

## **STRATEGIES TO INCREASE FERTILITY DURING FIXED-TIME ARTIFICIAL INSEMINATION IN BEEF CATTLE**

### **Estrategias para aumentar la fertilidad durante la inseminación artificial a tiempo fijo en ganado de carne**

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#### **ABSTRACT**

The application of Fixed Time Artificial Insemination (FTAI) protocols has strongly boosted artificial insemination in Brazilian bovine herds. The control of the estrous cycle with protocols based on progesterone and estrogen involves synchronizing the emergence of a follicular wave, controlling the progesterone phase, and inducing ovulation in a synchronized manner. The increase in pregnancy rates starts with the preparation of the breeding season and the choice of protocols and females. Thereafter, based on the characteristics of the property and the lots of animals, small adjustments can be made to the hormonal protocols as needed. Finally, resynchronization can increase usability by allowing multiple inseminations within short periods. The search for new strategies is of paramount importance for the development of the FTAI protocol and the increase in final pregnancy rates. Thus, this review sought to highlight the main critical points and adjustments of the FTAI protocols and their applicability in the reproduction of beef cattle.

**Keywords:** Artificial insemination; Cattle; Pharmacological synchronization of estrus; Nelore; protocol.

#### **RESUMEN**

La aplicación de los protocolos de Inseminación Artificial a Tiempo Fijo (IATF) ha impulsado fuertemente la inseminación artificial en los rebaños de bovinos brasileños. El control del ciclo estral con protocolos basados en progesterona y estrógeno implica sincronizar la aparición de una onda folicular, controlar la fase de progesterona e inducir la ovulación de forma sincronizada. El aumento de las tasas de preñez comienza con la preparación de la temporada de cría y la elección de protocolos y hembras. A partir de entonces, según las características de la propiedad y los lotes de animales, se pueden hacer pequeños ajustes a los protocolos hormonales según sea necesario. Finalmente, la resincronización puede aumentar el uso al permitir múltiples inseminaciones en períodos cortos. La búsqueda de nuevas estrategias es de suma importancia para el desarrollo del protocolo FTAI y el aumento de las tasas de preñez, finalmente. Así, esta revisión buscó resaltar los principales puntos críticos y ajustes de los protocolos del FTAI y su aplicabilidad en la reproducción del ganado vacuno.

**Palabras clave:** Inseminación artificial; Vacas; Sincronización del estro; Nelore; protocolo.

## INTRODUCTION

Fixed-Time Artificial Insemination (FTAI) is one of the most used reproductive biotechniques in commercial bovine herds (Bonato et al., 2021). The technique involves synchronizing the moment of ovulation, inseminating all registered animals at the same time, and obtaining a pregnancy rate ranging from 40 to 60% (Morotti et al., 2018; Rodrigues et al., 2019). Well-managed herds with good nutrition, health, and semen quality, and well-trained personnel lead to the best reproductive rates (Baruselli et al., 2018).

According to a comparison made by Sá Filho et al. (2013), the use of FTAI associated with estrus detection or the use of transfer bulls results in an increase in the proportion of pregnant cows at the end of the season relative to the use of conventional artificial insemination or bulls throughout the breeding season. In addition, by using FTAI at the beginning of the breeding season, a higher proportion of animals become pregnant in the early third of the period, generating a higher concentration of calving and a positive impact on subsequent seasons (Oosthuizen et al., 2018; Sá Filho et al., 2013).

With the control of the follicular and luteal phases, the protocols for the manipulation of the estrous cycle start to synchronize more satisfactorily at the moment of ovulation (Mapletoft; Bó; Adams, 2000). The FTAI protocols currently used for beef cattle are based on three important events of cycle manipulation: synchronized emergence of the follicular wave, control of the progesterone phase and growth of the dominant follicle, and synchronized induction of ovulation (Baruselli et al., 2018; Bonato et al., 2021).

