MATO GROSSO DO SUL STATE UNIVERSITY
AQUIDAUANA *Campus*
GRADUATE PROGRAM IN ANIMAL SCIENCE

IMPACTS OF DIFFERENT LEVELS OF FEED SUPPLEMENTATION ON AGE AT PUBERTY AND FERTILITY OF PRECOCIOUS BOVINE FEMALES

Student: Felipe de Oliveira Pedro

Aquidauana – MS
February / 2025

MATO GROSSO DO SUL STATE UNIVERSITY
AQUIDAUANA *Campus*
GRADUATE PROGRAM IN ANIMAL SCIENCE

IMPACTS OF DIFFERENT LEVELS OF FEED SUPPLEMENTATION ON AGE AT PUBERTY AND FERTILITY OF PRECOCIOUS BOVINE FEMALES

Student: Felipe de Oliveira Pedro
Advisor: Profa. Dra. Fabiana de Andrade Melo Sterza
Co-advisor: Profa. Dra. Andréa Roberto Duarte Lopes Souza

"Dissertation presented to the Postgraduate Program in Animal Science, concentrating on Animal Production in the Cerrado-Pantanal, at the Mato Grosso do Sul State University, as part of the requirements for obtaining a master's degree in Animal Science".

Aquidauana – MS
February / 2025

P413i Pedro, Felipe de Oliveira

Impacts of different levels of feed supplementation on age at puberty and fertility of precocious bovine females / Felipe de Oliveira Pedro. - Aquidauana, MS: UEMS, 2025. 79 p.

Dissertação (Mestrado) – Programa de pós-graduação em zootecnia (PGZOO): produção animal no cerrado e pantanal – Universidade Estadual de Mato Grosso do Sul, 2025.
Orientadora: Profa. Dra. Fabiana de Andrade Melo Sterza
Coorientadora: Andréa Roberto Duarte Lopes Souza

1. Breeding season 2. Dietary energy. 3. Ovum pick-up. 4. Partial budget. I. Título. II. Sterza, Fabiana de Andrade Melo.

CDD 23. ed. - 636.2

Ficha Catalográfica elaborada pela bibliotecária da Universidade Estadual de Mato Grosso do Sul (UEMS)
Susy dos Santos Pereira CRB1°1783

UNIVERSIDADE ESTADUAL DE MATO GROSSO DO SUL
PRÓ-REITORIA DE PESQUISA, PÓS-GRADUAÇÃO E INOVAÇÃO
UNIDADE UNIVERSITÁRIA DE AQUIDAUANA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA
ÁREA DE CONCENTRAÇÃO EM PRODUÇÃO ANIMAL

FELIPE DE OLIVEIRA PEDRO

Dissertação submetida ao Programa de Pós-Graduação em Zootecnia, área de concentração em Produção Animal, como requisito para obtenção do grau de Mestre em Zootecnia.

DISSERTAÇÃO APROVADA EM 25/02/2025.

gov.br Documento assinado digitalmente
FABIANA DE ANDRADE MELO STERZA
Data: 23/05/2025 13:55:29-0300
Verifique em https://validar.iti.gov.br

Dra. Fabiana de Andrade Melo Sterza (Orientadora)

gov.br Documento assinado digitalmente
JESSICA NORA DRUM
Data: 28/05/2025 17:21:22-0300
Verifique em https://validar.iti.gov.br

Dra. Jessica Nora Drum, South Dakota State University
(participação via videoconferência)

gov.br Documento assinado digitalmente
MARCELO VEDOVATTO
Data: 23/05/2025 18:30:23-0300
Verifique em https://validar.iti.gov.br

Dr. Marcelo Vedovatto, Louisiana State University
(participação via videoconferência)

EPIGRAPH

“Follow your heart and nothing else.”
(Lynyrd Skynyrd)

DEDICATION

To

God, family, friends, and partners in this journey!

ACKNOWLEDGMENTS

I thank God for everything because I have more than earned it.

To my family for their constant support and for the confidence of knowing that I will never be alone.

To the postgraduate program in zootechnics and the Mato Grosso do Sul State University for welcoming me.

To my advisor, Dr. Fabiana de Andrade Melo Sterza, for all the attention and teachings shared.

To all the PGZOO, especially my co-supervisor, Dr. Andrea Roberto Duarte, and Professor Dr. Andre Julian Ferraz (Splinter), who allowed me to enter the master's program.

To exchange program (UEMS International) for the possibility of travel to another country, university, and learn about different activities and cultures. Thanks also to South Dakota State University and Dr. Jessica Drum for welcoming me.

To my friends and all those who have contributed in some way to this achievement, which is certainly not mine alone.

To all members of GENTRA who helped a lot in the experiment execution and management.

To Fundect for the financial support to carry out my experiment.

To CAPES and PIBAP UEMS for scholarships.

The São Judas Tadeu farm, Claudio Zootesso, Reginaldo Figueiredo, Lucio Gomes, and Biomarte, for making it possible to carry out the experiment described in this work.

I am infinitely grateful to everyone.

SUMMARY

Summary

RESUMO **13**
ABSTRACT **14**
CHAPTER 1 - GENERAL REMARKS **15**
1 INTRODUCTION...**15**
2 LITERATURE REVIEW..**17**
2.1 Puberty physiology **17**
2.2 Precocity in *Bos taurus* Vs. *Bos indicus* heifers.......................................**19**
2.3 Effects of nutrition on puberty onset **20**
2.4 Biotechnologies and nutritional management applied to reproduction in cattle...**23**
2.4.1. Cattle reproductive biotechnologies development.............................**23**
2.4.2 Nutritional management and fixed-time artificial insemination (FTAI) **23**
2.4.3 Nutritional management and ovum pick-up / *In vitro* embryo production system...**24**
2.5 Dietary energy vs. oocyte quality..**26**
2.6 Cost-effectiveness **27**
2.7 Conclusion..**29**
3 AIMS **30**
3.1 Overall aim **30**
3.2 Specific aims **30**
REFERENCES..**31**
CHAPTER 2 - SCIENTIFIC PAPER, STUDY 1 (Livestock science). **39**
SCIENTIFIC PAPER, STUDY 2 (SEMINA). **58**
Summary **58**
INTRODUCTION **58**
MATERIALS AND METHODS...**60**
Site and study period **60**
Animals and treatments **61**
Age and body weight gain..**63**
Body condition score..**64**
Subcutaneous fat thickness **64**
Gynecological evaluation and pregnancy determination. **64**
Ovum pick-up (OPU) and *in vitro* embryo production (IVEP) **64**

Statistical analysis **65**
RESULTS **66**
DISCUSSION **68**
CONCLUSION **70**
ACKNOWLEDGMENTS **70**
REFERENCES **70**
CHAPTER 3 – TECHNICAL PAPER (ATP) **73**
CHAPTER 4 – FINAL REMARKS **78**

TABLE LIST

Table 1 - Composition (percentage of dry matter) of concentrates provided in different diets for Nelore heifers (Three different concentrates = 0.15%, 0.5%, and 1.0%). Chemical composition of concentrates (0.15%, 0.5%, and 1.0%), sugarcane bagasse, and BRS Capiaçu silage). Diets composition (percentage of each ingredient in dry matter)..**42**
Table 2 – Chemical composition (percentage of dry matter) of concentrates provided for Nelore heifers fed with three different diets (STP, S0.5%, and S1.0%). **43**
Table 3 - Guarantee level for macro and microminerals in concentrates (0.15%, 0.5%, and 1.0%) provided in three diets (STP, S0.5%, and S1.0%) for Nelore heifers. **44**
Table 4 - Physic-chemical properties of the water used for watering Nelore heifers fed with three different diets (STP, S0.5%, and S1.0%) in feedlots. The S0.5% and S1.0% groups shared the same water fountain (bunk). **44**
Table 5 – Body weight (kg) and average daily gain (kg/day) data as repeated measures over time of Nelore heifers housed in feedlots with three dietary strategies. Body weight data included treatment days 0, 30, 55, 64, and 94. Average daily gain data was divided into 2 periods, day 0 to 54 and day 55 to 94. ....**48**
Table 6 - Body maturation parameters (age, weight [kg], SCFT, and BCS) on artificial insemination day in Nelore heifers supplemented with three different treatments in feedlot..**48**
Table 7 – Puberty onset, artificial insemination fertility, service rate (% of inseminated), pregnancy losses, and calving rate of Nelore heifers supplemented with three different strategies in feedlots. **49**
Table 8 - Gynecological variables at day zero of fixed-time artificial insemination protocol of Nelore heifers fed in feedlots with three dietary strategies. **49**
Table 9 - Correlation among reproductive parameters (evaluated by ultrasound equipment) and body maturation parameters in Nelore heifers fed with three different diets in feedlots. **50**
Table 10 – Dietary consumption and costs of three treatments evaluated in Nelore heifers fed in a feedlot system. **50**
Table 11 – Production costs (Nutritional and reproductive protocol) and benefits (calf production and arroba value of cull heifers) of an intensive system of production with Nelore heifers supplemented in feedlots and submitted to a single fixed-time artificial insemination protocol. **51**

FIGURES LIST

Figure 1 - Illustration of the excitatory effect of leptin (produced by adipose tissue) on the kisspeptin neuron (right side), which occurs by stimulation of KNDy interneurons, finishing on GnRH neuron stimulation. The inhibitory effect of NPY (left side) occurs in negative energy balance (low adiposity and leptin concentration) by higher activity of the neurotransmitters GABA and AgRP that inhibit Kiss1 and KNDy neurons and reduce the GnRH liberation. **Source**: Sobrino et al. 2022..**18**
Figure 2 - Effect of prenatal (fetal programming) and postnatal nutrition on female bovine performance. Nutritional management in these periods can reduce the inhibitory factors (NPY) and increase the excitatory factors (Leptin, kisspeptin, KNDy, and POMC) resulting in greater GnRH and LH pulse frequency, and pubertal maturation. However, DNA methylation is affected, and higher dietary energy in the juvenile period may produce hypermethylation and oocyte incompetence. **Source**: Cardoso et al. 2020..**22**
Figure 3 - Experimental design showing the data collection period that begins after seventeen days of acclimatation on the post-weaning period. The treatment was different percentages of dietary concentrate inclusion (STP = Steir-step nutrition; S0.5% = Supplementation with 0.5% of body weight; S1.0% = supplementation with 1.0 % of body weight), providing more or less energy levels. CIP = Cyclicity induction protocol; FTAI = Fixed-time artificial insemination; AI = Artificial insemination..**42**
Figure 4 - Effect of the presence of corpus luteum (CL) at Day zero of fixed-time artificial insemination protocol on pregnancy rate of Nelore Heifers. The increase in the number of CL is followed by an increase in pregnancy rate ($p<0.05$)..**50**
Figure 5 – Experimental design showing the onset of treatment after 5 months of post-weaning. All females received a common diet (1.5% of body weight), and at the time of OPU, the groups (pregnant and nonpregnant) were divided. Data collection during the treatment period. OPU = Ovum pick-up; IVP = *in vitro* embryo production...**60**
Figure 6 - Effect of body weight (BW) on the number of cumulus-oocyte complexes (COCs) obtained after ovum pick-up in early pregnant and nonpregnant Nelore heifers fed with a high-energy diet. BW increase was followed by an increase in the number of COCs ($p=0.05$)............................**67**
Figure 7 - Effect of subcutaneous croup fat thickness (SCFT) on the number of cumulus-oocyte complexes (COCs) obtained after ovum pick-up in pregnant and nonpregnant Nelore heifers fed with a high-energy diet. SCFT over 7.30 mm reduces the number of COCs recovered at OPU. However, a quadratic effect was observed, and under 7.30 mm, the number of COCs recovered ha*s* been increased ($p<0.05$)..**67**
Figure 8 – Effect of subcutaneous croup fat thickness (SCFT) on the number of in vitro matured cumulus-oocyte complexes (COCs) derived from pregnant and nonpregnant young Nelore donors fed with a high-energy diet. A negative correlation was observed for the linear effect, when the SCFT increased the IVM-COCs decreased ($p<0.05$)...**68**
Figure 9 – Effect of subcutaneous croup fat thickness (SCFT) on the number of zygotes at in vitro embryo production with cumulus-oocyte complexes (COCs) derived from pregnant and nonpregnant Nelore donors fed with a high-energy

diet. A tendency for a negative correlation effect (P = 0.07) was observed when the SCFT increased the number of zygotes tended to decrease……………………………………………………………………………………**68**
Figure 10- Design of the reproductive management carried out, starting with the cyclicity induction protocol (CIP) 24 days before the start of the fixed-time artificial insemination protocol (FTAI). The FTAI protocol used was three treatments in nine days. Pregnancy diagnosis was carried out 30 days after insemination…………………………………………………………………………..**76**

RESUMO

Objetivou-se com este estudo identificar a estratégia de suplementação que melhor contribuísse para o desempenho produtivo e reprodutivo de novilhas super precoces da raça Nelore. O experimento foi dividido em dois estudos. O Estudo 1 contou com 60 novilhas com média de 9,05 ± 0,42 meses e peso médio de 259,3 ± 21,6 kg, mantidas em sistema de confinamento em baias coletivas com dieta e água *ad libitum*. As novilhas foram divididas em 3 grupos de forma randomizada em um delineamento inteiramente casualizado (DIC) considerando o animal como unidade experimental, sendo: S0,5% (N =20), S1,0% (N = 20) e STP (stair-step, N =20). A suplementação STP consistiu em aumentos graduais da oferta de concentrado, sendo: 0,07%; 0,15%; 0,3%; 0,5% e finalizando com 1,0% do peso vivo (PV). A suplementação dos grupos S0,5% e S1,0% foi de 0,5% do PV e 1,0% do PV, respectivamente. O período experimental foi de 111 dias, incluindo o período de adaptação de 17 dias. Como volumoso foi ofertado silagem de BRS Capiaçu e 2kg de bagaço de cana por animal. O ganho de peso médio diário (GMD), precocidade de puberdade, taxa de prenhez e perdas gestacionais, adiposidade, escore de estro (ESCT), presença de folículo dominante (FD), presença de corpo lúteo (CL), escore de contagem de folículos antrais (ECFA) e benefício custo foram analisados no experimento. O estudo 2 contou com 50 novilhas e consistiu em suplementação de 1.5% do PV iniciada ao término do estudo 1. O período de tratamento foi de 60 dias e ao final destes a aspiração folicular guiada por ultrassom (OPU) foi realizada em 5 fêmeas de cada grupo (20%), seguida da produção *in vitro* de embriões (PIVE). Os grupos foram divididos em função da taxa de prenhez do estudo anterior, sendo: gestantes (N=25) e não gestantes (N=25). O concentrado fornecido nesse período foi o 1.0% (utilizado no estudo anterior) com nível de inclusão de 1.5% do PV na dieta e a fonte de volumoso e água não diferiram do estudo 1, assim como, as condições de instalações. Foi avaliado o desempenho à OPU/PIVE por grupo, assim como a influência da energia dietética sobre a PIVE. As análises estatísticas foram realizadas pelo pacote estatístico SAS® (SAS Inst. Inc., Cary, NC, USA; SAS on demand) considerando o alfa de 5% de significância. A taxa de prenhez e perdas gestacionais avaliadas no estudo 1 não diferiram entre os grupos ($p>0.05$), assim como, o número de fêmeas púberes. O GMD foi superior para o grupo S1.0%, intermediário para o STP e inferior para o S0.5% ($p<0.05$). No estudo 2 não foi observada diferença entre fêmeas prenhas e vazias quanto a eficiência à OPU-PIVE. Observou-se, ainda, influência negativa da gordura subcutânea da garupa (EGP) sobre o número de complexos cúmulus-oócito (COCs)/OPU e embriões clivados/MIV. Concluiu-se no estudo 1, que os sistemas nutricionais implementados à IATF não interferiram na eficiência reprodutiva apesar de incrementar o ganho de peso e promover precocidade de puberdade e prenhez. Ademais, a dieta mais viável economicamente foi a utilizada para o grupo S0.5%, visto que apresentou uma maior produção por unidade de custo (US$ 2.64). No estudo 2, os grupos não diferiram na eficiência à OPU/PIVE, contudo, o nível de energia incluído na dieta apresentou efeitos negativos no número de COCs e embriões clivados, independente do grupo.

ABSTRACT

This study aimed to identify the supplementation strategy that would best contribute to the productive and reproductive performance of Nellore heifers. The experiment was divided into two studies. Study 1 included 60 heifers with an average age of 9.05 ± 0.42 months and an average weight of 259.3 ± 21.6 kg, kept in a feedlot system in collective stalls with food and water *ad libitum*. The heifers were randomly divided into 3 groups in a completely randomized design (CRD) considering the animal as the experimental unit: S0.5% (N = 20), S1.0% (N = 20), and STP (stair-step, N = 20). The STP supplementation consisted of gradual increases in concentrate supply: 0.07%, 0.15%, 0.3%, 0.5%, and ending with 1.0% of body weight (BW). The supplementation of the S0.5% and S1.0% groups was 0.5% of BW and 1.0% of BW, respectively. The experimental period was 111 days, including the acclimation period. BRS Capiaçu silage was offered as a roughage with 2.0 kg of sugarcane bagasse per animal. Variables such as average daily gain (ADG), onset of puberty, pregnancy rate, pregnancy loss, subcutaneous croup fat thickness (SCFT), estrus score (HTSC), gynecological variables (dominant follicle presence, *corpus luteum* presence, and antral follicle count score) on d0 of the fixed-time artificial insemination (FTAI) protocol, as well as the partial budget, were analyzed. In study 2, 50 heifers were used in a supplementation plan of 1.5% of BW, beginning by the end of study 1. The heifers were supplemented for 60 days, and at the end of this period, ovum pick-up (OPU) was performed on 5 females from each group (20%). The groups were divided according to the pregnancy rate in the previous study: pregnant (N=25) and open (N=25). For this study, the supplementation strategy of S1.0 (used in the previous study) was used with 1.5 of BW inclusion in the diet, and the same source of roughage and water from study 1. The performance at OPU and *in vitro* embryo production (OPU-IVEP) per group, and the influence of dietary energy, were evaluated. Statistical analyses were performed using the SAS® statistical package (SAS Inst. Inc., Cary, NC, USA; SAS on demand) with an alpha of 5% significance. The pregnancy rate and pregnancy loss evaluated in Study 1 did not differ among the groups ($p>0.05$), nor did the number of pubertal females in the entire study. ADG was higher for the S1.0% group, intermediate for STP, and lower for S0.5% ($p<0.05$). Otherwise, the S0.5% group showed a greater production ($2.64) per cost unit. In study 2, there was no difference between pregnant and non-pregnant females in terms of OPU-IVEP efficiency. There was also a negative influence of subcutaneous croup fat thickness (SCFT) on the number of cumulus-oocyte complexes (COCs/OPU) and cleaved embryos. In conclusion, the systems in this study did not interfere with reproductive efficiency, despite increasing weight gain and promoting early puberty and pregnancy. Furthermore, increased energy levels in the diet had negative effects on COCs and cleaved embryos, regardless of the group.

Keywords: Breeding season; dietary energy; ovum pick-up; partial budget

CHAPTER 1 - GENERAL REMARKS

1 INTRODUCTION

World beef production exceeded 76.7 million tons in 2023, representing an increase of 0.7% compared to 2022 (FAO, 2024). Brazil ranks second only to the United States of America (USA) in the world production context, but it has almost twice as many cattle in numbers. In 2023, there was an increase in national production and exports. The country crossed the 2-million-ton mark in meat exports, reaching 157 countries on 5 continents and generating revenues of 10.55 billion (US$). Among the country's states, Mato Grosso do Sul is an important player, as it was the sixth largest exporter with more than 200,000 tons and 956 million (US$) in revenue (ABIEC, 2024).

This scenario requires intensive technological improvement of the country's livestock production to supply the global demand for meat consumption, especially considering the country's size and productive potential.

With an area of 8,510,417,771 km^2 and a population of more than 213 million habitants, livestock farming is one of the main activities and directly impacts the economy, with a cattle population of approximately 200 million head (IBGE, 2022; ABIEC, 2024). On the other hand, crops such as soybeans (*Glycine max*) and eucalyptus (*Eucalyptus globulus*) have increasingly dominated the land space that used to be occupied by livestock. Livestock production has been confined to regions with poor soil quality, marshy and hilly areas, or directed towards intensive production in feedlots. These conditions present a challenge, especially in the breeding sector, which traditionally requires a greater demand for space.

As a result, the investment in livestock farming is growing exponentially. Strategies linking productivity with the cost-benefit of the activity and sustainability are indispensable, especially when it comes to maximizing reproductive efficiency by reducing age at puberty, calving interval, and increasing selection pressure for fertility and productivity.

In this sense, the use of biotechnologies applied to reproduction combined with appropriate nutritional management is essential to increase productivity in the country. Which, despite being one of the world's largest meat producers, has not reached its full productivity potential in the sector.

