

IMPACT OF PROGESTERONE DEVICE LOSSES ON THE SUCCESS OF TAI PROTOCOLS IN BUFFALOES: CHALLENGES AND SOLUTIONS



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ABSTRACT

A study was conducted in Oliveira/MG, Brazil, during the unfavorable reproductive period, with the objective of evaluating the loss of intravaginal progesterone devices in FTAI protocols in buffalo species. A total of 150 females (age 3.6 to 14.6 years, mean weight of 720 kg and body score of 4.5) were randomly distributed into three groups (G1 = 35, G2 = 55, G3 = 60). All groups followed the same FTAI protocol, which included the application of 2 mg of BE (Estrogin®) on D0, removal of the implant and application of 400 IU of eCG (Novormon®) + 2 mg of PGF2 α (Lutalyse®) on D9, 1 mg of BE (Estrogin®) on D10, and AI on D11. However, high rates of device loss were observed in D2 ($p < 0.05$): G1 = 51.4%, G2 = 31.7%, and G3 = 20%. Females showed signs of discomfort, such as restlessness and increased contractions, resulting in vaginal prolapses (G1 = 5, G2 = 7, G3 = 2).

Approximately 85% of the females responded to synchronization, manifesting heat until the day of the AI. The pregnancy diagnosis, performed 50 days after TAI, indicated 11% of gestation (15/136). Device losses in buffaloes were higher than those observed in other species of production animals, as possible causes attributed to greater sensitivity of the reproductive system, increased contractions due to and the use of oxytocin pre-milking.

Palavras-chave: Buffaloes. FTAI. Progesterone.

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INTRODUCTION

In Brazil, the buffalo herd is approximately 1,300,000 head (Matos *et al.*, 2020), distributed throughout the national territory, with the population consisting of the breeds (Jafarabadi, Mediterranean, Murrah and Carabao).

Female buffaloes (*Bubalus bubalis*) are short-day, seasonal polyestrous (Hafez, 1954; Gill *et al.*, 1973; Vale, 1988; Beg and Totey, 1999; Zicarelli and Vale, 2002; Almeida *et al.*, 2015; 2017; 2020a; 2021a; Costa *et al.*, 2023), presenting reproductive characteristics that hinder their reproduction compared to females of other production species. Among these characteristics, the difficulty in identifying estrus and the ideal time for artificial insemination (AI) stand out, due to the low frequency of homosexual behavior during estrus (Baruselli, 1992) and the variable periods of acceptance of breeding (Porto-Filho *et al.*, 1999; Baruselli *et al.*, 2009; Porto-Filho *et al.*, 2014; Almeida, 2018), causing difficulty and leading to higher labor costs (Baruselli, 1996).

Almeida (2018) found that the variation of estrus in buffaloes can be from 15 to 66 days, depending on the country, season and breed. This variability can also be attributed to different climatic and management conditions (Baruselli, 1992) and/or be related to the negative energy balance "measured by the body condition score - BCS" (Almeida, 2018), especially in the postpartum period.

These factors contribute to postpartum anestrus in buffalo herds, which increases the interval between calvings and reduces reproductive efficiency, due to decreased service rates (Almeida, 2018). Still according to the author, the postpartum anestrus promotes the reduction of the number of buffaloes inseminated annually by the conventional technique.

In recent years, these reproductive barriers have been minimized with the use of ovulation synchronization and the use of various types of semen (fresh, refrigerated and frozen), which has allowed higher fertility rates to be achieved (Almeida *et al.*, 2015; 2016a; 2017; Almeida, 2018; Almeida *et al.*, 2020b; 2021; 2023a, b, c; Costa *et al.*, 2023).

With FTAI programs it is possible to inseminate a large number of females on a predetermined day and time, without the need for heat observation. In addition to enabling the insemination of a large number of females at the beginning of the breeding season, reducing the interval between calvings and allowing the disposal of females that would be empty at the end of the breeding season (Baruselli *et al.*, 2004a). This technique also allows anticipating conception and calving within the respective reproductive seasons, in

addition to increasing the probability of a new pregnancy in the subsequent season and concentrating births (Gottschall *et al.*, 2008), optimizing fieldwork and reducing costs per pregnancy achieved (Almeida *et al.*, 2021).