Strategies that enhance the use of FTAI allow greater effectiveness of hormonal protocols and subsequent increase in pregnancy rates (Bonato et al., 2021). Genetics, nutritional status, sexual maturity, and antral follicle count are examples of characteristics to be observed in reproductive planning (Baruselli et al., 2018; Bonato et al., 2015; Morotti et al., 2019). Therefore, this review addresses the main strategies and adjustments of the FTAI protocols that can improve the results of the application of this biotechnique in beef cattle.

### 2. Female selection and breeding season preparation

The selection of females and the preparation of the breeding season can impact the expected results at the end of the breeding season (Rodrigues et al., 2017). The gynecological examination of all females prior to the beginning of the breeding season allows general information to be obtained on the flock and the addition of specific reproduction information through an examination of the reproductive system (López-Gatius and Hunter, 2017). This information is used for the selection of females for entry into reproductive management and for the choice of protocols according to the characteristics of the lot (Bonato et al., 2021)

#### 2.1. Nutrition

In periods of low availability of food with a period of high energy demand, it is common for females to lose weight before the beginning of the rainy season (Oliveira et al., 2009). Thus, the Body Condition Score (BCS) should be assessed in advance to ensure that there is time to carry out nutrition interventions or be used to determine the start of the breeding season to allow

most females to reach a reserve level of adequate energy (D'Occhio et al., 2019). For the annual planning of activities and reproductive efficiency, the recommendation is to carry out the breeding season in the period of greater availability of pastures, to ultimately obtain a positive energy balance of the females (Cooke et al., 2020; Oliveira et al., 2009).

Using a scale from 1 to 5 (Ferguson et al., 2019), some researchers have already established an important correlation between the BCS and a probability of pregnancy. According to Torres et al. (2016), every 0.5 points on the BCS increases the probability of a cow becoming pregnant by approximately 36%. According to Pereira et al. (2018), cows with BCS  $\geq 2.75$  had pregnancy rates twice as high as animals with BCS between 2.0 and 2.5 (Ayres et al., 2014; Pereira et al., 2018). However, animals that were excessively overweight may not obtain good results due to irregularities in the estrous cycle and greater risk of miscarriages and birth problems. Thus, it is recommended that the BCS of those used in FTAI programs is approximately 3.0 to 3.5 (Ayres et al., 2014).

#### 2.2. Female evaluation and selection

The evaluation of zootechnical indices is a practice that must be adopted when selecting females. Among the characteristics that determine the reproductive efficiency of beef cattle, the most relevant factors, among other zootechnical indices, include age at first calving, calving interval (Miranda et al., 2010; Perotto, Abrahão and Kroetz, 2006), period of service, conception rate, pregnancy rate, birth rate, weaning rate, and mortality rate (Campos et al., 2013). Age at first birth has a great economic impact on the system: the earlier the age, the faster the return on investment until reproductive age (Miranda et al., 2010).

The increase in calving intervals results in a lower return on the fixed and operational costs involved in the breeding herd owing to a longer service period and a lower birth rate and calving rate by cows (Perotto, Abrahão and Kroetz, 2006). In primiparous and multiparous cows, postpartum anestrus is one of the events that mainly interferes in the calving interval. Other factors that can interfere include body condition score (BSE) and the strength of the maternal bond between cow and calf (Diskin and Kenny, 2016). Properties with low efficiency have a low service rate; this index is associated with the lack of cyclicity of the herd or lack of estrus detection (Caetano and Caetano, 2015).

The physiological differences between the subspecies *Bos indicus indicus* and *Bos taurus taurus* must be highlighted. Zebu breed animals tend to have a greater number of waves of follicular growth, higher concentration of circulating progesterone and estrogen, in addition to puberty and later sexual maturity relative to breeds of European origin (Barros, 2011; Morotti et al., 2015; Sartori et al., 2016). These differences partially explain the importance of protocols based on progesterone and estrogen for predominantly Zebu herds (Baruselli et al., 2017, 2019). Accordingly, the entry of animals into the breeding season and the choice of biotechnologies to be used must take into account the racial characteristics of the females (Baruselli et al., 2019; Sartori et al., 2016).

