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Progesterone supplementation on Day 7 after TAI improves reproductive performance, productivity, and economic efficiency in beef cow-calf systems

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ABSTRACT - This study evaluated the technical and economic feasibility of long-acting progesterone (P4LA) supplementation in Nellore cows by assessing conception rates, pregnancy loss, cost per pregnancy, and progeny performance. A total of 452 cows (with a body condition score, BCS, ranging from 2.25 to 3.75) underwent a conventional three-handling timed artificial insemination (TAI) protocol. After TAI (D0), cows were allocated to a control group (n = 234; saline solution) or a P4LA group (n = 218; 150 mg, intramuscular, IM, administered on D7). Pregnancy diagnosis was performed by transrectal ultrasonography at 30 (D30) and 60 (D60) days of gestation. Cost per conception was calculated based on expenditures related to hormones, semen, labor, and supplies. P4LA-treated cows had higher conception rates at D30 (58.3% vs. 50.0%; P = 0.04) and D60 (52.7% vs. 42.3%; P = 0.02), with a tendency toward lower pregnancy loss (9.4% vs. 15.4%; P = 0.07). The cost per pregnancy was reduced in supplemented cows (CPC30: US$ 26.94 vs. 29.65; CPC60: US$ 29.65 vs. 35.08). Calves from supplemented cows had greater weaning weight (215.1 vs. 203.5 kg; P = 0.01), resulting in increased productivity per treated cow (95.7 vs. 72.2 kg of weaned calf). In conclusion, P4LA supplementation administered on Day 7 after TAI improved conception rates and was associated with a tendency toward reduced pregnancy loss, resulting in enhanced economic efficiency and productive performance, primarily driven by higher pregnancy rates and increased calf weaning weight in Nellore cows.

Keywords: economic analysis, livestock, profitability, reproduction

1. Introduction

Progesterone (P4) is a cholesterol-derived hormone essential for regulating the uterine environment, supporting conceptus development, and maintaining pregnancy in cattle (Wiltbank et al., 2014;

Couto et al., 2019). Its key functions include follicular modulation, early embryonic support, suppression of estrus and ovulation through negative hypothalamic feedback, and the preparation and maintenance of the endometrium during gestation (Clemente et al., 2009; Lonergan et al., 2016). P4 is synthesized by luteal cells of the corpus luteum and plays a central role regulating ovarian function and establishing pregnancy through its effects on gonadotropin secretion and uterine function (Rigoglio et al., 2013; Wiltbank et al., 2014; Graaff and Grimard, 2018).

In beef cows, although fertilization rates approach 90%, embryonic losses during the first three weeks of gestation may reach 30-40% (Martins et al., 2019a). Exogenous increases in P4 during early diestrus enhance conceptus elongation and interferon-τ production, which are essential for maternal recognition of pregnancy (Wiltbank et al., 2014; Couto et al., 2019; Martins et al., 2019b). Conversely, insufficient P4 during this period compromises embryonic survival and overall reproductive success (Wiltbank et al., 2014). However, it is important to note that most studies demonstrating these effects were conducted in dairy cattle, whose metabolic and endocrine characteristics differ from those of beef cattle.

Several progesterone supplementation strategies have been evaluated, including intravaginal devices (CIDR®), subcutaneous implants (Crestar®), long-acting intramuscular injections (P4LA®), and oral progestins such as melengestrol acetate (MGA®), all of which have shown the potential to improve pregnancy rates and reduce pregnancy losses (Carter et al., 2008; Pugliesi et al., 2016; Yan et al., 2016; Couto et al., 2019, 2022; Martins et al., 2019a). However, data regarding the economic impact of these interventions in Nellore herds remain scarce.

Progesterone regulates endometrial receptivity, conceptus elongation, and early embryo-maternal interactions during the early stages of gestation (Clemente et al., 2009; Wiltbank et al., 2014; Lonergan et al., 2016). There is evidence that elevated progesterone concentrations during early diestrus can improve embryonic development and enhance uterine support (Carter et al., 2008; Martins et al., 2019b). Since early embryonic and placental development is closely linked to subsequent fetal growth, progesterone supplementation after timed artificial insemination (TAI) may not only favor pregnancy establishment but also contribute to improved subsequent calf performance.

Given this gap, we hypothesized that P4LA supplementation in Nellore cows subjected to TAI would improve conception rates, reduce pregnancy losses, and enhance economic outcomes. Therefore, the objectives of this study were to: i) evaluate the effects of P4LA on conception rates and pregnancy losses; ii) assess its impact on the cost per pregnancy; and iii) determine its influence on calf weaning weight.