In buffaloes cycling during the favorable reproductive season (ERF) (autumn and winter), the synchronization of ovulation with hormonal methods (Ovsynch) has shown results of up to 50% (Baruselli, 1999; Chaikhun *et al.*, 2010; Almeida *et al.*, 2020a, 2023b), with rates higher than 60% for FTAI with frozen semen (Campanile *et al.*, 2013) and 66.7% with refrigerated semen (Almeida *et al.*, 2023b). However, according to Baruselli *et al.* (2003a), the efficacy of this protocol is compromised outside the reproductive season (spring-summer). In this context, protocols involving estradiol (E2), progesterone (P4) and equine chorionic gonadotropin (eCG) for FTAI have been developed and successfully tested in lactating buffalo females outside the breeding season (Baruselli *et al.*, 2002, 2003a; Porto-Filho *et al.*, 2004; Carvalho *et al.*, 2007; Monteiro *et al.*, 2016; Almeida *et al.*, 2017, 2020, 2021; Costa *et al.*, 2023).

After alternatives were created to solve reproductive problems in the buffalo species, such as ovulation synchronization through FTAI for favorable reproductive periods - PRF "autumn-winter - May to July" and unfavorable reproductive period - PRD "spring and summer - December to February" (Zicarelli, 2010; Almeida *et al.*, 2018) and the use of different types of semen in synchronization protocols (Almeida *et al.*, 2015; Almeida *et al.*, 2016; Almeida *et al.*, 2017; Almeida, 2018; Almeida *et al.*, 2020; 2021; 2023a,b,c; Costa *et al.*, 2023), other obstacles emerged that compromised the expected results. Among these obstacles, the loss of intravaginal progesterone implants stands out, which hinders the programming of FTAI activities, causing economic losses for the rural producer and generating environmental concerns. In view of these challenges, the present study aims to analyze the drop in the conception rate and investigate the possible causes of the losses of these devices, in addition to suggesting solutions to mitigate these problems.

MATERIAL AND METHODS

ETHICS

This study was approved by the Ethics Committee on Domestic Animals of the Federal University of Minas Gerais - UFMG (protocol CEUA UFMG 368/2015), following the ethical principles for animal experiments.

EXPERIMENT LOCATION

The experiment was carried out at the Bom Destino Farm, located in the district of Morro do Ferro in Oliveira/MG, Brazil (Latitude 20°41'45" South and Longitude 44°49'37" West), during the unfavorable reproductive period (URP, December).

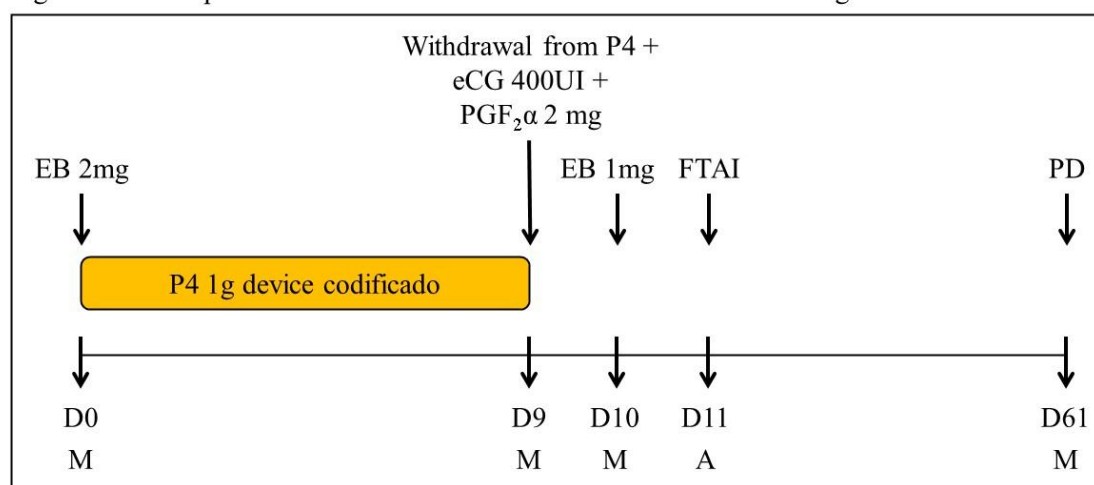
ANIMALS

A total of 150 Murrah and crossbred females (Murrah x Mediterranean), with ages ranging from 3.6 to 14.6 years, mean weight of 720 kg, BCS = 4.5 (1-5), multiparous and lactating (> 40 days postpartum) and kept in Tifton were used. (*Cynodon spp*) throughout the experiment, with free access to mineral salt and water *ad libitum*.