Regarding genetic grouping and nutritional management, sexual precocity has a great influence on the results of artificial

insemination in heifers (Claro et al., 2010). Taurine animals tend to reach puberty before the first year of life. Thus, with an adequate nutritional management, they are expected to first deliver at approximately 24 months of age (Claro et al., 2010; Filho et al., 2015). On the other hand, only approximately 16% of female Zebu females reach puberty naturally at 19 months old (Fortes et al., 2012). This difference is due to both genetic and environmental factors, and can be improved through hormonal and nutritional strategies (Sá Filho et al., 2015).

Using two ultrasound evaluations, Claro et al. (2010) classified prepubertal females that did not present corpus luteum (CL) in both exams and compared the conception rate in the first 7 days of the breeding season. Females that had already reached puberty had a conception rate of 43.8%, while prepubertal cows had a rate of only 27.3% (Claro et al., 2010). In a more detailed assessment, Holm et al. (2015) classified heifers as pubescent, peripubertal, or prepubertal using the reproductive tract score (RTS) proposed by Anderson et al. in 1991 (Table 1). In this study, animals considered pubescent (RTS 4 and 5) had shorter time to first conception, higher conception rate, and lower proportion of reproductive failures than peri-pubertal (RTS 3) and pre-pubertal (RTS 1 and 2) animals (Holm et al., 2015, 2016).

**Table 1.** Reproductive Tract Score (RTS) proposed by Anderson et al. (1991).

Score	Uterine Diameter and Tonus	Ovarian Structures
1	<20 mm, no tone	No palpable structures
2	20-25 mm, no tone	Follicles 8 mm
3	25-30 mm, little tone	Follicles 8-10 mm
4	30 mm, little tone	Follicles >10 mm sem CL
5	>30 mm, present tone	Follicles 10 mm com CL

Source: Adapted from Holm et al. (2016); Kasimanickan et al. (2020)

In the categories that did not reach puberty (RTS 1 to 4), a strategy to increase fertility involves the induction of puberty (Lima et al., 2020). There are different hormonal protocols based on exposure to exogenous progesterone, leading to sensitization of the hypothalamus and pituitary to reproductive hormones and maturation of the reproductive system, to ultimately stimulate the first ovulation and formation of the CL in the female (Kasimanickam et al., 2020; Lima et al., 2020). Kasimanickan et al. (2020) demonstrated that supplementation with progesterone is crucial for increasing pregnancy in animals that have not reached puberty. Claro et al. (2010) compared vaginal implants with different levels of progesterone and demonstrated that implants with low concentration (4th use) satisfactorily induced puberty and guaranteed conception rates similar to pubescent heifers at the beginning of the breeding season (Claro et al., 2010).

Zebu heifers present great challenges regarding the occurrence of puberty. In the primiparous and multiparous categories, the challenge is the return to cyclicity (Bonato et al., 2021). At the end of the gestational period, the follicles gradually increase in size by follicle-stimulating hormone (FSH) stimulation, while the luteinizing hormone (LH) pulsatility required for ovulation has not returned to normal. As a result, less than 30% of females have a CL at the beginning of the first protocol of FTAL

(Alves et al., 2021). Animals that present CL at the beginning of the protocol had higher estrus expression at the time of AI and higher pregnancy rates than acyclic animals at DO (Alves et al., 2021; Sá Filho et al., 2015). Presynchronization or exposure to exogenous progesterone prior to the protocol helps this resumption of cyclicity and has beneficial effects on the uterine environment and fertility in both heifers and postpartum cows (Sá Filho et al., 2015; Simões et al., 2018).

The counting of antral follicles (AFC) is another tool that is still in development, but aids in the classification of females (Morotti et al., 2018). Through ultrasound examination of the ovaries, it is possible to count the antral follicles greater than 3 mm due to the anechogenic aspect of the follicular antrum on examination (MORAES et al., 2019). Some studies have shown that CFA is related to genetic merit, the animal's ability to respond to exogenous hormones, and the influence of ovarian characteristics, such as size of the dominant follicle and CL (Moraes et al., 2019; Morotti et al., 2015, 2017, 2019). In large-scale studies with FTAL, an advantage of low CFA, which presents greater follicular growth, larger CL area, and higher pregnancy rates, has already been detected (Santos et al., 2016, 2013). However, depending on the subspecies, some divergent results were found, related to the productive longevity of the female (Jimenez-Krassel et al., 2017). Thus, there are still many questions to be answered on the subject; however, the CFA as a selection tool has promising aspects to add to reproductive technologies (Morotti et al., 2015, 2017).

