

Evolution of Fixed-Time Embryo Transfer protocols in cattle and their impacts on reproductive efficiency

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Abstract. Fixed-Time Embryo Transfer (FTET) has become one of the foremost reproductive biotechniques in cattle production, enabling the synchronization of recipients and the large-scale transfer of embryos independent of estrus detection. Despite considerable advancements over recent decades, pregnancy rates continue to exhibit variability across different protocols and management conditions, underscoring that the success of the technique is not solely contingent upon hormonal synchronization. This review aimed to synthesize the evolution of FTET protocols, discussing the principal hormonal advancements, protocol modifications, and the factors influencing reproductive efficiency. To this end, a comprehensive review of the scientific literature was conducted, encompassing studies on synchronization protocols, hormone utilization, recipient characteristics, and reproductive assessment tools. The analyzed literature demonstrates that protocol evolution has contributed to enhanced recipient utilization, improved herd management organization, and expanded reproductive opportunities. Nevertheless, conception and pregnancy outcomes may vary according to recipient category and physiological status, corpus luteum functionality, embryo quality, and management conditions. Studies evaluating distinct hormonal strategies have also yielded divergent results, indicating that recipient responses may differ depending on the specific program conditions. Thus, the evolution of FTET reflects a paradigm shift from protocols primarily focused on synchronization toward strategies that prioritize greater precision in recipient selection and rigorous control over the various stages of the reproductive process. Consequently, the integration of adequate synchronization, luteal functionality, embryo quality, and efficient management is essential for optimizing outcomes in FTET programs in cattle production.

Keywords: Fixed-Time Embryo Transfer; Embryo Recipients; Reproductive Biotechnologies; Cattle Production.

Introduction

In recent decades, reproductive biotechnologies have played a fundamental role in the development of cattle farming, contributing to increased productivity, genetic improvement of herds, and reproductive efficiency. In this context, Fixed-Time Embryo Transfer (FTET) has become established as a strategic biotechnology, capable of enhancing the genetic utilization of animals and offering greater predictability in reproductive management (Seneda et al., 2022; Moitinho, 2024). In recent years, the growing expansion of in vitro embryo production (IVP) has further driven the use of FTET, since this biotechnology depends on

efficient recipient synchronization protocols. According to Viana (2024), with data referring to the year 2023, 643 thousand bovine embryos were produced in vitro in South America, corresponding to a significant share of global IVP embryo production. Brazil played a prominent role in this scenario, ranking among the countries with the highest recorded production.

Since their introduction into production systems, FTET protocols have undergone several improvements, driven by advances in knowledge of reproductive physiology, endocrinology, and animal management (Seneda et al., 2022). This growth stimulated changes in hormonal protocols,

application intervals, recipient selection, and technical team training, making the process more efficient and safer, reducing losses.

Despite the advances achieved, the efficiency of this process does not depend exclusively on hormonal synchronization. Several studies demonstrate that factors such as breed, animal category, body condition, nutrition, heat stress, embryo quality, and reproductive management significantly affect the results. Thus, understanding how protocols have evolved over time becomes essential to identify which modifications contributed to increased reproductive efficiency and which challenges still limit the application of the technique.

Given this context, this review aims to present the evolution of Fixed-Time Embryo Transfer protocols in cattle, discussing the main changes introduced over the years and their impacts on reproductive efficiency, based on the scientific evidence available in the literature.

Contextualization and analysis

The early artificial insemination and conventional embryo transfer programs depended on the identification of estrus in females, making reproductive management more laborious, prone to failure, and less efficient in reproductive programs. This limitation hindered the large-scale application of biotechnologies, especially in extensive systems. With the advancement of knowledge on follicular dynamics and hormonal control of the estrous cycle, protocols capable of synchronizing follicular wave emergence and ovulation independently of estrus manifestation were developed. Initially, these advances were used in Fixed-Time Artificial Insemination (FTAI) programs, and shortly thereafter implemented in Fixed-Time Embryo Transfer (FTET) programs, generating greater standardization of reproductive management.

