell Biology**, v. 180, n. 4, p. 787–802, 25 fev. 2008. Disponível em: <http://www.jcb.org/cgi/doi/>. Acesso em: 22 fev. 2021.

BATESON, P. et al. Developmental plasticity and human health. **Nature**, v. 430, n. 6998, p. 419–421, 22 jul. 2004. Disponível em: <www.nature.com/nature>. Acesso em: 2 fev. 2021.

BENJAMINI, Y.; HOCHBERG, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. **Journal of the Royal Statistical Society: Series B (Methodological)**, v. 57, n. 1, p. 289–300, 1 jan. 1995. Disponível em: <http://doi.wiley.com/10.1111/j.2517-6161.1995.tb02031.x>. Acesso em: 8 fev. 2021.

BERG, A. H. et al. Adipocyte differentiation induces dynamic changes in NF-κB expression and activity. **American Journal of Physiology - Endocrinology and Metabolism**, v. 287, n. 6 50-6, dez. 2004. Disponível em: <http://www.ajpendo.orge1178>. Acesso em: 22 fev. 2021.

BOLLWEIN, H.; JANETT, F.; KASKE, M. **Impact of nutritional programming on the growth, health, and sexual development of bull calvesDomestic Animal Endocrinology**Elsevier Inc., , 1 jul. 2016. .

BURNS, K. H. et al. Roles of NPM2 in chromatin and nucleolar organization in oocytes and embryos. **Science**, v. 300, n. 5619, p. 633–636, 25 abr. 2003. Disponível em:

<www.sciencemag.orghttp://science.sciencemag.org/>. Acesso em: 22 fev. 2021.

CARMELO, V. A. O.; KADARMIDEEN, H. N. Genetic variations (eQTLs) in muscle transcriptome and mitochondrial genes, and trans-eQTL molecular pathways in feed efficiency from Danish breeding pigs. **PLoS ONE**, v. 15, n. 9 September, 1 set. 2020.

CARVALHEIRO, R. Genomic Selection in Nelore Cattle in Brazil. In: Proceedings, 10 th World Congress of Genetics Applied to Livestock Production, Vancouver. **Anais**... Vancouver: 2014.

CHEN, L. et al. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism influences the association of the methylome with maternal anxiety and neonatal brain volumes. **Development and Psychopathology**, v. 27, n. 1, p. 137–150, 2014. Disponível em: <http://www.singstat.gov.sg/publications>. Acesso em: 19 fev. 2021.

CHURCH, V. et al. Wnt regulation of chondrocyte differentiation. **Journal of Cell Science**, v. 115, n. 24, p. 4809–4818, 15 dez. 2002. Disponível em: <http://home.ncifcrf.gov/hivdrp/RCAS/plasmid.html>. Acesso em: 20 fev. 2021.

CLARK, J.; WHITELAW, B. **A future for transgenic livestockNature Reviews Genetics**Nature Publishing Group, , 1 out. 2003. . Disponível em: <http://www.nuffieldfoundation.org>. Acesso em: 3 fev. 2021.

COSTA, T. C. et al. Effect of maternal feed restriction in dairy goats at different stages of gestation on skeletal muscle development and energy metabolism of kids at the time of births. **Animal Reproduction Science**, v. 206, n. January, p. 46–59, 2019. Disponível em: <https://doi.org/10.1016/j.anireprosci.2019.05.006>.

COX, A. R. et al. STAT1 dissociates adipose tissue inflammation from insulin sensitivity in obesity. **Diabetes**, v. 69, n. 12, p. 2630–2641, 1 dez. 2020. Disponível em: <https://diabetes.diabetesjournals.org/content/69/12/2630>. Acesso em: 21 fev. 2021.

DEELEN, P. et al. Calling genotypes from public RNA-sequencing data enables identification of genetic variants that affect gene-expression levels. **Genome Medicine**, v. 7, n. 1, p. 30, 27 mar. 2015. Disponível em: <http://genomemedicine.com/content/7/1/30>. Acesso em: 3 fev. 2021.

DELMAS, V. et al. β-Catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. **Genes and Development**, v. 21, n. 22, p. 2923–2935, 15 nov. 2007. Disponível em: <http://www.genesdev.org.>. Acesso em: 20 fev. 2021.