### 3. Follicular growth and progesterone phase

The application of exogenous estradiol associated with the insertion of an intravaginal progesterone implant has a special emphasis in current protocols, as it induces follicular atresia at different stages and follicular recruitment at a fixed time (Bó and Baruselli, 2014). The permanence of the device allows the growth of the dominant follicle in a controlled manner, from recruitment during the diversion, to achievement of ovulation capacity (Morotti et al., 2018).

#### 3.1. Progesterone

High levels of progesterone (P4), exogenous or endogenous, throughout the protocol affect the recruitment and growth of the dominant follicle owing to the inhibition of progesterone in the release of Gonadotropin-releasing hormone (GnRH) and LH (Carvalho et al., 2008). In *Bos indicus* animals, the use of implants with a low concentration of progesterone improves final follicular growth and reduces the time between removal and the time of ovulation (Dadarwal et al., 2013).

A cost-cutting strategy with good results is the reuse of implants. Sales et al. (2015) showed no difference in conception rate from the first to the third use of 1.0 g P4 implants. In addition, the lower serum concentration allows greater final follicular growth, which is considered positive for improving ovulation rates (Sá Filho et al., 2009). In the category of pubescent heifers, first use implants lead to a reduction in the final growth of the follicle and reduction in the pregnancy rate, obtaining better results with low concentration implants or the third use of implants of 1 g or more (Dias et al., 2009). The use of a luteolytic agent at the beginning of the protocol also enables the maintenance of lower progesterone levels in cyclical flocks, such as pubescent heifers and single cows (Carvalho et al., 2008; Núñez-Oliveira; Cuadro and Menchaca, 2019).

The implant permanence time and the interval between removal and insemination also influence the pregnancy rate (Dadarwal et al., 2013). In protocols with higher concentrations of progesterone, implant permanence can be reduced to 7 days and the interval from removal to insemination can be increased to 54 hours (Dadarwal et al., 2013; Viziack et al., 2016). Another possible adjustment is the application of PGF2 $\alpha$  before implant removal (Peres et al., 2009a). The follicle diameter is directly related to ovulation and oocyte quality, which has direct consequences on the ovulation rate and conception, in addition to being related to the maintenance of pregnancy in early stages, which influences the size of the CL, serum levels of progesterone after FTAI, and the capacity to produce INF- $\gamma$  by the embryo (Pinto et al., 2020; Meneghetti et al., 2009b; Sá Filho et al., 2010).

### 3.2. Follicular growth and proestrus

To increase the final growth of the follicle and the quality of the CL after insemination, some studies have developed the application of equine chorionic gonadotropin (ecG) at the end of the progesterone phase. As a molecule with affinity for both FSH and LH receptors, an ecG can increase the final follicular growth and stimulate the luteinization of granulosa cells (Filho et al., 2010). In both heifers and cows, the ecG applied at the time of implant removal results in an increase in the growth rate and final diameter of the follicle, ovulation rate, and pregnancy rate (Dias et al., 2009; Sá Filho et al., 2010)

Primiparous cows and low BCS cows have a longer interval between the first ovulation. Further, a greater proportion of these animals do not have CL at the beginning of the breeding season (Alves et al., 2021; Claro et al., 2010). When the FTAI protocol is employed, these animals present smaller follicular growth, smaller dominant follicle, and lower ovulation rates (Sá Filho et al., 2011; Sales et al., 2015). Sales et al. (2016) and Bottino et al. (2021) highlight the importance of ecG in the most challenging postpartum categories. Despite affecting all categories, the increase in the pregnancy rate is more pronounced in primiparous cows than multiparous cows (Bottino et al., 2021; Sales et al., 2016).

