Integrative effects of long-acting progesterone and micronutrient-amino acid complex on pregnancy maintenance in Bos indicus females under field conditions

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Abstract: The present study aimed to evaluate the pregnancy rate following fixed-time artificial insemination (FTAI) in Nelore cattle supplemented with either long-acting progesterone (Experiment 1) or an injectable nutritional complex (Experiment 2). In Experiment 1, multiparous cows underwent estrus synchronization and were inseminated on day D10 (D0 = first day of synchronization). On D15, animals were assigned to a control group (n=166), which received an intramuscular injection of 0.5 mL of 0.9% NaCl solution, or to a treated group (n=147), which received 0.5 mL of long-acting progesterone (150 mg). In Experiment 2, heifers were divided into a control group (n=108), which received no treatment, and a treated group (n=99), which received an intramuscular injection of a nutritional supplement on D0 of synchronization. After 7 days of FTAI, all cows and heifers were maintained with bulls for natural mating (20 females per bull) for the following 45 days. Pregnancy diagnosis was performed 70 days after FTAI. The pregnancy rate was higher in the treated groups for both experiments (53.1% vs. 42.8% in Experiment 1, and 53.7% vs. 38.8% in Experiment 2), although differences were not statistically significant (p > 0.05). In animals that did not conceive, ovarian cyclicity was not suppressed, indicating that the use of long-acting progesterone or nutritional supplementation did not interfere with the return to estrus. In conclusion, supplementation with long-acting progesterone or an injectable nutritional complex tended to improve pregnancy rates in Nelore cattle subjected to FTAI, without residual inhibitory effects on subsequent ovarian activity.
Keywords: cow, estrus-synchronization, heifer, FTAI, Nelore.

1. Introduction

Nutritional status is a critical determinant of reproductive efficiency in beef cattle, influencing follicular development, luteal function, oocyte competence, and early embryonic survival. In tropical production systems, cows frequently experience mineral and vitamin deficiencies due to the seasonal variation in forage quality and high metabolic demands during lactation. These nutritional imbalances impair ovarian function and endocrine signaling, leading to suboptimal responses to synchronization and fixed-time artificial insemination (FTAI) protocols (Diskin & Kenny, 2014; Rizzo et al., 2019).

Beyond the correction of macronutrient deficits, targeted supplementation with nutraceuticals—bioactive compounds such as trace minerals, vitamins, and amino acids—has emerged as a promising strategy to enhance reproductive outcomes. Micronutrients including zinc, copper, manganese, and selenium act as essential cofactors for antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, protecting follicular cells from oxidative stress and improving steroidogenic activity (Arechiga et al., 1998; Bian et al., 2007). Vitamins A and E, along with β-carotene, contribute to follicular vascularization, luteal function, and conceptus development through retinoid and antioxidant mechanisms (Volker & Ludwig, 1995; Boxmeer et al., 2009). In mares, targeted nutritional supplementation with these compounds has been associated with improved follicular dynamics and higher embryo recovery rates, suggesting conserved physiological mechanisms across species (Aurich et al., 2018, 2019; Del Prete et al., 2024).

Injectable formulations of minerals and amino acids offer a rapid and bioavailable route to correct subclinical deficiencies, bypassing limitations of oral intake, ruminal degradation, and variable absorption. The metabolic support provided by such supplements may directly influence folliculogenesis and oocyte maturation, as well as modulate uterine receptivity through improved systemic oxidative balance. However, despite extensive studies on dietary supplementation, the reproductive effects of injectable nutraceutical complexes under field FTAI conditions remain poorly characterized.

Furthermore, hormonal modulation through exogenous progesterone administration represents another approach to optimize the endocrine environment during the peri-ovulatory period. Long-acting progesterone formulations may enhance luteal support and stabilize cyclicity in animals failing to conceive after FTAI, potentially sustaining ovarian activity and minimizing the anestrus period (Torres et al., 2021; Bó et al., 2023). When combined with targeted nutraceutical interventions, such strategies may synergistically improve follicular quality and pregnancy outcomes.

Therefore, this study was designed to test the hypothesis that injectable nutraceutical supplementation (trace minerals and amino acids) and long-acting progesterone administration can enhance reproductive performance in beef cows submitted to FTAI by improving ovarian function, luteal activity, and systemic metabolic balance.