DOBIN, A. et al. STAR: Ultrafast universal RNA-seq aligner. **Bioinformatics**, v. 29, n. 1, p. 15–21, 2013. Disponível em: <https://academic.oup.com/bioinformatics/article-abstract/29/1/15/272537>. Acesso em: 4 fev. 2021.

DU, M. et al. Fetal programming of skeletal muscle development in ruminant animals. **Journal of animal science**, v. 88, n. 13 Suppl, 2010.

FERNANDES JÚNIOR, G. A. et al. Whole-genome sequencing provides new insights into genetic mechanisms of tropical adaptation in Nellore (Bos primigenius indicus). **Scientific Reports**, v. 10, n. 1, p. 1–7, 1 dez. 2020. Disponível em: <https://doi.org/10.1038/s41598-020-66272-7>. Acesso em: 18 fev. 2021.

FOOTE, R. H. The history of artificial insemination: Selected notes and notables1. **Journal of Animal Science**, v. 80, n. E-suppl_2, p. 1–10, 2002.

FRANCO, P. N. et al. Maternal Undernutrition Modulates Neonatal Rat Cerebrovascular Structure, Function, and Vulnerability to Mild Hypoxic-Ischemic Injury via Corticosteroid-Dependent and -Independent Mechanisms. **International Journal of Molecular Sciences**, v. 22, n. 2, p. 680, 12 jan. 2021. Disponível em: <https://www.mdpi.com/1422-0067/22/2/680>. Acesso em: 27 jan. 2021.

FREHLICK, L. J.; EIRÍN-LÓPEZ, J. M.; AUSIÓ, J. New insights into the nucleophosmin/nucleoplasmin family of nuclear chaperones. **BioEssays**, v. 29, n. 1, p. 49–59, 1 jan. 2007. Disponível em: <http://doi.wiley.com/10.1002/bies.20512>. Acesso em: 22 fev. 2021.

FUNSTON, R. N.; LARSON, D. M.; VONNAHME, K. A. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production1. **Journal of Animal Science**, v. 88, n. suppl_13, p. E205–E215, 1 abr. 2010. Disponível em: <https://academic.oup.com/jas/article/88/suppl_13/E205-E215/4779794>. Acesso em: 27 jan. 2021.

GALLAGHER, M. D.; CHEN-PLOTKIN, A. S. **The Post-GWAS Era: From Association to FunctionAmerican Journal of Human Genetics**Cell Press, , 3 maio 2018. .

GAO, Y. et al. Loss of STAT1 in Bone Marrow-Derived Cells Accelerates Skeletal Muscle Regeneration. **PLoS ONE**, v. 7, n. 5, p. e37656, 23 maio 2012. Disponível em: <https://dx.plos.org/10.1371/journal.pone.0037656>. Acesso em: 21 fev. 2021.

GICQUEL, C.; EL-OSTA, A.; LE BOUC, Y. **Epigenetic regulation and fetal programmingBest Practice and Research in Clinical Endocrinology and Metabolism**Baillière Tindall, , 1 fev. 2008. .

GIONBELLI, T. R. S. et al. Foetal development of skeletal muscle in bovines as a function of maternal nutrition, foetal sex and gestational age. **Journal of Animal Physiology and Animal Nutrition**, v. 102, n. 2, p. 545–556, 2018.

GLEESON, M. Interleukins and exercise. **The Journal of Physiology**, v. 529, n. 1, p. 1–1, 15 nov. 2000. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/j.1469-7793.2000.00001.x>. Acesso em: 22 fev. 2021.

GLUCKMAN, P. D. et al. Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical

studies. **Proceedings of the Royal Society B: Biological Sciences**, v. 272, n. 1564, p. 671–677, 7 abr. 2005. Disponível em: <https://royalsocietypublishing.org/doi/10.1098/rspb.2004.3001>. Acesso em: 2 fev. 2021.

GREENWOOD, P. L.; BELL, A. W. **Developmental Programming and Growth of Livestock Tissues for Meat ProductionVeterinary Clinics of North America - Food Animal Practice**W.B. Saunders, , 1 jul. 2019. .