EXPERIMENTAL DESIGN

The animals were randomly distributed into three groups: G1 (n = 35), G2 (n = 55) and G3 (n = 60), which were submitted to FTAI on consecutive days. In the three groups, the FTAI protocol was used: D0 (8:00h), the animals received 2.0 mg i.m of BE (Estrogin®, Farmavet, SP, Brazil) and coded intravaginal implant (1st use), D9 (8:00h) removal of the implant and application of 400 IU i.m eCG (Novormon®, MSD Saúde Animal) + 2 mg of PGF₂α dinoprost i.m (Lutalyse®, Zoetis, SP, Brazil). On D10 (8:00 a.m.) 1.0 mg i.m of BE was applied (Estrogin®, Farmavet, SP, Brazil) and on D11 (2:00 p.m.) IA was performed (Figure 1).

Figure 1 – FTAI protocol for female buffaloes in na unfavorable breeding season.



Caption: A = afternoon; M = morning; D = day; EB = estradiol benzoate; P4 = progesterone; g = gram; eCG = equine chorionic gonadotropin; PGF₂α = prostaglandin; mg = milligram; FTAI = fixed-time artificial insemination and PD = pregnancy diagnosis.

Pregnancy diagnosis

The diagnosis of pregnancy was made by rectal palpation 50 days after FTAI (D61), females that allowed the visualization of the embryonic vesicle and/or confirmed with fetal heartbeats were considered pregnant.

STATISTICAL ANALYSIS

For the statistical analysis of the conception rate, descriptive analysis and the Z test ($p < 0.05$) were used.

RESULTS AND DISCUSSION

During hormonal treatment, the intravaginal progesterone implant is placed in the cranial portion of the vaginal canal and should remain for a period of between 7 and 9 days, depending on the protocol adopted. During this period, the loss of the implant has been reported by several colleagues who work with ovulation synchronization in the buffalo species, but so far, there is no scientific description of the percentage of losses and why they occur. However, it is known that device loss has been reported in a percentage between 2 to 10% in sheep (Ainsworth & Downey, 1986; Knight *et al.*, 1988, 2001 and Swelum *et al.*, 2018) and 2.5% to 5% in goats (Hashemi & Safdarian, 2017; Souza *et al.*, 2011).

The overall conception rate obtained was 11% (17/150), which is well below the expected for the conditions under which the study was conducted. One of the reasons for the poor result in the conception rate observed was the high loss of intravaginal implants of P4, which was observed in G1 = 51.4% (18/35)^a, G2 = 31.7% (19/60)^b and G3 = 20% (11/55)^b, showing a significant difference ($p < 0.05$) for the three groups evaluated. In these groups, it was also observed that the females showed signs of restlessness with the device, with an increase in contractions in an attempt to expel the device, with vaginal prolapses occurring (G1 = 5, G2 = 7 and G3 = 2), as shown in figures (2, 3, 4 and 5), and these animals were not inseminated.

Figure 2 - Buffalo trying to expel P4's device in the post-milking rest paddock.



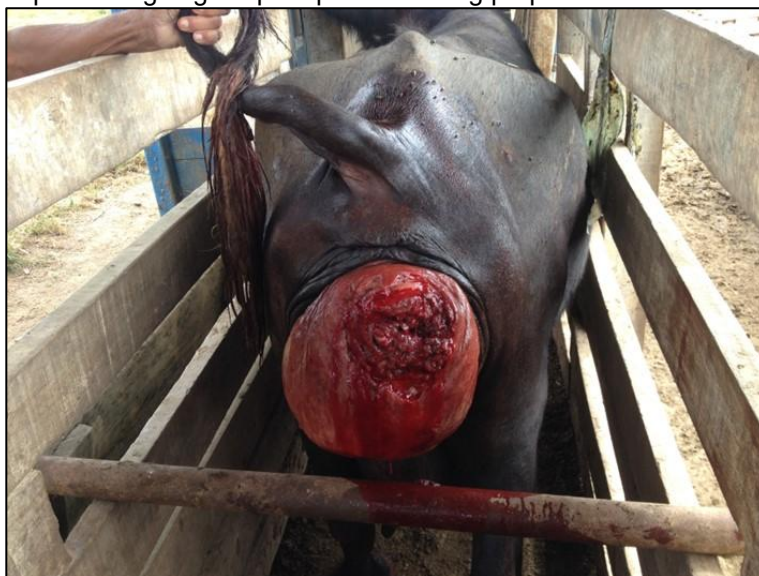
Figure 3 - Restless buffaloes, with contraction, forcing to expel the progesterone implant and presenting vaginal prolapse.



