ARTIFICIAL INSEMINATION PROTOCOLS IN CATTLE: TECHNIQUES TO OPTIMIZE CONCEPTION RATES

PROTOCOLOS DE INSEMINAÇÃO ARTIFICIAL EM BOVINOS: TÉCNICAS PARA OTIMIZAÇÃO DAS TAXAS DE CONCEPÇÃO

PROTOCOLOS DE INSEMINACIÓN ARTIFICIAL EN BOVINOS: TÉCNICAS PARA LA OPTIMIZACIÓN DE LAS TASAS DE CONCEPCIÓN

https://doi.org/10.56238/sevened2026.008-132

Leandro Giroux Gomes[1], Átila Bonfim Ferreira Cavalcante[2], Ana Edvirgens Vasconcelos de Souza[3], Jessica Saldanha Martins[4], Henrique Leite França Comes[5], Otávio Silveira Rizzi[6]

ABSTRACT

Artificial insemination (AI), and especially fixed-time artificial insemination (FTAI), have become established as central biotechnologies to enhance reproductive efficiency and accelerate genetic gain in cattle production systems by reducing dependence on estrus detection. Despite their widespread adoption, reproductive performance achieved under field conditions remains dependent on the interaction between the hormonal protocol, the physiological and metabolic status of the females, and the operational quality of the service. This narrative review synthesizes recent evidence (2021–2025) on estrous cycle synchronization strategies based on progesterone (P4), estrogens, and luteolysis with prostaglandin F2α (PGF2α), highlighting the importance of maintaining adequate P4 concentrations during dominant follicle development for oocyte competence and subsequent luteal functionality. Additionally, the use of equine chorionic gonadotropin (eCG) as a supportive tool in cows with low body condition and higher probability of anestrus is discussed, as well as the impact of inseminator training and semen handling (storage, thawing, and intrauterine deposition) on conception rates. Finally, emerging perspectives related to omics sciences are addressed, with emphasis on circulating microRNAs as potential biomarkers for predicting pregnancy success and enabling early diagnosis, with implications for reducing the interval between services and optimizing system profitability. It is concluded that maximizing conception rates in AI/FTAI programs depends on an integrated approach combining protocol design, nutritional management, and technical excellence, in addition to the critical and gradual incorporation of higher-resolution diagnostic tools.

Keywords: Artificial Insemination. FTAI. Estrus Synchronization. Progesterone. PGF2α. eCG. Biomarkers.

[1] Undergraduated student in Veterinary Medicine. Universidade Federal da Bahia (UFBA).
[2] Graduated in Veterinary Medicine. Centro Universitário Católica do Tocantins (UNICATÓLICA).
[3] Undergraduate student in Animal Science. Universidade Estadual Vale do Acaraú (UVA).
[4] Undergraduated student in Veterinary Medicine. Universidade Norte do Paraná (UNOPAR).
[5] Graduated in Veterinary Medicine. Universidade Federal de Sergipe (UFS).
[6] Master's degree in Genetics and Biochemistry. Universidade Federal de Uberlândia (UFU).

RESUMO
A inseminação artificial (IA) e, sobretudo, a inseminação artificial em tempo fixo (IATF) consolidaram-se como biotecnologias centrais para elevar a eficiência reprodutiva e acelerar o ganho genético em sistemas de produção bovina, ao reduzir a dependência da detecção de estro. Apesar da ampla adoção, o desempenho reprodutivo obtido em campo permanece condicionado à interação entre o protocolo hormonal, o estado fisiológico/metabólico das fêmeas e a qualidade operacional do serviço. Esta revisão narrativa sintetiza evidências recentes (2021–2025) sobre estratégias de sincronização do ciclo estral baseadas em progesterona (P4), estrógenos e luteólise com prostaglandina F2α (PGF2α), destacando a relevância da manutenção de concentrações adequadas de P4 durante o desenvolvimento do folículo dominante para a competência oocitária e a subsequente funcionalidade luteal. Discute-se, adicionalmente, a utilização da gonadotrofina coriônica equina (eCG) como ferramenta de suporte em vacas com baixa condição corporal e maior probabilidade de anestro, bem como o impacto da capacitação do inseminador e do manejo do sêmen (armazenamento, descongelação e deposição intrauterina) sobre as taxas de concepção. Por fim, são abordadas perspectivas emergentes associadas às ciências ômicas, com ênfase em microRNAs circulantes como potenciais biomarcadores para predição do sucesso gestacional e diagnóstico precoce, com implicações para a redução do intervalo entre serviços e otimização da rentabilidade do sistema. Conclui-se que a maximização das taxas de concepção em programas de IA/IATF depende de abordagem integrada, combinando desenho protocolar, manejo nutricional e excelência técnica, além de incorporação crítica e gradual de ferramentas diagnósticas de maior resolução.

