

# PREGNANCY RATE OF NELORE HEIFERS UNDER ARTIFICIAL INSEMINATION COMBINED WITH MINERAL AND VITAMIN SUPPLEMENTATION

Tasa de preñez de novillas Nelore bajo Inseminación Artificial combinada con suplementación mineral y vitamínica

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**ABSTRACT::** The objective of this study was to evaluate the pregnancy rate of Nelore heifers subjected to artificial insemination combined with mineral and vitamin supplementation. The study was conducted in a rural property in the municipality of Uruará in the state of Pará. Two hundred forty-two Nelore heifers, 24 months old, with an average weight of  $320\pm2.5$  kg, were used. Heifers were divided into a control group (n = 121 animals) and a group (n = 121 animals) treated with vitamin and mineral supplementation (Anabolic<sup>®</sup>), at a dose of 10 mL per animal, by subcutaneous route, at the beginning (D0) of the Fixed-Time Artificial Insemination (FTAI) protocol. Pregnancy diagnosis was made by transvaginal ultrasonography (TVUS). The pregnancy rate of the groups was contrasted by comparison analysis of expected proportion using the chi-square test, with continuity corrections at 5 % significance. Pregnancy rate results were 44,62 % for the control group and 55,38 % for the treated group (p > 2373). However, regarding economic terms, the supplemented cows generated 13 more calves than the control group, with a gross profit of 38,805.60. Vitamin and mineral supplementation administered before FTAI did not promote a significant increase in the pregnancy rate of Nelore cows with moderate body condition score. However, regarding the economic bias, the implementation of the supplementation protocol provided considerable gains to the producer.

Key words: Calves, economic gains, hormonal protocols, livestock.

**RESUMEN::** El objetivo de este estudio fue evaluar la tasa de preñez de novillas Nelore sometidas a inseminación artificial combinada con suplementación mineral y vitamínica. El estudio se realizó en una propiedad rural del municipio de Uruará, en el estado de Pará. Se utilizaron un total de 242 novillas Nelore, de 24 meses de edad, con un peso promedio de  $320\pm2.5$  kg. Las novillas se dividieron en un grupo control (n = 121 animales) y un grupo tratado (n = 121 animales) tratados con suplementación vitamínico mineral (Anabolic®) a una dosis de 10 mL por animal, por vía subcutánea, al inicio (D0) del protocolo de Inseminación Artificial a Tiempo Fijo (IATF). El diagnóstico de embarazo se realizó mediante ecografía. Se calculó la tasa de embarazo, así como el análisis económico del uso del suplemento. La tasa de preñez de los grupos se contrastó mediante análisis de comparación de proporciones esperadas mediante la prueba de chi-cuadrado con correcciones de continuidad al 5 % de significancia. Los resultados de las tasas de embarazo fueron del 44,62 % para el grupo control y del 55,38 % para el grupo tratado (p > 2373). Sin embargo, en términos económicos, las vacas suplementadas generaron 13 terneros más que el grupo control, con una ganancia bruta de 38.805,60. La suplementación con vitaminas minerales administrada antes de la IATF no promovió un aumento significativo en la tasa de preñez en vacas Nelore con una condición corporal moderada. Sin embargo, en el sesgo económico, la adopción del protocolo de suplementación proporcionó ganancias considerables al productor.

Palabras clave: Terneros, ganancias económicas, protocolos hormonales, rumiantes.

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# **INTRODUCTION**

Reproductive efficiency of cattle herds is influenced by several factors, such as the breeding system, the breed used, the management employed on the farm, the sanitary status and nutritional status of the animals (1,2), as well as the animal welfare associated with heat stress indices (3,4). Despite this variety of factors that interfere with animal reproduction, the use of biotechniques applied to reproduction has shown positive results, being of fundamental importance for the economic growth of national livestock (5,6,7,8,9,10).

The live weight of the animal, the category and the physiological conditions are parameters that vary according to the nutritional, vitamin and mineral conditions of the cattle. In this context, the reproductive phase in bovine females is established as the one with the highest requirement for vitamin and nutritional supplementation and that can affect reproductive efficiency. Thus, the use of vitamins is necessary to maximize reproductive efficiency (11, 12).

The use of vitamins is essential for the growth, reproduction and health of animals and their deficiencies in the body can lead to the emergence of negative symptoms (13). Associated with this, the suplementation of minerals ensures the proper development of the organism and, when administered by injection, it does not suffer interference from dietary antagonists (14), which causes an increase in these molecules in the animal organism (15).