One of the first advances was the introduction of intravaginal progesterone (P4) devices, which reduced dependence on estrus detection and increased management predictability by allowing control of the recipients' endocrine environment. Nevertheless, the isolated use of P4 still presented limitations regarding the uniformity of follicular emergence. The incorporation of estradiol then allowed the regression of the dominant follicle and synchronization of a new follicular wave, while the addition of prostaglandin F2 α (PGF2 α) enabled luteolysis of functional corpora lutea, further refining ovulation synchronization (Bó et al., 2024).

Another important milestone was the incorporation of equine chorionic gonadotropin (eCG), mainly in recipients in anestrus or with low body condition. In these categories, the response to conventional protocols was often limited; eCG administration stimulates the growth of the dominant follicle, favors the formation of more functional corpora lutea, and increases progesterone concentrations after ovulation, potentially resulting in better pregnancy rates under certain conditions.

One aspect related to the evolution of synchronization protocols was the increased utilization of recipients. In a study, Reineri et al. (2023) evaluated the effect of different ovulation synchronization protocols in beef heifers. Although pregnancy rates did not differ between protocols, the comparison between strategies that depend on estrus observation and those that use fixed-time synchronization demonstrates the impact of these technologies on the standardization and efficiency of reproductive management. These advances consolidated FTET as an important reproductive biotechnology in modern cattle farming, conferring greater predictability and organization to programs.

In addition to greater recipient utilization, the evolution of protocols provided greater organization of reproductive management, reduced need for estrus observation, better use of labor, and greater predictability of embryo transfer programs. These advances contributed to consolidating FTET as an important reproductive biotechnology employed in modern cattle farming.

Currently, the incorporation of new tools allows a more precise assessment of the reproductive condition of females. In recent decades, the advancement of reproductive biotechnologies in cattle has been related not only to the improvement of synchronization protocols but also to the expansion of in vitro embryo production, the development of resynchronization strategies, and the use of Doppler ultrasonography in reproductive assessment (Pugliesi et al., 2024). Thus, the evolution of programs has come to involve not only hormonal control of the estrous cycle but also the use of tools capable of assisting in the identification of females with greater reproductive potential.

The recent evolution of FTET programs is also related to the use of Doppler ultrasonography for functional assessment of the corpus luteum. Unlike conventional assessment, based mainly on the presence and morphological characteristics of the corpus luteum, Doppler allows estimation of its blood perfusion, providing information related to luteal activity. In a study conducted with 1,700 Brangus recipients submitted to FTET, the final conception rate was 44.2%, being higher among females with high luteal perfusion (48.4%) compared to those with low perfusion (37.8%). On the other hand, corpus luteum size did not present a significant association with conception. These results reinforce the importance of functional assessment of the corpus luteum in recipient selection and demonstrate how FTET has been incorporating tools capable of providing information beyond morphological assessment (Santos et al., 2023).

The incorporation of these tools represents a new stage in the evolution of FTET protocols, in which the objective is no longer only to synchronize recipients but also to involve the more precise selection of females with a greater probability of establishing pregnancy. Thus, technological

advances contribute to the better utilization of recipients and available embryos, favoring greater efficiency of embryo transfer programs. In addition to luteal functionality assessment, resynchronization strategies have also contributed to the improvement of FTET programs. After the first transfer, part of the recipients that do not establish pregnancy need to be submitted again to a reproductive protocol. The evolution of these strategies allowed reducing the interval between the first transfer and a new reproductive opportunity. Associated with ultrasonographic assessment, earlier identification of non-pregnant females enables the initiation of a new protocol in a shorter interval, increasing the number of reproductive opportunities within a given period (Pugliesi et al., 2024).

Concurrently with the evolution of transfer protocols, a significant expansion of in vitro production (IVP) of bovine embryos is observed. More recent data from the International Embryo Technology Society (IETS) show this expansion in 2024, where 2.02 million bovine embryos produced in vitro were produced worldwide, representing an 8.1% increase compared to the previous year. In South America, 714,277 bovine IVP embryos were produced and 654,936 were transferred in 2024. These data evidence the current dimension and importance of this biotechnology (Viana, 2024).