2. Material and methods

2.1. Animals

All animal procedures were conducted in accordance with the Ethics Committee on Animal Use of the Instituto de Zootecnia, Universidade Federal Rural do Rio de Janeiro (protocol no. 23.083.008085/2018–34).

The experiment was conducted on a commercial beef cattle farm located in São Paulo State, Brazil. A total of 452 multiparous, non-pregnant Nellore cows (*Bos taurus indicus*), between 40 and 60 days postpartum, with body condition scores (BCS) ranging from 2.25 to 3.75 (on a 1–5 scale) (Siddiqui et al., 2002), were enrolled. Prior to the beginning of the experiment, all animals were evaluated for general health status and underwent a gynecological examination. Only healthy cows were included. Cows were maintained on *Brachiaria* spp. pasture with *ad libitum* access to water and mineral supplementation at a stocking rate of 1.25 AU/ha.

2.2. Ovulation synchronization, TAI, and P4LA treatment

On Day −10, an intravaginal device containing 0.5 g of progesterone (Primer®, Tecnopec, Brazil) was inserted, and cows received 2.0 mg of estradiol benzoate (Sincrodiol®, Ouro Fino, Brazil) intramuscularly (IM). The intravaginal device was removed on Day −2, when cows received 500 µg of cloprostenol (Sincrocio®, Ouro Fino, Brazil), 400 IU of equine chorionic gonadotropin (Folligon®, MSD, Brazil), and 1.0 mg of estradiol cypionate (SincroCP®, Ouro Fino, Brazil). TAI was performed 48 h after device removal (Day 0). On Day 7 post-TAI, cows in the P4LA group received 150 mg of long-acting injectable progesterone (Sincrogest Injetável®, Ouro Fino, Brazil), whereas cows in the control group received an equivalent volume of saline solution.

2.3. Experimental design and treatments

Ovarian status was evaluated by transrectal ultrasonography on Day −10 to determine the presence or absence of a *corpus luteum* (CL). Cows were then allocated into two treatment groups using stratified randomization based on ovarian status to ensure a balanced distribution of cows with and without CL between groups (control: 80/234 cows with CL; P4LA: 74/218 cows with CL). A small number of cows were excluded after initial allocation due to operational reasons under commercial farm conditions (e.g., identification inconsistencies, absence during handling, or incomplete records), resulting in minor differences in final group size. These exclusions occurred prior to pregnancy diagnosis and were unrelated to treatment assignment. All remaining cows underwent a single insemination without resynchronization. Following insemination, cows received either saline solution (control) or 150 mg of long-acting injectable progesterone administered intramuscularly on Day 7 post-TAI (P4LA). The overall experimental design and timeline are illustrated in Figure 1.

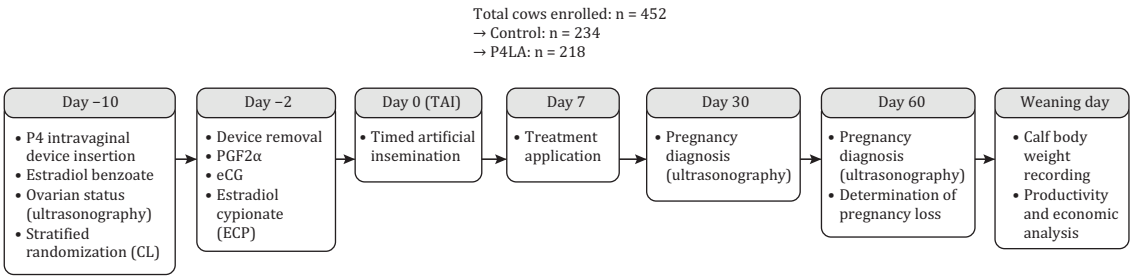

Figure 1 - Experimental design and timeline of the synchronization protocol, treatment allocation, and pregnancy diagnosis in Nellore cows subjected to timed artificial insemination.

2.4. Pregnancy diagnosis and pregnancy loss

Pregnancy diagnosis was performed using transrectal ultrasonography (Mindray D2200 Vet, Shenzhen, China) on Days 30 (D30) and 60 (D60) after TAI, based on the visualization of a viable embryo or fetus with a detectable heartbeat. Pregnancy loss was assessed by repeat transrectal ultrasonography on Day 60 and was defined as the loss of pregnancies diagnosed at D30 and subsequently classified as non-pregnant at D60.