Palavras-chave: Inseminação Artificial. IATF. Sincronização do Estro. Progesterona. PGF2α. Ecg. Biomarcadores.

RESUMEN
La inseminación artificial (IA) y, especialmente, la inseminación artificial a tiempo fijo (IATF) se han consolidado como biotecnologías centrales para aumentar la eficiencia reproductiva y acelerar el progreso genético en los sistemas de producción bovina, al reducir la dependencia de la detección del estro. A pesar de su amplia adopción, el desempeño reproductivo obtenido en campo sigue condicionado por la interacción entre el protocolo hormonal, el estado fisiológico y metabólico de las hembras y la calidad operativa del servicio. Esta revisión narrativa sintetiza evidencias recientes (2021–2025) sobre estrategias de sincronización del ciclo estral basadas en progesterona (P4), estrógenos y luteólisis con prostaglandina F2α (PGF2α), destacando la relevancia de mantener concentraciones adecuadas de P4 durante el desarrollo del folículo dominante para la competencia ovocitaria y la posterior funcionalidad luteal. Asimismo, se discute la utilización de la gonadotropina coriónica equina (eCG) como herramienta de apoyo en vacas con baja condición corporal y mayor probabilidad de anestro, así como el impacto de la capacitación del inseminador y del manejo del semen (almacenamiento, descongelación y deposición intrauterina) sobre las tasas de concepción. Finalmente, se abordan perspectivas emergentes asociadas a las ciencias ómicas, con énfasis en microARN circulantes como potenciales biomarcadores para la predicción del éxito gestacional y el diagnóstico precoz, con implicaciones para la reducción del intervalo entre servicios y la optimización de la rentabilidad del sistema. Se concluye que la maximización de las tasas de concepción en programas de IA/IATF depende de un enfoque integrado que combine el diseño protocolar, el manejo nutricional y la excelencia técnica, además de la incorporación crítica y gradual de herramientas diagnósticas de mayor resolución.

Palabras clave: Inseminación Artificial. IATF. Sincronización del Estro. Progesterona. PGF2α. eCG. Biomarcadores.

1 INTRODUCTION

Productivity in modern livestock farming is strongly linked to the reproductive efficiency of the herd, with Artificial Insemination (AI) and, more specifically, Fixed Time Artificial Insemination (TAI), being the most impactful biotechnological tools for genetic and operational progress (Sales et al., 2024). In the last 40 years, the evolution of these techniques has made it possible to overcome one of the biggest obstacles to bovine reproduction: the need for continuous observation of estrus, which often has low detection rates and efficiency in the field (Sartori et al., 2023; Sales et al., 2024).

However, the present presents new challenges. Increased milk production and metabolic changes in high-performance cows have been associated with a reduction in fertility, requiring that the control of the estrous cycle, through the manipulation of follicular development and ovulation, be increasingly precise (Sartori et al., 2023; Ayantoye et al., 2025). The success of these technologies does not depend only on robust hormonal protocols, but on a multifactorial triad that involves nutritional management, semen quality, technical competence, and inseminator training, a variable that is often underestimated, but which determines the correct deposition of semen in the reproductive tract (Dalton et al., 2021; Mathewos et al., 2023).

At the same time, the field of animal reproduction is moving towards a new frontier with the integration of omics technologies. The identification of specific biomarkers, such as serum microRNAs, emerges as a promising tool to predict the success of conception and diagnose pregnancy early, reducing the interval between inseminations and optimizing the profitability of the system (Ayantoye et al., 2025; Tzelos et al., 2023).

In view of this scenario of technical evolution and persistence of production problems, the present narrative review aims to analyze the current synchronization protocols and hormonal manipulation strategies for fertility optimization. In addition, it seeks to discuss critical management factors and explore how new biotechnological tools can transform reproductive prognosis in cattle, offering an integrated vision for maximizing conception rates.

The association of Fixed Time Artificial Insemination (TAI) combined with methods that allow the identification of the estrus period (heat), can enhance reproductive performance, increase productivity and consequently, increasing profits for dairy cow producers, in addition to maximizing reproductive programs, (TAI) also allows contributing to advances in genetic improvement and herd management, being factors that impact profitability and sustainability in dairy production (Ayantoye, et al., 2025).