From this perspective, postnatal nutrition with high dietary energy can accelerate adipose tissue formation, increase leptin release, and reduce neuropeptide Y levels, ultimately leading to early pubertal maturation (Cardoso et al., 2020; Freitas et al., 2021).

Thus, the objective of this study was to evaluate the influence of different dietary energy levels on puberty age, fertility, and embryo production in Nellore heifers, considering the economic feasibility of the systems. In addition, we aimed with the literature review to clarify the pivotal factors related to nutritional management enrolled in Fixed Time Artificial Insemination (FTAI) and *in vitro* embryo production (IVEP) programs in precocious bovine females to promote early puberty and pregnancy.

2 LITERATURE REVIEW

2.1 Puberty physiology

Puberty is defined as when ovulation occurs, accompanied by visual signs of estrus and normal luteal function (Perry, 2016). Its onset depends on complex endocrine processes. These events are variable depending on factors such as genetics, environment, nutritional status, and year season (Freitas et al. 2021).

Factors such as nutritional restriction in post-natal development increase the age at puberty (Day et al. 1984), while supplementation with concentrate-rich diets in the juvenile period, during the first life year, tends to lead to earlier puberty and, consequently, earlier calving (Gasser et al. 2006a). In addition, puberty is a highly heritable trait (h2 = 0.42) and can be selected to improve this trait in the herd (Lau et al. 2020).

Puberty is dependent on the maturation of the reproductive neuroendocrine axis, with high episodic frequency release of Gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) (Cooke et al. 2020). It is known that high rates of body weight gain anticipate pubertal maturation due to the programming of the hypothalamic centers that underline this process (Tahir et al. 2021; Canal et al. 2020). Even maternal nutrition during pregnancy can modulate the development of the fetal neuroendocrine axis, thus influencing the progenies' puberty (Cardoso et al. 2020).

Several mechanisms and metabolic products are involved in the process of sexual maturation in the bovine female. Of these, leptin is indispensable to stimulate (excitatory effects) the GnRH secretion by action on Kisspeptin neurons (Sartori et al. 2024). Proopiomelanocortin (POMC), a precursor of alpha melanocyte-stimulating hormone (aMSH), plays a synergistic role with leptin in pubertal activation. On the other hand, neuropeptide Y (NPY) produces an inhibitory stimulus for GnRH neurons. In this scenario, nutritional management in the juvenile period follows organizational changes with an increase in excitatory factors and a reduction in inhibitory factors (Cardoso et al. 2020).

Hormones such as insulin, Insulin-like growth factor 1 (IGF-1), and growth hormone (GH), as well as nutrients (glucose and fatty acids), are dependent on the nutritional status of the female and act in the hypothalamus regulating food intake, energy expenditure, and neuroendocrine mechanisms such as reproduction (Sartori et al. 2024.; Gasser et al. 2006a). It is also known that the main neurons related to metabolism are located in the arcuate nucleus (ARC) of the hypothalamus (Allen et al. 2017).

The expression of neuropeptide Y increases during negative energy balance (McShane et al. 1993), stimulating food intake and inhibiting reproductive processes through mechanisms such as the antagonistic effect of GnRH and LH release (Gazal et al. 1998; Thomas et al. 1999). However, during a physiological state of positive energy balance, its expression decreases (Sanacora et al. 1990).

On the other hand, POMC, the gene that encodes the precursor molecule of alpha-melanocyte-stimulating hormone (α-MSH), has increased action in animals on high-weight-gain diets after high adiposity and leptin concentrations, reducing food intake (Figure 1). Similarly to POMC, leptin, IGF-1, glucose, and insulin levels increase in an adequate energy balance, stimulating reproductive functions (Sartori, R.; Guardieiro, 2010).

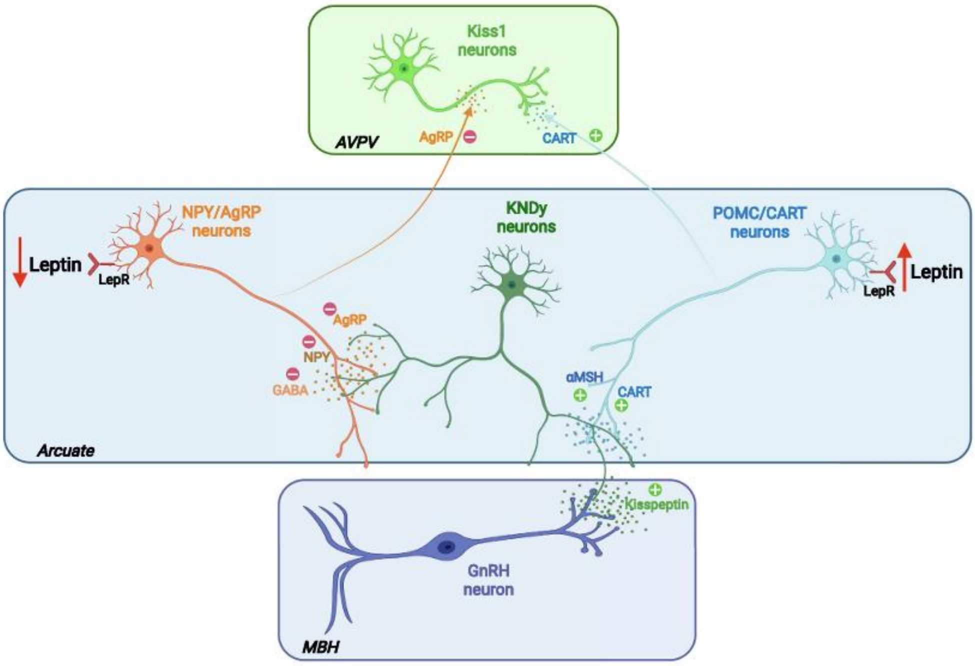

Figure 3 - Illustration of the excitatory effect of leptin (produced by adipose tissue) on the kisspeptin neuron (right side), which occurs by stimulation of KNDy interneurons, resulting in GnRH neuron stimulation. The inhibitory effect of NPY (left side) occurs in negative energy balance (low adiposity and leptin concentration) due to the higher activity of the neurotransmitters GABA and AgRP, which inhibit Kiss1 and KNDy neurons and reduce the GnRH release. **Source**: Sobrino et al. 2022.

With the onset of puberty, hypothalamic estrogen feedback becomes positive, enabling the production of GnRH. Kisspeptin, gamma-aminobutyric acid (GABA), glutamatergic, and cholinergic neurons interact with GnRH neurons, activating signaling cascades, including calcium signaling, cyclic adenosine monophosphate (cAMP) signaling and Mitogen-activated protein kinase (MAPK) signaling, culminating in the secretion of GnRH and the subsequent release of LH and Follicle-stimulating hormone (FSH) by the pituitary gland, which will act at the gonadal level for growth, follicular maturation, and ovulation, as well as luteinization (Tahir et al. 2021; Kinder. et al 2019).

On the other hand, the action of NPY in the pituitary gland suppresses the release of LH and tends to stimulate the release of GH and consumption. This occurs in negative energy balance to improve the maintenance energy (Thomas et al. 1999).

In a proteomic analysis of the adipose tissue of Brahman heifers, Nguyen et al (2018) observed that gluconeogenesis was increased as puberty approached. In addition, the study identified four central proteins that showed the highest degrees of connection in puberty-related metabolism processes: phosphoglycerate kinase 1 (PGK1), aldehyde dehydrogenase 5 family member A1 (ALDH5A1), eukaryotic translation elongation factor 2 (EEF2), and lactate dehydrogenase B (LDHB). These proteins, especially PGK1 and LDHB, were involved in metabolic processes like the glycolysis pathway in the abdominal adipose tissue, which are essential to reach the leptin concentration required for puberty onset. Leptin concentrations over 4 ng/mL promote more females to reach puberty (Cardoso et al. 2014).

2.2 Sexual precocity in *Bos taurus* Vs. *Bos indicus* heifers

The domestication of aurochs (*Bos primigenius*) culminated in the formation of two distinct but cross-fertile cattle subspecies, taurine cattle (*B. taurus*) and indicine or zebu cattle (*Bos indicus*). Each subspecies was important in human history, with several implications on the diet, culture, and socioeconomic structure of the farming populations worldwide (Chen et al. 2023). The *Bos taurus* subspecies was domesticated first, around 10,000 years before present (YBP) in the Fertile Crescent region (Mesopotamia), followed by *Bos* *indicus* domestication 2,000 years later in the Indus Valley of Pakistan (Utsunomiya et al. 2019).

Thus, the origin, selection, and climate conditions modulate the reproductive efficiency of each subspecies over time. In this context, *Bos taurus* cattle that have early reproductive maturation were selected for reproductive efficiency and precocity, while *Bos indicus* prioritized survival in tropical environments, which led to a delay in puberty onset. These differences reflect the evolutionary pressures and adaptive requirements of each subspecies (Utsunomiya et al. 2019; Nogueira, 2004).

On the other hand, the European breeds have been selected over centuries for milk and meat production, with a focus on precocity and reproductive efficiency. This resulted in genetic selection for earlier sexual maturation (Zhang et al. 2020). Zebu breed evolution occurs in tropical and subtropical regions, being selected for resistance to climatic conditions (Duittoza et al. 2023).

Thus, Zebu cattle are most commonly raised in extensive systems, knowing that nutrition and body condition can influence the reproductive performance of heifers (D'Occhio et al. 2019), when the environment is challenging (tropical regions), reproduction may be delayed, and survival (maintenance) would be the priority (Utsunomiya et al. 2019).

In this sense, Nellore females have an average puberty age of around 22-36 months while *Bos taurus* heifers are around 16 months (Cooke et al. 2020; Ferraz JR et al. 2018). However, there is a natural variation in age at puberty depending on genetics and environment, but under an intensive management system, puberty can be reached earlier, and the first pregnancy may occur before 15 months in Nellore cattle (Viana et al. 2024).

2.3 Effects of nutrition on puberty onset

Pre and postnatal feed supplementation can positively or negatively impact the productive and reproductive performance of female cattle. Gasser et al. (2006a,b,c,d) showed that *Bos taurus* heifers weaned at around 3 months of age and supplemented with concentrate-rich diets for high body weight gain between 3 and 7 months of age reached puberty early by 300 days of age.

Cardoso et al. (2014), using a different strategy, evaluated two stair-step nutritional systems. The stair-step 1 method consisted of *ad libitum* feeding of concentrate from weaning (week 0) that occurs around 3.5 months of age, to week 10 (6.5 months of age), followed by a diet rich in forage for average daily gain (ADG) of 0.35 kg/d until week 20 (9 months of age) with subsequent *ad libitum* consumption of a diet rich in concentrate until week 30 (11.5 months) followed by another forage-based diet until week 40. The Stair-step 2 diet was the reverse sequence of 1. The authors observed a linear increase in leptin levels with *ad libitum* consumption, as a result of the increase in body weight and adipose tissue, as well as a reduction in the age at puberty.

In the same way, Freitas et al. (2021) evaluated different parameters of body maturation with puberty age and pregnancy by FTAI in Nellore heifers induced to puberty, and subcutaneous fat (SCFT) contributes to both variables. This contribution is because subcutaneous adipose tissue is a determining factor for puberty since it is the source of leptin. Puberty is highly correlated with leptin circulating levels (R = 0.82) (Willians et al. 2002). Thus, heifers reach puberty when leptin production is sufficient to stimulate reproductive function, resulting in ovulation.

Allen et al. (2017), also working with nutritional modulation of reproduction, evaluated the effects of diet type (high forage and high concentrate) and body weight gain rate (low gain [LG] 0.45 kg/d and high gain [HG] 0.91 kg/d) on the main hormones and age at puberty, and observed that average daily gain (ADG) was higher ($P < 0.04$) in the high gain groups (HG = 0.81 ± 0.06 kg/d; LG = 0.44 ± 0.05 kg/d), but the rate of gain was not affected by dietary energy source (HC-LG vs. HF-LG and HC-HG vs. HF-HG, respectively). The leptin levels were greater for the HG than for the LG groups (5.0 ng/mL vs. 2.5 ng/mL [$p<0.01$]). In addition, the mean age at puberty did not differ between dietary types (HF or HC), however, HG heifers reached puberty 6 weeks earlier ($P < 0.04$) than LG heifers. On the other hand, mean BW at puberty did not differ among groups.

Maternal nutrition during the gestational phase can also modulate the progenies' reproductive performance. According to Weller et al. (2016), maternal overnutrition in the second half of pregnancy affects the growth and number of ovarian follicles, with an increase in the number of primaries, preantral, and antral follicles, as well as a reduction in the number of primordial and total follicles. The authors associate this change in follicle population with the altered expression of genes related to folliculogenesis (MPR2, TGFBR1, GDF9, and FSHR), steroidogenesis (P450arom and StAR), and apoptosis (Bax, CASP3). These gene alterations may be causing decreased proliferation and increased germ cell atresia.

On the other hand, maternal undernutrition tends to reduce the number of antral follicles at the onset of puberty, compromise metabolic status, weight gain, and therefore age at puberty, since age and weight are components of puberty (Noya et al. 2019; Wiltbank et al. 1969).

Therefore, the productive and reproductive performance of female cattle can be defined even before they are born, depending on their mother's energy intake and balance (Weller et al. 2016). However, post-natal supplementation also has positive effects on reproductive performance (Figure 2) and can be used to promote early puberty and pregnancy despite maternal nutrition (Freitas et al. 2021).

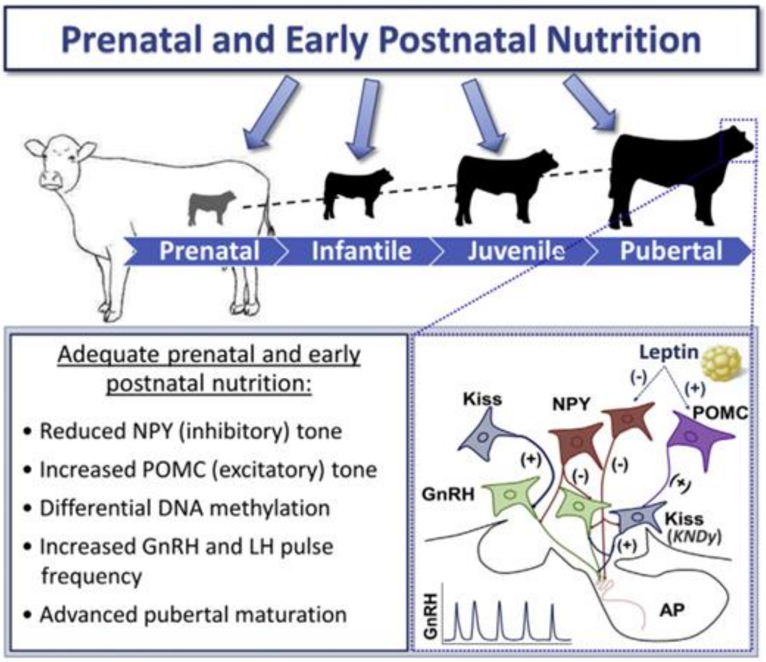

Figure 4 - Effect of prenatal (fetal programming) and postnatal nutrition on female bovine performance. Adequate nutritional management in these periods can reduce the inhibitory factors (NPY) and increase the excitatory factors (Leptin, kisspeptin, KNDy, and POMC), resulting in greater GnRH and LH pulse frequency, and pubertal maturation. However, DNA methylation is affected, and higher dietary energy in the juvenile period may produce hypermethylation and oocyte incompetence. **Source**: Cardoso et al. 2020.

2.4 Biotechnologies and nutritional management applied to reproduction in cattle

2.4.1. Cattle reproductive biotechnologies development

Biotechnologies applied to cattle reproduction are constantly being improved. Artificial insemination (AI) is the most widely used reproductive biotechnology, contributing greatly to the genetic evolution of herds. Around 20 to 22% of cattle are inseminated worldwide (Baruselli et al. 2022).

The use of other biotechnologies, such as embryo transfer (ET) and *in vitro* embryo production (IVEP), has been increased (Moore; Hasler, 2017; Rodrigues; Bertolini, 2019, and promotes an increase in the reproductive efficiency of both beef and dairy herds.

Far beyond biotechnologies, reproductive efficiency depends primarily on genetic and environmental factors, among which nutrition is the basis. Although complex, the relationship between nutrition and reproduction has been increasingly studied. However, the answers obtained for different fed strategies are generally variable and inconsistent in reproductive parameters (Sartori et al. 2024). One of the most important reproductive characteristics is the age at puberty, which can have an impact throughout the female's reproductive life, can be accelerated by nutritional management, and is indispensable to achieve a high pregnancy rate at FTAI.

2.4.2 Nutritional management and fixed-time artificial insemination (FTAI)

Reproductive biotechnologies, especially FTAI, have contributed to productivity improvement and sustainability in cattle farming (Moore; Hasler. 2017; Baruselli et al. 2021; Rodrigues, Bertolini. 2019). FTAI increases the number of pregnancies at the beginning of the breeding season and reduces the age at the first conception of heifers, increasing productivity and economic turnaround (Baruselli et al. 2021).

Breeding systems that use FTAI efficiently can reduce CO_2 equivalent emissions by up to 37.7% by obtaining calves with better genetic potential and using fewer cows, thus contributing to the sustainability of livestock farming (Baruselli et al. 2021).

The FTAI can be used in prepubertal Zebu heifers (Sá Filho et al. 2006; Freitas et al. 2021) to reduce the age at first service and, consequently, the age at first conception and first calving (Day; Nogueira, 2013). In this way, it is possible to promote the extension of the female's reproductive life, increasing the number of calves produced per cow (Short et al. 1994) and reducing the interval between generations (Yokoo et al. 2012; Baruselli et al. 2017).

Nutritional management is a critical factor in increasing the reproductive efficiency of early heifers in FTAI. Diets with high-quality forage and supplementation that provide an average daily gain (ADG) between 150 and 700 g^{-1} are essential for ensuring that the female reaches the breeding season weighing at least 260 kg and promoting pregnancy rates above 50.0% at the end of the breeding season, for Nellore breed (Nogueira et al. 2023).

In addition, to improve the efficiency of FTAI in heifers, cyclicity induction is a pivotal management. An example was done by Sá Filho et al. (2009) who demonstrated that Nellore heifers submitted to a cyclicity induction protocol (CIP) showed a higher cyclicity rate on d0, as well as a higher pregnancy rate when compared to the untreated heifers (40.0% vs. 61.9%).

In this sense, it is important to emphasize that, for biotechnology to provide all the potential benefits in heifers, adequate nutrition and hormonal induction of estrus are essential.

Thus, several studies have focused on elucidating the effects of feed restriction and overfeeding on the reproductive performance of heifers. Mollo et al. (2007) conducted a study with nulliparous Nellore heifers evaluating dry matter intake (DMI) with one group on feed restriction (0.70 of maintenance) and the other overfed (1.70 of maintenance). It was observed that the heifers subjected to high DMI ovulated larger follicles with the formation of larger CLs, but the circulating concentrations of estradiol and progesterone did not differ. In this sense, nutritional modulation occurs especially in the first year of life of the female (Freitas et al. 2021; Cardoso et al. 2014). However, to completely elucidate this theme, more studies are necessary.

2.4.3 Nutritional management and ovum pick-up / *In vitro* embryo production system

The ovum pick-up (OPU) and *in vitro* embryo production (IVEP) system has accelerated the genetic improvement and cattle productivity. The success of OPU-IVEP depends on the quantity and quality of oocytes, which can be influenced by factors such as breed, puberty, nutrition, and reproductive status.

Sales et al. (2015) observed that the IVEP success was dependent on the breed of cattle and found that the quality of oocytes recovered by OPU was greater in *Bos indicus* than in *Bos taurus* cattle. This is presumably due to greater numbers of ovarian follicle population and plasma anti-Mullerian concentration (AMH), and zebu donors also have been shown to yield greater amounts of viable oocytes compared to Bos taurus donors (Batista et al. 2014; Gimenes et al. 2015).

Viana et al. (2024) evaluated the results of 3,030 OPU-IVEP sessions performed in Gir donors and observed that the number of viable oocytes recovered and embryos produced from prepubertal heifers was lower than those observed in peripubertal and pubertal donors (12.3±2.8 vs. 28.0±0.9 oocytes, and 1.8±0.5 vs. 4.4±0.3 embryos, respectively).

The nutritional approach to reproductive management is an important tool, however, the effect of a high-energy diet on *in vitro* embryo production is not yet completely understood (Tomita et al. 2023).

In this scenario, energy consumption can modulate the IVEP efficiency, and the high energy dietary intake could affect fertility, especially in young females. Animals with high body condition scores (BCS) or consuming diets with high energy content may present hyperinsulinemia or insulin resistance, which is related to a drop in oocyte quality. In addition, these females generally have high circulating concentrations of glucose and Insulin-like growth factor 1 (IGF-I), which can interfere with glucose transport in embryos and increase apoptosis (Santos et al. 2008). Another effect, in Nellore heifers, is the reduction in circulating concentrations of steroid hormones, mainly estradiol and progesterone, affecting follicular growth (Sartori et al. 2010).