2.5. Economic analysis

The economic analysis considered costs associated with hormones, semen, labor, supplies (disposable gloves, sheaths, and liquid nitrogen), and veterinarian travel. The cost per conception (CPC) was

calculated based on these components. Hormone and supplies were obtained from the records of actual expenditures at the experimental farm where the trial was conducted. Labor costs were estimated according to the official salary standards for farm workers and veterinarians for 2023. Detailed unit costs of hormones, semen, labor, and consumable supplies are presented in Table 1. Economic return was estimated using the average market price of weaned calves (US$ 2.23/kg; CEPEA, 2024). Revenue per treated cow was calculated as the product of the total kilograms of weaned calf produced per cow exposed and the average market price.

Net profitability was calculated on a per-cow basis as:

$$\text{Net profitability (US\$/cow)} = \text{Gross income} - \text{Total reproductive cost.}$$

The percentage difference in net profitability between treatments was calculated as:

$$[(\text{P4LA} - \text{Control}) / \text{Control}] \times 100.$$

A partial budget analysis (PBA) was performed to evaluate the net economic impact of the progesterone supplementation protocol (Rodgers et al., 2012). The analysis considered only the items directly affected by the intervention, grouped into four categories: (i) increased returns, represented by the additional kilograms of weaned calf per treated cow multiplied by the average selling price (US$ 2.23/kg, CEPEA 2024); (ii) decreased costs, which were not applicable in this study since both treatments involved fixed-time AI with similar management structure; (iii) increased costs, corresponding to the additional hormonal treatment (P4LA) and the associated labor and supplies; and (iv) decreased returns, which were not applicable in this context. The net benefit was calculated as:

(Increased returns + Decreased costs) – (Increased costs + Decreased returns), expressed in US$/cow exposed.

Table 1 - Unit costs of hormonal treatments and per-service reproductive expenses included in the economic analysis

Hormonal treatment	Unit cost (US$)	Cost per dose (US$)
Intravaginal progesterone device (P4 device)	22.47	2.25
Estradiol benzoate	3.94	0.08
Prostaglandin F2α (PGF2α)	18.94	0.76
Equine chorionic gonadotropin (eCG)	28.09	2.25
Estradiol cypionate	8.95	0.09
Long-acting progesterone (P4LA)	41.07	0.82
Cost per TAI service (US$)	**Control**	**P4LA**
Hormonal protocol	5.42	6.24
Semen	4.84	4.84
Labor	3.88	3.88
Consumables (needle, syringe, glove, sheath etc.)	0.20	0.20

TAI - timed artificial insemination.
Prices correspond to actual expenses recorded at the experimental farm during the study period (2023). Hormone and consumable supply costs were obtained from farm purchase records. Labor costs were estimated according to official 2023 salary standards for farm workers and veterinarians. All values are expressed in US dollars (US$), converted from Brazilian reais (BRL) at an exchange rate of 5.16 BRL per US$.

2.6. Progeny performance

The calves were weaned at 7 months of age and weighed using an electronic scale. The productivity per treated cow was calculated as the total kilograms of weaned calf produced per treatment group divided by the total number of treated cows in that group.

2.7. Statistical analysis

Conception and pregnancy loss rates (binary outcomes) were analyzed using logistic regression models in SPSS (IBM SPSS Statistics). The initial model included treatment, ovarian status at allocation [presence or absence of a corpus luteum (CL)], body condition score (BCS), and all relevant two-way interactions. Non-significant terms (P>0.20) were sequentially removed based on Wald's test to obtain a parsimonious model. Ovarian status and BCS did not remain in the final model (P>0.20) and were therefore excluded. Because all cows originated from the same farm and all inseminations were performed by a single technician, "farm" and "inseminator" were not included as sources of variation.

Continuous outcomes, including calf weaning weight and kilograms of calf weaned per cow exposed, were analyzed using general linear models (GLM) in SPSS. Initial models contained treatment, ovarian status, BCS, and their two-way interactions. As observed for the binary outcomes, ovarian status and BCS were not significant (P>0.20) and were removed, resulting in final models including only treatment. Residual normality was evaluated using the Shapiro–Wilk test, and homogeneity of variances was assessed with Levene's test. Model assumptions were verified prior to interpretation of the results.

Continuous variables are presented as mean ± standard error (SE), whereas categorical outcomes are expressed as percentages or predicted probabilities. Statistical significance was declared at $P<0.05$, and tendencies were considered for $0.05 \leq P \leq 0.07$.

The model for continuous variables was:

$$y_{ij} = \mu + \alpha_i + e_{ij},$$

In this model, y_{ij} is the continuous response variable (calf weaning weight or kilograms of calf weaned per cow) measured in the j-th cow that received the i-th treatment (i = 1, 2). The term μ represents the overall mean, α_i is the fixed effect of treatment, and e_{ij} is the random error term, assumed to be normally distributed with mean 0 and variance σ^2.

