

REDUCING THE TIME THE PROGESTERONE IMPLANT STAYS DOES NOT AFFECT THE REPRODUCTIVE PERFORMANCE OF EMBRYO RECEIVER HEIFERS

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ABSTRACT

The Brazilian beef production chain stands out in the global market and, as a result, the search for better production rates is constant. Reproductive biotechnologies, such as embryo transfer, foster this chain and, therefore, must be continuously improved. The objective with this study was to evaluate the reproductive performance of receiver heifers synchronized with a hormonal protocol for embryo transfer, with the intravaginal progesterone device. Crossbred receiver heifers were subjected to a synchronization protocol for embryo transfer, with the intravaginal progesterone implant remaining in place for 7 and 8 days. The manifestation of estrus, follicle and corpus luteum size, and the utilization rates, conception at 40 days and gestation at 60 days were analyzed. Reducing the time spent using the intravaginal progesterone device in the embryo receiver heifer synchronization protocol by one day did not affect the variables studied. Therefore, it is concluded that the reduction is viable, since the 7-day protocol improves the work and production schedule of the personnel involved, as it allows all stages to be carried out on weekdays.

KEYWORDS: beef cattle; cattle reproduction; embryo transfer; hormonal protocol.

1. INTRODUCTION

The beef production chain is of great importance in the international market, and places Brazil in a prominent position in the market, since the country has the largest commercial cattle herd and is the world's largest exporter (ABIEC, 2023).

In the constant search for greater efficiency in this chain, health and nutritional control, genetic improvement, and reproductive performance must be considered, and to promote reproductive efficiency, the use of reproductive biotechnologies is essential (BLONDIN, 2015). Artificial insemination (AI), embryo transfer (ET), and *in vitro* embryo production (IVEP), among other biotechnologies, are efficient commercial practices to accelerate genetic improvement and production.

Among them, ET stands out in exploring the reproductive potential of genetically superior bovine females (VARAGO et al., 2008). ET performed with embryos from *in vitro* embryo production (IVEP) involves subjecting donor cows to follicular aspiration (*ovum pick-up* - OPU) protocols, followed by *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC), at which point the structures reach the blastocyst form and are transferred to the receiver cows (ET) (SOUZA; ABADE, 2019; PAZZIM, 2021).

However, one of the most important factors to be considered for the success of ET is the quality of the receiver that will receive the embryo produced *in vitro*. To achieve this, both receiver and donors must be synchronized to present simultaneous development of the corpus luteum (CL), necessary to guarantee the viability of the transferred embryo, ensuring good results in the conception and gestation rate (PAZZIM, 2021).

The use of progestogens in protocols allows the synchronization of these females, with progesterone (P4) devices being the usual choice, which are kept in the vagina for a period of 8 to 9 days, according to the protocol used for beef cows (SANTOS, 2016).

However, reducing the implant's permanence time to 7 days may be an interesting alternative, and several studies conducted with females synchronized for Fixed-Time Artificial Insemination (FTAI) have shown that there is no negative influence of reducing the P4 device's permanence period on the fertility of beef cows (BÓ et al., 2001; YELICH et al., 2002; CHESTA et al., 2003; VASCONCELOS & SÁ FILHO, 2008). In addition to not compromising the female's performance, other benefits are pointed out, such as the reduction in time and costs with the protocol. The practical and operational advantage is also highlighted, since the 7-day protocol allows for a better workflow schedule for those involved, resulting in the distribution of the protocol stages throughout the week, without involving weekends. Although, studies on the synchronization of receivers for ET using intravaginal P4 implants for 7 days are scarce in the literature.

Aiming for more data about this subject, this project evaluated the size of the follicle and CL and the utilization, conception and pregnancy rates of receivers heifers synchronized with a hormonal protocol for ET, with the intravaginal progesterone device (P4) remaining in place for seven and eight days.