The ecG is obtained from the plasma of pregnant mares through a purification process. Thus, both the ethical dilemma and the costs of available commercial products fostered a search for alternatives to ecG that have similar biological activity. FSH was tested as an aid to follicular growth by Bottino et al. (2021); however, they did not obtain satisfactory results.

Selection and growth of the dominant follicle after the shift is highly dependent on LH (Gomez-León et al., 2020). Thus, another applicable molecule with high affinity for LH receptors is human chorionic gonadotropin (hcG). Prata et al. (2018) tested the application of hcG at different doses on day 8 of the protocol, in addition to the removal of the P4 implant. In this study, a dose of 150 IU of hcG did not differ from the control, while a dose of 300 IU of hcG presented a follicular growth rate similar to the application of ecG, leading to 44.4% of cows experiencing early ovulation (Prata et al., 2018). In another study, Souza et al. (2019) compared the application of hcG and ecG together with implant removal on day 8.5 of the protocol and insemination at 36 to 40 h later. In this work, pregnancy rates were similar between treatments, indicating hcG as a possible substitute for ecG as long as the time of

application of the drug and artificial insemination are applicable (Souza et al., 2019).

## 4. Ovulation and Artificial Insemination

Ovulation is a consequence of a rapid increase in serum LH in the absence of a CL and in the presence of a follicle of sufficient size and maturity to ovulate. In the FTAI protocols, ovulation induction can be performed directly with the application of LH or analogs, or indirectly by the application of gonadotropins such as GnRH, hcG or other hormones such as estradiol esters and prostaglandin (Baruselli et al., 2019; Pfeifer et al., 2018).

### 4.1. Ovulation induction

The estrogen analogs include estradiol benzoate (EB) and estradiol cypionate (EC). EB has a shorter half-life and induces ovulation at approximately 60 to 70 hours after its application (Ayres et al., 2008). Different researchers have demonstrated that EB is effective at synchronously inducing ovulation in FTAI when applied 24 hours after progesterone implant removal (Ayres et al., 2008; Bó; Baruselli and Martínez, 2003; Cipriano et al., 2011; Sá Filho et al., 2011, 2009). However, the use of EB is associated with additional management of animals beyond the day of removal and insemination. An alternative to EB is EC, which has a longer half-life (Sá Filho et al., 2011; Sales et al., 2012).

The application of EC at the time of removal of the progesterone implant causes an interval until ovulation, with maximum follicle diameter and ovulation rate similar to EB applied 24 hours after implant removal (Sales et al., 2012). Furthermore, the application of cypionate induces estrus behavior, increases gene expression in ovary and uterus, and improves luteal function after ovulation (Basolasco et al., 2021; Pfeifer et al., 2020).

For other inducers, the use of LH is usually restricted to ovarian superovulation protocols owing to its high cost. The same limiting factor is considered for hcG. GnRH has been widely used alone or with estrogens in TAI protocols (Rodrigues et al., 2019). In conventional FTAI protocols, ovulation induction is usually achieved by cheaper alternatives, such as the use of estrogens. However, the use of GnRH has markedly increased in recent years.

### 4.2. Manifestation of heat and moment of insemination

The main objective of FTAI is the synchronization of ovulation and not of estrus; however, the onset of estrus has an important impact on the results of biotechnology (Richardson et al., 2016; Sá Filho et al., 2011). Nogueira et al. (2019) demonstrated that the expression of estrus is related to conception rates up to 20% higher than those in animals that do not manifest estrus. In addition, the investigation sought to detect the influence of BCS on the expression of estrus and conception (Nogueira et al., 2019). The same researchers suggest the use of removable paintings at the base of the tail as a substitute method for direct observation of the mount, which is laborious and inefficient.

Using newly calved Nellore cows, Sá Filho et al. (2011) compared the application of EC or GnRH or both as ovulation inducers. As a result, the researchers found a higher occurrence of estrus and higher pregnancy rates in animals treated with cypionate than those administered only GnRH. By employing

Nellore animals, Rodrigues et al. (2019) used a protocol with EC as an ovulation inducer and painting at the base of the tail to detect estrus. The application of GnRH was only performed at the time of insemination for animals that did not manifest estrus. Further, adjustment of the protocol for these animals increased the pregnancy rate of animals who did not manifest estrus compared to the control; however, the same rate as animals that presented estrus was not achieved (Rodrigues et al., 2019).