In this context, the use of supplements improves the pregnancy rates of cows in the postpartum period (16,17), as well as promoting the reduction of oxidative stress, due to inadequate management (18). Based on this information, the objective of this study was to evaluate the pregnancy rate of Nelore heifers subjected to artificial insemination combined with mineral and vitamin supplementation.

### MATERIAL AND METHODS

# **Ethical aspects**

This study was submitted to and approved by the Ethics Committee on Research and Use of Animals of the Federal University of Western Pará, protocol number No. 0120230238.

#### Local

The study was conducted on a rural property in the municipality of Uruará, in the state of Pará. The climate is hot and humid (Am4) with annual precipitation between 1900 and 2100 mm, average annual air temperature of 25.6 °C, and relative humidity ranging from 84 to 86 %. The wettest quarter occurs between the months of February and April and the least rainy between the months of August and October (18).

# **Experimental animals**

Two hundred forty-two Nelore heifers (*Bos taurus indicus*), 24 months old, with an average weight of  $320\pm2.5$  kg, were used in a semi-intensive system. All heifers were clinically healthy, with no cyclical reproductive

changes, evidenced by palpation, with a body condition score of 2.85 to 3.00 (19).

All heifers were raised in an extensive system under the same conditions and received mineral salt (Bellman<sup>®</sup> 80 ready to the trough) and *Panicum maximum* cultivar Mombaça grass with access to *ad libitum* water. They were vaccinated against foot-and-mouth disease, brucellosis and rabies. In addition, they were dewormed subcutaneously with Moxidectin 1mg (Cydectin<sup>®</sup>), 1ml per 50 kg, 35 days before the start of the experiment.

#### **Experimental design**

Heifers were randomly divided into two groups: Control group (n = 121 animals - group I) and Treatment group (n = 121 animals - group II), treated with vitamin and mineral supplementation (Anabolic<sup>®</sup>. Noxon Animal Health, Rod. Anhanguera, km 296 Distrito Industrial, São Paulo, Brazil) (20), at a dose of 10 mL per animal, subcutaneously, at the beginning (D0) of the FTAI protocol. In the control group, the same protocol was carried out without the administration of the vitamin-mineral- supplement. FTAI protocol in both groups was performed by the same applicator.

All heifers were submitted to the FTAI protocol, which consisted of the application of 2ml Estradiol (BE; mention the commercial name and the laboratory) intramuscularly (IM) and the implantation of progesterone (P4, Primer Monodose<sup>®</sup>, Tecnopec) intravaginally. On day 8, all cows had the implants removed and received 0.5 ml of estradiol cypionate (ECP), 2 ml of Estrom<sup>®</sup> (Chlorprotenol 24.1 mg) and 1.5 ml of Novormom<sup>®</sup> (Equine Choreonic Gonadotropin). On day 10, Fixed Time Artificial Insemination (FTAI) was performed (Figure 1).



**Figure 1.** Experimental design infographics. BE - estradiol benzoate. ECP - FTAI - fixed-time artificial insemination. eCG - equine chorionic gonadotropin. PD - pregnancy diagnosis. MI - intramuscular. SC = Subcutaneous. / Infografía del diseño experimental. BE - benzoato de estradiol. ECP - IATF - inseminación artificial a tiempo fijo. eCG gonadotropina coreónica equina. PD - diagnóstico de gestación. MI intramuscular. SC = subcutánea.

Ultrasound (Mindray<sup>®</sup>, DP-10 VET) was used to diagnose pregnancy 30 days after FTAI performance, and the presence of the viable embryo with a heartbeat indicated pregnancy.

Pregnancy rate was calculated using the following formula:

$$PR = NCP/NCT * 100$$

Where: PR = pregnancy rate; NCP = number of pregnant cows; NCT = number of cows treated.

It was assumed that the heifers considered pregnant in this study would also give birth and, consequently, produce calves, which was expressed in the results of the economic rate calculation.

# **Economic analysis**

For the economic analysis, expense composition was made with products and services, considering real market values corresponding to the analysis period (June/2023) (Table 1).

# Statistical analysis

The individual information of each cow (experimental unit) was filled in spreadsheets for statistical analysis. Statistical analysis for the equality of expected proportions was performed using the chi-square test with continuity corrections at 5 % significance. Data were analyzed using SAS procedures (SAS Inst. Inc., Cary, NC, version 9.3).