The reproductive status of the donor is also shown as important. As Pfeifer et al. (2009) observed, oocyte donor cows with very low circulating concentrations of progesterone had lower cleavage rates and blastocyst yield than those with higher blood concentrations of progesterone. Some studies have diverged concerning the results on IVEP from COCs derived from females with or without

CL. Barbosa et al. (2013) evaluated the efficiency of OPU-IVEP and found no significant differences in production between pregnant and non-pregnant cows. However, non-pregnant cows had better blastocyst quality at d7 from oocytes derived from ovaries with CL, whereas the opposite was observed in pregnant cows. Baruselli et al. (2016) observed that pregnant Holstein heifers had a greater number of embryos produced per OPU and ultimately a greater blastocyst rate when compared to pubertal and prepubertal nonpregnant heifers ($p<0.05$).

In this way, the quality of the oocyte for both pregnant and nonpregnant females can be affected by nutritional management, especially the energy level of the diet.

2.5 Dietary energy vs. oocyte quality

Although nutrition can accelerate reproductive maturation (Davis et al. 2023), a high level of energy intake can have a negative effect on embryonic development (Sartori et al. 2017).

In this sense, one pivotal cause of reduced fertility is related to oocyte maturation. This maturation depends on the interaction between the steroids in the granulosa and theca cells of the oocyte. Tahir et al. (2021) identified a series of genes involved in oocyte maturation, such as MAPK3, ADAMs, EGFR, MAPK13/14, PDE10A, CDC25A, ADCY7, SPEDY, MAPK8, ESR2, PLCB, MOS, KIT, PABPC, and KISS. These genes act in a coordinated way for oocyte maturation in the presence of hormones such as estrogen, LH, and progesterone, and altering their expression can lead to maturation failures and the production of non-competent oocytes.

The effects of nutrition are exerted even before fertilization during the acquisition of developmental competence of the oocyte (Boland et al. 2000). There is evidence that embryos from younger donors, 8 to 14 months, tend to be hypermethylated in some targets with genes regulating the cellular tumor antigen p53, transforming growth factor β1, tumor necrosis factor and hepatocyte nuclear factor 4α (Morin-Doré et al. 2020). Thus, the quality of the oocyte and subsequently the embryo has a direct impact on its ability to develop and maternal recognition of pregnancy, directly impacting gestational losses, which occur most in the first 90 days of pregnancy (Wiltbank et al. 2016).

2.6 Cost-effectiveness of nutritional and reproductive management

The calving system is one of the key sectors of beef cattle farming, directly impacting land use. This system occupies approximately 70% of pastures and overall farm productivity in Brazil. The primary indicators of system efficiency include the number and weight of calves produced per cow and the age at first calving for heifers (Baruselli et al., 2021).

In Brazil, the predominant breed in herds is *Bos indicus*, specifically Nellore cattle. Nelore cows utilize between 65% and 75% of the total energy required in a full-cycle production system. Compared to *Bos taurus* breeds, Nellore cattle demand significantly less energy, allowing them to survive and reproduce in more challenging environments (Fonseca et al., 2009; Valadares Filho & Paulino, 2005).

In this context, several breeding system models can be applied in Brazil, with extensive grazing being the most prevalent. This system requires large pasture areas but involves lower costs in terms of technology and mineral supplementation. However, to enhance productivity and environmental sustainability, intensive (feedlot) and semi-intensive (semi-confinement) systems are becoming increasingly common, particularly as crop expansion reduces available pastureland (Portes et al., 2021; Neto et al., 2018).

In this sense, in Brazil, the FTAI market represents approximately R$567 million (~US$ 175 million), with 3,500 veterinarians directly involved with this biotechnology. The FTAI is currently performed in approximately 8.2 million beef cows, promoting an 8% increase in calf production, which represents approximately 656 thousand more calves per year or an additional income of R$820 million/year (~US$ 253 million) compared with natural service breeding (Baruselli et al. 2018).

Overton et al. (2005) conducted an economic evaluation comparing the use of FTAI and bull mating in dairy cattle, observing that the use of sires resulted in approximately US$10 more in cost per cow per year than the AI program. The use of AI-proved sires resulted in a gain of US$ 89-101 in value of milk production per year as a result of genetic improvement that results in progeny's milk production increase.

Rodgers et al. (2014), in a study with beef cows (*Bos taurus*), observed that calves derived from estrus synchronization (ES) programs were estimated to be 10 days older than calves not derived from ES. As a result, on an assumed daily growth rate of 0.91 kg/d, the investigators determined that calves born from ES gained an extra 9.1 kg, which results in an extra US$16.23.

Lardner et al. (2020) studied the profitability of a natural-service breeding (NSB) program compared to a single fixed-time artificial insemination (FTAI), on beef cattle reproductive efficiency, breeding costs, and economic return. Eighty Black Angus lactating beef cows (5-6 yrs of age; n = 80; BW = 599.4±78.6 kg) were randomly assigned to the FTAI or NSB. The FTAI cows received a CIDR for 7 d and a 100 µg (2 mL) Intramuscular (i.m.) injection of GnRH, following this 25 mg (5 mL) i.m. of PGF2α with CIDR removed. Then a second 25 mg (5 mL) i.m PGF2α dose on day 10 to ensure luteal regression, and was artificially inseminated by a trained technician. The NSB cows were exposed to bulls on a bull:cow ratio of 1:25 for a 63-day breeding season. Results indicated that an NSB program can be a lower cost (US$85 vs. US$123) compared to the FTAI program. But, if improvements in conception rate, calf weaning rate, and total 205-d adjusted wean weights are incorporated, a partial budget analysis reveals that FTAI can increase net profit by US$284 per cow.

In Nellore beef cattle, timed AI also hastens parturition and adds genetic gain to commercial herds, generating an average gain of 20 kg on the weaning weight of calves, which 248 represents 3.3 million weaned calves with an extra 20 kg or, extra R$400 million (~US$ 123 million). Also, from weaning to slaughtering, TAI calves gain an additional 15 kg of carcass, generating extra R$482.2 million (~US$ 149 million). Thus, FTAI aggregates to the bovine beef chain around R$1.7 billion (more than half a billion US$) per year (Baruselli, 2018).

In this context, the main distinction between breeding systems lies in investment costs and financial returns. In beef cattle production, feed expenses are crucial, often accounting for 70% to 90% of total operating costs, depending on the production stage and the target output level. However, optimizing feed management can significantly impact the economic performance of meat production systems (Nogueira et al., 2023; Neto et al., 2018; Valadares Filho & Paulino, 2005; Silva et al., 2002).

2.7 Conclusion

In conclusion, there are many tools that can be used to improve the productivity of young heifers after using FTAI or OPU/IVEP. Nutritional management, environmental conditions, and the breed can modulate the reproductive maturation and promote early puberty in bovine females, especially in systems with FTAI. In this sense, this early puberty is a prior factor to increase the profitability in the beef cattle system by obtaining at least one more calf per cow throughout her reproductive life. However, it is necessary to pay attention to the type of nutrition strategy to improve both production and reproduction in an economically sustainable form. In addition, the OPU-IVEP can be used to produce more than one calf per cow per year, including in pregnant donors. However, it is dependent on environmental conditions, especially nutrition, and the oocyte quality that can be affected by the breed, age, and reproductive status of the donor.

3 AIMS

3.1 Overall aim

The purpose of this study was to evaluate the influence of different dietary energy levels on age at puberty, fertility, and embryo production in Nellore heifers, considering the economic feasibility of the systems.

3.2 Specific aims

To evaluate puberty and pregnancy in Nellore females fed with different supplementation strategies in feedlots.

To evaluate the follicular development of heifers fed in feedlots.

Evaluate body maturation parameters (Weight, ADG, BCS, and subcutaneous croup fat thickness [SCFT]) with different dietary energy levels.

Evaluate the partial budget of supplementation levels and income revenue.

To evaluate the efficiency of embryo production in pregnant and non-pregnant heifers fed with high dietary energy.

REFERENCES

ALLEN, C. C. et al. Interaction of dietary energy source and body weight gain during the juvenile period on metabolic endocrine status and age at puberty in beef heifers. **Journal of Animal Science**.v. 95, p. 2080-2088, 2017.

ABIEC – Associação Brasileira das Indústrias Exportadoras de Carnes. **Exportações**. Disponível em: https://www.abiec.com.br/exportacoes/. Acesso em: 15 de maio. 2024.

ABIEC – Associação Brasileira das Indústrias Exportadoras de Carnes. **Beef Report 2024**. SP, 2024.

ANDERSON, K. J. D. et al. The Use of Reproductive Tract Scoring in Beef Heifers. **Agri-Practice**. v. 12, p. 19-26, 1991.

BARUSELLI, P. S. et al. Efeito de diferentes protocolos de inseminação artificial em tempo fixo na eficiência reprodutiva de vacas de corte lactantes. **Revista Brasileira de Reprodução Animal**, v.26, p.218-221, 2002.

BARUSELLI, P. S. et al. Factors that interfere with oocyte quality for in vitro production of cattle embryos: effects of different developmental & reproductive stages. **Animal Reproduction**, v.13, n.3, p.264-272, Jul./Sept. 2016.

BARUSELLI, P. S. et al. Review: Using artificial insemination v. natural service in beef herds. **Animals**, v.12, n.1, p. 45-52, 2018.

BARUSELLI, P. S. et al. Genetic market in cattle (Bull, AI, TAI, MOET, IVP). Animal Reproduction, v.15, n.3, p.247-255, Jul./Sept. 2018.

BARUSELLI, P. S. et al. Evolução e perspectivas da inseminação artificial em bovinos. **Revista Brasileira de Reprodução Animal**, v.43, p.308 314, 2019b.

BARUSELLI, P. S. et al. IATF em números: evolução e projeção futura. **Revista Brasileira de Reprodução Animal**, v.46, n.2, p. 76-83, abr./jun. 2022.

BARUSELLI, P. S. et al. Mitos e realidades sobre a inseminação artificial em tempo fixo (IATF) em bovinos de corte. **Revista Brasileira de Reprodução Animal**, v.45, n.4, p.625-646, out./dez. 2021.

BAGLEY, C. P. Nutritional management of replacement beef heifers: A review. **Journal of Animal Science**. v.71, p. 3155-3163, 1993.

BARBOSA, C.P. et al. Produção in vitro de embriões bovinos da raça Nelore oriundos de oócitos de ovários com e sem corpo lúteo. **Ciência Animal Brasileira**. Goiânia, v.14, n.1, p. 81-90, jan./mar. 2013.

BATISTA, E. O. S. et al. Plasma Antimullerian Hormone as a Predictor of Ovarian Antral Follicular Population in Bos indicus (Nelore) and Bos taurus

(Holstein) Heifers. **Reproduction in Domestic Animals**, v.49, p.448-452, 2014.

BYERLEY, D. J. et al. Pregnancy rates of beef heifers bred either on pubertal or third estrus. **Journal of Animal Science**. v. 65, p. 645-650, 1987. doi:10.2527/ jas1987.653645x.

BÓ, G. A. et al. Technologies for fixed-time artificial insemination and their influence on reproductive performance of Bos indicus cattle. **Society of Reproduction and Fertility**, Supplement v. 64, p.223-236, 2007.

BOLAND, M. P.; LONERGAN, P.; O'CALLAGHAN. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. **Theriogenology**. v. 55, p. 1323-1340, 2001.

BORCHARDT, S. et al. Economic impact of adding a second prostaglandin F2α treatment during an Ovsynch protocol using a meta-analytical assessment and a stochastic simulation model. **Journal of Dairy Science**, v. 104, n. 11, 2021.

CARDOSO, R. C. Reciprocal changes in leptin and NPY during nutritional acceleration of puberty in heifers. **Journal of Endocrinology**, n.223, v.3, p.289-98, 2014.

CARDOSO, R. C. et al. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. **Journal of Animal Science**. Published online, 2014.

CARDOSO, R. C. et al. Nutritional control of puberty in the bovine female: prenatal and early postnatal regulation of the neuroendocrine system. **Domestic Animal Endocrinology** (2020).

CHEN, N. et al. Global genetic diversity, introgression, and evolutionary adaptation of indicine cattle revealed by whole genome sequencing. **Nature Communications**, v.14, p.1-13, 2023.

COOKE, R. F. et al. Cattle adapted to tropical and subtropical environments: genetic and reproductive considerations. **Journal of Animal Science**, v. 98, n.2, p.1-14, 2020.

DAY, M. L. et al. Endocrine Mechanisms of Puberty in Heifers - Estradiol Negative Feedback-Regulation of Luteinizing-Hormone Secretion. **Biology of Reproduction**. v.31, p. 332-341, 1984.

DAY, M. L.; Nogueira, G. P. Management of age at puberty in beef heifers to optimize efficiency of beef production. **Animal Frontiers: The Review Magazine of Animal Agriculture,** v.3, p.6-11, 2013.

DAVIS, T. C. et al. Effect of dietary energy source on pregnancy rates and reproductive physiology of pastured beef heifers. **Frontiers of Animal Science.** v. 4, 2023.

D'OCCHIO, M. J.; BARUSELLI, P. S.; CAMPANILI, G. 2019. Influence of nutrition, body condition, and metabolic status on reproduction in female beef cattle: a review. **Theriogenology**. v. 125, p. 277-284, 2019.

DUITTOZA, A. H.; KENNY, D. A. Review: Early and late determinants of puberty in ruminants and the role of nutrition. **Animals**, v.17, 2023.

EDMONSON, A. J. et al. A Body Condition Scoring Chart for Holstein Dairy Cows. **Journal of Dairy Science**. v. 72, n. 1, p. 68-78, jan. 1989.

FERRAZ JR, M. V. C. et al. A combination of nutrition and genetics is able to reduce age at puberty in Nellore heifers to below 18 months. **Animal**, v.12, n.3, p. 569–574, 2018.

FERRELL, C.L., JENKINS, T.G. Cow type and the nutritional environment: nutritional aspects. **Journal of Animal Science**, v.61, n.2, p.725741, 1985.

Food and Agriculture Organization of the United Nations – FAO. **MEAT MARKET REVIEW**: Overview of market and policy developments 2021. FAO 2022. Disponível em: https://www.fao.org/3/cc0984en/cc0984en.pdf. Acesso em: 31 de maio de 2023.

FONSECA, M. A. **Exigências Nutricionais de Vacas e Bezerros Nelore, do nascimento à desmama**. Viçosa, MG: UFV, 2009. 87p. Dissertação (Mestrado em Zootecnia) – Universidade Federal de Viçosa, 2009.

FUNSTON, R. N. et al. Alpharma beef cattle nutrition symposium: implications of nutritional management for beef cow-calf systems. **Journal of Animal Science**. 2012.

FREITAS, B. G. et al. Relationship of body maturation with response to estrus synchronization and fixed-time AI in Nellore (Bos indicus) heifers. **Livestock Science**. v. 251, 2021.

GASSER, C. L. et al. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. **Journal of Animal Science**. v. 84, p. 3118-3122, 2006a.

GASSER, C. L. et al. Induction of precocious puberty in heifers III: Hastened reduction of estradiol negative feedback on secretion of luteinizing hormone. **Journal of Animal Science**. v. 84, p. 2050–2056, 2006b.

GASSER, C. L. et al. Induction of precocious puberty in heifers II: Advanced ovarian follicular development. **Journal of Animal Science**. v. 84, p. 2042-2049, 2006c.

GASSER, C. L. et al. Induction of precocious puberty in heifers I: Enhanced secretion of luteinizing hormone. **Journal of Animal Science**. v. 84, p. 2035-2041, 2006d.

GAZAL, O. S., L. S. et al. Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: Correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. **Biology and Reproduction**. v.56, p. 676–683.1998.

GIMENES, L. U. et al. The interval between the emergence of pharmacologically synchronized ovarian follicular waves and ovum pickup does not significantly affect in vitro embryo production in Bos indicus, Bos taurus, and Bubalus bubalis. **Theriogenology**, v.83, p.385-393, 2015.

GINTHER, O. J. et al. Luteal blood flow and progesterone production in mares. **Animal Reproduction Science**, v. 99, p. 213-220, 2007.

IBGE – Instituto Brasileiro de Geografia e Estatística. **Brasil, cidades e estados**. 2021. Disponível em: https://cidades.ibge.gov.br/brasil/ms/anastacio/panorama. Acesso em: 01 de jul. 2023.

KINDER, J. E. et al. Endocrine regulation of puberty in cows and ewes. **Journal of reproduction and fertility**. v.34, p.167-86, 2019.

LAU et AL. Gene regulation could be attributed to TCF3 and other key transcription factors in the muscle of pubertal heifers. **Veterinary Medicine Science**. v.6, p.695–710, 2020.

KANAL, L. B. et al. Relationships between feed efficiency and puberty in Bos taurus and Bos indicus-influenced replacement beef heifers. **Journal of Animal Science**, v.98, n.10, p.1-9, 2020.

LARDNER, H. et al. Comparison of FTAI and Natural Service Breeding Programs on Beef Cow Reproductive Performance, Program Cost, and Partial Budget Evaluation. **Journal of Agricultural Science**, v. 12, n. 9, 2020.

LESMEISTER, J. L.; BURFENING, P. J.; BLACKWELL, R. L. Date of first calving in beef cows and subsequent calf production. **Journal of Animal Science**. v. 36, n. 1-6, 1973.

McSHANE, T. M. et al. Influence of food restriction on neuropeptide-Y, proopiomelanocortin, and luteinizing hormone-releasing hormone gene expression in sheep hypothalami. **Biology and Reproduction**. v. 49, p.831–839, 1993.

MOORE, S. G.; HASLER, J. F. A 100-Year Review: Reproductive technologies in dairy Science. **Journal of Dairy Science.** v. 100, n.12, 2017.

MOLLO, M.R.; RUMPF, R.; MARTINS, A.C. et al. Função ovariana em novilhas Nelore submetidas a alta ou baixa ingestão alimentar. **Acta Scientiae Veterinariae**, v.35(Supl 3), p.958, 2007.

MORIN-DORÉ, L. et al. Transcriptomic evaluation of bovine blastocysts obtained from peri-pubertal oocyte donors. **Theriogenology**, 2017.

MORIN-DORÉ, L. et al. DNA methylation status of bovine blastocysts obtained from peripubertal oocyte donors. **Molecular Reproduction and Development**. n.1, v. 15, 2020.

National Research Council (NRC). Nutrient requirements of beef cattle. 7th rev. ed. Washington, DC: National Academy Press, 2000.

NETO, E. S. et al. Reproductive performance of Nelore heifers raised in an extensive system undergoing different vaccination protocols in fixed-time artificial insemination (FTAI). **Ciência Rural**. v. 49, n.11. 2019.

NGUYEN et al. Adipose tissue proteomic analyses to study puberty in Brahman heifers. **Journal of Animal Science**. v.96, p. 2392–2398, 2018.

NOGUEIRA, G. P. Puberty in South American Bos indicus (Zebu) cattle. **Animal Reproduction Science**, v.83, p.361-372.

NOGUEIRA, E. et al. Protocolo Embrapa +Precoce P14. **EMBRAPA gado de corte**. Comunicado técnico, 1ª edição. 2023.

NOYA. et al. Effects of Developmental Programming Caused by Maternal Nutrient Intake on Postnatal Performance of Beef Heifers and Their Calves. **Animals**, v. 9, 2019.

OVERTON, M. W. et al. Cost comparison of natural service sires and artificial insemination for dairy cattle reproductive management. **Theriogenology**, v.64, p.589–602, 2005.

PENTEADO, L. et al. **Eficiência reprodutiva em vacas Nelore (Bos indicus) lactantes submetidas a diferentes manejos durante a estação de monta**. In: Anais do XVI Congresso Brasileiro de Reprodução Animal, 2005, Goiânia, GO, 2005.

PEREIRA, D. M. et al. Production of in vitro bovine embryos supplemented with L-carnitine in different oxygen tensions and the relation to nitric oxide. **Zygote**, v. 28, n. 5, p. 403-408, 2020.

PERRY, G. A. Factors affecting puberty in replacement beef heifers. **Theriogenology**. v.86, n.1, p.373-378, 2016.

PFEIFER, L.F.M. et al. Effect of circulating progesterone on in vitro developmental competence of bovine oocytes. **Animal Reproduction**, v. 6, n. 3, p. 473-480, 2009.

PORTES, J. V. et al. Selection indexes for Nelore production system in the Brazilian Pantanal. **Revista Brasileira de Zootecnia**, v. 50. 2021.

PINHEIRO, A. K. et al. Parâmetros produtivos e genéticos da produção in vitro de embriões Nelore no Estado do Acre. **Research, Society and Development**, v. 11, n. 7, 2022.

RODGERS, J. C. et al. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. **Journal of Animal Science**, v.90, p.4055–4062, 2012.

RODRIGUES, J. L.; BERTOLINI, M. Biotecnologias da reprodução animal: de Aristóteles à edição gênica. **Revista Brasileira de Reprodução Animal**. v.43, n.2, p.204-208, abr./jun. 2019.