HEMMRICH, K. et al. Monocyte Chemoattractant Protein-1 and Nitric Oxide Promote Adipogenesis in a Model That Mimics Obesity**. **Obesity**, v. 15, n. 12, p. 2951–2957, 1 dez. 2007. Disponível em: <http://doi.wiley.com/10.1038/oby.2007.352>. Acesso em: 22 fev. 2021.

HOGAN, J. C.; STEPHENS, J. M. The identification and characterization of a STAT 1 binding site in the PPARγ2 promoter. **Biochemical and Biophysical Research Communications**, v. 287, n. 2, p. 484–492, 21 set. 2001.

HUBER, E. et al. **Fetal programming in dairy cows: Effect of heat stress on progeny fertility and associations with the hypothalamic-pituitary-adrenal axis functionsAnimal Reproduction Science**Elsevier B.V., , 1 maio 2020. .

HWANG, S. G. et al. Wnt-3a regulates chondrocyte differentiation via c-Jun/AP-1 pathway. **FEBS Letters**, v. 579, n. 21, p. 4837–4842, 29 ago. 2005.

JOHNSON, M. L.; RAJAMANNAN, N. **Diseases of Wnt signalingReviews in Endocrine and Metabolic Disorders**Springer Netherlands, , 31 ago. 2006. . Disponível em: <http://www.stanford.>. Acesso em: 20 fev. 2021.

JONES, R. H.; OZANNE, S. E. Fetal programming of glucose-insulin metabolism. **Molecular and Cellular Endocrinology**, v. 297, n. 1–2, p. 4–9, 15 jan. 2009.

KENGAKU, M. et al. Distinct WNT pathways regulating AER formation and dorsoventral polarity in the chick limb bud. **Science**, v. 280, n. 5367, p. 1274–1277, 22 maio 1998. Disponível em: <http://science.sciencemag.org/>. Acesso em: 20 fev. 2021.

KIM, H. S.; LEE, M. S. **STAT1 as a key modulator of cell deathCellular Signalling**Pergamon, , 1 mar. 2007. .

LANGEN, R. C. J. et al. Tumor necrosis factor-alpha inhibits myogenic differentiation through MyoD protein destabilization. **The FASEB Journal**, v. 18, n. 2, p. 227–237, 1 fev. 2004. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1096/fj.03-0251com>. Acesso em: 22 fev. 2021.

LARUE, L.; DELMAS, V. **Secrets to developing Wnt-age melanoma revealedPigment Cell and Melanoma Research**, 2009. . Disponível em: <https://europepmc.org/article/med/19558582>. Acesso em: 20 fev. 2021.

LINGENFELTER, B. M. et al. Molecular cloning and expression of bovine nucleoplasmin 2 (NPM2): A maternal effect gene regulated by miR-181a. **Reproductive Biology and Endocrinology**, v. 9, n. 1, p. 1–9, 29 mar. 2011. Disponível em: <https://link.springer.com/articles/10.1186/1477-7827-9-40>. Acesso em: 22 fev. 2021.

LITTLEJOHN, M. D. et al. Sequence-based Association Analysis Reveals an MGST1 eQTL with Pleiotropic Effects on Bovine Milk Composition. **Scientific Reports**, v. 6, n. 1, p. 1–14, 5 maio 2016. Disponível em: <www.nature.com/scientificreports/>. Acesso em: 3 fev. 2021.

LIU, L. et al. Differential network analysis of bovine muscle reveals changes in gene coexpression patterns in response to changes in maternal nutrition. **BMC Genomics**, v. 21, n. 1, p. 684, 2 out. 2020. Disponível em: <https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-020-07068-x>. Acesso em: 20 fev. 2021.

LONG, J. M. et al. Maternal nutrient restriction alters endocrine pancreas development in fetal heifers. **Domestic Animal Endocrinology**, v. 74, p. 106580, 1 jan. 2021.

LONG, N. M. et al. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. **Journal of Animal Science**, v. 87, n. 6, p. 1950–1959, 1 jun. 2009. Disponível em: <https://academic.oup.com/jas/article/87/6/1950-1959/4731173>. Acesso em: 28 nov. 2019.