# **RESULTS AND DISCUSSION**

Pregnancy rate results were 44,62 % (54/121) for group I and 55,38 % (67/121) for the group to which Anabolic® was applied (Figure 2). There were no statistical differences between the supplemented and control groups (p > 0.2373). Although there were no significant differences between the groups, the use of Anabolic® showed promising results in increasing the pregnancy rate of heifers. There were no studies aimed to evaluate the effect of increasing doses of the use of vitamin-mineral supplements (Anabolic®) on the fertility of dairy cattle. However, it is noted that in the present study, the cows evaluated presented satisfactory results in relation to the pregnancy rate after the administration of Anabolic<sup>®</sup>. According to Ren et al. (21), it is important to note that cows injected with the lowest dose of vitamins, despite having lower concentrations of progesterone, were able to sustain similar pregnancy rates, 30 and 45 days after FTAI compared to the other groups evaluated.

It is also noted that the pregnancy rate in the group receiving mineral-vitamin supplements increased by 10,76 %, in relation to the control group. Thus, it was possible to identify that the use of Anabolic<sup>®</sup> before FTAI tended to increase embryonic survival and pregnancy rate. These data corroborate the studies carried out by Pinheiro *et al.* (22), who observed an increase in the pregnancy rate only in multiparous cows compared to primiparous cows, when subjected to multivitamin-based FTAI protocols, as it was observed only in heifers in this study. In addition, the aforementioned authors also highlighted that the increase

in nutrient demand in primiparous cows associated with the inhibitory effect of sucking may promote a lower frequency of LH pulses in these animals, leading to a lower pregnancy rate.

Binelli *et al.* and Vasconcelos *et al.* (23) state that the efficacy of Anabolic<sup>®</sup> steroids use varies with the body condition score (BCS), the number of days postpartum, and the association with other management strategies, such as temporary weaning.

Sá Filho *et al.* (16) and Baruselli *et al.* (17) describe that the use of supplements improves the pregnancy rates of cows in the postpartum period, with a more pronounced effect in those with a lower anestrus or in those that were in anestrus.

Corroborating the above, Rodrigues *et al.* (12) evaluated the use of different vitamins in the fertility of zebu cows and described that the association between them caused greater ovarian activity, as well as better pregnancy rate indexes. Souza et al. (24) also found better fertility rates in cows that received vitamin supplementation when comparing the control group and the supplemented group.

With results similar to those of the present study, Gouvêa *et al.* (25) administered  $\beta$ -carotene and vitamins to cows 30 days before FTAI, and observed an increase in the pregnancy rate.

The use of minerals and vitamins in cow reproduction is inconsistent, in which it is observed that some studies show an increase in the pregnancy rate (26,27,28) and others not (29,30,31,32). This can be linked to factors such as breed, category, diet provided, reproductive management, and environmental factors.

The economic analysis showed that there was a gross profitability of R\$ 38.805,60, considering the calf value of R\$ 3.026,00, in which the difference was 13 calves born after FTAI associated with the administration of vitamin-mineral supplements when compared to the non-supplemented group, as observed in Figure 2.

Based on the results found in this study, future research using variable doses, associated with the study of physiological mechanisms and hormone concentrations in cattle, could provide further information capable of pointing to efficient strategies for the use of these products at the time of FTAI. In addition, evaluating other breeds using this protocol could be useful when making efficient choices for producers.

Table 1. Expenses per animal according to the products and services performed, Uruará, Pará, Brazil. / Gastos por animal según productos y servicios realizados, Uruará, Pará, Brasil.

Product/Procedure	Unit	Quantity	Cost/animal
Protocol	1	1	28.50
Supplement	ml	5	4.40
Dose of semen	ml	1	20.00
Labor	Animal	1	25.00
Total	-	-	77.90



Figure 2. Cost dynamics and economic gains for the producer when comparing the control group and the supplemented group. / *Dinámica de costos y ganancias económicas para el productor al comparar el grupo control y el grupo suplementado*.

### CONCLUSION

Mineral-vitamin supplementation administered before FTAI did not promote a significant increase in the pregnancy rate in Nelore cows with moderate body condition score. However, in the economic bias, the supplementation protocol provided considerable gains to the producer.

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