SAMADI, F. et al. Nutrition, metabolic profiles and puberty in Brahman (Bos indicus) beef heifers. **Animal Reproduction Science**. v. 146, p. 134–142, 2014.

SÁ FILHO, M. F. et al. Effect of ciclicity induction in Nelore heifers treated with a previously used intravaginal progesterone device on conception rate after fixed time artificial insemination. **Acta Scientiae Veterinarie**, v.34, p.403, 2006.

SÁ FILHO, M. F. et al. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows II: Strategies and factors affecting fertility. **Theriogenology**, v.72, p.210-218, 2009.

SÁ FILHO, M. F. et al. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. **Animal Reproduction Science**, v.129, n. 3-4, p.132-139, 2011.

SÁ FILHO, M. F. et al. Timed artificial insemination earlier during the breeding season improves the reproductive performance of suckled beef cows. **Theriogenology**, v.79, p.625–632, 2013.

SALES, J. N. et al. Effects of a high-energy diet on oocyte quality and in vitro embryo production in Bos indicus and Bos taurus cows. **Journal of Dairy Science**. v. 98, p.3086–3099, 2015.

SANACORA, G. M. et al. Increased hypothalamic content of proneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food privation. **Endocrinology** v.127, p.730–737. 1990.

SANTOS, J. E., R. L. Cerri, and R. Sartori. 2008. Nutritional manage ment of the donor cow. **Theriogenology**. v.69, p.88–97, 2008.

SARTORI, R., M. R. et al. Physiological differences and implications to reproductive management of Bos taurus and Bos indicus cattle in a tropical environment. **Society of Reproduction and Fertility**. Suppl. v.67, p.357–375, 2010.

SARTORI, R; GUARDIEIRO, M. M. Fatores nutricionais associados à reprodução da fêmea bovina. **Revista Brasileira de Zootecnia**., v.39, p.422–432, 2010.

SARTORI, R. et al. 2024. Induction of puberty vs. induction of ovulation using steroid hormones in beef heifers: a comprehensive review. **Animal Reproduction**, v.21, n.3, 2024.

SCHILLO, K. K.; HALL, J. B.; HILEMAN, S.M. Effects of Nutrition and Season on the Onset of Puberty in the Beef Heifer. **Journal of Animal Science**. v. 70, p.3994-4005, 1992.

SOUSA, G.G.T. et al. Características reprodutivas de bovinos da raça Nelore do meio Norte do Brasil. **PUBVET**, v. 6, n. 21, 2012.

SHORT, R. et al. Breeding Heifers at one year of age: biological and economic considerations. **Factors Affecting Calf Crop**. ed. M.J. Fields R.S. Sand. p. 55–68, 1994.

SILVA, F. F. et al. Exigências líquidas e dietéticas de energia, proteína e macro-elementos minerais de bovinos de corte no Brasil. **Revista Brasileira de Zootecnia**, v. 31, n. 2, p. 776-792, 2002.

TAHIR, M.S. et al. Meta-Analysis of Heifer Traits Identified Reproductive Pathways in Bos indicus Cattle. **Genes**, v.12, n.768. 2021.

TOMITA, K. et al. Effects of short-term dietary supplementation on the number of ovarian follicles, quantity and quality of oocytes, and in vitro embryo production in Japanese Black cows. **Journal of Reproduction and Development**, v. 69, n. 2, 2023.

THOMAS, M. G. et al. Injection of neuropeptide Y into the third cerebroventricle differentially influences the pituitary secretion of luteinizing hormone and growth hormone in ovariectomized cows. **Domestic Animal Endocrinology**. v.16, p.159-169, 1999.

UTSUNOMIYA, Y. T. et al. Genomic clues of the evolutionary history of Bos indicus cattle. **Animal Genetics**, v.50, p.557–568, 2019.

VALADARES FILHO, S. C.; PAULINO, P. V. R.; MAGALHÃES, K. A. **Exigências Nutricionais de Zebuínos e Tabelas de Composição de Alimentos BR CORTE**. 1. ed. – Viçosa: UFV, DZO, 2006. 142p.

VIANA, J. H. M. et al. Oocyte developmental potential and embryo production before puberty in cattle. **Animal Reproduction**. v.21, n.3, 2024.

YOKOO, M. J. et al. Características reprodutivas e suas associações com outras características de importância econômica na raça Nelore. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**. Universidade Federal de Minas Gerais, Escola de Veterinária, v. 64, n.1, p.91-100, 2012.

WELLER 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, 2016.

WILTBANK, M. C. et al. Puberty in crossbred and straightbred beef heifers on two levels of feed. **Journal of Animal Science**. v.29, p. 602. 1969.

WILTBANK, M. C. et al. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows, **Theriogenology**. 2016.

WILLIANS, G. L. et al. Leptin and its role in the central regulation of reproduction in cattle. **Domestic Animal Endocrinology**, v.23, p. 339–349, 2002.

ZHANG, K. et al. Evolution and domestication of the Bovini species. **Animal Genetics**, v.51, p.637–657, 2020.

CHAPTER 2 - SCIENTIFIC PAPER, STUDY 1 (Livestock Science).

Impact of different dietary energy levels on the productive and reproductive performance of Nellore heifers

Author: Felipe de Oliveira Pedro[1], Juan Fuentes Sebastian[2]; Aldair Félix da Silva[3], Thais Ferreira Lima[1]; João Victor Bezerra Machado[1]; André Rosemberg Peixoto Simões[1], Andréa Roberto Duarte Lopez de Souza[1], Jéssica Nora Drum[4]; Fabiana de Andrade Melo Sterza[1]

[1] Mato Grosso do Sul State University; Aquidauana, Mato Grosso do Sul, Brazil
[2] University of The Llanos, Villavicencio, Meta, Colombia
[3] Mato Grosso do Sul Federal University; Campo Grande, Mato Grosso do Sul, Brazil
[4] South Dakota State University, Brookings, South Dakota, United States

ABSTRACT

This study aimed to identify the supplementation strategy that best enhances the productive and reproductive performance of Nellore heifers. The experiment included 60 heifers with an average age of 9.05 ± 0.42 months and weighing 259.3 ± 21.6 kg, maintained in a feedlot system in collective stalls with free access to feed and water. The heifers were randomly divided into 3 groups in a completely randomized design: S0.5% (N = 20), S1.0% (N = 20), and STP (Stair-step nutrition, N = 20). The STP group received a gradual increase in concentrate supply: 0.07%, 0.15%, 0.3%, 0.5%, and ending with 1.0% of body weight (BW). The S0.5% and S1.0% groups received 0.5% and 1.0% of BW, respectively. The experimental period lasted 111 days, including 17 days of adaptation. BRS Capiaçu silage and sugarcane bagasse were provided as roughage. Variables such as average daily gain (ADG), early puberty onset, pregnancy rate, pregnancy loss, subcutaneous croup fat thickness (SCFT), estrus score (HTSC), gynecological parameters (uterus score, dominant follicle presence, corpus luteum presence, and antral follicle count score) at D0 of the fixed-time artificial insemination (FTAI), and partial budget were analyzed in the experiment. Statistical analyses were performed using SAS® (SAS Inst. Inc., Cary, NC, USA; SAS on demand), considering an alpha of 5% significance. No differences were observed in pregnancy rates and losses, or the number of pubertal females among groups ($p>0.05$) during the entire experimental duration. However, the S1.0% group showed higher average daily gain (ADG) compared to STP and S0.5% ($p<0.05$). The partial budget was greater for the S0.5% group ($2.64) than the others, which did not differ between each other ($2.55). It was concluded that the supplementation energy levels provided did not differ for reproductive outcomes but influenced weight gain.

Keywords: Bovine reproduction; biotechnology; feedlot; nutrition; puberty.

INTRODUCTION

Reproductive efficiency is a critical factor in achieving high productivity in beef cattle (Noya et al. 2019), with age at first calving being a key parameter for assessing heifer fertility (Sá Filho et al. 2011). In this way, age at puberty is a prior variable for a herd's good reproductive performance. It is controlled by genetic and environmental factors such as nutritional intake, which is one of the main tools for reducing the age at puberty (Allen et al. 2017).

Puberty involves several behavioral and physiological changes related to the activation of the hypothalamic-pituitary-ovarian axis, with great importance given to hypothalamic activity, which is responsible for the release of gonadotropin-releasing hormone (GnRH) with a subsequent increase in the frequency of luteinizing hormone (LH) pulses that provides the final development of follicles and the steroidogenesis necessary for the first ovulation and consequent establishment of reproductive cyclicity (Cardoso et al. 2020).

Early puberty between 12 and 14 months of age is essential to ensure conception and calving at 2 years of age (Cardoso et al. 2014; Cardoso et al. 2018; Freitas et al. 2021). Early first calving influences reproductive performance throughout the female's life (Short et al. 1994). In this context, there is a period of greater importance during the early life of the bovine female where sensitivity to the quality and quantity of the diet is greater (Allen et al. 2017).

From this perspective, diets rich in energy during the juvenile period promote a higher body weight gain (BW) and fat, culminating in earlier puberty (Cardoso et al. 2014). Similarly, to obtain satisfactory pregnancy rates by fixed-time artificial insemination (FTAI), supplementation is essential, whether in an intensive system in confinement or on pasture, promoting gains of more than 300 (g/d) to provide a pregnancy rate of more than 50.0% at the end of the breeding season in the case of Nellore young females (Nogueira et al. 2023). Thus, adopting dietary strategies is an essential tool for expressing the animal's genetic potential and subsequently increasing efficiency, especially in the initial phase of reproductive development. However, this is not a reality in Brazil's livestock, where 60% of the total pasturelands, estimated at 109.7 million hectares, have some level of degradation, and more than 30 % of the farms still offer only cattle sodium-based supplements that are poor in macrominerals, microminerals, energy, and protein (Bolfe et al. 2024).

Therefore, it is necessary to understand the best nutritional strategy to provide earlier puberty and reproductive performance in heifers. Several studies have found positive effects of high dietary energy on early puberty (Cardoso et al. 2020; Freitas et al. 2021; Sales et al. 2015; Toledo et al. 2023). On the other hand, high energy consumption may affect fertility and oocyte quality in heifers by promoting incompetent oocytes as a result of hypermethylation of genes involved in oocyte competence acquisition (Morin-doré et al. 2021; Toledo et al. 2023). However, it has not been fully elucidated how these effects occur on reproduction, especially for the Nellore breed.

This study hypothesized that the highest dietary energy would reduce the fertility of Nellore heifers despite promoting early puberty attainment. The aim is to evaluate the impact of different dietary energy levels on the productive and reproductive performance of Nellore heifers, as well as the partial budget of each strategy.

MATERIALS AND METHODS

This study adhered to animal welfare principles and was approved by the Ethics Committee for Animal Use (ECAU) of Mato Grosso do Sul State University (protocol N°. 039/2022).

Site and study period

The experiment was conducted in Anastácio, Mato Grosso do Sul, Brazil. The farm is located in the biome Cerrado (IBGE, 2023), and the region has a tropical climate with a rainy season in the summer (November to April) and a dry season in the winter (May to October). The experimental period lasted 111 days, starting in August 2023, with 17 days of acclimation and 94 days of supplementation. For this period, the regional temperature has varied between 14.60 to 41.50 Celsius degrees (°C), and the accumulated rain precipitation was 248.90 mm (CENTEC, 2023).

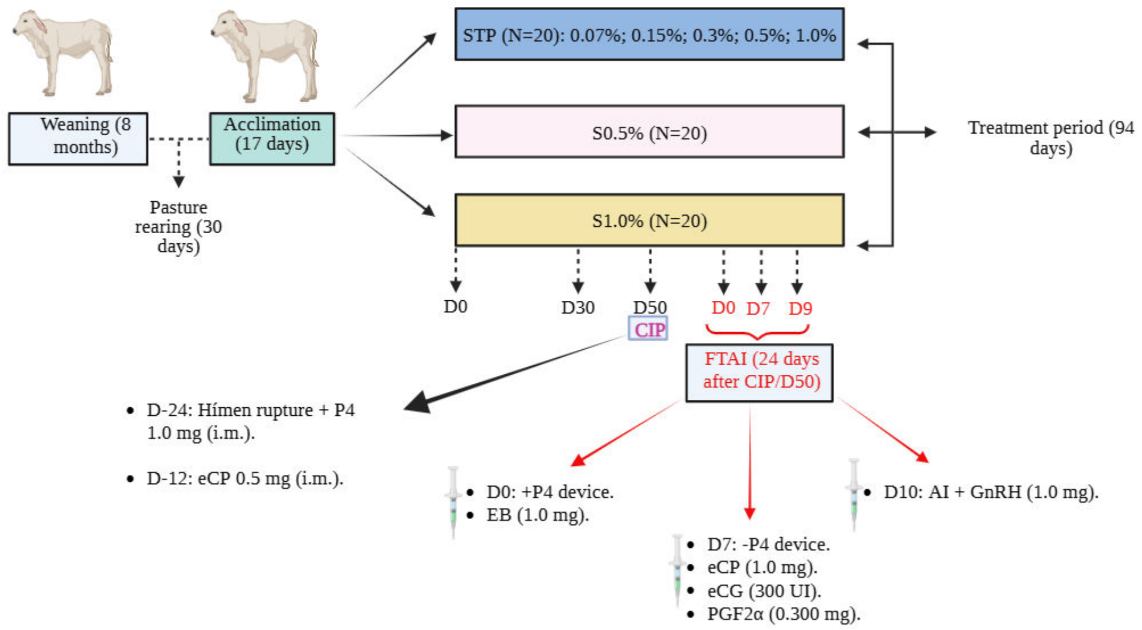

Figure 1 - Experimental design showing the data collection period that began after 17 days of acclimation during the post-weaning period. Different percentages of dietary concentrate inclusion (STP = Stair-step nutrition; S0.5% = Supplementation with 0.5% of body weight; S1.0% = supplementation with 1.0 % of body weight), providing more or less energy levels, were studied. The data collection was performed on each management day till the d0 of FTAI. CIP = Cyclicity induction protocol (FTAI = Fixed-time artificial insemination; AI = Artificial insemination.

Animals and treatments

Sixty Nellore heifers were randomly assigned to three treatments in feedlots: STP (stair-step supplement), S0.5% (0.5% BW supplementation), and S1.0% (1.0% BW supplementation). STP supplementation consisted of gradual increases, every 15 days, in concentrate supply, as follows: 0.07% of BW, 0.15% of BW, 0.3% of BW (0.15% concentrate), 0.5% of BW, and ending with 1.0% of BW. Supplementation for groups S0.5% and S1.0% was 0.5% of BW and 1.0% of BW, respectively, during the entire experimental period. BRS Capiaçu silage was provided as roughage, with 2.0 Kg of sugarcane bagasse addition per animal in each group. Diets were formulated using RLM software (RLM Pesquisa em Otimização Agropecuária e Ambiental Ltda) following NRC Tropicalizado ESALQ recommendations (NRC, 2001). The feed ingredients composition (Table 1), diet chemical composition (Table 2), and mineral concentration (Table 3) were defined to be different in each treatment.

Table 1 - Composition (percentage of dry matter) of concentrates provided in different diets for Nelore heifers (Three different concentrates = 0.15%, 0.5%, and 1.0%). Chemical composition of concentrates (0.15%, 0.5%, and 1.0%), sugarcane bagasse, and BRS Capiaçu silage). Diets composition (percentage of each ingredient in dry matter).

DM (%)	STP (0.15%)	0.5%	1.0%	Sugarcane bagasse	BRS Capiaçu silage
NaCl	10.73	3.00	1.00	-	-
Cottonseed meal	17.77	20.00	21.00	-	-
Sorghum (grain)	46.81	65.70	73.30	-	-
Urea (NNP)	10.73	4.00	2.00	-	-
Probiotic (MultSacch)	1.07	0.30	0.20	-	-
Zoofós 150 nucleus (Zoomix)	12.88	7.00	2.50	-	-
DM	86.70	87.80	86.10	73.20	27.50
CP	53.20	27.90	25.70	1.60	5.00
MM	23.90	14.90	6.70	2.30	6.60
dCP	53.10	27.80	25.68	1.10	3.23
dFAT	0.90	1.30	1.80	0.20	0.70
dNFC	10.78	40.67	54.49	14.20	16.07
dNDF	3.95	6.28	3.45	42.00	42.87
TDN	69.84	77.15	87.67	57.75	66.74
Diet STP	5.00	-	-	7.50	87.50
Diet S0.5	-	20.00	-	15.5	64.50
Diet 1.0	-	-	37.50	13.5	49.00

Legend: The 0.15% was utilized in the STP treatment in the 0.07% and 0.3% concentration inclusion. And after this, the concentrates 0.5% followed by 1.0% were fed to STP in their respective diet phase. DM = Dry matter; CP = Crude Protein; MM = Mineral Matter; dCP = Digestible Crude Protein; dFAT = Digestible Fat; dNFC = Digestible Non-Fiber Carbohydrates; dNDF = Digestible Neutral Detergent Fiber Carbohydrates; TDN = Total Digestible Nutrients. Probiotic composition: MultSacch (Biomart, Martinópolis, São Paulo, Brazil) = *Bacillus subtilis* (CCT 0089) *Bifidobacterium bifidum* (DSM 20456), *Enterococcus faecium* (CCT 6646), *Lactobacillus acidophilus* (CCT 2949), *Lactobacillus buchneri* (CCT 3746), *Lactobacillus casei* (CCT 1465), *Lactobacillus lactis* (CCT 1344), *Saccharomyces cerevisiae* (CCTCC M207177 / NCYC 996).

Table 2 – Chemical composition (percentage of dry matter) of concentrates provided for Nelore heifers fed with three different diets (STP, S0.5%, and S1.0%).

Ingredients (% DM)	STP			S0.5%	S 1.0%
	0.15	0.5	1.0		
DM	34.00	41.20	39.20	40.60	39.20
CP	6.12	7.00	9.00	7.13	8.14
FAT	1.30	1.60	2.00	1.50	1.70
NFC	11.96	14.10	19.50	12.84	18.23
NDF	42.65	41.06	36.00	40.94	38.63

TDN	63.65	65.76	69.00	64.28	68.82
DE mcal/Kg/DM	2.80	2.90	3.04	2.83	3.03
mE mcal/kg/DM	2.30	2.37	2.49	2.32	2.50
DMI Kg/animal/d	8.50	9.92	9.98	10.03	9.95
mEI Kg/DM/animal/d	19.55	23.51	24.87	23.26	24.77

Legend: DM = Dry matter; CP = Crude Protein; MM = Mineral Matter; CP = Crude Protein; FAT = Fat; CNF = Non-Fiber Carbohydrates; NDF= Neutral Detergent Fiber Carbohydrates; TDN = Total Digestible Nutrients; DE = Digestible Energy; ME = Metabolic Energy; DMI = Dry Matter Intake; mEI = Metabolic Energy Intake.

Table 3 - Guarantee level for macro and microminerals (Zoofós 150 nucleus, Zoomix) in concentrates (0.15%, 0.5%, and 1.0%) provided in three diets (STP, S0.5%, and S1.0%) for Nelore heifers.

		Supplements		
		0.15%	0.5%	1.0%
Macro (% DM)				
	Calcium	2.82	1.56	0.67
	Phosphorus	2.23	1.39	0.82
	Magnesium	0.30	0.23	0.20
	Potassium	0.42	0.51	0.62
	Sodium	3.72	1.14	0.45
	Chlorine	6.11	1.88	0.74
	Sulfur	0.62	0.39	0.25
Micro (ppm/DM)				
	Cobalt	20.66	11.26	4.51
	Copper	270.45	154.01	71.99
	Iron	50.44	56.92	68.22
	Iodine	14.21	7.76	3.12
	Manganese	214.33	121.85	56.33
	Selenium	4.89	2.92	1.25
	Zinc	738.27	444.50	196.48

Legend: DM = Dry Matter; ppm = parts per million; 0.15% = concentrate fed for STP on the levels 0.07%, 0.15%, and 0.3%; 0.5% = concentrate provided for S0.5% group, and STP on 0.5% level; 1.0% = concentrate provided for S1.0% group, and STP on 1.0% level. Minerals obtained from Zoofós 150 nucleus (Zoomix, Campo Grande, Mato Grosso do Sul, Brazil).

At weaning, the heifers were treated for ecto-parasites (Colosso® pour on, Ourofino, Cravinhos, São Paulo, Brazil) and endo-parasites (Treo® ACE, Zoetis, Campina, São Paulo, Brazil). The females were weaned at 8±0.4 months and 254.0 ± 30.0 kg on average, and went through a 30-day post-weaning pasture period until they reached 9±0.4 months and 259.3 ± 21.6 kg on average. They were then allocated and managed in the proposed stall systems, with free access to feed and water. The quality of the drinking water was previously assessed in terms of its physical and chemical properties (Table 4). Supplements were provided twice a day (morning and afternoon).