Estrus expression is directly related to serum estrogen levels and the size of the dominant follicle. Thus, an adjustment

proposed by Pfeifer et al. (2015) involves blocking FTAI based on the size of the dominant follicle. These researchers found that animals with a follicle larger than 10 mm had ovulation rates close to 100%; however, the time until ovulation was inversely proportional to the diameter of the follicle. Thus, the animals were evaluated for follicle diameter and divided into different insemination periods (Table 2). Owing to this distribution, the researchers found an increase of up to 35% in pregnancy in animals with follicles smaller than 13 mm (Pfeifer et al., 2015).

**Table 2.** Distribution of insemination blocks over time according to the size of the dominant follicle.

Block	Diameter DF	IA in relation to implant removal	Additional waiting time
B0	≥15 mm	48 h	0 h
B1	13,0-14,9 mm	54 h	+6 h
B2	10,1-12,9 mm	72 h	+24 h
B3	≤10 mm	78 h	+30 h

Source: Adapted from Pfeifer et al. (2015).

Other results show that FTAI in blocks has better results when hyperactivated semen is employed to reduce the interval between insemination and ovulation (Pfeifer; Oliveira Júnior and Potiens, 2019). For the application of sexed semen, the use of EC as an ovulation inducer is recommended, and the association of estrus observation with the evaluation of the follicle diameter favors fertility (Freitas et al., 2018; Nogueira et al., 2019; Pfeifer et al., 2015; Pfeifer; Oliveira Júnior and Potiens, 2019).

## 5. Diestrus and resynchronization

Serum progesterone levels and CL volume are related to the establishment of pregnancy after the FTAI protocol. Thus, some hypotheses have been proposed regarding hormonal supplementation after insemination. Couto et al. (2019) studied supplementation with long-acting injectable P4 at a dose of 150 mg and demonstrated a slight increase in the pregnancy rate when applied at 5 days after insemination. However, with the application of the same dose at 11 days, this effect was not observed. At higher doses of 300 mg, Pugliesi et al. (2014) revealed that the treatment caused early luteolysis by inhibiting the LH needed for maturation and the correct establishment of the CL. To date, the feasibility of supplementation after AI has not been fully established and seems to markedly depend on the timing of application and the molecular weight of the analog used (Couto et al., 2019; Martins et al., 2017; Motta et al., 2021; Pugliesi et al., 2014; Wiltbank et al., 2014).

In addition to genetic gains, the FTAI allows an increase in reproductive efficiency by increasing service fees and concentrating conception at the beginning of the breeding season (Kasimanickam; Kasimanickam and Kappes, 2021; Sá Filho et al., 2013). To enable the best use of biotechnology, resynchronization protocols were developed for females that did not become pregnant after the first protocol. Traditional resynchronization is performed after the pregnancy diagnosis, which, due to the popularization of the B-mode ultrasound, can be easily performed between 28 and 30 days after insemination (Purcell et al., 2005; Sá Filho et al., 2014). This method allows the performance of three inseminations in 80

days; however, this period can be reduced after the protocol begins at 22 days after insemination (Sá Filho et al., 2014).

### 5.1. Color Doppler and super-early resynchronization

The development of pregnancy diagnosis with the color Doppler ultrasound mode enabled further anticipation of resynchronization (Pugliesi et al., 2017; Vieira et al., 2014). By evaluating the volume and vascularization of the CL at 21 to 22 days after insemination, pregnancy can be diagnosed with 100% sensitivity and 91% specificity using the scale proposed by Pugliesi et al. in 2017 (Figure 1). With the application of this technology, it is possible to start the protocol between 13 and 14 days after the first insemination and perform three inseminations in 48 days (Pugliesi et al., 2017; Vieira et al., 2014) (Figure 2). However, Vieira et al. (2014) reported early luteolysis and pregnancy losses with the use of 1.5 mg EB in the resynchronization protocol. In a recent study, Palhão et al. (2020) found that the 1-mg dose did not have the same effect, demonstrating that the technique can be safely used for resynchronization.