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The specific objectives of this study were: (1) to evaluate whether the administration of long-acting progesterone five days after FTAI influences cyclicity and pregnancy rate in multiparous cows, and (2) to determine the effect of injectable nutraceutical supplementation initiated at the onset of synchronization on follicular dynamics and conception rate in heifers.

2. Material and methods

2.1. Animals and management

Experiment 1 was carried out in the municipality of Porto Acre, Brazil (latitude −9.588° S, longitude −67.448° W), located in an equatorial climate with abundant rainfall. A total of 313 multiparous Nelore cows (Bos indicus) were selected, with a mean body weight of 430.0 ± 12.9 kg and an average age of 6.0 ± 0.6 years. Body condition score (BCS) averaged 3.0 ± 0.2 on a 1-to-5 scale (1 = emaciated; 5 = obese) according to Moraes et al. (2013). Animals grazed on Brachiaria brizantha mixed with MG-5 Vitória pasture and had ad libitum access to water and a commercial mineral supplement (PhósPrime®, Arantes Nutrição Animal, Ji-Paraná, Rondônia, Brazil) throughout the experimental period.

2.2. Estrus synchronization and progesterone treatment

Cows were enrolled on a random day of the estrous cycle, approximately 90 days postpartum, and synchronized according to the protocol illustrated in Figure 1.

On Day 0 (D0), each cow received an intravaginal progesterone device (1 g progesterone; Sincrogest®, Ouro Fino, Cravinhos, São Paulo, Brazil) and an intramuscular (IM) injection of 2 mL estradiol benzoate (2 mg; Sincrodiol®, Ouro Fino). On D8, the device was removed and the cows were treated IM with 1 mL estradiol cypionate (1 mg; SincroCP®, Ouro Fino), 1.5 mL eCG (300 IU; Sincro eCG®, Ouro Fino), and 2 mL sodium cloprostenol (0.530 mg; Sincrocio®, Ouro Fino). Fixed-time artificial insemination (FTAI) was performed on D10.

Five days later (D15), cows were randomly allocated into two groups:
- Control group (n = 166): received 0.5 mL of 0.9% NaCl IM.
- Treated group (n = 147): received 0.5 mL of long-acting progesterone (300 mg/mL; P4-300®, Botupharma, São Paulo, Brazil) IM.

To evaluate potential long-term effects of exogenous progesterone, all cows were maintained with fertile Nelore bulls (ratio = 1 bull per 20 cows) for 45 days following FTAI. Bulls (~5 years old) met breeding-soundness standards recommended by the Brazilian College of Animal Reproduction (CBRA, 2013): subjective motility ≥ 60%, minor defects ≤ 20%, major defects ≤ 10%, and ≥ 70% morphologically normal spermatozoa.

2.3. Experiment 2 – Effect of injectable nutraceutical supplementation on FTAI pregnancy rate

2.3.1. Animals and management

Experiment 2 was conducted in a herd located at latitude 11° 46′ 10″ S and longitude 61° 42′ 77″ W, also under an equatorial climate with abundant rainfall. A total of 207 Nelore heifers (Bos indicus) were enrolled, with an average body weight of 311.0 ± 48.1 kg and a mean BCS of 3.0 ± 0.5 (1–5 scale; Moraes et al., 2013). Heifers grazed on Brachiaria brizantha pastures and had unrestricted access to water and a mineral supplement (PhósPrime®, Arantes Nutrição Animal, Ji-Paraná, Brazil). Animals were randomly assigned to two groups:
- Control group (n = 108): received 5 mL of 0.9% NaCl IM.
- Treated group (n = 99): received 5 mL IM of a nutraceutical supplement containing amino acids, vitamins, and minerals. The supplement provides vitamins A, D3, E, B1, B5, B6, nicotinic and pantothenic acids, and multiple trace elements (Zn, Mn, Cu, Se, I, Fe, Co, Mg, Ca, P) together with amino acids (methionine, arginine, lysine, glycine, tryptophan) and inositol, with a recommended re-application interval of 3–4 months (Figure 1).
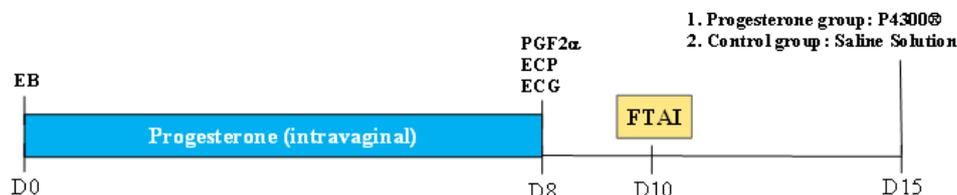