The logistic regression model was:

$$\text{logit}(\pi_{ij}) = \mu + \alpha_i$$

In the logistic model, π_{ij} denotes the probability of success (e.g., conception) for the j-th cow subjected to the i-th treatment (i = 1, 2). The logit link function is defined below. In this model, μ is the general constant and α_i is the fixed effect of treatment.

$$\text{logit}(\pi_{ij}) = \ln\left(\frac{\pi_{ij}}{1 - \pi_{ij}}\right)$$

3. Results

Cows receiving P4LA had higher conception rates than the control group, both at day 30 (58.3% vs. 50.0%; P = 0.04) and day 60 post-TAI (52.7% vs. 42.3%; P = 0.02; Table 2). A tendency toward reduced pregnancy loss was also observed in the supplemented group (9.4% vs. 15.4%; P = 0.07; Table 2).

Table 2 - Conception rates and pregnancy losses in Nellore cows supplemented or not with P4LA, 7 days after timed artificial insemination

Parameter	P4LA	Control	P-value
Conception rate at 30 days	58.3% (127/218)	50.0% (117/234)	0.04
Conception rate at 60 days	52.7% (115/218)	42.3% (99/234)	0.02
Pregnancy loss	9.4% (12/127)	15.4% (18/117)	0.07

P4LA - long-acting injectable progesterone.

The CPC was lower in P4LA-supplemented cows, being 10% lower at 30 days and 18% lower at 60 days than in control cows, indicating that achieving pregnancy was more cost-effective when P4LA was used (Table 3).

Calves from supplemented cows had a higher weaning weight (215 vs. 203 kg; P = 0.01). Consequently, productivity per treated cow was higher in the P4LA group (95.7 vs. 72.2 kg of weaned calf), leading to greater gross income per cow (US$ 213 vs. US$ 160; Table 4). Considering conception costs, the net profitability from calf sales was 46% higher in the P4LA group, demonstrating the economic feasibility of this supplementation strategy.

The partial budget analysis indicated that progesterone supplementation (P4LA) resulted in an additional 23.5 kg of weaned calf per exposed cow, corresponding to an increased return of US$ 52.38/cow (at a mean selling price of US$ 2.23/kg; Table 5).

Table 3 - Cost per conception (CPC) at 30- and 60-days post-timed artificial insemination in Nellore cows supplemented or not with P4LA

Parameter	P4LA	Control
CPC at 30 days (US$)	26.94	29.65
CPC at 60 days (US$)	29.65	35.08

P4LA - long-acting injectable progesterone.

Table 4 - Productivity per cow, and profitability in Nellore cows supplemented or not with P4LA

Parameter	P4LA	Control
Kg of weaned calf per cow	95.7	72.2
Gross income per cow (US$)	213	160
Net profitability (US$)	183.63	125.83
Total net profitability (US$)	40,031.34	29,444.22

P4LA - long-acting injectable progesterone.

Table 5 - Partial budget analysis of progesterone supplementation in timed artificial insemination (US$/cow exposed)

Category	Item	Value (US$/cow)
Increased returns	23.5 kg additional calf × US$ 2.23/kg	+52.38
Decreased costs	–	0
Increased costs	P4LA	–0.88
Decreased returns	–	0
Net benefit		+51.50

P4LA - long-acting injectable progesterone.

4. Discussion

The hypothesis that P4LA supplementation in Nellore cows subjected to TAI would improve conception rates, reduce pregnancy losses, reduce cost per conception, and increase the kilograms of weaned calf produced per treated cow was partially supported by the results, as pregnancy loss showed only a statistical trend. Although the influence of progesterone on reproductive processes in cattle has been extensively investigated, few studies have simultaneously evaluated its biological and economic impacts within an integrated production context. Therefore, our findings contribute new evidence regarding both reproductive efficiency and system-level productivity in *Bos indicus* herds.

The P4LA administration on Day 7 after TAI increased conception rates at both early (D30) and late (D60) pregnancy diagnoses, while pregnancy loss showed only a statistical trend toward reduction. This pattern suggests that the primary effect of P4LA supplementation was associated with improved pregnancy establishment rather than a clearly demonstrated enhancement of subsequent embryonic maintenance. Progesterone plays a central role in promoting uterine receptivity and supporting early embryonic development by modulating endometrial secretory activity and embryo-maternal signaling (Robinson et al., 2006). Therefore, increased luteal support during the early post-insemination period may have optimized the uterine environment during the critical window of maternal recognition of pregnancy. Although the numerical reduction in pregnancy loss is biologically plausible, given that insufficient luteal function has been linked to early embryonic mortality, this effect was not statistically significant in the present study. Similar improvements in pregnancy outcomes following progesterone supplementation have been reported in Bos indicus females receiving embryos (Neto et al., 2024), reinforcing the importance of adequate progesterone exposure during early gestation. However, environmental, nutritional, and cow-related factors may modulate luteal function and embryonic survival, potentially explaining the modest magnitude of the observed effect on pregnancy maintenance.