Recent studies show that the reproductive efficiency obtained with FTET may vary according to the characteristics of recipients and embryo management. Ambrozini (2024), when evaluating 4,987 embryo transfers in Girolando females, observed a higher conception rate in heifers (56.56%) compared to cows (46.62%), in addition to lower pregnancy loss in heifers (27.18%) compared to cows (34.94%). However, no significant differences were observed in conception rates and pregnancy loss regarding the use of fresh or vitrified embryos. These results demonstrated that FTET efficiency is not related exclusively to the synchronization protocol, being also influenced by the recipient category and other factors associated with the establishment and maintenance of pregnancy.

In addition to recipient category and embryo characteristics, studies have also been evaluating modifications in hormonal protocols aiming to improve recipient response and increase pregnancy rates. Moscoso (2022) compared two FTET protocols in Nelore recipients submitted to the transfer of in vitro-produced embryos. In the first protocol, 400 IU of eCG were used on day six, while in the second, 300 IU of eCG were used on day eight, aiming to evaluate whether different timing and doses of hormone application could influence reproductive results.

The results show that the recipient utilization rate was similar between the two protocols, being 82.04% in the first and 80.04% in the second. However, differences were observed in conception and pregnancy rates. The protocol using 400 IU of eCG on day six presented a conception

rate of 43.4%, while the protocol with 300 IU of eCG on day eight presented 35.9%. For the final pregnancy rate, the values were 35.60% and 28.90%, respectively. These results show that changes in the timing and dose of hormones used may influence the reproductive response of recipients. Furthermore, the study demonstrated that corpus luteum characteristics are also related to conception and pregnancy outcomes, reinforcing the importance of a good ovarian and luteal response for FTET success.

However, not all modifications made to protocols result in a significant increase in pregnancy rates. Carôso (2022) evaluated the effect of progesterone exposure ten days before the initiation of the ovulation synchronization protocol in zebu recipients. In the study, 192 recipients were evaluated, of which 160 were effectively submitted to embryo transfer. The mean pregnancy rate was 48.75% at 30 days and 43.75% at 60 days, while pregnancy loss between 30 and 60 days was 10.25%. When the groups were evaluated separately, the pregnancy rate at 30 days was 44.73% in the control group and 52.38% in the group that received progesterone before the protocol. At 60 days, the rates were 40.80% and 46.42%, respectively. Although numerically higher results were observed in the group that received progesterone, no significant differences were observed between treatments. No differences were also observed in corpus luteum characteristics evaluated at the time of transfer.

The comparison between the studies by Moscoso (2022) and Carôso (2022) demonstrates that the use of different hormonal strategies may present distinct results between studies. While Moscoso (2022) found better conception and pregnancy results with the alteration of eCG dose and timing of application, Carôso (2022) did not observe a significant effect of prior progesterone exposure. Thus, the results indicate that protocol efficiency depends not only on the hormones used but also on the individual response of recipients and the conditions under which the protocol is performed.

Overall, more recent studies show that the evolution of FTET is related to the pursuit of greater reproductive control and better utilization of recipients. Protocols have evolved from strategies aimed mainly at ovulation synchronization to systems that also consider corpus luteum condition, recipient category, embryo quality, and female management. Thus, FTET efficiency depends on the association between good recipient synchronization, adequate corpus luteum formation and functionality, embryo quality, and correct animal selection, demonstrating that protocol evolution is not related only to the inclusion of new hormones but to the pursuit of greater precision and efficiency in the different stages of the reproductive process.

Conclusion

The evolution of Fixed-Time Embryo Transfer (FTET) protocols has provided greater control over follicular dynamics and recipient ovulation, reducing dependence on estrus identification and making reproductive management more predictable. The incorporation of different hormonal strategies, associated with the use of tools such as Doppler ultrasonography and resynchronization, has contributed to better recipient utilization and to the increase of reproductive opportunities in embryo transfer programs.

However, the analyzed studies demonstrate that conception and pregnancy outcomes may vary according to the protocol used and factors related to recipients, embryos, and management. Thus, the evolution of FTET has contributed to the improvement of reproductive efficiency, but the results do not depend exclusively on hormonal synchronization. The association between adequate synchronization, corpus luteum functionality, recipient selection, embryo quality, and appropriate management is fundamental to increase the efficiency of FTET programs in cattle farming.

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