## 6. Anestrus and fertility reduction

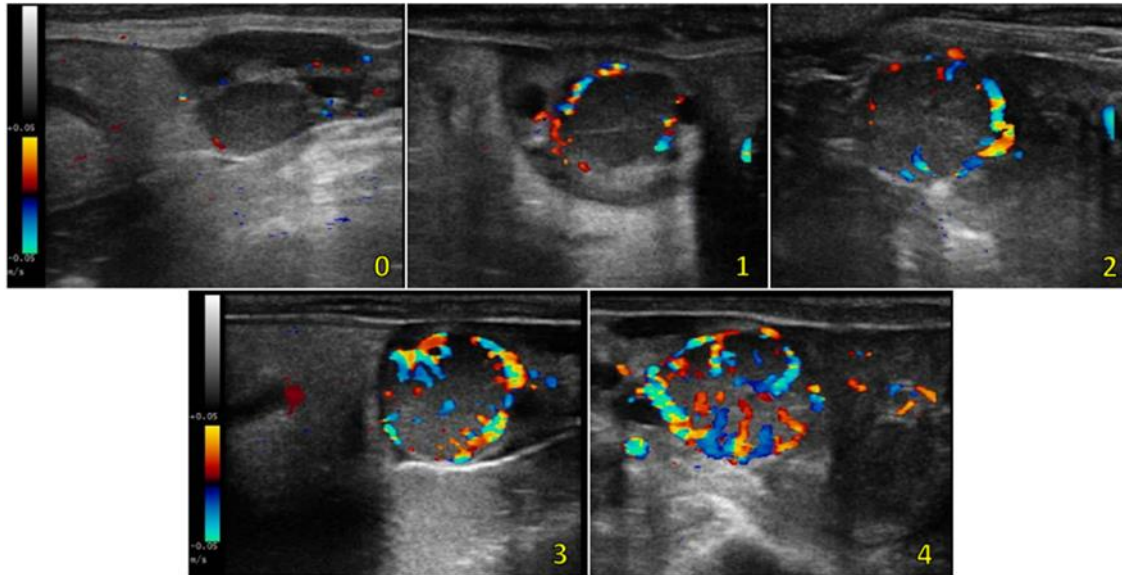
There are several causes of anestrus in bovine females. Postpartum anestrus represents one of the biggest challenges for beef cattle raising, mainly due to endocrine imbalance in terms of adequate LH secretion in this period, which is considered the main factor for anestrus in *Bos indicus indicus* cattle (Baruselli et al., 2004). Even with a hormonal protocol for FTAI, fertility can be affected if hormonal adjustments or management practices are not performed appropriately.

### 6.1. Fertility enhancement strategies for cows in anestrus

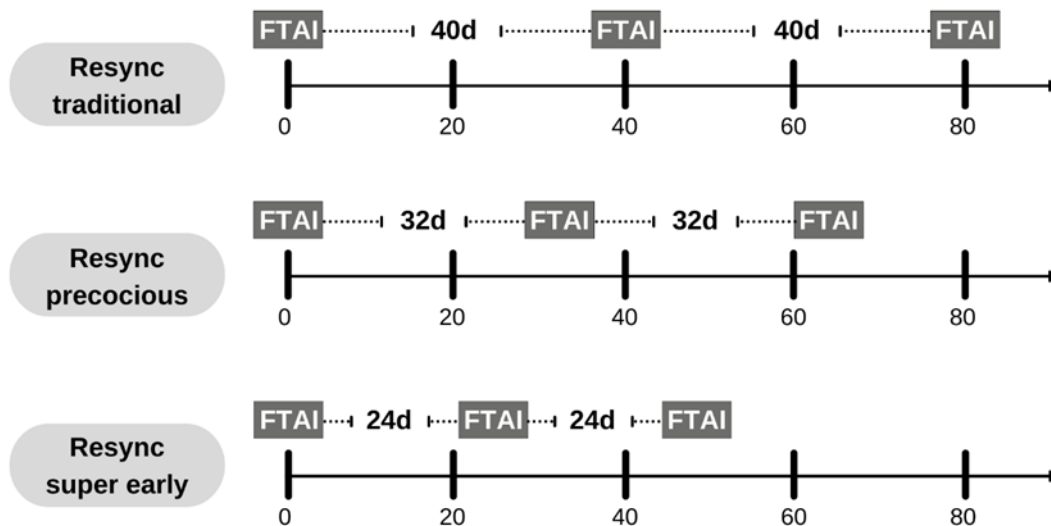
The administration of eCG and temporary calf removal (TCR) are strategies that can provide adequate gonadotropin support for the growth and induction of the dominant follicle in females destined for FTAI (Yavas and Walton, 2000). These practices are commonly associated with FTAI protocols and improve follicular activity and lead to a consequent increase in fertility in postpartum cows (Barreiros et al., 2014).