The economic analysis demonstrated that P4LA supplementation reduced cost per conception at both pregnancy diagnosis time points, reflecting the biological advantage of higher pregnancy rates. This reduction indicates improved reproductive efficiency because total reproductive costs were distributed across a greater number of successful pregnancies. In addition, the partial budget analysis evaluated the incremental economic impact of supplementation, showing that supplemented cows weaned an additional 23.5 kg of calf per exposed cow, corresponding to an increased return of US$ 52.38/cow at prevailing market prices. After accounting for the additional supplementation cost of US$ 0.88/cow, the net incremental benefit reached US$ 51.50 per treated cow. Together, these analyses indicate that P4LA supplementation improved reproductive efficiency while generating a positive economic return.

The improvement in offspring performance further highlights the system-wide productivity gains associated with enhanced reproductive success. Calves from supplemented cows were heavier at weaning, and the total kilograms of weaned calf produced per treated cow were substantially greater in the P4LA group. This response may be associated with enhanced embryonic and placental development, likely due to increased progesterone concentrations during early gestation. Progesterone plays a central role in regulating endometrial function and conceptus elongation during the early luteal phase (Wiltbank et al., 2014; Lonergan et al., 2016) by promoting a uterine environment that supports placental establishment and fetal development. Although placental development and fetal programming were not directly assessed in this study, improved uterine support during this critical period may have contributed to the differences in calf growth. Calves from both treatment groups were born during the same calving season, making it unlikely that seasonal factors influenced the observed differences in weaning weight.

These findings have broader implications for the profitability and sustainability of beef production systems. Improved reproductive efficiency increases calf crop percentage, reduces reproductive waste, and enhances uniformity of weaning groups. Additionally, by increasing the kilograms of weaned calf produced per cow, overall production efficiency is enhanced, potentially improving productivity per unit of output (Baruselli et al., 2025). Thus, P4LA supplementation represents a management strategy capable of simultaneously improving biological performance and overall production efficiency under commercial conditions.

Some limitations should be acknowledged. The study was conducted in a single commercial herd, and progesterone concentrations were not directly measured, which limits the ability to fully characterize the endocrine responses underlying the observed effects. Future studies should validate these findings across different environments, genetic groups, and management systems while incorporating broader economic analyses, including feed, health, and environmental components, to more comprehensively assess long-term system sustainability.

5. Conclusions

In conclusion, supplementation with long-acting progesterone on Day 7 after TAI improved reproductive performance and increased the kilograms of weaned calf produced per cow, resulting in higher economic returns in Nellore beef cows. Although P4LA increased the cost per treated cow, the improvement in pregnancy outcomes reduced the cost per conception and generated a positive net return under the conditions of this study.

Data availability

The entire dataset supporting the results of this study was published in the article itself.

Author contributions

Conceptualization: Couto, S. R. B.; Maysonnave, G. S.; Barbero, R. P. and Mello, M. R. B. **Data curation:** Vieira, T. S.; Couto, S. R. B.; Dias, H. R. S.; Caparelli, N. M. P. M. and Freitas, D. C. R. **Investigation:** Vieira, T. S.; Couto, S. R. B.; Dias, H. R. S.; Caparelli, N. M. P. M. and Freitas, D. C. R. **Resources:** Barbero, R. P. **Supervision***:* Maysonnave, G. S.; Barbero, R. P. and Mello, M. R. B. **Funding acquisition***:* Mello, M. R. B. **Project administration:** Mello, M. R. B. **Visualization***:* Vieira, T. S.; Dias, H. R. S.; Caparelli, N. M. P. M. and Freitas, D. C. R. **Writing – original draft:** Vieira, T. S. and Freitas, D. C. R. **Writing – review & editing:** Maysonnave, G. S. and Mello, M. R. B.

Conflict of interest

The authors declare no conflict of interest.

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Declaration of generative AI in scientific writing

Generative artificial intelligence tools were used solely to enhance the English language quality, improve textual fluency, and support content organization, under strict human supervision. All scientific interpretations, conclusions, and substantive content were entirely conceived, verified, and validated by the authors, who take full responsibility for the final manuscript.

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