Figure 1 – Figure 1. Experimental design of Experiment 1 evaluating the effect of long-acting progesterone administration after fixed-time artificial insemination (FTAI) in Bos indicus cows. Legend: On Day 0 (D0), cows received estradiol benzoate (EB, 2 mg, IM) and an intravaginal progesterone device (1 g P_4), maintained until Day 8 (D8). On D8, the devices were removed and animals were treated intramuscularly with $PGF_2\alpha$ (0.530 mg), estradiol cypionate (1 mg), and equine chorionic gonadotrophin (300 IU). Fixed-time artificial insemination was performed on Day 10 (D10). On Day 15 (D15), cows were allocated into two groups: (1) Progesterone-treated group, receiving long-acting natural progesterone (300 mg; P4-300®); or (2) Control group, receiving physiological saline solution (0.9% NaCl).

2.3.2. Estrus synchronization and nutraceutical administration

The synchronization protocol began on a random day of the estrous cycle (Figure 2). On D0, all heifers received an intravaginal progesterone device (1 g; Sincrogest®, Ouro Fino) and 2 mL estradiol benzoate IM (2 mg; Sincrodiol®, Ouro Fino). At the same time, treated heifers received 10 mL IM of the nutraceutical supplement.

On D7, all heifers received 25 mg PGF$_2\alpha$ (dinoprost tromethamine; Lutalyse®, Zoetis, Guarulhos, Brazil). On D9, the devices were removed and animals were administered 0.3 mL estradiol cypionate (ECP®, Zoetis) and 300 IU eCG (Novormon®, Zoetis). FTAI was performed on D11. Seven days after FTAI, all heifers were exposed to fertile Nelore bulls (1 bull per 20 heifers) for 45 days to allow natural breeding. Bulls were ~5 years old and had been evaluated for breeding soundness according to CBRA (2013) guidelines.

2.4. Pregnancy diagnosis and evaluation of cyclicity

In both experiments, pregnancy diagnosis was performed 70 days after FTAI (D80) using transrectal ultrasonography with a 5 MHz linear probe (SD-20, Mindray do Brasil, São Paulo, Brazil; Figure 2).
Gestational age was used to differentiate pregnancies resulting from FTAI versus natural mating.

Additionally, the reproductive status of non-pregnant females was evaluated indirectly during the 45-day natural mating period following FTAI. The re-establishment of normal estrous cyclicity was inferred from comparable post-mating pregnancy rates between treated and control groups, indicating that neither long-acting progesterone nor injectable nutraceutical supplementation impaired ovarian function or estrous resumption.
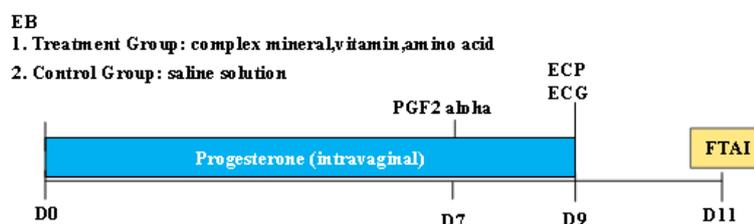

Figure 2 – Figure 2. Experimental design of Experiment 2 evaluating the effect of injectable mineral–vitamin–amino acid supplementation on the pregnancy rate after fixed-time artificial insemination (FTAI) in Bos indicus heifers. Legend: On Day 0 (D0), heifers received estradiol benzoate (2 mg, IM) and an intravaginal progesterone device (1 g P$_4$), maintained until Day 9 (D9). At the same time, animals in the treated group received an injectable mineral–vitamin–amino acid complex containing vitamins A (1.176 g), D$_3$ (0.025 g), E (0.5 g), B$_1$ (0.09 g), B$_5$ (1.0 g), and B$_6$ (0.09 g); nicotinic acid (0.65 g); pantothenic acid (1.0 g); trace minerals—selenium (0.06 g), zinc (0.15 g), manganese (0.1 g), copper (0.1 g), cobalt (0.1 g), iodine (0.2 g), magnesium (0.5 g), calcium (0.8 g), phosphorus (0.2 g), and iron (0.35 g); and amino acids—methionine (0.25 g), arginine (0.03 g), lysine (0.03 g), glycine (0.2 g), and tryptophan (0.06 g). On Day 7 (D7), all heifers were treated with PGF$_2\alpha$ (25 mg). On Day 9 (D9), after device removal, animals received estradiol cypionate (0.3 mg) and equine chorionic gonadotrophin (300 IU). Fixed-time artificial insemination was performed on Day 11 (D11).