2. MATERIALS AND METHODS

The experiment was carried out at the Cesumar University School Farm - Unicesumar, Maringá, Paraná, Brazil (23°25'S, 51°57'W and altitude of 550 meters), from March to November 2022. The biotechniques used in this research were submitted for approval by the Animal Use Ethics Committee of the Maringá University Center - Unicesumar.

A total of 111 crossbred receiver heifers (Nelore x Angus) were used, kept in *Brachiaria brizantha* cv MG-5 paddocks, with mineral supplementation and water *ad libitum*, subjected to hygienic-sanitary management adopted on the property.

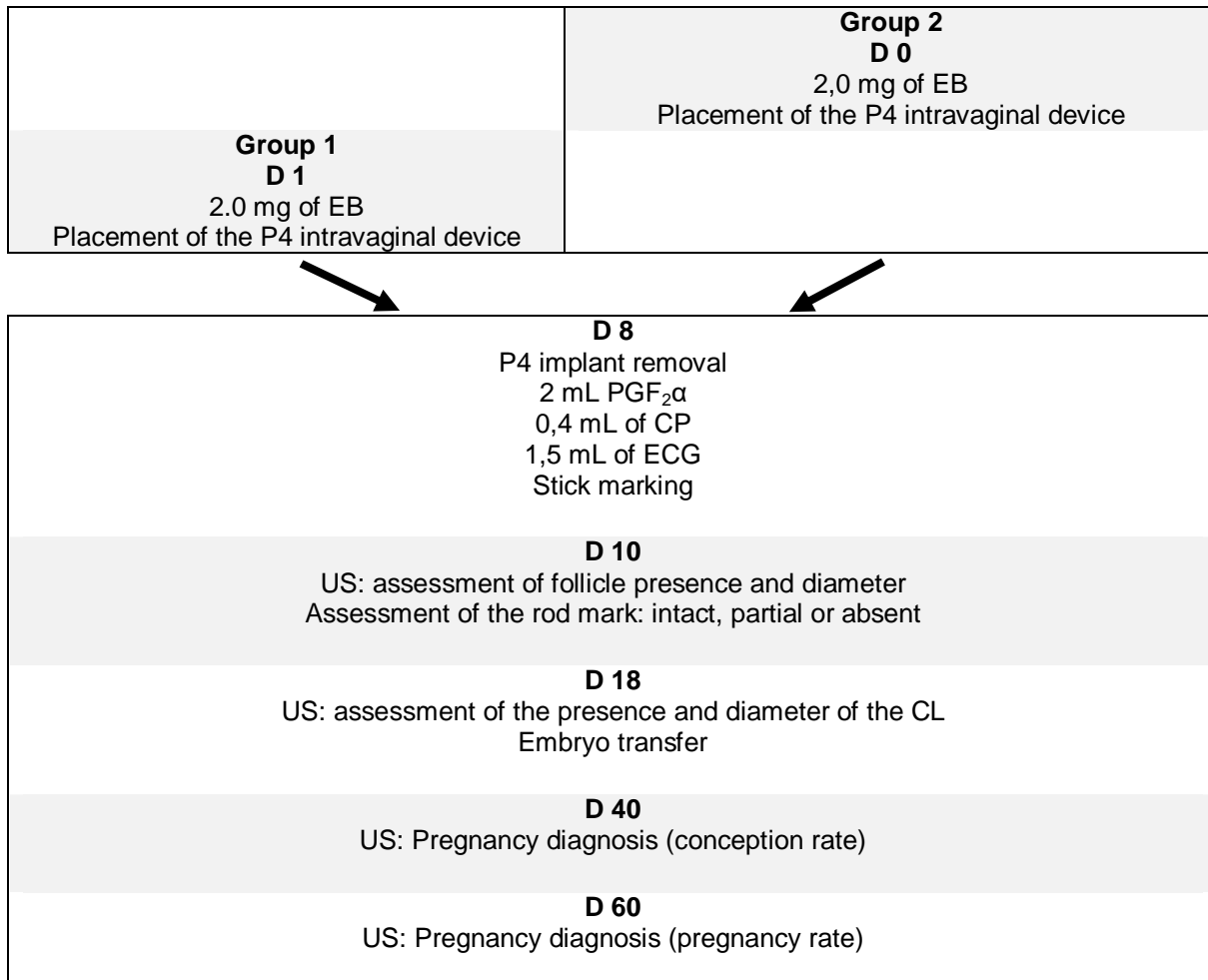
The heifers were divided into two groups:

- **Group 1:** 55 synchronized receiver heifers that received an intravaginal P4 device on D1 of the protocol - 7 days of device retention.
- **Group 2:** 56 synchronized receiver heifers that received an intravaginal P4 device on D0 of the protocol - 8 days of device retention.

In the protocol used, the receiver heifers, on a random day of the estrous cycle (Group 1 = D1; Group 2 = D0), received 2.0 mg of Estradiol Benzoate (EB) (Zoetis[®], São Paulo, SP) intramuscularly and the insertion of the multipurpose P4 device (Zoetis[®], São Paulo, SP). On day 8 (D8), 2 mL of PGF₂α (Zoetis[®], São Paulo, SP) were applied, the P4 implant was removed, 0.4 mL of Estradiol Cypionate (ECP) (Zoetis[®], São Paulo, SP) and 1.5 mL of Equine Chorionic Gonadotropin (ECG) (Zoetis[®], São Paulo, SP) were applied (Figure 1).

On day 10 (D10), the estimated day of estrus, all receivers had their ovaries assessed by ultrasound with an Ultrasound (US) device (Aloka SSD-500™) and, at this time, the diameter of the dominant follicle was identified and measured.

Figure 1. Diagram of the experimental protocol.



Source: elaborated by the authors (2024).

On day 18 (D18), all receivers had their ovaries assessed by US (Aloka SSD-500™), observing the presence and diameter of the CL and, at this time, regardless the diameter, all receivers that presented CL had an embryo transferred (ET). On day 40 (D40 –

conception rate) the first pregnancy diagnosis was performed and, on day 60 (D60 – pregnancy rate), the second, using an US (Aloka SSD-500™).

To obtain the embryos, 25 Wagyu donors were aspirated, and their oocytes were quantified, classified, matured (IVM), and fertilized (IVF) with sexed female semen from a Wagyu bull. After fertilization, the zygotes were cultured *in vitro* (IVC) and transferred to the receiver on D18 of the protocol.

The variables were analyzed using the PROC GLM procedure of the SAS® On Demand for Academics (2024) statistical program. The means were analyzed using the least squares method (LSM).

3. RESULTS & DISCUSSION

The results showed that reducing the time the P4 device remained in place intravaginally did not compromise the expression of estrus (Table 1), the diameter of the dominant follicle (Table 2) and the diameter of the CL (Table 3).

Table 1. Rate of estrus expression as a function of the time the intravaginal progesterone device (P4) remained in place, in crossbred receivers heifers (Nelore x Angus).

Variable	Stay time (days)		P value
	7	8	
Estrus expression (%)	(n=55) 98.18	(n=56) 98.21	0.9999

Table 2. Diameter of the ovulatory follicle as a function of the time the intravaginal progesterone device remained in place, in crossbred receivers heifers (Nelore x Angus).

Variable	Stay time (days)		P value
	7	8	
Diameter of the ovulatory follicle (mm) (M±MSE)	(n=55) 9.94 ± 0.41	(n=56) 9.36 ± 0.40	0.3175

Table 3. Diameter of the corpus luteum as a function of the time the intravaginal progesterone device remained in place, in crossbred receivers heifers (Nelore x Angus).

Variable	Stay time (days)		P value
	7	8	
Diameter of the corpus luteum (mm) (M±MSE)	(n=43) 18.58 ± 0.65	(n=40) 18.82 ± 0.67	0.7922

The estrus and ovulation synchronization protocols comprise three stages. The first consists of initiating a new wave of follicular growth in treated females; the second is based on reducing serum P4 concentrations, initiating the proestrus period and development of the ovulatory follicle; and the last stage of estrous cycle manipulation is the induction of ovulation of the dominant follicle (SANTOS, 2016), which, according to its development, is related to the manifestation of estrus. To evaluate the reproductive performance of lactating Nelore cows, inseminated 55 or 65 hours after removal of the P4 implant in a 7-day protocol, Ferraz Junior et al. (2023) also found no differences in the estrus detection rate.