Table 4 - Physico-chemical properties of the water used for watering Nelore heifers fed with three different diets (STP, S0.5%, and S1.0%) in feedlots. The S0.5% and S1.0% groups shared the same water fountain (bunk).

ITEM	STP	S0.5%	S1.0%
Turbidity	3	4	4
pH	7.3	7.25	7.25
Conductivity (uS/cm)	476.1	485.7	485.7
N-NH3(mg/mL)	0.099	0.211	0.211
TNVS (mg/L)	493.6	543.59	543.59

Legend: pH = Potential of hydrogen; uS = micro siemens; TNVS = total non-volatile solids; N-NH3 = ammoniacal nitrogen.

All heifers underwent a cyclicity induction protocol (CIP) at the start of the breeding season, 30 days after the treatment start, as well as blood sampling, body parameter measurements, gynecological assessment, and FTAI.

Body weight gain (BW), body condition score (BCS), and subcutaneous croup fat thickness (SCFT)

The animals were weighed during every management on an automatic scale (Tru-Test®) attached to a trunk (Beckhauser®, Maringá, Paraná, Brazil) to obtain the average daily gain (ADG), which was determined by the equation (Final weight - Initial weight/number of experimental days).

The body condition score (BCS) was measured and recorded at each handling by 3 technicians to mitigate the subjectivity of the assessment. The BCS scale adopted was 1 to 5, with 1 being extremely lean and 5 being extremely fat, following Edmonson et al (1989).

To monitor carcass development and SCFT, the animals underwent carcass ultrasound (CSUS), with images collected using an ultrasound (KAIXIN®, Xuzhou Kaixin Electronic Instrument CO., Ltd., Xuzhou, Jiangsu, China) with a 17 cm linear transducer and a frequency of 3.5 MHz with bi-dimensional (B-mode) images. Fat thickness in the croup (EGP8) was measured between the *ileum* and *ischium* bones at the intersection of the *Gluteus medius* and *Biceps femoris muscles*. For better image quality, vegetable oil was used as a conductor. Measurements were taken at the beginning (d0) and end (d94) of the confinement feeding period using the Lince software (USP, SP, Brazil).

Gynecological assessment of follicular dynamics and puberty determination.

The heifers underwent monthly gynecological evaluation for the presence of *corpus luteum* (CL) and dominant follicle (DF) using an ultrasound equipment (SonoScape E1v®, SonoScape, Nanshan District, Shenzhen, Guangdong, China) and a 7.5 MHz rectal linear transducer. The cross-sectional areas of the FD (ATFD) and CL

(ATCL) were measured using the device itself. For analysis purposes, the entire area measured was the area of the FD (AFD) and CL (ATCL). An antral follicle count (FC) was also carried out on both ovaries using a score of classification of 1 to 3: 1 (0-10 follicles), 2 (11-20 follicles), and 3 (more than 21 follicles). The uterus score (US) was defined as: 1 (with tonus), 2 (medium), and 3 (flaccid). Puberty was considered to have occurred when a *corpus luteum* was detected in ultrasound analysis.

Cyclicity induction protocol (CIP) and synchronization of heifers

On the day of the start of the CIP (d-24), a gynecological evaluation was carried out, observing the following parameters: antral follicle count (≥ 3 mm), uterine diameter, largest follicle, and CL.

The cyclicity induction protocol consisted of: d-24 = intramuscular administration of 1mg of progesterone and breaking of the hymen, d-12 = intramuscular administration of 0.5 mg of estradiol cypionate. The FTAI protocol consisted of: d0 = application of a vaginal progesterone (P4) device (Progestar®, Biogenesis Bagó, Campo Largo, Paraná, Brazil) and 1.0 mg of estradiol benzoate (Biostrogen®, Biogenesis Bagó, Campo Largo, Paraná, Brazil); d7 = removal of the P4 implant and parenteral (intramuscular) application 1.0 mg of estradiol cypionate (Croni-cip®, Biogenesis Bagó, Campo Largo, Paraná, Brazil), 300 UI of equine chorionic gonadotrophin (eCG, ecegon®, Biogenesis Bagó, Campo Largo, Paraná, Brazil) and 0.300 mg of prostaglandin 2α (PGF2α, croniben®, Biogenesis Bagó, Campo Largo, Paraná, Brazil); on d9 artificial insemination was carried out reading of heat score score (HTSC), and application of 1.0 mg of GnRH (MaxRelin®, Globalgen vetscience, Jaboticabal, São Paulo, Brazil) in all females.

Service rate, pregnancy diagnosis, and calving rate

The service rate was obtained by the formula: Service rate = number of animals that underwent the FTAI protocol/number of animals per group x 100.

Detection of pregnancy was performed by rectal ultrasound evaluation on days 30, 60, and 90 after AI. Conception rate (CR) was calculated using the formula: CR = number of pregnant heifers/number of inseminated heifers x 100. The pregnancy loss rate (PL) was calculated using the formula: PL = number of open heifers at dg90/number of pregnant heifers at dg30 x 100.

After the last pregnancy detection, the females were removed from the feedlot and underwent a pasture supplementation form with 0.3% of body weight till parturition. The calving rate was obtained by the equation: Calving rate = number of births/CR x 100.

Partial Budget

The treatment cost-benefit ratio (CB) was obtained by the formula: CB = benefit/cost, following Mulualem et al (2021). The costs evaluated included the supplementation (cost of each component of diet*total fed), and the reproductive management protocol (Hormones and semen). The considered benefits were calf production (calf value at weaning) and the current market value of the arroba of non-pregnant females (cull heifers). However, the slaughter weight was considered, the main objective of the treatments was the reproductive variable, and it was not established based on slaughter weight.

The initial values were obtained in Reais (BRL) and then converted to United States dollars (US$) using the conversion value available at the time of the experiment.

Statistical analysis

The completely randomized design (CRD) was used to evaluate performance and reproductive efficiency, considering the animal as the experimental unit. The treatments evaluated were the three levels of supplementation (STP, S0.5, and S1.0 % BW). All analyses were performed on SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4).

One heifer from S1.0% and four from STP did not reach the weight expected to start the breeding season (260 kg) and were removed from the study.

The data were submitted to the univariate procedure to check that it adhered to the normal distribution curve. The data collected was submitted to analysis of variance, breaking down the treatment effect into polynomial regression components with supplementation levels, adopting a 5% probability level. The continuous variables were analyzed for normality (Shapiro–Wilk) and homogeneity of variance (Welch test) before analysis with the MIXED procedure with or without repeated measures over time, and the Satterthwaite approximation was used to determine the denominator degrees of freedom for the treatment effect. The means were obtained by the LSMEANS command, and mean comparisons were performed by the PDIFF option. The categorical variables were analyzed by the GLIMMIX procedure using the binomial option. To evaluate the correlation between the variables, the CORR procedure was used. Further, linear regressions were made between variables, using the PROC logistic for binary parameters and the PROC reg for data with normal distribution, and tested for linear and quadratic effects.

RESULTS

Weight gain and ADG differed among groups ($P<0.05$), with the S1.0% group showing the highest gain and the S0.5% group the lowest. At the beginning of treatment, the STP group had a lower gain than S1.0% and S0.5%, but with the increase of the level of concentrate supplementation, the performance in weight gain also increased. At the final of the treatment phase, and for both variables, the S0.5% group underperformed compared to the others, as shown in Table 5.

Table 5 – Body weight (Kg) and average daily gain (Kg/day). Data as repeated measures over time of Nellore heifers housed in feedlots with three dietary strategies. Body weight data included treatment days 0, 30, 55, 64, and 94. Average daily gain data was divided into 2 periods, day 0 to 54 and day 55 to 94.

Item	Treatments			SEM	*P* – values	
	STP	S0.5%	S1.0%		Treat	Treat × Dia
Body weight (kg)					0.0001	0.0001
d 0 (Treatments start)	266.820	267.111	266.844	2.815		
d 30	255.320[c]	270.951[b]	287.492[a]	2.815		
d 55 (FTAI protocol start)	279.425[b]	275.489[b]	300.847[a]	2.815		
d 64 (AI day)	280.872[b]	274.486[b]	294.891[a]	2.815		
d 94 (Pregnancy check)	306.274[b]	287.323[c]	325.628[a]	3.028		
Average daily gain (kg/d)					<0.01	<0.01
d 0 to 54	0.278[b]	0.201[b]	0.452[a]	0.058	<0.01	
d 55 to 94	0.485[b]	0.365[c]	0.683[a]	0.048	<0.01	

[a-b-c] Within the line differs ($P \leq 0.05$). STP = Stair-step supplementation; S0.5% = Supplementation with 0.5% of body weight; S1.0% = Supplementation with 1.0% of body weight; SEM = Standard error of the mean; FTAI = Fixed-time artificial insemination; AI = Artificial insemination; treat = treatment. D0 values were used as a covariate.

Body weight differed between the groups at the time of FTAI ($p<0.05$), being higher for the S1.0% group than the others, which did not differ from each other. The BCS had the same behavior. Subcutaneous fat thickness at AI was higher for the S0.5% and S1.0% groups compared to the STP (Table 6).

Table 6 - Body maturation parameters (age, weight [Kg], SCFT, and BCS) on artificial insemination day in Nellore heifers supplemented with three different treatments in feedlot.

Item	Treatments			SEM	P-value
	STP	S0.5%	S1.0%		
Age AI (Mo)	12	12	12	0.5	-
Weight AI (Kg)	280.87[b]	274.48[b]	294.89[a]	2.815	<0.01
SCFT AI (mm)	2.53[b]	3.52[a]	3.01[a]	0.870	0.01
BCS AI	2.97[b]	2.95[b]	3.20[a]	0.068	0.02

[a-b-c] Within the line differs, $P \leq 0.05$. STP = Stair-step supplementation; S0.5% = Supplementation with 0.5% of body weight; S1.0% = Supplementation with 1.0% of body weight; SEM = standard error of the mean. SCFT AI = subcutaneous croup fat thickness on the day of artificial insemination; BCS AI = body condition score on the day of AI; AI = Artificial insemination.

The females were randomized for the closest age among the groups possible at the beginning of the study. The onset of puberty rate was not different among treatments before and after the CIP ($p>0.05$). The fertility observed with a single AI did not differ among the treatments, as well as the losses and calving numbers (Table 7).

Table 7 – Puberty onset, artificial insemination fertility, service rate (% of inseminated), pregnancy losses, and calving rate of Nellore heifers supplemented with three different strategies in feedlots.

Item	Treatments			SEM	P-value
	STP	S0.5%	S1.0%		
Puberty before CIP (%)	5.00 (1/20)	0.0 (0/20)	10.0 (2/20)	0.04	0.71
Puberty after CIP (%)	41.17 (7/17)	30.0 (6/20)	36.8 (7/19)	0.18	0.50
Service rate (%)	80.0(16/20)	100 (20/20)	90.0(18/20)	0.10	0.60
Single AI fertility (%)	56.2 (9/16)	40.0 (8/20)	44.4 (8/18)	0.17	0.54
Pregnancy Loss rate (%)	11.11 (1/9)	12.50 (1/8)	14.29 (1/7)	0.15	0.80
Calving rate (%)	88.88 (8/9)	87.5 (7/8)	85.7 (6/7)	0.11	0.86

Legend: STP = Stair-step supplementation; S0.5% = Supplementation with 0.5% of body weight; S1.0% = Supplementation with 1.0% of body weight; SEM = standard error of the mean; Puberty before CIP = the number of heifers that presented CL before the cyclicity induction protocol; Puberty after CIP = the number of heifers that presented CL after the cyclicity induction protocol; Service rate = number of inseminated animal per group; Single AI fertility = pregnancy rate after 1 artificial insemination; calving rate = (amount of birth/number of pregnant heifers); pregnancy losses = (pregnancy loss/pregnancy rate).

The gynecological variables at d0 of the FTAI protocol, being antral follicle count score (AFCS), uterus score (US), dominant follicle presence (DF), and *corpus luteum* presence (CL), did not differ between treatments. The estrus expression verified by HTSC was higher for the STP group ($p<0.05$), as shown in Table 8. In addition, the CL presence showed a positive correlation with the pregnancy rate (Figure 7), while the other variables were not significantly correlated (Table 9).

Table 8 - Gynecological variables at day zero of the fixed-time artificial insemination protocol of Nellore heifers fed in feedlots with three dietary strategies.

Item	Treatments			SEM	P-value
	STP	S0.5%	S1.0%		
AFCS D0 (n°)	1.96	2.07	2.40	0.28	0.29
US D0 (n°)	2.55	2.41	2.11	0.25	0.19
FD D0 (%)	62.5 (10/16)	55.0 (11/20)	57.0 (11/19)	0.30	0.70
CL D0 (% cyclical)	35.0 (6/17)	30.0 (6/20)	31.6 (5/19)	0.15	0.92
HTSC (%)	75.0 (12/16)[a]	50.0 (10/20)[b]	57.8 (11/19)[b]	0.11	0.01

Legend: STP = Stair-step supplementation; S0.5% = Supplementation with 0.5% of body weight; S1.0% = Supplementation with 1.0% of body weight; SEM = standard error of the mean; Antral follicle count score (AFCS); Uterus Score (US); Dominant follicle presence (DF); *Corpus luteum* presence (CL); heat score (HTSC). AFCS was classified as 1 (0-10 follicles), 2 (11-20 follicles), and 3 (More than 21 follicles). The US was 1 (tonus), 2 (medium), 3 (flaccid).

Table 9 - Correlation among reproductive parameters (evaluated by ultrasound equipment) and body maturation parameters in Nellore heifers fed with three different diets in feedlots.

-	P/AI	BCS AI	HTSC	CLD0	PL
P/AI	1	0.294 (0.57)	0.190 (0.37)	**0.680 (0.005)**	0.09 (0.52)
BCS AI		1	0.26 (0.24)	0.17 (0.19)	0.004 (0.97)
HTSC AI			1	0.12 (0.37)	0.14 (0.31)
CL D0				1	0.008 (0.94)
PL					1

Legend: Values within each cell represent the correlation coefficient, and numbers in brackets, the significance level. STP = Stair-step supplementation; S0.5% = Supplementation with 0.5% of body weight; S1.0% = Supplementation with 1.0% of body weight; BCS = Body Condition Score; HTSC = Heat Score; CL D0 = *corpus luteum* presence on day zero of fixed-time artificial insemination protocol; PL = Pregnancy Loss.

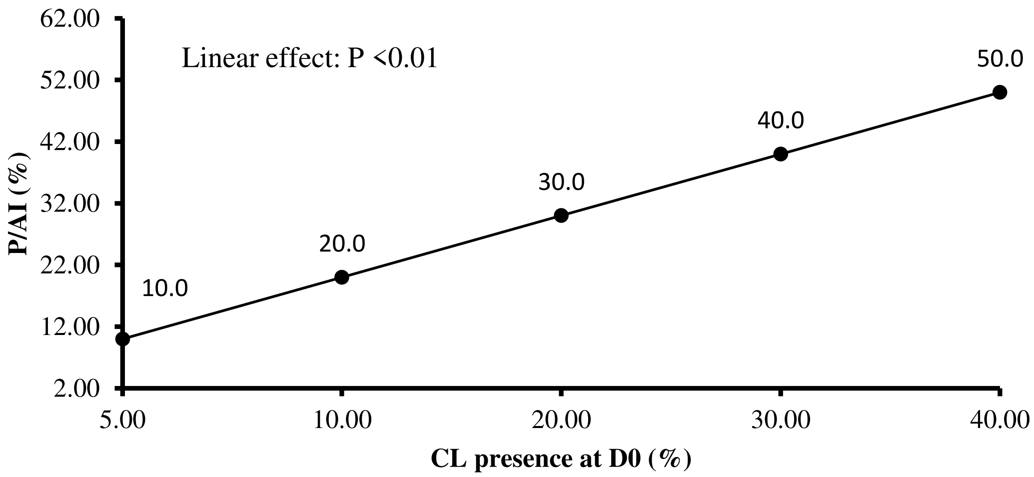

Figure 2 - Effect of corpus luteum (CL) presence at day zero of fixed-time artificial insemination protocol on pregnancy rate of Nellore heifers. The increase in CL number is followed by an increase in pregnancy rate (p<0.05).

Although the costs of supplementation and FTAI protocol were higher for the treatment with a greater energy level (S1.0%) compared to the others (Table 11), the benefit was greater (Table 12), especially due to the higher body weight of the non-pregnant females sold. However, the S0.5% group showed a better benefit per unit of cost (US$ 2.64).

Table 10 – Dietary consumption and costs of three treatments evaluated in Nellore heifers fed in a feedlot system.

Treatment	Diet consumption (kg)	Diet costs (US$)	Costs per Kg (US$/kg)
STP	41,775,630	2,380,511	0.057

S0.5%	43,625,610	2,439,915	0.056
S1.0%	43,144,310	2,696,377	0.062

Legend: Diet consumption = Total consumption (kg) per group; Diet costs = (concentrate costs + forage costs); Costs per Kg = (diet costs/diet consumption); US$ = United States Dollar.

Table 11 – Production costs (Nutritional and reproductive protocol) and benefits (calf production and arroba value of cull heifers) of an intensive system of production with Nellore heifers supplemented in feedlots and submitted to a single fixed-time artificial insemination protocol.

Item	STP	S0.5%	S1.0%
Benefit (US$)	6,700,00	7,271,75	7,539,17
Costs (US$)	2,627,93	2,749,19	2,959,26
Net value (US$)	4,072,07	4,522,56	4,579,92
Ratio (CB)	2.55	2.64	2.55

Legend: Benefit = value generated by calf and arroba price; Costs = value generated by nutrition and reproductive protocol costs; Net value = (benefit – costs); Ratio = (benefit/cost); US$ = United States Dollar.

DISCUSSION

It is important to clarify that most of the scientific data on the effects of higher energy nutritional treatments on fertility and reproductive performance originates from studies of cross-bred cattle (Sales et al. 2015). Aligning with that, according to the NRC (2000), *Bos indicus* animals have maintenance energy requirements that are 10% lower than those required by animals of European breeds influence (*Bos taurus*) and 20% lower than milk production breeds, and that can contribute to achieving early puberty. However, the pasture-based systems of Brazil are not enough to promote early puberty onset, and the adoption of nutritional supplementation is necessary.

The body weight and ADG follow the expected, being higher in groups with greater dietary energy sources (S1.0%, and STP vs. S0.5%). The same was observed for Allen et al (2017) where beef heifers (n = 48; 1/2 Angus × 1/4 Hereford × 1/4 Brahman) had a greater ADG ($P < 0.001$) when fed with high concentrate/high gain (HC/HG) than heifers fed with high forage low gain (HF/LG) diet.

In this study, the STP strategy had a better efficiency of BW gain than the low gain group (S0.5%), but lower than the high gain group (S1.0%). Similar results were observed by Cardoso et al (2014) with a stair-step compensatory gain nutritional regimen when forty crossbred beef heifers (½ Angus, ¼ Hereford, ¼ Brahman) that showed body weight gain lower than the high concentrate group (HC) but higher than the low concentrate group (LC) (LC = 0.55; STP1 0.84 STP2 0.76; HC 0.90 [$p<0.05$]). In the same perspective, Toledo et al (2023) evaluated two different systems of nutrition for early Nellore heifers of approximated 9 mo age (NP1 = ME of 1.4kg/d, and NP2 = 19%

more ME than NP1) considering the difference between the initial average weight and the final average weight, they observed that NP2 animals showed a higher average weight gain than NP1 animals (284.88±20.19 vs 298.19±25.15, respectively).

The number of cyclical heifers before the CIP treatment was low and did not differ among groups (STP = 5.00% [1/20]; S0.5% = 0.00% [0/20]; and S1.0% = 10.0% [2/20]). After the CIP, an increase in the number of puberty heifers was observed (STP = 41.17 [7/17]; S0.5% = 30.0% [6/20]; and S1.0% = 36.8% [7/19]). These results are common for Nellore heifers, which present low cyclicity before induction, under 20.0% (Freitas et al. 2021).

The importance of the CIP protocol has already been shown in the literature. Sá Filho et al. (2009) demonstrated higher cyclicity and higher pregnancy rate (61.9% vs. 40.0%) in heifers submitted to cyclicity induction. The same effect of CIP was observed by Alves et al. 2023b. This occurs because treatment with P4 reduces estrogen receptors in the hypothalamus, followed by a decrease in the negative feedback of estradiol on GnRH release (Day; Anderson, 1998) and efficiently induces puberty in heifers. In addition, estrogen at the end of progesterone treatment can simulate physiological proestrus and increase the results of the cyclicity induction protocol (Sá Filho et al., 2015). These results reinforce that body maturation by nutrition alone is not enough to trigger the functioning of the hypothalamic-pituitary-gonadal axis.

Although Freitas et al. (2021) evaluated body maturation indicators of Nellore heifers observed that the parameter that most contributed to P/AI was subcutaneous fat thickness (SCFT) ($P = 0.003$), with almost twice the number of heifers with SCFT > 2.5 mm pregnant compared with heifers with SCFT ≤ 2.5 mm ($P = 0.003$), our results showed no correlation between the subcutaneous croup fat thickness (SCFT) and pregnancy rate in a single AI. This is probably because all the females had subcutaneous fat greater than 2.5 mm, independent of the treatment strategy.