2 METHODOLOGY

The present investigation is a narrative literature review, structured with the purpose of synthesizing and critically analyzing the main scientific evidence related to the optimization strategies of artificial insemination in cattle. The search for theoretical subsidies was carried out in the PubMed database, using the MeSH descriptors "Artificial Insemination" and "Cattle", articulated by the Boolean operator AND, respecting the terminology of the Medical Subject Headings (MeSH) system. Additionally, filters for period (2021–2025), language (Portuguese or English), and full-text availability were applied, when available on the platform.

Studies published in the period between 2021 and 2025, available in full text and written in Portuguese or English, which dealt directly with synchronization and reproductive efficiency protocols, were selected. Brief communications, duplicate articles, studies without robust methodological foundation, or studies that were outside the central thematic scope were excluded. The selection process involved the preliminary analysis of titles and abstracts, followed by the full analytical reading of the manuscripts to ensure the consistency of the data presented. As this is a narrative review, the selection of studies was based on convenience and thematic relevance, with no prior registration protocol (e.g., PROSPERO) or application of formal instruments for assessing risk of bias. The synthesis of information was conducted in a descriptive and integrative manner, prioritizing convergent findings on: (i) hormonal synchronization protocols (P4, estradiol, PGF2α and eCG); (ii) management factors (BCS, nutrition and animal category); (iii) operational variables related to semen and insemination technique; and (iv) emerging perspectives with biomarkers (microRNAs).

3 RESULTS AND DISCUSSION

The search for the optimization of conception rates in cattle is based on the strict control of follicular dynamics and the synchronization of endocrine events that determine the emergence of follicular wave, luteolysis and ovulation, pillars that support the efficiency of Fixed Time Artificial Insemination (TAI) programs. Current protocols predominantly use the association between intravaginal progesterone (P4) devices and estrogens (e.g., estradiol benzoate) to synchronize the emergence of a new wave of follicular growth and reduce follicular stage variability at the time of insemination (Sales et al., 2024).

A critical point identified in the literature is that the success of this process depends not only on the presence of progesterone, but also on its circulating concentration during the growth of the dominant follicle. Adequate levels of P4 during follicle development are of great importance to avoid frequent pulses of luteinizing hormone (LH), which could lead to early

oocyte maturation and compromise of its viability (Sartori et al., 2023). Thus, the maintenance of an "adequate progesteronic environment" is interpreted as a determining component for the performance of the protocol, especially in categories with greater physiological variability.

According to the findings of (Mathewos et al., 2023) with the acquisition of semen, contributing to Artificial Insemination (AI), enhances the use of reproductive males, this technology favors the proliferation of superior genetics, as well as accelerates the reproductive process efficiently, (AI) is consolidated as it is the main reproductive biotechnique in developing countries, In dairy farming, it is a strategic management tool, favoring and enabling access to breeding males of high genetic quality.

3.1 EFFICIENCY OF LUTEOLYSIS AND PRE-OVULATORY PROGESTERONE DROP

Complementing this control, the effectiveness of luteolysis, which is the regression of the corpus luteum, emerges as one of the main factors to ensure fertility. Recent strategies indicate that the application of a second dose of prostaglandin F2α (PGF2α) can significantly increase conception rates, especially in cows that have more resistant corpora luteum. This practice allows progesterone levels to drop dramatically before insemination, allowing ovulation to occur in a perfectly synchronized manner after the removal of the devices (Sartori et al., 2023; Sales et al., 2024).

From an applied point of view, luteolytic failure should be interpreted as a frequent cause of well-executed protocols with pregnancy below expectations, since the persistence of P4 in the pre-AI period can reduce the LH peak and compromise ovulatory timing.

3.2 METABOLIC STATUS, CCS, AND GONADOTROPHIC SUPPORT WITH ECG

However, the biological response to hormones is directly influenced by the metabolic state of the animal. In scenarios of nutritional restriction, the body condition score (BCS) becomes a limiting factor, often keeping the animals in deep anestrus. To reduce this problem, the use of equine chorionic gonadotropin (eCG) has been shown to be a very efficient optimization technique, as it provides the necessary support for the final growth of the dominant follicle and for the formation of a functional corpus luteum in cows with low energy reserve and lower ovarian responsiveness (Mathewos et al., 2023).

Dominant follicle growth is elevated by eCG due to its affinity for gonadotropin receptors (LH and FSH) present in follicular granulosa cells (Murphy and Martinuk, 1991). Physiologically, LH binds to granulosa cell receptors, causing a cascade of reactions that synthesize catalytic enzymes that promote steroid production and, consequently, stimulate the final growth of the dominant follicle (Carroll et al., 1992). Thus, eCG would act as a

gonadotrophic support, similar to LH, stimulating the growth of the dominant follicle, especially in primiparous females and in anestrus with low body condition score (BCS) (Sales et al., 2024).