The effect of TCR and eCG administration on the conception rate in FTAI has already been tested. Ereno et al. (2007) found that *Bos indicus* cows with high anestrus rate showed a 22% increase in conception rates when exposed to TCR compared to unexposed animals. After the FTAI programs, females that do not become pregnant can undergo hormonal treatment-induced

cyclicity, resulting in pregnancy rates of approximately 60 to 65% in the first 45 days of the breeding season (Baruselli et al., 2004).



**Figure 1.** Scale for evaluating the bovine ovary at 21 to 22 days after insemination using B-mode and color Doppler ultrasound proposed by Pugliesi et al. (2017). 0 and 1: non-pregnant animals; 2 to 4: pregnant animals. (Pugliesi et al., 2017).



**Figure 2.** Schematic of the fixed-time artificial insemination (FTAI) programs with different types of resynchronization.

Variables, such as BCS and postpartum period, can be used for final conception rates. For animals between 30 and 60 days postpartum, eCG administration is recommended, regardless of BCS. However, in cows with a high postpartum period and BCS < 3, the use of eCG is recommended to obtain conception rates of approximately 50% (Baruselli et al., 2004). Treatment with TCR for 56 hours was effective at increasing the conception rate of cows undergoing FTAI with BCS < 2.5 compared to untreated cows (48.2% versus 28.2%;  $P < 0.05$ ) (Peña, 2007).

Other studies have demonstrated the effect of eCG and TCR after removal of the intravaginal P4 implant on the growth of the dominant follicle in *Bos indicus* animals. Meneghetti (2001) demonstrated increased dominant follicle diameter and increased ovulation rate (85%) in Nellore cows treated with GnRH associated with 48 hours of TCR compared to cows injected with GnRH without TCR (51%). The effects on the growth of the dominant follicle may be partially related to the percentage of cyclicity and the postpartum period at the beginning of the FTAI protocol. Follicles with high maximum

diameter have been reported to promote a linear increase in both follicular diameter and conception rates in *Bos indicus* and *Bos taurus* cattle (Baruselli et al., 2018; Borsato, 2004; Sá Filho et al., 2010).

## CONCLUSION

The Fixed-Time Artificial Insemination has been highlighted as one of the most applicable biotechnologies for cattle production, driving the improvement of herds. There are many factors that influence the outcome of the FTAI; thus, different strategies can be adopted to increase pregnancy at the end of the breeding season. The preparation of the station, knowledge of the lots, and gynecological evaluation allow veterinarians and other technicians to make important decisions for the breeding season. To date, there is no single protocol that meets the needs of all herds; however, several possible adjustments can be made to better meet the needs of a lot or property. It is very important to understand the different moments of the protocol and the impact of each on the result of the breeding season.

## Conflict of Interest

Authors declare no conflict of interest.

## Author Contribution

A.C.C.S. prepared the first text. G.D.G. and A.O.S.F. contributed to the figures and references. D.N.Y. and D.V.B. performed the final adjustments. M.M.S. was the supervisor of all steps.

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