The BCS, BW, uterus score, score of antral follicle count, and dominant follicle presence did not exert an effect on the pregnancy rate at AI, and also were not different among groups. This suggests that all diets provide the necessary nutrients for reproductive status establishment. As expected, the presence of CL on d0 of the FTAI protocol showed a strong correlation with the pregnancy rate ($p<0.05$), which corroborates other studies showing the importance of the number of pubertal and cyclic females for the pregnancy rate at AI (Allen et al. 2017; Baruselli et al. 2021; Cardoso et al. 2018).

Puberty in heifers was reported to be influenced by age and body weight (Freitas et al. 2021; Allen et al. 2017; Byerley et al. 1987). But, in our study, the average age was the same among treatments, and the BW does not affect the puberty and pregnancy rate, once all females reach the BW of 260.00 Kg-1 recommended to Nellore heifers at the beginning of the breeding season (Nogueira et al. 2023). In this view, all treatment strategies provided the required conditions for heifers to achieve a BW and SCFT enough to stimulate reproductive functions and promote puberty as well as pregnancy. On the other hand, there was no correlation between BCS, BW, and P/AI. The number of animals per group may have caused a dissociation between BCS, BW, and P/AI observed in previous studies (Ayres et al. 2014). In addition, the FTAI protocol may have exerted a stimulus enough to affect cyclicity and pregnancy rate despite these parameters.

The HTSC differed among groups, being greater for de STP than others. However, there was no effect on P/AI. The probable reason for no correlation between HTSC and fertility at AI should be the use of GnRH at the moment of insemination, which may contribute to ovulation in females with lower heat expression (Berean et al. 2023). In addition, the number of heifers may not be enough to show the difference in pregnancy rate that was numerically greater for the STP group.

The fertility rate obtained with a single TAI was close to that obtained for Nogueira et al (2023), who achieved results between 40.0 and 50.0 % in Nellore heifers with an average age of 14 months. However, a final pregnancy rate of over 50.0% is expected. To achieve this index, three or more TAI or bull matching should be performed. There was no difference in calving rate among treatments, and it was close to the results of 80.0 % obtained by Lardner et al. (2020). In this sense, the calving rate is the parameter that most shows the efficiency of the cow-calf system, where a rate over 60.0 % is essential for the profitability of the system.

The benefit observed in the treatment strategies was dependent, especially on the BW at the end of the experiment, as the calving rate was not different among treatments. The costs were higher for the S1.0% group. However, the benefit is also greater for the S1.0%. In this perspective, the S1.0% and STP groups showed a similar cost-benefit ratio (US$ 2.55), while the S0.5% group presented a greater ratio (US$2.64), which indicates a greater production per unit cost. These results confirmed the observed from different authors evaluating body weight gain and the costs of supplementation (Fonseca et al. 2009; Valadares Filho, Paulino 2005), considering that, the costs involved in feeding the animals are important and can account for 70% of 90% of total operating costs, depending on the desired level of production.

Finally, the management in feedlots is a strategy for intensifying the weight gain after weaning, especially with the heifers that are born at the beginning of the calf season in the attempt to ensure a weight at least of 260 kg at the beginning of the breed season and promote a greater pressure of selection for Nellore heifers (Nogueira et al. 2023; Sá Filho et al. 2009).

The results obtained from the treatment strategies demonstrate that it is possible to decrease the puberty age in Nellore heifers to 12 months with nutritional management, matching with the breeding season period in tropical countries (Nepomuceno et al. 2017). This represents a reduction of 6 months compared with the puberty age of 18 months obtained by Ferraz et al. (2018) and 26 months compared with the mean of the breed in Brazil (ABCZ, 2016), improving the rentability and sustainability of the system.

CONCLUSION

We can conclude that the dietary energy treatments evaluated (STP, S0.5%, and S1.0%) did not differ in early puberty onset and reproductive performance in Nellore heifers. However, the strategies with greater energy improve body weight gain. Finally, considering the partial budget, the treatment with the low costs, S0.5%, showed a greater cost-benefit ratio with a great production per cost unit.

ACKNOWLEDGMENTS

We thank Fundect for financial support, CAPES and PIBAP UEMS for scholarships, and São Judas Tadeu Farm for providing the animals and resources.

REFERENCES

Associação Brasileira dos Criadores de Zebuínos. **Sumário de touros.** ABCZ 2016. Disponível em: https://www.abczstat. com.br/comunicacoes/sumario/apresentacao/Sumario-racas-NEL.htm. Acesso em: 02/15/2025.

ALLEN, C. C. et al. Interaction of dietary energy source and body weight gain during the juvenile period on metabolic endocrine status and age at puberty in beef heifers. **Journal of Animal Science**.v. 95, p.2080-2088, 2017. doi:10.2527/jas2016.1002.

ALVES, R. L. O. R. et al. Reproductive outcomes of 11-month-old Nelore (Bos indicus) heifers submitted or not to ovulation induction protocols prior to the timed-AI protocol. **Animal Reproduction**. v.20, n.12 2023b.

AYRES, H. et al. Inferences of body energy reserves on conception rate of suckled Zebu beef cows subjected to timed artificial insemination followed by natural mating. **Theriogenology**, v.82, p.529–536, 2014. https://doi.org/10.1016/j.theriogenology.2014.04.026.

BARUSELLI, P. S. et al. Mitos e realidades sobre a inseminação artificial em tempo fixo (IATF) em bovinos de corte. Myths and realities of timed artificial insemination in beef cattle. **Revista Brasileira de Reprodução Animal**, v.45, n.4, p.625-646, out. /dez. 2021.

BEREAN, D. et al. Economic implications and the impact of gonadotropin-releasing hormone administration at the time of artificial insemination in cows raised in the extensive system in North Romania. **Frontiers in Veterinary Science**, v.10. 2023. Doi: https://doi.org/10.3389/fvets.2023.1167387.

BOLAND, M. P.; LONERGAN, P.; O'CALLAGHAN. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. **Theriogenology**. v. 55, p. 1323-1340, 2001.

BOLFE, E. L. Potential for Agricultural Expansion in Degraded Pasture Lands in Brazil Based on Geospatial Databases. **Land**, v.13, p. 200, 2024. https://doi.org/ 10.3390/land13020200.

BYERLEY, D.J. et al. Pregnancy Rates of beef heifers bred either on puberal or third estrus. **Journal of Animal Science**, v.65, p.645–650, 1987.

BUSKIRK, D. D. et al. Growth, Reproductive Performance, Mammary Development, and Milk Production of Beef Heifers as Influenced by Prepubertal Dietary Energy and Administration of Bovine Somatotropin. **Journal of Animal Science**, v.74, p.2649–2662, 1996.

CARDOSO, R. C. Reciprocal changes in leptin and NPY during nutritional acceleration of puberty in heifers. **Journal of Endocrinology**, n.223, v.3, p.289-98, 2014 doi: 10.1530/JOE-14-0504.

CARDOSO, R. C. et al. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. **Journal of Animal Science**. Published online, 2018.

CARDOSO, R. C. et al. Nutritional control of puberty in the bovine female: prenatal and early postnatal regulation of the neuroendocrine system. **Domestic Animal Endocrinology** xxx (2020) 106434.

CENTEC – Centro de Monitoramento do Tempo e do Clima de Mato Grosso do Sul. **Banco de dados**. https://www.cemtec.ms.gov.br/bancodedados/. Acess:03/02/2025.

DAY, M. L; ANDERSON, L. H. Current concepts on the control of puberty in cattle. **Journal of Animal Science** v.76 (Suppl. 3), p.1–15.1998.

EDMONSON, A. J. et al. A Body Condition Scoring Chart for Holstein Dairy Cows. **Journal of Dairy Science**. v. 72, n. 1, p. 68-78, jan. 1989.

FONSECA, M. A. **Exigências Nutricionais de Vacas e Bezerros Nelore, do nascimento à desmama**. Viçosa, MG: UFV, 2009. 87p. Dissertação (Mestrado em Zootecnia) – Universidade Federal de Viçosa, 2009.

FERRAZ JR, M. V. C. et al. A combination of nutrition and genetics is able to reduce age at puberty in Nellore heifers to below 18 months. **Animal**, v.12, n.3, p. 569–574, 2018.

FREITAS, B. G. et al. Relationship of body maturation with response to estrus synchronization and fixed-time AI in Nellore (Bos indicus) heifers. **Livestock Science**. v. 251, 2021, 104632.

IBGE – Instituto Brasileiro de Geografia e Estatística. **Brasil, cidades e estados**. 2021. Disponível em: https://cidades.ibge.gov.br/brasil/ms/anastacio/panorama. Acesso em: 01 de jul. 2023.

LARDNER, H. et al.Comparison of FTAI and Natural Service Breeding Programs on Beef Cow Reproductive Performance, Program Cost, and Partial Budget Evaluation.Journal of Agricultural Science; Vol. 12, No. 9; 2020.

NATIONAL RESEARCH COUNCIL – NRC. **Nutrient requirements of beef cattle**. update7 ed. National Academic Press. Washington, D.C.:2000. 248 p.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of dairy cattle** 7. ed. National Academic Press. Washington, D.C.: 2001. 381p.

NEPOMUCENO, D. D. et al. Effect of prepartum dam supplementation, creep-feeding, and post-weaning feedlot on age at puberty in Nellore heifers. **Livestock Science** 195, 58–62, 2017.

NOGUEIRA, E. et al**. Protocolo Embrapa +Precoce P14**. EMBRAPA gado de corte. Comunicado técnico, 1ª edição. 2023.

NOYA. et al. Effects of Developmental Programming Caused by Maternal Nutrient Intake on Postnatal Performance of Beef Heifers and Their Calves. **Animals**, v.9, 1072, 2019. doi:10.3390/ani9121072.

SALES, J. N. et al. Effects of a high-energy diet on oocyte quality and in vitro embryo production in Bos indicus and Bos taurus cows. **Journal of Dairy Science**, v.98, p.3086–3099, 2015.

SÁ FILHO, O. G. et al. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows II: Strategies and factors affecting fertility. **Theriogenology**, v.72, p.210-218, 2009.

SÁ FILHO, M. F. et al. Impact of progesterone and estradiol treatment before the onset of the breeding period on reproductive performance of Bos indicus beef heifers. **Animal Reproduction Science**, v.160, p.30-39, 2015.

SÁ FILHO, M. F. et al. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. **Animal Reproduction Science**, v.129, n. 3-4, p.132-139, 2011.

SCHILLO, K. K.; HALL, J. B.; HILEMAN, S.M. Effects of Nutrition and Season on the Onset of Puberty in the Beef Heifer. **Journal of Animal Science**. v. 70, p.3994-4005, 1992.

SOUSA, G.G.T. et al. Características reprodutivas de bovinos da raça Nelore do meio Norte do Brasil. **PUBVET**, v. 6, n. 21, 2012.

SHORT, R. et al. Breeding Heifers at one year of age: biological and economic considerations. **Factors Affecting Calf Crop**. ed. M.J. Fields R.S. Sand. p.55–68.

MULUALEN, T. et al. Examining the Impact of Polyacrylamide and Other Soil Amendments on Soil Fertility and Crop Yield in Contrasting Agroecological Environments. **Journal of Soil Science and Plant Nutrition**, v.21, p.1817–1830, 2021. https://doi.org/10.1007/s42729-021-00482-4

TOLEDO, R. B. et al. Effect of food supplementation on in vitro embryo production and growth performance in prepubertal Nellore heifers. **Animal Biotechnology**, v.34, n.9, p.5087-5096, 2023. DOI: 10.1080/10495398.2023.2279612.

VALADARES FILHO, S. C.; PAULINO, P. V. R.; MAGALHÃES, K. A. **Exigências Nutricionais de Zebuínos e Tabelas de Composição de Alimentos BR CORTE**. 1. ed. – Viçosa: UFV, DZO, 2006. 142p.

SCIENTIFIC PAPER, STUDY 2 (SEMINA).

In vitro embryo production (IVEP) performance of pregnant and nonpregnant Nelore heifers fed with a high-energy diet in a feedlot system.

Authors: Felipe de Oliveira Pedro[1], Taynara dos Santos Santana[1], Wallery Caroliny Costa Da Costa[1], Thais Ferreira Lima[1]; Jéssica Nora Drum[2], Fabiana de Andrade Melo Sterza[1]

[1] Animal Science Pos Graduation Program, Mato Grosso do Sul State University, Aquidauana, Mato Grosso do Sul State, Brazil
[2] South Dakota State University, Brookings, South Dakota State, United States

Summary

This study aimed to investigate the ovum pick-up / in vitro embryo production (OPU/IVEP) performance of pregnant and non-pregnant Nellore heifers fed with a high-energy diet in the feedlot. Fifty (50) heifers with an average age of 12.04 ± 0.42 months and an average weight of (300.80 ± 80.0 kg), were housed in a feedlot system in collective stalls (three pens) with food and water *ad libitum*. All heifers received a common diet with 1.5% of body weight (BW) of concentrate. BRS Capiaçu silage was offered as roughage. The treatment period was sixty (60) days, which starts ten (10) days after a fixed-time artificial insemination (FTAI) protocol, and at the end of this period, the OPU was carried out, divided into 2 groups: pregnant (N=5) and non-pregnant (N=5). The body composition (weight [kg], body condition score [BCS], and subcutaneous croup fat thickness [SCFT]) on OPU day was analyzed comparing the groups. The performance at OPU-IVEP per group was evaluated in the following parameters: Antral follicle count score, cumulus-oocyte complexes (COCs) at OPU, number of cleaved embryos, cleavage rate, and blastocyst yield on day 7. Statistical analyses were conducted using the SAS® statistical package (SAS Inst. Inc., Cary, NC, USA; SAS on demand), considering an alpha of 5% significance. Linear and quadratic regression evaluations were performed to identify the effect of SCFT over the OPU/PIVE. efficiency in both groups. The results showed no difference between pregnant and non-pregnant females regarding OPU-IVEP efficiency. There was also a negative influence of subcutaneous croup fat thickness (SCFT) on the number of COCs/OPU and blastocyst number, despite the group. It can be concluded that the OPU/IVEP performance did not differ between pregnant and non-pregnant Nellore donors in a high-energy diet system, despite the SCFT exerting a negative impact on the oocyte number.

Keywords: biotechnologies; fat; heifers; nutrition, reproduction

INTRODUCTION

Ovum pick-up (OPU) and *in vitro* embryo production (IVEP) management have accelerated the genetic improvement and productivity of cattle. The success of OPU-IVEP depends, especially, on the quantity and quality of oocytes (Tomita et al. 2023).

Among the factors that modulate the oocytes' quantity and quality, the reproductive status of the oocyte donor is of great importance, as is the dietary energy reflected in adiposity.

In this context, some studies have found different results on IVEP efficiency from oocytes derived from donors with or without a corpus luteum (CL), pregnant or non-pregnant. Reis et al. (2006) have observed that cows with CL performed better than cows without CL in terms of the number of oocytes collected from both ovaries, the number of greater Cumulus oocyte complex (COCs), the cleavage rate, and the number of blastocysts on day 7 after *in vitro* fertilization (IVF). Similarly, Pfeifer et al. (2009) found that cows treated with progesterone implants showed a greater number of follicles/oocytes, greater cleavage rate, and blastocyst yield than non-treated cows.

Batista et al. (2016b) have demonstrated that neither exposure to lower levels of luteinizing hormone (LH) nor cycles of progesterone (P4) are limiting to oocyte viability and development to the blastocyst stage.

On the other hand, Machatková et al. (2004) suggested that the best results in all stages of IVEP came from oocytes derived from donors without CL presence. The opposite was observed by Baruselli et al. (2016) when pregnant Holstein heifers had a greater number of embryos produced per OPU and ultimately a greater blastocyst rate when compared to pubertal and prepubertal nonpregnant heifers ($p<0.05$).

Despite the reproductive status of the donor, nutrition can impact fertility in situations of feed restriction or overnutrition. High concentrate diets have been shown as a tool to reduce de first calving age (Gasser et al. 2006a; Cardoso et al. 2014; Cardoso et al. 2018). In this context, several studies have focused on understanding the real impact of dietary energy on reproductive efficiency, especially concerning oocyte quality and embryo production efficiency (Tomita et al. 2023; Morin-doré et al. 2020; Sales et al. 2015). Some studies have demonstrated a relationship between nutrition and reproduction (Garnsworthy et al. 2008; Sales et al., 2011; Thatcher et al., 2011). There is evidence that energy excess reduces the response to superovulation and embryo production, as well as altering the expression of some genes in embryonic development. (Morin-doré et al. 2017). The quality of the oocytes appears to be compromised, but there is no evidence of how this occurs (Morin-doré et al. 2017; Boland et al. 2000).

Therefore, it is necessary to understand the effects of the reproductive status of the donor on oocyte quality and efficiency in embryo production, as well as the effects of adipose tissue, for pregnant or non-pregnant Nellore donors.

The objective of this study was to investigate the efficiency at OPU/IVEP of pregnant and nonpregnant Nellore heifers, as well as the impact of the subcutaneous fat thickness (SCFT) in both groups. We hypothesized that even pregnant and nonpregnant donors can be used in in vitro embryo production without differences in efficiency of this biotechnology, and that high adipose tissue can decrease its efficiency.

MATERIALS AND METHODS

This study followed the principles of animal welfare, and all the procedures carried out were approved by the Ethics Committee for the Use of Animals (CEUA) of Mato Grosso do Sul State University (protocol N°. 039/2022).

Site and study period

The experiment was conducted in Anastácio, Mato Grosso do Sul State, Brazil. The farm is located in the Cerrado biome (IBGE, 2023), and the region's climate is tropical, with a rainy season in the summer (November to April) and a dry season in the winter (May to October). The region's temperature has varied between 14.60 to 41.50 °C, and the accumulated rain precipitation was 345.0 mm between December to February (CENTEC, 2023).

This study started after a 5-month post-weaning period, and a single FTAI protocol in early Nellore heifers was carried out after a cyclicity induction protocol (PIC). The experiment consisted of a 60-day treatment period (December to February) and ovum pick-up at the end (Figure 1). The animals were housed in feedlots in collective stalls.

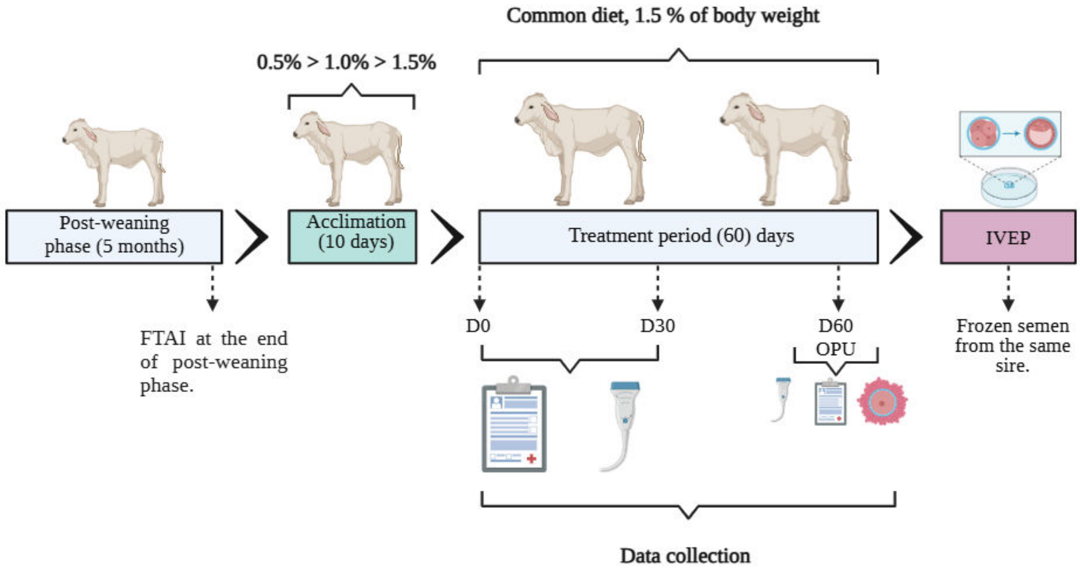

Figure 1 – Experimental design showing the onset of treatment after 5 months of post-weaning. All females received a common diet (1.5% of body weight) that was gradually increased in the acclimation phase, beginning with 0.5%, followed by 1.0% after 5 days, and finishing in 1.5% in 10 days. At the time of OPU, the groups (pregnant and nonpregnant) were divided. Data collection was performed on days 0, 30, and 60 during the treatment period. OPU = Ovum pick-up; IVEP = *in vitro* embryo production.