2.5. Pregnancy diagnosis

In both Experiments, the pregnancy diagnosis was performed 70 days after the FTAI (D80) by ultrasonography, using a 5 MHz probe (SD.20, Mindray do Brasil, São Paulo, Brazil). The gestational age of each pregnancy was used to determine the pregnancy rate from FTAI and the natural service. In addition to pregnancy diagnosis, the reproductive status of non-pregnant females was indirectly assessed through their fertility response during the 45-day natural service period following FTAI. The re-establishment of normal estrous cyclicity was inferred from the comparable pregnancy rates between treated and control groups after natural mating, indicating that neither long-acting progesterone nor the micronutrient-amino acid complex interfered with ovarian function or estrous resumption.

2.6. Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS®, version 9.4; SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used to summarize animal characteristics (mean ± standard deviation for continuous variables and percentages for categorical variables). Pregnancy rate after FTAI and cumulative pregnancy rate after natural mating were analyzed as binary outcomes (pregnant = 1; non-pregnant = 0) using logistic regression models (PROC LOGISTIC). The models included treatment group (control or treated) as a fixed effect, while body condition score (BCS) and days postpartum at the start of synchronization were included as covariates to account for individual variability. The odds ratio (OR) and 95% confidence interval (CI) were estimated to quantify the strength of association between treatments and pregnancy outcomes.

Continuous variables such as body weight and BCS were compared between groups using Student's t-test, after confirming data normality with the Shapiro–Wilk test. When assumptions of normality were not met, the Wilcoxon rank-sum test was applied. Statistical significance was declared at P < 0.05, and tendencies were discussed when 0.05 ≤ P ≤ 0.10. Results are presented as least squares means ± standard error of the mean (SEM) unless otherwise specified. To assess the potential influence of long-acting progesterone and nutraceutical supplementation on cyclicity resumption, a comparative analysis of post-FTAI natural-service pregnancy rates was performed using Pearson's chi-square test, evaluating whether treatment influenced the re-establishment of normal estrous activity among non-pregnant females.

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3. Results

In Experiment 1, treatment with long-acting progesterone five days after FTAI did not affect pregnancy rate (P > 0.05). The proportion of pregnant cows was 53.1% (78/147) in the treated group and 42.8% (71/166) in the control group (Figure 3A). Similarly, in Experiment 2, no significant difference was observed between heifers supplemented with the injectable mineral–vitamin–amino acid complex (53.7%; 36/67) and control heifers (38.8%; 26/67) (P > 0.05; Figure 3B).

Bootstrap violin plots were used to illustrate the uncertainty distribution of pregnancy rates across treatment groups. Dots represent the observed pregnancy proportions, vertical bars indicate 95% Wilson confidence intervals, and violin shapes display the bootstrap density (20,000 resamples) for visual comparison between treated and control animals.
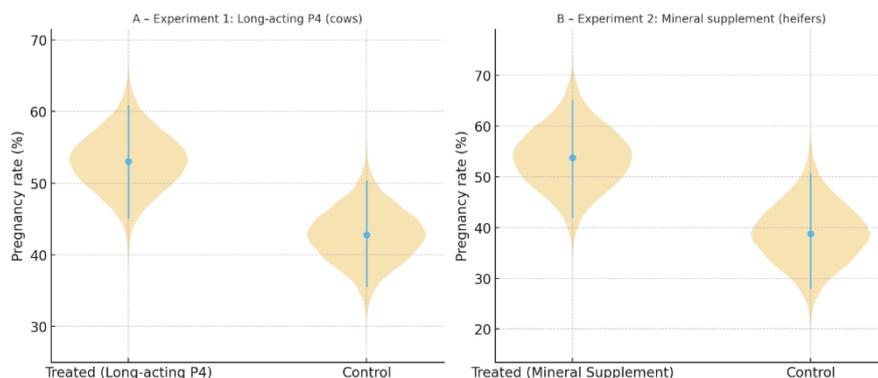

Figure 3 – Figure 3A. Bootstrap violin plots of pregnancy rates (FTAI) in Bos indicus cows and heifers. Panel A shows Bos indicus cows treated with long-acting injectable progesterone (P4) and controls (Experiment 1). Panel B shows Bos indicus heifers supplemented with a micronutrient and amino acid complex and controls (Experiment 2). Dots indicate observed pregnancy rates (%), and vertical bars represent 95% Wilson confidence intervals. Violins display the bootstrap distribution (20,000 resamples) of pregnancy outcomes, illustrating uncertainty around group proportions.