MAGNABOSCO, C.; CORDEIRO, C.; TROVO, J. Catálogo de linhagens do germoplasma zebuíno: raça Nelore. 1997. Disponível em: <http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=AGB.xis&method=post&formato=2&cantidad=1&expresion=mfn=180025>. Acesso em: 18 fev. 2021.

MARCONDES, C. R. et al. Breeders and family effects in stayability in Nellore herds. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 59, n. 4, p. 977–982, 2007. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-09352007000400025&lng=en&nrm=iso&tlng=pt>. Acesso em: 18 fev. 2021.

MUDADU, M. A. et al. Genomic structure and marker-derived gene networks for growth and meat quality traits of Brazilian Nelore beef cattle. **BMC Genomics**, v. 17, n. 1, p. 1–16, 15 mar. 2016. Disponível em: <https://link.springer.com/articles/10.1186/s12864-016-2535-3>. Acesso em: 18 fev. 2021.

MULLINIKS, J. T. et al. Supplementation strategy during late gestation alters steer progeny health in the feedlot without affecting cow performance. **Animal Feed Science and Technology**, v. 185, n. 3–4, p. 126–132, 2013. Disponível em: <http://dx.doi.org/10.1016/j.anifeedsci.2013.07.006>.

NOVAKOFSKI, J. Adipogenesis: Usefulness of in vitro and in vivo experimental models. In: Journal of Animal Science, 3, **Anais**...American Society of Animal Science, 1 mar.

2004. Disponível em: <https://academic.oup.com/jas/article/82/3/905/4790555>. Acesso em: 20 fev. 2021.

O'DONNELL, K. J. et al. Maternal prenatal anxiety and child COMT genotype predict working memory and symptoms of ADHD. **PLOS ONE**, v. 12, n. 6, p. e0177506, 14 jun. 2017. Disponível em: <https://dx.plos.org/10.1371/journal.pone.0177506>. Acesso em: 19 fev. 2021.

PAN, W. et al. β-catenin regulates myogenesis by relieving I-mfa-mediated suppression of myogenic regulatory factors in P19 cells. **Proceedings of the National Academy of Sciences of the United States of America**, v. 102, n. 48, p. 17378–17383, 29 nov. 2005. Disponível em: <www.pnas.orgcgidoi10.1073pnas.0505922102>. Acesso em: 20 fev. 2021.

PEDERSEN, B. K. et al. **Role of myokines in exercise and metabolismJournal of Applied Physiology**American Physiological Society, , set. 2007. . Disponível em: <http://www.jap.org>. Acesso em: 22 fev. 2021.

QIU, A. et al. Effects of Antenatal Maternal Depressive Symptoms and Socio-Economic Status on Neonatal Brain Development are Modulated by Genetic Risk. **Cerebral Cortex**, v. 27, n. 5, p. 3080–3092, 1 maio 2017. Disponível em: <https://academic.oup.com/cercor/article/27/5/3080/3074416>. Acesso em: 19 fev. 2021.

RAMÍREZ, M. et al. Maternal energy status during late gestation: Effects on growth performance, carcass characteristics and meat quality of steers progeny. **Meat Science**, v. 164, p. 108095, 1 jun. 2020.

REYNA, S. M. et al. Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. **Diabetes**, v. 57, n. 10, p. 2595–2602, 1 out. 2008. Disponível em: <http://diabetes.diabetesjournals.org>. Acesso em: 22 fev. 2021.

ROBINSON, M. D.; MCCARTHY, D. J.; SMYTH, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. **Bioinformatics**, v. 26, n. 1, p. 139–140, 1 jan. 2010. Disponível em: <https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btp616>. Acesso em: 4 fev. 2021.

ROHART, F. et al. mixOmics: An R package for 'omics feature selection and multiple data integration. **PLOS Computational Biology**, v. 13, n. 11, p. e1005752, 3 nov. 2017. Disponível em: <https://dx.plos.org/10.1371/journal.pcbi.1005752>. Acesso em: 3 fev. 2021.

RUDNICKI, J. A.; BROWN, A. M. C. Inhibition of chondrogenesis by Wnt gene expression in vivo and in vitro. **Developmental Biology**, v. 185, n. 1, p. 104–118, 1 maio 1997.