Animals and treatments

Fifty Nellore, pregnant and non-pregnant heifers, received a common diet with 1.50% of body weight (BW) of concentrate. At Ovum pick-up (OPU), the animals were divided into 2 groups: pregnant (N=5) and non-pregnant (N=5). BRS Capiaçu silage was offered as roughage with the addition of 2.0 kg of sugarcane bagasse per animal. The diets were formulated using RLM software (RLM Pesquisa em Otimização Agropecuária e Ambiental Ltda) following the recommendations of the NRC Tropicalizado ESALQ (NRC, 2001). The feed composition (Table 1), diet chemical composition (Table 2), and Mineral concentration (Table 3) were defined to be the same for all heifers.

Table 1 – Chemical composition (% in dry matter) of feed components (concentrate, sugarcane bagasse, and BRS Capiaçu silage) provided in the diet of pregnant and open Nellore heifers.

DM (%)	Concentrate (1.50)	Sugarcane bagasse	BRS Capiaçu silage
NaCl	1.00	-	-
Cottonseed meal	21.00	-	-
Sorghum (grain)	73.30	-	-
Urea (NNP)	2.00	-	-

Probiotic (Multsacch)	0.20	-	-
Zoofós 150 nucleus (Zoomix)	2.50	-	-
DM	86.10	73.20	27.50
CP	25.70	1.60	5.00
MM	6.70	2.30	6.60
dCP	25.68	1.10	3.23
dFAT	1.80	0.20	0.70
dNFC	54.49	14.20	16.07
dDNF	3.45	42.00	42.87
TDN	87.67	57.75	66.74
Diet (1.50)	37.50	13.50	49.00

Legend: DM = Dry matter; CP = Crude Protein; MM = Mineral Matter; dCP = Digestible Crude Protein; dFAT = Digestible Fat; dNFC = Digestible Non-Fiber Carbohydrates; dNDF= Digestible Neutral Detergent Fiber Carbohydrates; TDN = Total Digestible Nutrients.

Table 2 – Chemical composition (percentage of dry matter) of concentrate provided for pregnant and open Nellore heifers in feedlots, and maintenance reference levels (FONSECA, 2009).

Ingredients (% DM)	Maintenance reference	Concentrate (1.50)
DM	-	39.20
dCP	15.8	8.14
dFAT	1.40	1.70
dNFC	-	18.23
dNDF	41.8	38.63
TDN	66.2	68.82
DE mcal/Kg/DM	1.46	3.40
mE mcal/kg/DM	1.64	2.70
DMI Kg/animal/d	8.1	10.25
mEI Kg/DM/animal/d	11.0	28.59

Legend: DM = Dry matter; CP = Crude Protein; MM = Mineral Matter; dCP = Digestible Crude Protein; dFAT = Digestible Fat; dCNF = Digestible Non-Fiber Carbohydrates; dNDF= Digestible Neutral Detergent Fiber Carbohydrates; TDN = Total Digestible Nutrients; DE = Digestible Energy; ME = Metabolic Energy; DMI = Dry Matter Intake; mEI = Metabolic Energy Intake.

Table 3 -Guarantee level for macro and microminerals in concentrate (1.50%) provided for pregnant and open Nellore heifers in the feedlot system.

	Minerals	1.50
Macro (% DM)		
	Calcium	0.67
	Phosphorus	0.82
	Magnesium	0.20
	Potassium	0.62
	Sodium	0.45
	Chlorine	0.74
	Sulfur	0.25
Micro (ppm/DM)		
	Cobalt	4.51
	Copper	71.99
	Iron	68.22
	Iodine	3.12
	Manganese	56.33
	Selenium	1.25
	Zinc	196.48

Legend: DM = Dry Matter; ppm = parts per million; 1.50% = concentrate fed for all heifers.

At weaning, the heifers were treated for ecto-parasites (Colosso® pour on, ourofino, Cravinhos, São Paulo, Brazil) and endo-parasites (Treo® ACE, Zoetis, Campinas, São Paulo, Brazil). The females were weaned at 8±0.4 months of age on average and went through a post-weaning period (30 days of pasture, and 111 days of feedlot) until they reached 12±0.4 months on average with an average body weight of 300.80±80 kg^{-1}. Ten days after the AI, the females were allocated and managed in the proposed stall systems (three replicates with the same diet). All the stalls had a bunk for supplementation and drinking fountains in adequate quantity and size (1m linear *per* animal) for *ad libitum* feeding.

Age and body weight gain

The animals were weighed at the start (d0) and end (d60) on an automatic scale (Tru-Test®, Datamars, Porto Alegre, Brazil) attached to a trunk (Beckhauser®, Maringá, Paraná, Brazil) to obtain the average daily gain (ADG), which was determined by the equation (Final weight - Initial weight/number of experimental days).

Body condition score

The body condition score (BCS) was measured and recorded at each handling (d0 and d60) by 3 assessors to mitigate the subjectivity of the assessment. The BCS scale adopted was 1 to 5, with 1 being extremely lean and 5 being extremely fat, following Edmonson et al (1989).

Subcutaneous fat thickness

To monitor carcass development, the animals underwent carcass ultrasound (CSUS) on d0 and d60 with images collected using an ultrasound (KAIXIN®, Xuzhou Kaixin Electronic Instrument CO., Ltd., Xuzhou, Jiangsu, China) with a 17 cm linear transducer and a frequency of 3.5 MHz with bi-dimensional (B-mode) images. Fat thickness in the croup (SCFT) was measured between the *ileum* and *ischium* bones at the intersection of the *Gluteus medius* and *Biceps femoris muscles*. For better image quality, vegetable oil was used as a conductor. Measurements were taken at the beginning, middle, and end of the confinement feeding period.

Gynecological evaluation and pregnancy determination.

The heifers underwent, in each management, a gynecological evaluation for the presence of *corpus luteum* (CL) and dominant follicle (DF) using an ultrasound equipment (SonoScape E1v®, SonoScape, Nanshan District, Shenzhen, Guangdong, China) and a 7.5 MHz rectal linear transducer. The cross-sectional areas of the FD (ATFD) and CL (ATCL) were measured using the device itself. For analysis purposes, the entire area measured was the area of the FD (AFD) and CL (ATCL). An antral follicle count (FC) was also carried out on both ovaries using a score of classification of 1 to 3: 1 (0-10 follicles), 2 (11-20 follicles), and 3 (More than 21 follicles).

On the OPU day, the animals underwent pregnancy detection and were divided into pregnant and non-pregnant. The pregnancy age was 80 days, once the females were introduced in the acclimation period, 10 days after FTAI, they underwent 10 days of acclimation and 60 days of treatment.

Ovum pick-up (OPU) and *in vitro* embryo production (IVEP)

At the end of the experiment, 20.0% (10/50) of females underwent OPU according to previously described methods (SILVA et al. 2020). Before the OPU sessions, the population of follicles on the ovarian surface was measured using transrectal ultrasound with the SonoScape E1v device with a linear transducer. The system used for OPU consisted of a digital vacuum pump (WTA®, Cravinhos, São Paulo, Brazil), an ultrasound device (Mindray®, São Paulo, Brazil) connected to an 8 MHz micro convex transducer coupled to a silicone hose with an internal diameter of 2 mm and a length of 80 cm and disposable hypodermic needles (20G (50 mm) × 9 mm; Terumo®, Brazil). The aspiration medium consisted of Dulbecco's phosphate-buffered saline (DPBS) plus 20,000 IU/L of heparin sodium, kept at 30 °C during the aspiration. Before each follicular aspiration, the females received epidural anesthesia (3-5 mL of 2% lidocaine) to reduce peristalsis and the discomfort of manipulation. All follicles measuring 3-8 mm in diameter were aspirated.

The aspirated COCs were classified, and the viable ones were selected for *in vitro* embryo production. The viable COCs rate was obtained as a function of the number of aspirated COCs and the number of COCs that were selected and underwent maturation. *In vitro* fertilization (IVF) was performed using semen from a single bull to minimize the sire effect. All stages of IVEP were carried out following Pereira et al. (2020). The variables analyzed were: oocyte maturation rate, cleavage rate, and blastocyst rate on day 7 (d7).

Statistical analysis

The OPU/IVEP data of one non-pregnant heifer was lost for the accomplishment of IVEP. For statistical analysis, nine animals were considered.

In all evaluations, the animal was considered the experimental unit. The data was submitted to the univariate procedure to check that it adhered to the normal distribution curve. The data collected was submitted to analysis of variance, breaking down the treatment effect into polynomial regression components, adopting a 5% probability level. All results were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4). The proc mixed was used to analyze the parameters with a normal distribution. Further, regression tests were made between variables using the PROC reg procedure of SAS and tested for linear and quadratic effects.

RESULTS

On the OPU day, the BW was not different between the groups (p>0.05). The BCS was greater for the pregnant cows (P=0.008), and a tendency for higher SCFT by the pregnant cows (p=0.07) was observed.

There was no difference for the following variables: number of COCs at OPU, number of maturated COCs, number of cleaved embryos, cleavage rate, and blastocyst yield at day 7 (p>0.05). On the other hand, the AFCS was greater for non-pregnant cows (p=0.006). These effects are shown in Table 4.

Table 4 - Corporal and in vitro embryo production parameters observed on pregnant and non-pregnant Nellore heifers submitted to a high-energy diet in a feedlot system.

Item	treatments		SEM	P-value
	Pregnant	Non-pregnant		
Weight at OPU	395.81	382.74	12.53	0.51
BCS at OPU	4.30a	3.25b	0.28	**0.008**
SCFT at OPU	10.47	7.62	0.94	0.07
AFCS at OPU	1.40b	2.75a	0.26	**0.006**
COCs at OPU	21.40	23.75	5.63	0.76
Cleaved embryos	15.20	17.33	6.81	0.81
Cleavage (%)	66.78	61.55	9.94	0.69
Bla d7 yield (%)	49.65	43.30	11.66	0.68

[a-b] Within the line differs ($P \leq 0.05$). SEM = Standard error of the mean; BCS = Body condition score; OPU = Ovum pick-up; SCFT = subcutaneous croup fat thickness; AFCS = antral follicle count score; COCs = Cumulus oocyte complex; Bla = blastocysts.

From the non-pregnant group, just one heifer presented CL at the OPU moment. Despite the group, a positive correlation was observed between body weight and the number of COCs at OPU (Figure 2). On the other hand, the opposite situation was observed for the SCFT at the OPU vs. COCs number (Figure 3), and SCFT vs. viable COCs rate (Figure 4) (p<0.05). A tendency was observed in a negative correlation between SCFT vs. cleaved embryos (Figure 5) (p=0.07).

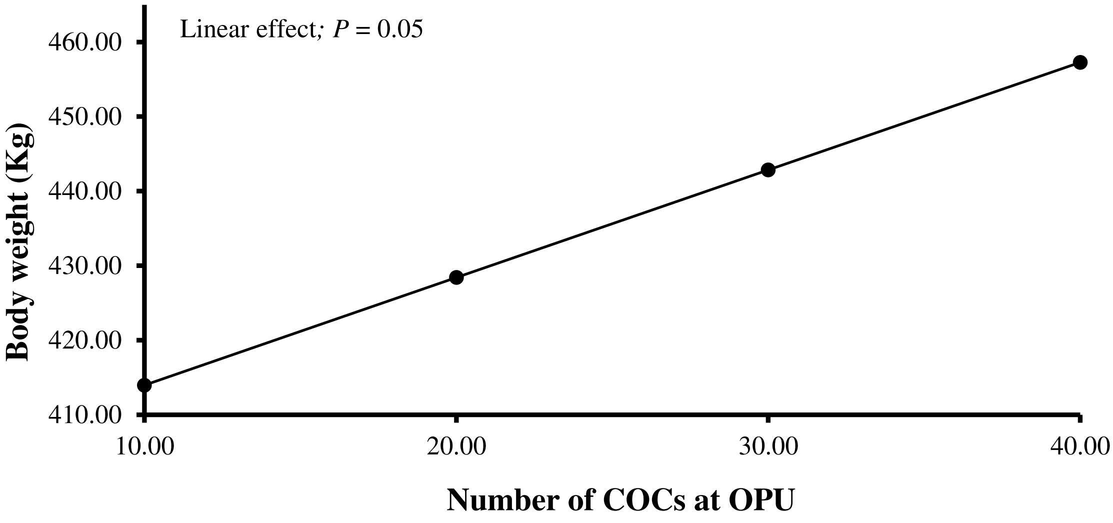

Figure 2 - Effect of body weight (BW) on the number of cumulus-oocyte complexes (COCs) obtained after ovum pick-up in pregnant and nonpregnant Nellore heifers fed with a high-energy diet. BW increase was followed by the number of COCs (p=0.05).

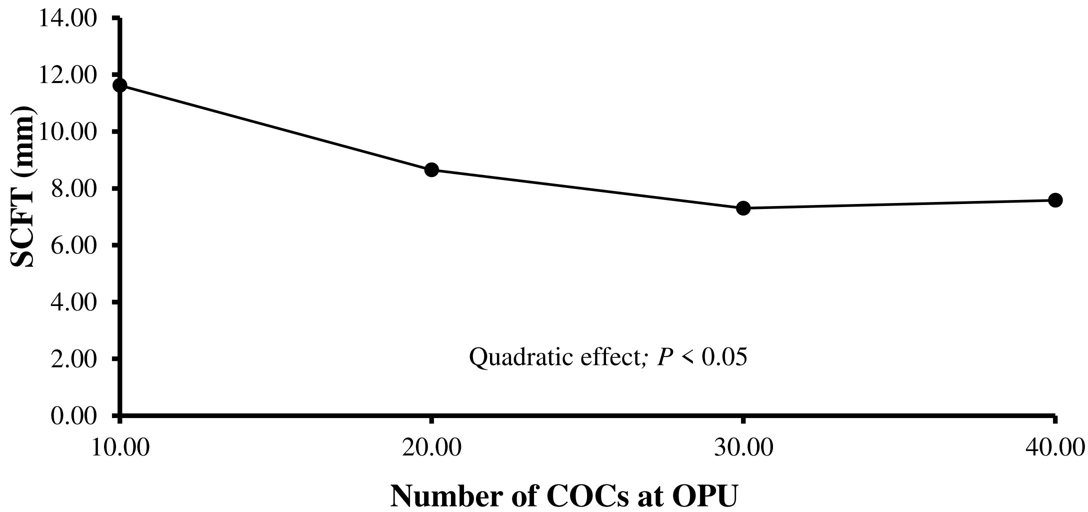

Figure 3 - Effect of subcutaneous croup fat thickness (SCFT) on the number of cumulus-oocyte complexes (COCs) obtained after ovum pick-up in pregnant and nonpregnant Nellore heifers fed with a high-energy diet. SCFT over 7.30 mm reduces the number of COCs recovered at OPU. However, a quadratic effect was observed, and under 7.30 mm, the number of COCs recovered increased ($p<0.05$).

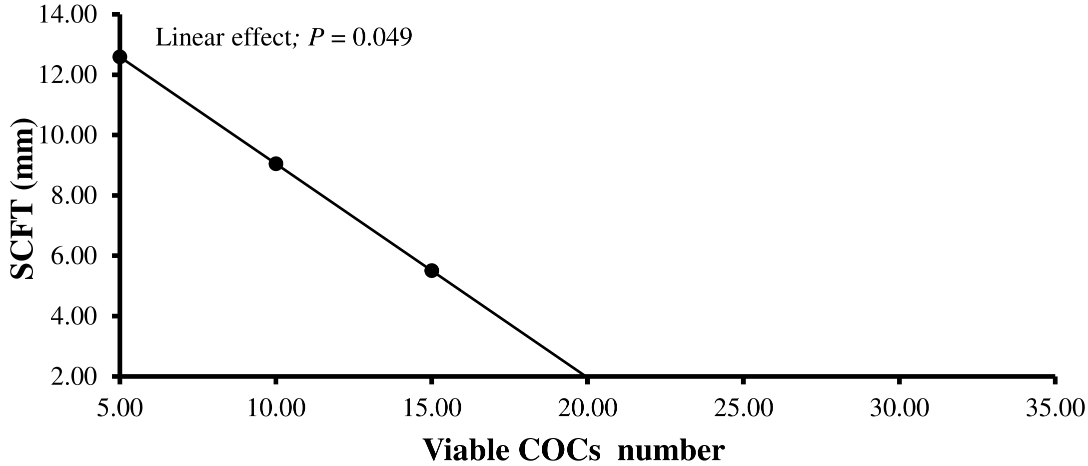

Figure 4 – Effect of subcutaneous croup fat thickness (SCFT) on the number of viable cumulus-oocyte complexes (COCs) derived from pregnant and nonpregnant Nellore donors fed with a high-energy diet. A negative correlation was observed for the linear effect, when the SCFT increased, the viable COCs decreased ($p<0.05$).

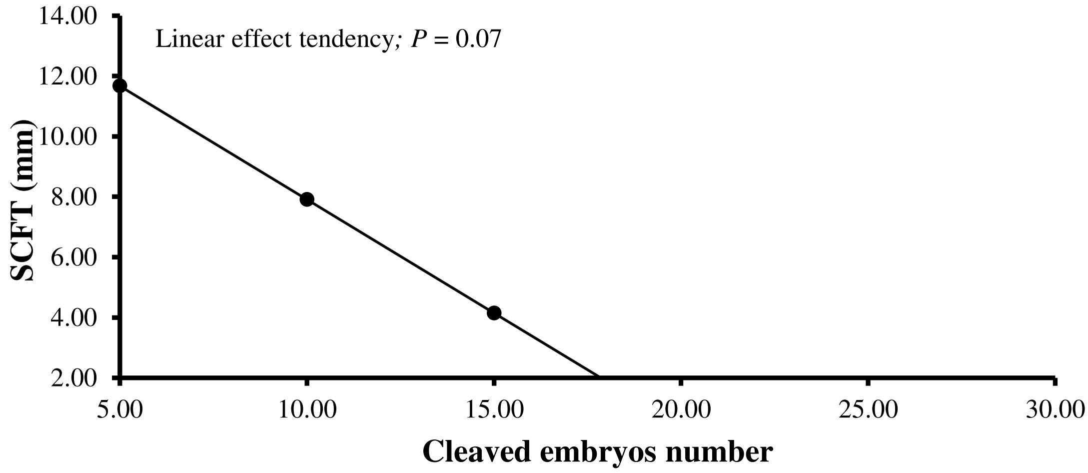

Figure 5 – Effect of subcutaneous croup fat thickness (SCFT) on the number of cleaved embryos at in vitro embryo production with cumulus-oocyte complexes (COCs) derived from pregnant and nonpregnant Nellore donors fed with a high-energy diet. A negative correlation effect tendency ($P = 0.07$) was observed for the linear effect; when the SCFT increased, the number of cleaved embryos tended to decrease.

DISCUSSION

The BW did not differ between 80th-day pregnant and non-pregnant heifers. At first, this is what was expected once they received the same diet. However, pregnant cows usually have a greater BW gain, higher BCS, and adiposity (Rasby et al. 1990). This can be a progesterone effect, supported by our results showing higher BCS ($p>0.05$) and a trend towards higher adiposity (SCFT) in pregnant heifers. In this context, the BW can be influenced by withers high (hWITHERS) and bone tissue (Freitas et al. 2021). This should be the reason for BW did not differ as observed for BCS and SCFT.

There was no difference in IVEP efficiency between pregnant and non-pregnant females, which corroborates Batista et al. (2016b), who have demonstrated that neither exposure to lower levels of LH, nor cycles of P4, are limiting to oocyte viability and development to blastocyst stage. However, there are different considerations regarding oocytes derived from pregnant and non-pregnant donor cows in the literature. Reis et al. (2006) have observed that cows with CL performed better than cows without CL in terms of the number of oocytes collected from both ovaries, the number of greater COCs, the cleavage rate, and the number of blastocysts at IVF on day 7 because of progesterone concentration.

The same was observed by Pfeifer et al. (2009), who found that cows treated with progesterone implants showed a greater number of follicles and oocytes, greater cleavage rate, and blastocyst yield than non-treated cows. Baruselli et al. (2016) observed that pregnant Holstein heifers had a greater number of embryos produced per OPU and ultimately a greater blastocyst rate when compared to pubertal and prepubertal nonpregnant heifers.

On the other hand, Machatková et al. (2004) observed a greater performance ($p<0.05$) for oocytes derived from donors with no CL presence. In addition, it is postulated that the reason why oocytes derived from pregnant donors or non-pregnant donors differed in IVEP efficiency is the concentrations of P4 affecting the oocyte development, by exerting negative feedback to estrogen receptors in the hypothalamus. However, this did not occur in our study. That indicates that the concentrations of P4 in pregnant Nellore heifers don’t exert a negative effect on OPU-IVEP efficiency till the blastocyst stage.