After the natural mating period, neither treatment significantly influenced the subsequent pregnancy rate among females that had failed to conceive after FTAI (P > 0.05; Table 1). In Experiment 1, pregnancy rates after natural service were 58.9% (56/95) for treated cows and 49.3% (34/69) for controls (Absolute Risk Difference = +9.7 pp; 95% CI: −5.7 to +25.1; OR = 1.48; one-sided Fisher's P = 0.142).

In Experiment 2, the corresponding rates were 75.6% (31/41) in supplemented heifers and 58.0% (18/31) in controls (Absolute Risk Difference = +17.6 pp; 95% CI: −4.2 to +39.3; OR = 2.24; one-sided Fisher's P = 0.093).

Supplementation type	Pregnancy rate Treated N (%)	Pregnancy rate Control N (%)	Absolute Risk Difference (95% CI)	Odds Ratio (95% CI)	Fisher P
Long-acting progesterone (P4-300)	56/95 (58.9)	34/69 (49.3)	+9.7 (−5.7 to +25.1)	1.48 (0.79–2.76)	0.142
Injectable mineral–vitamin complex	31/41 (75.6)	18/31 (58.0)	+17.6 (−4.2 to +39.3)	2.24 (0.82–6.14)	0.093

Table 1 – Pregnancy rate (%) at natural service of non-pregnant females (Bos indicus) after fixed time artificial insemination according to supplementation type.

4. Discussion

The violin plots presented in Figure 3 (A–B) illustrate the distribution and variability of pregnancy rates following fixed-time artificial insemination (FTAI) in Bos indicus females subjected to hormonal or nutraceutical interventions. The visual symmetry and substantial overlap between the violin densities of treated and control groups clearly indicate that neither long-acting progesterone administration nor injectable micronutrient supplementation exerted a significant influence on conception outcomes. The bootstrap distributions, reinforced by the 95% Wilson confidence intervals, reveal that the observed differences fall within the range of random variation, supporting the absence of treatment effects detected in the statistical models.

The use of long-acting progesterone five days post-FTAI did not translate into improved pregnancy rates (Figure 3A). This finding suggests that the exogenous hormone, while capable of transiently elevating circulating progesterone, did not substantially modify luteal function or uterine receptivity during the early luteal phase. Similar results were reported by Torres et al. (2021) and Bó et al. (2023), who observed that post-insemination progesterone supplementation failed to increase fertility in Bos indicus cows under adequate luteal function. Physiologically, the lack of benefit may be explained by the high endogenous luteal progesterone concentrations typically achieved after synchronized ovulations in cyclic females. Once a functional corpus luteum is established,

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additional exogenous progesterone is unlikely to enhance embryo survival or endometrial receptivity beyond the physiological threshold required for maternal recognition of pregnancy (Diskin & Kenny, 2014; Mann & Lamming, 2001).

Conversely, Figure 3B depicts the effect of the injectable mineral–vitamin–amino acid complex administered at the onset of synchronization in heifers. Although the violin shape of the treated group shows a slightly higher central tendency and broader distribution—suggesting greater biological variability—the overlap between groups confirms that the supplementation did not yield a statistically meaningful advantage in pregnancy rate. These results align with prior field studies indicating that short-term parenteral supplementation may not immediately translate into measurable reproductive gains (Rizzo et al., 2019; Arechiga et al., 1998). The reproductive effects of micronutrients are often cumulative and depend on the correction of subclinical deficiencies over time rather than on acute supplementation during a single estrous cycle (Boxmeer et al., 2009; Del Prete et al., 2024).