SALINAS, P. C.; ZOU, Y. Wnt Signaling in Neural Circuit Assembly. **Annual Review**

of Neuroscience, v. 31, n. 1, p. 339–358, 17 jul. 2008. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.neuro.31.060407.125649>. Acesso em: 20 fev. 2021.

SAMPAIO, C. B. et al. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Tropical Animal Health and Production**, v. 42, n. 7, p. 1471–1479, 23 abr. 2010. Disponível em: <https://link.springer.com/article/10.1007/s11250-010-9581-7>. Acesso em: 19 fev. 2021.

SARGOLZAEI, M.; CHESNAIS, J. P.; SCHENKEL, F. S. A new approach for efficient genotype imputation using information from relatives. **BMC Genomics**, v. 15, n. 1, p. 478, 17 jun. 2014. Disponível em: <http://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-15-478>. Acesso em: 8 fev. 2021.

SHABALIN, A. A. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. **Bioinformatics**, v. 28, n. 10, p. 1353–1358, 15 maio 2012. Disponível em: <https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bts163>. Acesso em: 8 fev. 2021.

SHANG, Y. C. et al. Activated β-catenin induces myogenesis and inhibits adipogenesis in BM-derived mesenchymal stromal cells. **Cytotherapy**, v. 9, n. 7, p. 667–681, 2007.

STEENSBERG, A. et al. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. **The Journal of Physiology**, v. 529, n. 1, p. 237–242, 15 nov. 2000. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/j.1469-7793.2000.00237.x>. Acesso em: 22 fev. 2021.

SUN, L. et al. JAK1-STAT1-STAT3, a key pathway promoting proliferation and preventing premature differentiation of myoblasts. **Journal of Cell Biology**, v. 179, n. 1, p. 129–138, 8 out. 2007. Disponível em: <http://www.jcb.org/cgi/doi/10.1083/jcb.200703184>. Acesso em: 21 fev. 2021.

TEH, A. L. et al. The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. **Genome Research**, v. 24, n. 7, p. 1064–1074, 1 jul. 2014. Disponível em: <http://creativecommons.org/licenses/by/4.0.http://www.genome.org/cgi/doi/10.1101/gr.171439.113.www.genome.org;www.genome.org>. Acesso em: 19 fev. 2021.

THOMA, A.; LIGHTFOOT, A. P. Nf-kb and inflammatory cytokine signalling: Role in skeletal muscle atrophy. In: **Advances in Experimental Medicine and Biology**. [s.l.] Springer New York LLC, 2018. 1088p. 267–279.

TOCA, M. D. C.; TONIETTI, M.; VECCHIARELLI, C. Nutrición pre y posnatal: impacto a largo plazo en la salud. **Archivos Argentinos de Pediatria**, v. 113, n. 3, p.

248–253, 1 jun. 2015. Disponível em: <http://www.sap.org.ar/docs/publicaciones/archivosarg/2015/v113n3a10.pdf>. Acesso em: 19 jan. 2021.

WADHWA, P. D. et al. Variation in the Maternal Corticotrophin Releasing Hormone-Binding Protein (CRH-BP) Gene and Birth Weight in Blacks, Hispanics and Whites. **PLoS ONE**, v. 7, n. 9, p. e43931, 11 set. 2012. Disponível em: <https://dx.plos.org/10.1371/journal.pone.0043931>. Acesso em: 19 fev. 2021.

WANG, H. et al. NF-κB Regulation of YY1 Inhibits Skeletal Myogenesis through Transcriptional Silencing of Myofibrillar Genes. **Molecular and Cellular Biology**, v. 27, n. 12, p. 4374–4387, 15 jun. 2007. Disponível em: <http://mcb.asm.org/>. Acesso em: 22 fev. 2021.

WEST-EBERHARD, M. J. Developmental plasticity and the origin of species differences. **Proceedings of the National Academy of Sciences of the United States of America**, v. 102, n. SUPPL. 1, p. 6543–6549, 3 maio 2005. Disponível em: <www.pnas.orgcgidoi10.1073pnas.0501844102>. Acesso em: 2 fev. 2021.

WOOD, A. R.; ESKO, T.; YANG, J. et al. Defining the role of common variation in the genomic and biological architecture of adult human height. **Nature Genetics**, v. 46, n. 11, p. 1173–1186, 5 nov. 2014. Disponível em: <https://www.nature.com/articles/ng.3097>. Acesso em: 3 fev. 2021.