Several studies have found negative effects of high energy intake on oocyte quality (Toledo et al. 2023; Morin-doré et al. 2020; Sales et al. 2015; Armstrong et al. 20001). We could not see any difference in IVEP parameters between pregnant and non-pregnant heifers, however, BCS was greater, and SCFT had a tendency to be greater, and a negative correlation between the SCFT and the number of COCs at OPU, viable COCs, and cleaved embryos was observed. In the same way, Shingu et al. (2002) observed that Holstein heifers showed a decreased IVEP when the BCS increased from 3.5 to 4. It is postulated that the high-energy diets promote a long-term hyperinsulinemic state and downregulation of cellular metabolism genes in the Nelore breed with effects on oocyte quality and embryo development (Tomita et al. 2023; Sartori et al. 2017). Another role of reduction in fertility in cattle overnutrition is increased metabolism of the hormone progesterone. Oocyte donor cows with very low circulating concentrations of progesterone had lower cleavage rates and lower in vitro embryo production than those with higher blood concentrations of progesterone (Pfeifer et al. 2009). From this perspective, a balanced diet is essential to obtain high efficiency in OPU/IVEP systems.

CONCLUSION

In conclusion, the IVEP efficiency was not affected by pregnancy in Nellore heifers, and this can be used to optimize the production system and generate more calves from the same cow. On the other hand, the SCFT has exerted a negative impact on IVEP efficiency, especially on the number of COCs OPU, and the number of cleaved embryos at IVEP for both pregnant and nonpregnant Nellore heifers, illustrating that high dietary energy can reduce the success of this biotechnology.

ACKNOWLEDGMENTS

We thank Fundect for financial support, CAPES and PIBAP UEMS for scholarships, and São Judas Tadeu Farm for providing the animals and resources.

REFERENCES

ARMSTRONG, D.G. et al. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. **Biology of Reproduction**, v.64, p.1624-1632, 2001.

BARUSELLI, P. S. et al. Factors that interfere with oocyte quality for in vitro production of cattle embryos: effects of different developmental & reproductive stages. **Animal Reproduction**, v.13, n.3, p.264-272, Jul./Sept. 2016.

BATISTA, E. O. et al. Ovarian follicular growth suppression by long-term treatment with a GnRH agonist and impact on small follicle number, oocyte yield, and in vitro embryo production in Zebu beef cows. **Theriogenology**, v.85, p.1680-1687, 2016b.

BOLAND, M. P. et al. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. **Theriogenology**. v. 55, p. 1323-1340, 2001.

CARDOSO, R. C. Reciprocal changes in leptin and NPY during nutritional acceleration of puberty in heifers. **Journal of Endocrinology**, n.223, v.3, p.289-98, 2014 doi: 10.1530/JOE-14-0504.

CARDOSO, R. C. et al. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. **Journal of Animal Science**. Published online, 2018.

CENTEC – Centro de Monitoramento do Tempo e do Clima de Mato Grosso do Sul. **Banco de dados**. https://www.cemtec.ms.gov.br/bancodedados/. Acess:03/02/2025.

GARNSWORTHY, P. C., et al. Effect of dietary-induced changes in plasma insulin concentrations during the early postpartum period on pregnancy rate in dairy cows. **Reproduction**, v.137, p.759–768, 2009. doi:10.1530/REP-08-0488

EDMONSON, A. J. et al. A Body Condition Scoring Chart for Holstein Dairy Cows. **Journal of Dairy Science**. v. 72, n. 1, p. 68-78, jan. 1989.

FONSECA, M. A. **Exigências Nutricionais de Vacas e Bezerros Nelore, do nascimento à desmama**. Viçosa, MG: UFV, 2009. 87p. Dissertação (Mestrado em Zootecnia) – Universidade Federal de Viçosa, 2009.

FREITAS, B. G. et al. Relationship of body maturation with response to estrus synchronization and fixed-time AI in Nelore (Bos indicus) heifers. **Livestock Science**. v. 251, 2021, 104632.

GASSER, C. L. et al. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. **Journal of Animal Science**. v. 84, p. 3118-3122, 2006a.

IBGE – Instituto Brasileiro de Geografia e Estatística. **Brasil, cidades e estados**. 2021. Disponível em: https://cidades.ibge.gov.br/brasil/ms/anastacio/panorama. Acesso em: 01 de jul. 2023.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of dairy cattle**.7. ed. National Academic Press. Washington, D.C.: 2001. 381p.

PEREIRA, D. M. et al. Production of in vitro bovine embryos supplemented with L-carnitine in different oxygen tensions and the relation to nitric oxide. **Zygote**, v. 28, n. 5, p.403-408, 2020. doi: 10.1017/S0967199420000258.

PFEIFER, L.F.M. et al. Effect of circulating progesterone on in vitro developmental competence of bovine oocytes. **Animal Reproduction**, v.6, p.473-480, 2009.

MACHATKOVÁ, M. et al. Developmental competence of bovine oocytes: effects of follicle size and the phase of follicular wave on in vitro embryo production. **Theriogenology**, v.61, n.3, p.329-335, 2004.

MORIN-DORÉ, L. et al. Transcriptomic evaluation of bovine blastocysts obtained from peri-pubertal oocyte donors. **Theriogenology**, 2017. doi: 10.1016/j.theriogenology.2017.01.005.

MORIN-DORÉ, L. et al. DNA methylation status of bovine blastocysts obtained from peripubertal oocyte donors. **Molecular Reproduction and Development**. n.1, v.15, 2020.

REIS, A. et al. Efeito da estrutura ovárica e da idade de bovinos da raça Holstein Friesian na quantidade e qualidade de ovócitos e de embriões produzidos in vitro. **Brazilian Journal of Veterinary Research and Animal** Science, v. 43, n. 5, p. 629-636, 2006.

RASBY, R. J. et al. Body condition and reproduction in beef cows: fetal and placental development, and estrogens and progesterone in plasma. **Journal of Animal Science**. v.68, n.12, p.4267-76.

SALES, J. N. S. et al. Effect of injectable copper, selenium, zinc, and manganese on the pregnancy rate of crossbred heifers (Bos indicus×Bos Taurus) synchronized for timed embryo transfer. **Livestock Science**, v.142, p.59–62, 2011.

SALES, J. N. S. et al. Effects of a high-energy diet on oocyte quality and in vitro embryo production in Bos indicus and Bos taurus cows. **Journal of Dairy Science,** v.98, p.3086–3099, 2015.

SARTORI, R.; GUARDIEIRO, M. M. Fatores nutricionais associados à reprodução da fêmea bovina. **Revista Brasileira de Zootecnia**, v.39, p.422-432, 2010 (supl. especial).

SARTORI, R.; SPIES, C.; WILTBANK, M. C. Effects of dry matter and energy intake on quality of oocytes and embryos in ruminants. **Reproduction and Fertility Development**. v.29, v.1, p.58–65.

SILVA, L. G. **Produção in vitro de embriões de novilhas Nelore (Bos indicus) de 12 e 24 meses de idade tratadas ou não com FSH** [dissertation]. São Paulo: Universidade de São Paulo; 2020. http://doi.org/10.11606/D.10.2020.tde-13052020-093248.

SILVA, M. O. et al. Effect of Nelore (Bos indicus) donor age on in-vitro embryo production and pregnancy rate. **Reproduction in Domestic Animals**. v.57, n.9, p.980-8, 2016. http://doi.org/10.1111/rda.14164. PMid:35612981.

SHINGU, H. et al. Breed differences in growth hormone and insulin secretion between lactating Japanese Black cows (beef type) and Holstein cows (dairy type). **Comparative Biochemistry & Physiology (CBP),** v.132, p.493–504, 2002.

THATCHER, W. J. E. et al. Dietary manipulations to improve embryonic survival in cattle. **Theriogenology**, v.76, p.1619–1631, 2011.

TOLEDO, R. B. et al. Effect of food supplementation on in vitro embryo production and growth performance in prepubertal Nelore heifers. **Animal Biotechnology**, v.34, n.9, p.5087-5096, DOI: 10.1080/10495398.2023.2279612.

TOMITA, K. et al. Effects of short-term dietary supplementation on the number of ovarian follicles, quantity and quality of oocytes, and in vitro embryo production in Japanese Black cows. **Journal of Reproduction and Development**, v. 69, n 2, 2023.

CHAPTER 3 – TECHNICAL PAPER (ATP)

– Publicado no VIII Workshop de Pós-graduação em Zootecnia e Ciência Animal do Estado de Mato Grosso do Sul.

Impacto de diferentes estratégias nutricionais sobre o desempenho produtivo e reprodutivo de novilhas precoces confinadas.

Felipe de Oliveira Pedro[1], Andréa Roberto Duarte Lopes Souza [1], Aldair Félix da Silva[2], Thais Ferreira Lima[1], Sílvio Isler[1], Dauydson Gonzalez[3], Claudio Luiz Ferreira Ribeiro[1], Fabiana de Andrade Melo Sterza[1]

[1]Universidade Estadual de Mato Grosso do Sul, Unidade Universitária de Aquidauana, MS, Brasil
[2] Universidade Federal de Mato Grosso do Sul, Unidade Universitária de Chapadão do Sul, MS, Brasil
[3] Universidade Federal de Mato Grosso do Sul, Unidade Universitária de Campo Grande, MS, Brasil

Autor para correspondência*: felipedro2301@gmail.com

A busca por sustentabilidade na produção de carne exige a implementação de estratégias que otimizem a produtividade em menor espaço.

Nesse contexto, destaca-se a busca pelo aumento da eficiência reprodutiva de fêmeas bovinas, índice este que é dependente da interação de fatores genéticos e ambientais. Entre os fatores ambientais a nutrição tem importância de destaque, no entanto, também representa a maior parcela do custo de produção, fato que torna ainda mais importante o estabelecimento de estratégias eficientes, economicamente viáveis, que respeitem o bem estar animal e o meio ambiente.

Uma das principais estratégias para a intensificação da produção consiste na redução da idade à puberdade. Assegurar a precocidade sexual de novilhas Nelore entre 12 e 14 meses, possibilita que o primeiro parto aconteça, aproximadamente, 12 meses antes, ou seja por volta dos dois anos. Esse cenário, ainda que distante da realidade do pantanal Sul-matogrossense, é um vislumbre de uma pecuária mais tecnificada e rentável.

A atividade pecuária no pantanal é caracterizada por uma produção extensiva de raças zebuínas, em especial Nelore, e apresenta índices ainda aquém do desejado, com idade média do primeiro parto aos 40 meses (PORTES et al. 2021). Esse cenário impacta a produção em todo o estado, visto que a cria é a principal atividade desenvolvida no bioma, proporcionando bezerros para recria e engorda nas áreas de planalto e cerrado (NETO et al. 2018).

A fisiologia que regula a puberdade e aptidão à reprodução na fêmea bovina é complexa. Todavia, o mecanismo basal é dependente especialmente da condição corporal, uma vez que em bovinos a mantença é prioridade, ficando então, a produção e a reprodução em segundo plano.

Em conformidade com Nogueira et al. (2023) alguns requisitos necessários para a redução da idade a puberdade são:

- Peso de 260 Kg no início da estação de monta.
- Seleção das fêmeas do início da estação de nascimento.
- Manejo sanitário adequado.
- Manejo alimentar, com forragem de qualidade e suplementação que forneça ganho médio diário (GMD) entre 150 e 700 g.

Nesse cenário, buscando uma melhor compreensão do efeito nutricional sobre parâmetros produtivos e reprodutivos de novilhas super precoces da raça Nelore, 60 novilhas desmamadas com média de 8,05 ± 0,42 meses foram objeto de estudo. Após breve recria (30 dias), as novilhas, com média de 259,3 ± 21,6 Kg foram introduzidas em confinamento. As novilhas foram divididas em 3 grupos de forma randomizada em um delineamento inteiramente casualizado (DIC) considerando o animal como unidade experimental, sendo: S0,5% (N =20), S1,0% (N = 20) e STP (N =20). A suplementação STP (Stair-step) consistiu de aumentos graduais da oferta, sendo: 0,07% do peso vivo (PV), 0,15% do PV, 0,3% do PV, 0,5% do PV, 1,0% do PV e finalizando com 1,5% do PV. A suplementação dos grupos S0,5% e S1,0% consistiu da oferta de 0,5% do PV e 1,0% do PV, respectivamente, de concentrado.

O período experimental foi de 130 dias e, ao final do experimento, todos os grupos receberam uma dieta comum de 1,5% do PV por 60 dias. Todos os grupos tiveram a mesma fonte de volumoso, sendo esta, silagem de BRS Capiaçu. O ganho de peso foi acompanhado a cada 30 dias. Quatro novilhas do grupo STP e uma do grupo S1,0% não atingiram o peso para entrada na estação de monta e foram retiradas do experimento.

Após 55 dias do início do experimento foi realizado o protocolo de indução de ciclicidade (D-24 e D-12), seguido do início do protocolo para IATF (D0). No dia da inseminação artificial (IA) foi aferido o escore de condição corporal (ECC, escala de 1 a 5), bem como, realizada aplicação intramuscular de $1mg^{-1}$ de GnRH em todas as fêmeas. Foi performada apenas uma IA, e 10 dias após as fêmeas foram entouradas. O diagnóstico gestacional foi realizado por ultrassonografia transretal 30 dias após a inseminação, seguida de confirmação com 60 dias. O manejo reprodutivo está evidenciado na figura 1.

Figura 1 – Design do manejo reprodutivo.

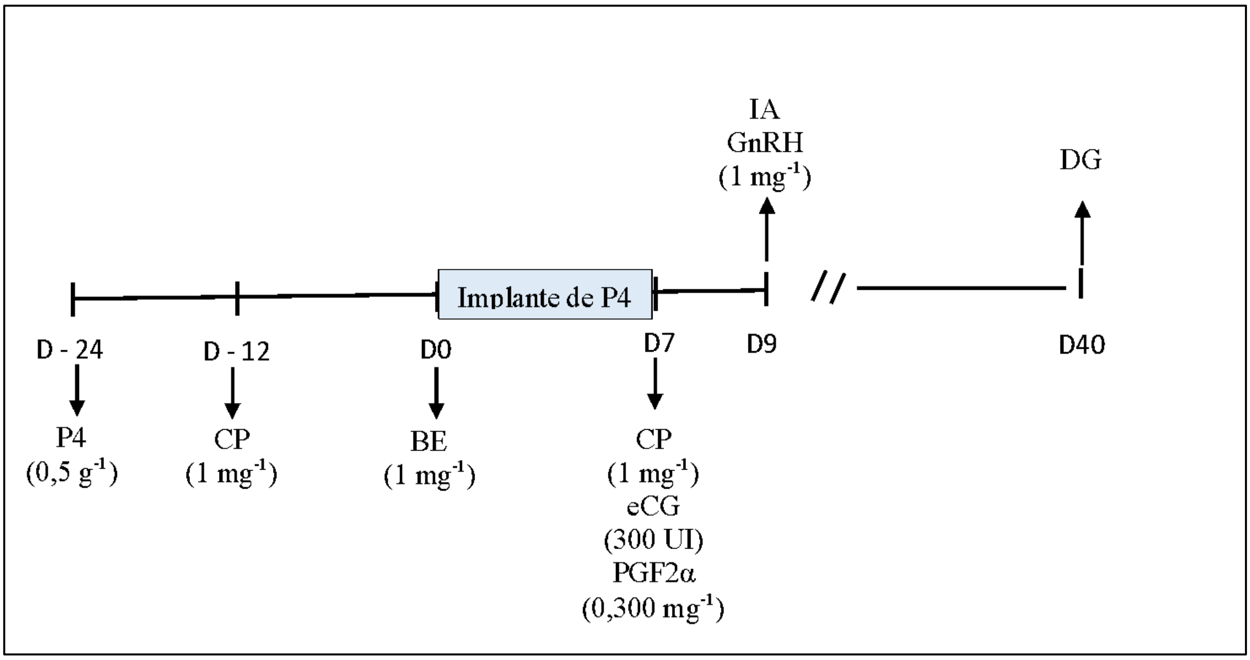

Figure 5- Design do manejo reprodutivo realizado, iniciando com o protocolo de indução de ciclicidade (PIC) 24 dias antes andes do inicio do protocolo de inseminação artificial em tempo fixo (IATF). O protocolo de IATF utilizado foi o de três manejos em nove dias. 30 dias após a inseminação foi realizado o diagnóstico gestacional.

As análises estatísticas foram feitas utilizando os procedimentos proc mix e glimmix do SAS® (SAS Inst. Inc., Cary, NC, USA) considerando o alfa de 5% de significância. O peso médio à indução, peso final e GMD de todo o experimento diferiram entre os grupos ($p<0,05$), (Tabela 1).

.

Table 1 - Peso final e GMD médio por grupo avaliado.

Variável	STP	S0,5%	S1,0%
Peso à indução a ciclicidade (Kg^{-1})	250,5 ± 27,86b	280,2 ± 16,85a	283,0 ± 35,70a

Peso final (Kg^{-1})	366 ± 29,2b	359 ± 28,1b	382 ±40,2a
GMD g^{-1}/d	811b	777b	935a

Letras minúsculas diferentes indicam significância estatística entre as colunas (p<0,05).

O desempenho em ganho de peso é essencial para uma maior deposição de gordura subcutânea. Esta deposição, por sua vez, é indispensável para a puberdade precoce, uma vez que, é a fonte de leptina, hormônio responsável pela ativação da cascata hormonal que culminará na ovulação.

Ademais, o ECC é fator determinante para a obtenção de bons índices por IA sendo preconizado um limite intermediário, por volta de 3 (NOGUEIRA et al. 2023). Nas condições deste experimento, o ECC médio à IA foi superior para o grupo S1,0% (p<0,05) em comparação ao demais, como mostra a tabela 2.

Table 2 - ECC médio por grupo no dia da IA.

Grupos	ECC IA
STP	2,97 ± 0,25b
S0,5%	2,93 ± 0,30b
S1,0%	3,20 ± 0,19a

Letras minúsculas diferentes indicam significância estatística entre as colunas (p<0,05).

Não foram observadas diferenças na taxa de prenhez por IA, na taxa de prenhez final, bem como, nas perdas gestacionais (p>0,05), tabela 3. As taxas de prenhez estiveram dentro do esperado para categoria, que é de ao menos 50 % de prenhez ao final da estação de monta, em conformidade com Nogueira et al. (2023). A variação da taxa de prenhez final pode ser atribuída ao touro utilizado no repasse, que, foi diferente entre os grupos observada a taxa de lotação das baias.

Table 3 - Dados de desempenho reprodutivo.

Variável	STP	S0,5%	S1,0%	Valor de p

Prenhez por IA (%)	56,2	40,0	44,4	p>0,05
Prenhez final (%)	66,7	85,0	89,5	p>0,05
Perdas gestacionais (%)	12,5	10,0	11	p>0,05

É postulado que um dos principais riscos da utilização de protocolos nutricionais de alto aporte energético para precocidade de puberdade consiste na alteração da qualidade embrionária e aumento de perdas gestacionais. Contudo, nas condições desse estudo as perdas foram semelhantes entre os grupos de baixo, médio e alto ganho.

As estratégias nutricionais propostas neste estudo foram eficientes em garantir que mais de 40% das novilhas se tornassem prenhes após uma única inseminação artificial em tempo fixo no início da estação de monta, bem como, uma taxa de prenhez final acima de 50%.

Agradecimentos: À fazenda São Judas Tadeu pela parceria e à Fundect – MS pelo financiamento do projeto.

Referências

PORTES, J. V. et al. Selection indexes for Nelore production system in the Brazilian Pantanal. **Revista Brasileira de Zootecnia**, v. 50. 2021.

NETO, E. S. et al. Reproductive performance of Nelore heifers raised in extensive system undergoing different vaccination protocols in fixed-time artificial insemination (FTAI). **Ciência Rural** v. 49, n. 11. 2019.

NOGUEIRA, E. et al. Protocolo Embrapa +Precoce P14. **EMBRAPA gado de corte.** Comunicado técnico, 1ª edição. 2023.

CHAPTER 4 – FINAL REMARKS

This study contributes to identifying the nutritional level and strategy that best improve productivity, cost-benefit, and reproductive efficiency in Nellore heifers that underwent an FTAI protocol. Promote also an understanding of the effects of a high-energy diet on the ovum pick-up (OPU) and *in vitro* embryo production (IVEP) system results. In this sense, it is hoped that the results and information obtained in this work will be shared with the scientific community and society, focusing on cattle farmers and rural livestock companies.

However, the energy levels studied did not affect reproductive variables associated with early puberty and fixed-time artificial insemination (FTAI) pregnancy, and further studies are needed to identify the level of dietary energy at which these effects become apparent. On the other hand, this study shows that pregnant and open heifers did not differ in the efficiency at OPU/IVEP on a high-energy diet.

Was identified as well, the level of supplementation that provided the best economic return, with a great production per cost unit.

So, this work has the potential to provide the information that livestock demands in Brazil, about the intensification of production systems by the nutritional management applied to reproductive biotechnologies regarding sustainability and profitability.

Finally, this study raises a question of how the subcutaneous fat thickness (SCFT) affects the IVEP efficiency, cause if this occurs due to oocyte quality, accomplishment can also affect de pregnancy establishment in FTAI or natural breeding, increasing the pregnancy losses as well. In this scenario, the level of supplementation and SCFT that promotes these results needs to be clarified, especially in the pasture system, which is the most usual for livestock production in Brazil.