Nevertheless, the numerical trend toward higher conception rates among supplemented heifers, visible in the violin median shift, may reflect subtle improvements in oxidative balance and follicular competence induced by the nutraceutical formulation. Trace minerals such as zinc, copper, manganese, and selenium act as cofactors for antioxidant enzymes—superoxide dismutase and glutathione peroxidase—that protect granulosa and luteal cells against oxidative stress (Bian et al., 2007; Aurich et al., 2018). Likewise, vitamins A and E, along with β-carotene, enhance follicular vascularization and luteal steroidogenesis, supporting oocyte maturation and embryo viability (Volker & Ludwig, 1995; Boxmeer et al., 2009). Although such metabolic adjustments may require repeated dosing to manifest in reproductive performance, the observed positive tendency corroborates the concept that nutraceutical support contributes to the physiological optimization of the ovarian microenvironment.

Collectively, the visual interpretation of Figure 3 highlights the biological stability of reproductive outcomes across treatments. The absence of statistical significance, combined with overlapping violin densities, underscores that both interventions were physiologically safe—neither disrupting cyclicity nor compromising fertility. From a methodological standpoint, the violin plot approach provides a more nuanced visualization than bar charts or box plots, revealing not only central tendencies but also the shape of the probability distribution and the degree of uncertainty inherent to reproductive data collected under field conditions.

The data summarized in Table 1 complement the violin plots by illustrating the outcomes of the natural mating period that followed FTAI. Among non-pregnant females, neither long-acting progesterone administration nor injectable nutraceutical supplementation produced statistically significant differences in subsequent conception rates ($P > 0.05$). However, the numerical tendency toward higher pregnancy proportions in both treated groups suggest a potential carry-over effect on ovarian function and fertility recovery.

In Experiment 1, cows receiving long-acting progesterone displayed a pregnancy rate of 58.9%, compared with 49.3% in controls, corresponding to an absolute risk difference of +9.7 percentage points and an odds ratio of 1.48. Although these differences were not significant, the trend is biologically plausible. Exogenous progesterone administered after FTAI may contribute to transient endocrine stabilization, supporting the re-establishment of cyclicity and maintenance of luteal sensitivity during the subsequent follicular wave (Mann & Lamming, 1999; Stronge et al., 2005). Nonetheless, as indicated by the similar overall pregnancy rates at 70 days, the hormone did not adversely affect ovulation or corpus luteum formation in the next estrous cycle. This finding reinforces that long-acting progesterone formulations can be safely used in non-pregnant cows without disrupting the hypothalamic–pituitary–ovarian axis, a critical consideration in large-scale reproductive management of Bos indicus herds.

In Experiment 2, heifers supplemented with the injectable mineral–vitamin–amino acid complex achieved a pregnancy rate of 75.6% after natural service, compared with 58.0% in controls (risk difference = +17.6 pp; OR = 2.24). Although the effect did not reach conventional statistical significance (one-sided Fisher's $P = 0.093$), this positive tendency may reflect improved metabolic readiness and oocyte competence induced by the micronutrient formulation. Trace minerals such as selenium, zinc, and manganese enhance antioxidant capacity and steroidogenic efficiency (Bian et al., 2007), while amino acids including methionine and arginine act as precursors for nitric-oxide-mediated follicular vascularization, favoring luteal development (Arechiga et al., 1998; Aurich et al., 2018). Therefore, the observed trend supports the hypothesis that nutraceutical supplementation can modulate the endocrine milieu and facilitate conception during subsequent natural mating, particularly under tropical pasture conditions where latent mineral deficiencies are common.

Taken together, the findings from Table 1 reinforce the interpretation drawn from Figure 3: both interventions were physiologically neutral to beneficial, promoting reproductive stability without inducing endocrine suppression or luteal dysfunction. While the statistical no significance reflects the inherent variability of field data, the consistent direction of effect—favoring treated animals—suggests that combining endocrine support with targeted nutritional supplementation could yield incremental gains in fertility when applied repeatedly or under nutritionally challenged environments. These results warrant further investigation using longitudinal designs and hormonal monitoring to quantify the mechanistic pathways through which nutraceutical and hormonal strategies may converge to optimize post-AI fertility in Bos indicus cattle.

5. Conclusion

In summary, both long-acting progesterone and injectable nutraceutical supplementation were physiologically safe and did not compromise ovarian function or fertility. Although neither treatment significantly increased conception rates, both showed a consistent numerical trend toward improved reproductive performance, suggesting that endocrine and nutritional support strategies may contribute to reproductive stability under tropical field conditions.