XIA, W. et al. Integrative multi-omics analysis revealed SNP-lncRNA-mRNA (SLM) networks in human peripheral blood mononuclear cells. **Hum Genet**, v. 136, p. 451–462, 2017. Disponível em: <https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/content/pdf/10.1007/s00439-017-1771-1.pdf&casa_token=qGsqRaC77P4AAAAA:XGTtg3ynPHQBm68dtHgCO5RpXDgXBBskcYOti4azwDdews5QntQmz4eg4_uIFTsm2QNu182tNHtDQ6M6k5c>. Acesso em: 8 fev. 2021.

YAMANOUCHI, K. et al. Myogenic and Adipogenic Properties of Goat Skeletal Muscle Stem Cells. **Journal of Reproduction and Development**, v. 53, n. 1, p. 51–58, fev. 2007. Disponível em: <http://joi.jlc.jst.go.jp/JST.JSTAGE/jrd/18094?from=CrossRef>. Acesso em: 20 fev. 2021.

ZHBANNIKOV, I. Y. et al. Seqyclean: A pipeline for high-throughput sequence data preprocessing. In: ACM-BCB 2017 - Proceedings of the 8th ACM International Conference on Bioinformatics, Computational Biology, and Health Informatics, New York, NY, USA. **Anais**... New York, NY, USA: ACM, 2017. Disponível em: <http://github.com/ibest/seqyclean>. Acesso em: 4 fev. 2021.

5. General Conclusions and perspectives

Throughout this thesis, we presented findings that contributed to the enrichment of the field of fetal programming and novel insights about the gene mechanisms that comprise this phenotype.

As demonstrated in this thesis, there are still many gaps regarding the mechanisms that comprise fetal programming, regardless of the species analyzed. However, the impacts on beef cattle production indexes can show great advances in the production of Brazilian and worldwide meat. Thus, this thesis encourages more work to be carried out in this area of research with different nutritional levels and approaches during pregnancy so that the effects of fetal programming are fully elucidated.

APPENDIX A – Supplementary Material of Chapter 1

Table S1. Genomic windows with explained genetic variance greater than or equal to 1% for SC at 12 months, NP treatment.

Chromossome	Location (bp)	Genetic variance (%)	Genes
10	37800788-37806570	1.05480	ZNF106
14	43924204-43953144	1.42040	ZNF704
14	76898047-76912769	1.80006	
16	32556879-32564071	1.01510	DESI2
16	37286221-37290307	1.30401	
18	47933146-47936668	1.08335	SIPA1L3
22	13891229-13914987	1.12460	ULK4
23	2861718-2879438	2.01555	PRIM2
23	12423847-12440485	1.47894	BTBD9

Table S2. Genomic windows with explained genetic variance greater than or equal to 1% for SC at 18 months, NP treatment.

Chromossome	Location (bp)	Genetic variance (%)	Genes
2	57642251-57649344	1.08480	
8	27557552-27565476	1.08766	BNC2
8	105299245-105307167	1.37200	
9	97906449-97913459	1.38566	PRKN
14	80736254-80746691	2.22560	
16	32556879-32564071	1.71763	DESI2
18	47933146-47936668	1.00608	SIPA1L3
22	13891229-13914987	1.11366	ULK4
23	27399609-27411352	3.61961	DXO SKIV2L NELFE

Table S3. Genomic windows with explained genetic variance greater than or equal to 1% for SC at 12 months, PP treatment.

Chromossome	Location (bp)	Genetic variance (%)	Genes
7	92659035-92667652	1.84305	
8	58124778-58132887	1.23570	
14	3247912-3255976	3.34678	TRAPPC9
14	3258945-3275592	1.50346	TRAPPC9; 5S_rRNA
17	63622450-63628020	2.80010	MVK

Table S4. Genomic windows with explained genetic variance greater than or equal to 1% for SC at 18 months, PP treatment.