Figure 4 - Buffalo in idleness with contraction, forcing to expel the progesterone implant and presenting vaginal prolapse.



Figure 5 - Buffalo presenting vaginal prolapse and being prepared to receive surgical correction.



Among the animals that remained with the intravaginal device until the day of removal, 68.0% (102/150), 85.0% had heat with mucus on the day of TAI.

DISCUSSION

In recent years, FTAI has been widely adopted by buffalo technicians and producers due to its advantages. The technique makes it possible to anticipate conception and childbirth in the reproductive seasons, in addition to increasing the chance of a new pregnancy in the following season and concentrating births (Baruselli *et al.*, 2004b). In addition, animals that did not become pregnant during the ovulation synchronization

program and artificial insemination returned to earlier cyclicity, which speeds up subsequent reproduction and reduces costs for the producer (Almeida *et al.*, 2021).

Pharmacological protocols for synchronizing the estrous cycle aim to adjust the luteal phase, follicular growth, and ovulation, enabling FTAI even in animals without signs of estrus or cyclicity (Almeida, 2018). However, Almeida *et al.* (2015) when synchronizing buffaloes in an unfavorable reproductive period (PRD - acyclic animals), using protocols with BE, P4, eCG and PGF2 α , they obtained a pregnancy rate of 11%. According to the researchers, the low pregnancy rate observed suggests that, despite exogenous hormonal stimulation, a significant part of the buffalo females remained with a pronounced reproductive seasonality.

Almeida *et al.* (2017) report that, subsequently, on the same property and using the same synchronization protocol for the PRD, they achieved a design rate of more than 50%. However, it was observed that even before the beginning of the protocol, the buffaloes had 57.0% of seasonal anestrus.

Factors such as low BCS, malnutrition, heat stress, inappropriate use of hormones (in terms of dosage, route of application and specific concentrations for each animal category), in addition to the lack of qualification and commitment of the professionals involved, can influence the response of buffalo females to synchronization protocols. It is known that a percentage of buffalo females do not respond to ovulation synchronization protocols, with variations between 15% and 30% (unpublished data), depending on factors such as breed, category, BCS (Almeida, 2018), geographic location "in relation to the equator" Garcia (2006), with latitude being a determinant in the reproductive behavior of this species (Vale and Ribeiro, 2005) and seasonality (Hafez, 1954; Gill *et al.*, 1973; Vale, 1988; Beg and Totey, 1999; Zicarelli and Vale, 2002; Almeida *et al.*, 2015; 2017; 2020c; 2021a; Costa *et al.*, 2023), nutrition, climatic conditions, and proper management (Baruselli, 1992).

During the conduct of this study, it was observed that several females showed signs of restlessness due to the device, with an increase in uterine contractions in an attempt to expel the implant, which resulted, in some cases, in vaginal prolapses. The hypotheses raised to explain these episodes include: 1) the buffalo species has greater sensitivity in the reproductive system compared to other species, such as the female bovine; 2) the increase in contractions, possibly due to the administration of oxytocin before milking (in the morning and afternoon), being observed, in some cases, the application of overdose or

doses higher than those recommended by the manufacturer - a common situation in dairy farms that exploit the species using this drug; 3) the use of rigid implants with varied anatomical shapes, inadequate to the anatomy of the reproductive system of buffalo females (figure 6), which have a smaller reproductive system than that of bovine females (Vale and Ribeiro, 2005), for which most P4 implants were developed.

Figure 6 - Different anatomical shapes of progesterone implants found in the national market.