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Briefing notes: All experimental procedures were conducted in accordance with institutional guidelines for animal research and were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Acre (protocol no. 23107.025574/2018-35).

6. References

Arechiga, C. F., Ealy, A. D., Hansen, P. J., & Thatcher, W. W. (1998). Evidence that glutathione protects preimplantation bovine embryos from heat shock by maintaining embryonic viability and reducing oxidative stress. Biology of Reproduction, 59(5), 1409–1416. https://doi.org/10.1095/biolreprod59.5.1409

Aurich, C., Budik, S., & Aurich, J. E. (2018). Nutritional factors and antioxidant status in relation to fertility in horses. Journal of Equine Veterinary Science, 68, 79–85. https://doi.org/10.1016/j.jevs.2018.04.002

Aurich, C., Budik, S., & Aurich, J. E. (2019). Effects of antioxidant supplementation on reproductive performance in mares. Theriogenology, 132, 169–175. https://doi.org/10.1016/j.theriogenology.2019.04.014

Bian, X., Huang, J., Liang, J., Liu, L., & Li, Y. (2007). Zinc, copper and manganese superoxide dismutase activities and mRNA expression in the bovine corpus luteum. Theriogenology, 68(6), 979–985. https://doi.org/10.1016/j.theriogenology.2007.07.001

Bó, G. A., Cutaia, L., Baruselli, P. S., Menchaca, A., & Mapletoft, R. J. (2023). Advances in reproductive management of beef cattle in South America: Synchronization, embryo technologies, and field fertility. Animal Reproduction, 20(2), e20230012. https://doi.org/10.1590/1984-3143-AR2023-0012

Boxmeer, J. C., Macklon, N. S., Lindemans, J., Beckers, N. G. M., Eijkemans, M. J. C., Laven, J. S. E., Steegers, E. A. P., & Steegers-Theunissen, R. P. M. (2009). Antioxidants and the reproductive system: Impact on sperm, oocytes and embryos. Human Reproduction Update, 15(6), 667–682. https://doi.org/10.1093/humupd/dmp025

CBRA – Colégio Brasileiro de Reprodução Animal. (2013). Manual para Exame Andrológico e Avaliação de Sêmen Animal (3rd ed.). Belo Horizonte: CBRA.

Del Prete, C., Di Palo, R., Salzano, A., & Neglia, G. (2024). Effects of antioxidant vitamin supplementation on reproductive performance and oxidative balance in dairy cows and mares. Animals, 14(3), 455. https://doi.org/10.3390/ani14030455

Diskin, M. G., & Kenny, D. A. (2014). Optimising reproductive performance of beef cows and replacement heifers. Animal, 8(S1), 27–39. https://doi.org/10.1017/S175173111400086X

Mann, G. E., & Lamming, G. E. (1999). The influence of progesterone during early pregnancy in cattle. Reproduction in Domestic Animals, 34(3–4), 269–274. https://doi.org/10.1111/j.1439-0531.1999.tb01250.x

Mann, G. E., & Lamming, G. E. (2001). Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction, 121(1), 175–180. https://doi.org/10.1530/rep.0.1210175

Moraes, J. C. F., Hoff de Souza, C. J., Brauner, C. C., Pimentel, M. A., Vieira-Benavides, M., & Sterman-Ferraz, J. B. (2013). Utilização do escore de condição corporal pós-parto na identificação de vacas de corte mais férteis criadas em sistemas extensivos. Archivos Latinoamericanos de Producción Animal, 21(3), 149–155.

Rizzo, A., D'Occhio, M. J., Kaczmarek, M. M., & Williams, S. A. (2019). Nutritional modulation and reproductive efficiency in beef cattle: Impacts on ovarian physiology and endocrine signaling. Animal Reproduction Science, 207, 123–132. https://doi.org/10.1016/j.anireprosci.2019.06.005

Torres, C. A., Pereira, M. H. C., Martins, C. M., & Bó, G. A. (2021). Post-AI progesterone supplementation and fertility in Bos indicus cattle: A meta-analytical perspective. Theriogenology, 170, 1–9. https://doi.org/10.1016/j.theriogenology.2021.04.010

Volker, D., & Ludwig, M. (1995). Role of vitamin A and β-carotene in reproduction. Theriogenology, 43(3), 795–801. https://doi.org/10.1016/0093-691X(94)00022-Y