Chromossome	Location (bp)	Genetic variance (%)	Genes
1	40594860-40610060	5.03081	
1	63963815-63967488	1.36131	
2	60912867-60932690	1.63289	
4	51134478-51141809	2.82907	WNT2; ASZ1
5	32228224-32235430	1.67640	SENP1
18	5548583-5553095	1.51384	WWOX
22	13849687-13862818	1.10565	ULK4
22	13891229-13914987	1.21504	ULK4
23	9967641-9991227	5.11950	SLC26A8 MAPK14
27	24957747-24978301	3.58867	MFHAS1

Table S5. Genomic windows with explained genetic variance greater than or equal to 1% for SC at 12 months, FP treatment.

Chromossome	Location (bp)	Genetic variance (%)	Genes
1	40594860-40610060	4.03684	
1	63963815-63967488	1.24719	
2	60912867-60932690	2.58775	
2	60937098-60952745	1.00089	
4	51134478-51141809	3.10899	WNT2; ASZ1
5	32228224-32235430	1.43437	SENP1
18	5548583-5553095	1.19052	WWOX
22	13849687-13862818	1.24474	ULK4
22	13891229-13914987	1.38084	ULK4
23	9967641-9991227	6.20713	SLC26A8; MAPK14
27	24957747-24978301	3.32982	MFHAS1;

Table S6. Genomic windows with explained genetic variance greater than or equal to 1% for SC at 18 months, FP treatment.

Chromossome	Location (bp)	Genetic variance (%)	Genes
7	92659035-92667652	3.02971	
11	76002231-76009997	1.16541	
14	3247912-3255976	4.49624	TRAPPC9
14	3258945-3275592	1.94728	TRAPPC9 5S_rRNA
22	58218919-58227914	1.43112	

APPENDIX B – Supplementary Material of Chapter 3

Table S1. Sequencing data samples with initial filters and multimapping reads, uniquely mapped and unmapped reads rates.

samples	initial_reads	reads_seqyclean	unique_reads	%	multi_mapping_reads	unmapped_reads
6781_R1	17003194	15401127	13871676	90.07%	2.33%	7.60%
6781_R2	17003194	15401127				
6795_R1	15573847	14180094	12477956	88.00%	2.40%	9.60%
6795_R2	15573847	14180094				
6796_R1	15991588	14543844	13258301	91.16%	2.28%	6.55%
6796_R2	15991588	14543844				
6797_R1	13476944	12189860	10785747	88.48%	2.49%	9.03%
6797_R2	13476944	12189860				
6808_R1	18036472	16396448	13849280	84.47%	2.16%	13.36%
6808_R2	18036472	16396448				
6816_R1	17358504	15755934	13755476	87.30%	2.32%	10.38%
6816_R2	17358504	15755934				
6821_R1	16851018	15341064	13588125	88.57%	2.34%	9.08%
6821_R2	16851018	15341064				
6822_R1	25475309	23151556	20143456	87.01%	2.34%	10.65%
6822_R2	25475309	23151556				
6979_R1	18916426	17089334	14805027	86.63%	2.28%	11.09%
6979_R2	18916426	17089334				
7045_R1	17746685	16193467	13985121	86.36%	2.39%	11.23%
7045_R2	17746685	16193467				
7063_R1	20830071	18972948	17032178	89.77%	2.35%	7.88%
7063_R2	20830071	18972948				
7072_R1	19879937	18033617	15786674	87.54%	2.40%	10.05%
7072_R2	19879937	18033617				
7074_R1	17097975	15510348	13986701	90.18%	2.61%	7.22%
7074_R2	17097975	15510348				
7075_R1	21365369	19213600	17499102	91.08%	2.53%	6.40%
7075_R2	21365369	19213600				
7085_R1	21022549	19132252	16802028	87.82%	2.27%	9.90%
7085_R2	21022549	19132252				

Table S2. Genes from the differential expression analysis with your respectively p values between the treatments (NP, PP and FP).

Due to the large extension of the table S2, it was attached in separate material.

Table S3. Significant muscle cis-eQTLs from young Nellore bulls regardless the prenatal treatment submitted.

Due to the large extension of the table S3, it was attached in separate material.

Table S4. Significant muscle trans-eQTLs from young Nellore bulls regardless the prenatal treatment submitted.

Due to the large extension of the table S4, it was attached in separate material.

Additional file 1. Quick reference guide for figure 3.

This reference guide is attached as a separate material.