It has already been shown that eCG, added to the hormonal protocol at the time of PGF application, helps to increase: follicle size, estrus manifestation, ovulation rate and fertility in beef cattle (Baruselli et al., 2004; Sales et al., 2016). However, it is essential to highlight that the positive effect of the use of eCG on FTAI when used in lactating Bos taurus cows is minimal or non-existent, probably because lactating cows have a higher frequency of LH pulses, which provides sufficient stimulus for follicular development (Vasconcelos et al., 2003; Ferreira et al., 2015).

Thus, it can be said that eCG should be understood as an excellent strategic tool for "rescue" in critical categories (e.g., low BCS, recent postpartum, primiparous women challenged), especially in Bos Indicus beef cows, rather than a universal addition to all protocols.

3.3 SEMEN QUALITY AND TECHNICAL EXECUTION: IMPACT OF THE INSEMINATOR

In addition to pharmacological rigor and nutritional management, precision in technical execution is a differential in the results obtained. The technical training of the inseminator, involving everything from the correct thawing of the semen to its exact deposition in the body of the uterus, is a variable that can cause considerable variations in pregnancy rates (Dalton et al., 2021). Operational aspects such as time between thawing and insemination, keeping the straw at the appropriate temperature, applicator hygiene, and efficient transposition of the cervix are critical points, as failures in this step can nullify the benefits of a well-planned hormonal protocol (Dalton et al., 2021; Mathewos et al., 2023). Therefore, the standardization of the procedure (checklists, periodic recycling, and technical supervision) constitutes a cost-effective intervention to increase reproductive efficiency in the field.

To achieve success in reproduction, the health and physiology of the cow, the time for insemination and the quality of the semen are conditioned (Ayantoye et al., 2025).

3.4 BIOMARKERS AND PRECISION REPRODUCTION: MICRORNAS AS EMERGING TOOLS

Finally, the transition to a phase of precision reproduction is being driven by the sciences. The discovery of molecular biomarkers, such as serum microRNAs, represents a significant advance in pregnancy monitoring. The increase in miR-26a levels around the eighth day after insemination has been shown to be directly correlated with pregnancy

maintenance (Tzelos et al., 2023). The integration of these early diagnosis tools with traditional protocols allows for early identification of failures in maternal signaling, enabling rapid and individualized interventions and reducing the interval between services in non-pregnant females (Ayantoye et al., 2025). However, the broad applicability of these markers still depends on analytical standardization, validation in different herds/breeds and cost-benefit evaluation under commercial conditions, so that their use goes beyond the experimental environment.

3.5 INTEGRATIVE SYNTHESIS

In general, the studies analyzed indicate that the result of FTAI does not depend only on the hormonal protocol, but on the combination of several factors. Among the main ones are: (i) the proper functioning of the endocrine protocol, with adequate levels of progesterone and efficient luteolysis; (ii) the metabolic status and category of the animal, especially the body condition score and the presence or absence of cyclicity; and (iii) the correct execution of the insemination, including semen management and deposition in the appropriate place. Thus, improving conception rates requires joint attention to these points, as hormonal adjustments may have little effect if the overall management and technique applied in the field are not satisfactory.

4 CONCLUSION

When analyzing the current panorama of bovine reproduction, it is clear that the optimization of conception rates does not depend on a single solution, but rather on fine-tuning between the animal's biology and the precision of human interventions. The advancement of FTAI protocols has brought essential predictability to the sector, however, the review of the data shows that we still face fundamental problems. Hormonal control, although increasingly sophisticated with the strategic use of PGF2α and eCG, often comes up against issues such as animal nutrition and the technical preparation of those who perform the insemination at the end of the process.

The integration of omics sciences and the use of biomarkers such as miR-26a open a valuable door to what we can call precision reproduction. This technology should not be seen only as a futuristic concept, but as a practical tool that will soon allow the producer to identify gestational failures in record time, drastically reducing the waste of time and resources with empty animals.

In short, the path to maximizing reproductive indices requires us to look at the cow in a holistic way. It is not enough to apply the most modern protocol if the animal does not have

metabolic support or if the semen is not deposited with the necessary technical rigor. The future of efficient livestock farming must be built by mastering the basic physiology of animals, ensuring excellence in management in the corral and intelligently adopting new biotechnological frontiers to transform early diagnosis into real profitability.

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