Regarding the diameter of the ovulatory follicle, contrary to our findings, some researchers have reported that keeping the implant in place for 7 days reduces the follicular diameter. Smaller follicular diameter in the 7-day protocol compared to the 9-day protocol was observed in pluriparous Nelore cows (VASCONCELOS & SÁ FILHO, 2008), in Nelore heifers treated with CIDR[®] previously used for 18 days (MARTINS et al., 2014), and in lactating Nelore cows between 40 and 60 days postpartum subjected to FTAI (SANTOS, 2016).

Regarding the diameter of the CL, Santos et al. (2018) evaluated the effects of reducing the period of permanence of P4 implants from 9 to 7 days in an FTAI protocol and found no differences ($P>0.05$) in relation to the diameter of the CL in Nelore cows.

The utilization rate of receiver subjected to ET, with the P4 intravaginal device remaining in place for 7 and 8 days, did not differ between the groups ($P>0.05$) (Table 4). Likewise, there was no effect ($P>0.05$) regarding the time the intravaginal progesterone device remained in place on the conception and pregnancy rates of the heifers evaluated (Table 5).

Table 4. Proportion of receiver that presented corpus luteum (Utilization rate) at the time of embryo transfer according to the time the intravaginal progesterone device remained in place, in crossbred receivers heifers (Nelore x Angus).

Variable	Stay time (days)		P value
	7	8	
Utilization rate (%) (M \pm MSE)	(43/55) 78.18 \pm 0.16	(40/56) 71.43 \pm 0.19	0.7654

Table 5. Conception rate at 40 and gestation rate at 60 days, according to the time the intravaginal progesterone device (P4) remained in place, in crossbred receivers heifers (Nelore x Angus).

Variable	Stay time (days)		P value
	7	8	
Conception rate at 40 days (%) (M \pm MSE)	(19/43) 44.19 \pm 0.07	(18/40) 45.00 \pm 0.08	0.9406
Conception rate at 60 days (%) (M \pm MSE)	(17/43) 39.53 \pm 0.06	(17/40) 42.50 \pm 0.07	0.8074
Embryo losses (%) (M \pm MSE)	10.53 \pm 0.07	5.55 \pm 0.05	0.6743

Some authors suggest that young follicles may have a greater potential to secrete estradiol (BURKE et al., 2001), therefore, there is a hypothesis that reducing the time of exposure to P4 may increase the production of estradiol by the ovulatory follicle and, consequently, increase the pregnancy rate (VALDEZ et al., 2005; BRIDGES et al., 2008), a fact not observed in this study.

Results similar to this research were also reported by Vasconcelos & Sá Filho (2008), who evaluated pluriparous Nelore cows subjected to FTAI and observed that the

conception rate did not differ between treatments with 7 or 9 days of P4 device permanence. Santos et al. (2018) stated that cows subjected to the 7- or 9-days protocols had similar reproductive performance. Aiming to evaluate the reproductive performance of lactating Nelore cows inseminated 55 or 65 hours after removal of the P4 implant in a 7-day protocol, Ferraz Junior et al. (2023) did not find differences in ovulation rate and pregnancy rate, evidencing that this protocol is efficient with the implant remaining for 7 days.

4. CONCLUSIONS

Reducing the permanence time using the intravaginal progesterone device in the embryo receivers heifer's synchronization protocol by one day did not affect the reproductive performance of the females studied.

The evidence that the 7-day protocol is like the 8-day protocol allows, in addition to reduce the total time of the protocol, practical benefits, since it improves the workflow and production schedule of the personnel involved, since 7-day protocols allow all stages to be carried out on weekdays.

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