In Brazil currently, there are several P4 implants, with varying concentrations of the hormone, among them are: CIDR® 1.9g (Zoetis BR); DIB® 0.5g and 1.0g (Zoetis BR); PRIMER® 1.0g (União Química); CRONIPRES® 1.0g (Biogénesis Bagó Saúde Animal LTDA); SINCROGEST® 1.0g Ourofino Animal Health); PROCYCLE® 750mg (CEVA); PROGESTAR® 0.96g (Boehringer Ingelheim); BIPROGEST® 1.0g (Bimeda Tag); REPRO ONE® 0.5g, REPRO NEO® 1g and REPRO SYNC® 2g (GlobalGen); FERTILCARE 600® and FERTILCARE 1200® (MSD Animal Health).

The intravaginal implants currently used in Brazil were developed years ago to serve adult cows, such as dairy cows, which can weigh up to 800 kg. Considering that the average weight of Murrah, Mediterranean and crossbred female buffaloes in Brazil varies from 500 to 600 kg (Andrade and Garcia, 2005), it is possible to get an idea of the discomfort that these females could feel. In addition to the size, rigidity and varied anatomical shape of intravaginal silicone implants used in buffalo females may contribute to the loss of the devices during the ovulation synchronization period. However, in the present experiment, this was probably not the main cause of implant losses, since the

females involved had, on average, 720 kg. The most probable cause identified in this study was related to the reproductive system of the buffalo species, which is smaller than that of the bovine species, corroborating Vale and Ribeiro (2005).

In view of the above, some options have emerged to correct the problems of loss of the device and vaginal prolapse. Among them, the cut of the nylon cord (Figure 7) stands out, used to remove the device from the female's vagina at the end of the protocol, in order to minimize the discomfort caused by nylon, which frequently slips into the animal's tail. However, our group tested this technique in later experiments, and although there was a reduction in the percentage of losses, there were still cases of loss, which indicates that the problem was probably related to the size and anatomical design of the implants.

Figure 7 - Loss of conventional progesterone implants and with a cut in the nylon cord.



Another option would be the recommendation not to use oxytocin in the pre-milking of synchronized animals, as oxytocin promotes the contraction of the muscles of the uterus, which can intensify the animals' attempts to expel the P4 implants. According to Ferreira (2010), oxytocin stimulates the production of prostaglandin in the uterus, a hormone that can induce regression (lysis) of the corpus luteum formed after heat. The author suggests that the daily use of this drug should be avoided in cows, as a safety measure, at least until the 20th or 22nd day after artificial insemination, when the mechanism of blocking luteolysis by the embryo would possibly already be established. As the reproductive cycle of the female buffalo is similar to that of the bovine species, it is believed that this recommendation also applies to this species. However, dairy farmers

argue that the withdrawal of oxytocin during pre-milking would cause great damage to milk production, which leads them to be reluctant to adopt this practice.

Finally, a solution that seems to be quite effective, although there are few specific studies for the buffalo species, is the use of a smaller P4 implant. In order to offer an alternative to technicians and owners, the group of Professor Baruselli (USP) developed an intravaginal device of reduced size and with a lower concentration of P4 (Figure 8), compared to the conventional devices available in the national market (Primer PR - Agener/Tecnopec®), for Nellore breed precocinhas. This device is only 7 cm (instead of the 17 cm of the common device) and contains a lower concentration of progesterone (0.3 g, compared to 0.5 g in commonly used devices), which can result in improvements in ovulation synchronization results and the conception rate of the buffalo species.

Figure 8 - Intravaginal progesterone implantation with only 7 cm (compared to 17 cm for the conventional device).



Source: Rodrigues, C. (2018).

In addition, another benefit of the smaller implant is the reduction in the drop in food intake, a problem observed in animals that used the conventional implant, possibly due to the discomfort caused. Additionally, this new implant will certainly provide animal welfare and increase reproductive efficiency.

CONCLUSION

Despite significant advances in FTAI strategies for buffalo reproduction, there are still aspects that need further investigation, especially with regard to the adaptation of synchronization devices to the anatomical characteristics of buffaloes and to the environmental factors that impact the reproductive response. It is recommended that additional studies be carried out to explore alternatives to minimize device losses, as well as research on nutritional management and the reduction of heat stress, aiming to optimize synchronization protocols. Such investigations are essential to improve conception rates, ensure animal welfare, and promote the sustainability of production, contributing to the advancement of buffalo farming.

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CONFLICT OF INTEREST

There were no conflicts of interest for the preparation of this work.

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