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Effects of a reusable progesterone device on conception rates and estrus cycle re-synchronization in Nelore cows

Efeito de implante de progesterona reutilizado na taxa de concepção e na ressincronização do estro em vacas Nelore

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Abstract

The objective of this study was to evaluate the reuse of an intravaginal progesterone (P4) device during diestrus of Nelore cow embryo recipients in order to improve conception rate, accelerate estrus cycle re-synchronization of non-pregnant animals, and reduce the amount of waste generated by animal breeding biotechnologies. Two experiments were performed using 268 multiparous Nelore cows. In experiment 1, all animals were subjected to a timed embryo transfer (TET) procedure, but at the time of embryo transfer, two treatment groups were established: T1 - the control treatment (N = 132) and T2 - animals receiving a second-use CIDR® device for 12 days (N = 136). Experiment 2 was performed on cows that had not remained pregnant after experiment 1 using two groups: G1 - a control group (N = 69) and G2 -re-synchronized cows that received a P4 device for 12 days for the first TET (N = 74). In experiment 1, no significant effect of the P4 treatment was observed on conception rate (T1 = 37.9%, T2 = 39.7%; P = 0.50) and corpus luteum (CL) diameter (T1 = 17.5 ± 3.4 mm, T2 = 18.1 ± 3.4 mm; P = 0.61). In experiment 2, no significant effect of the treatment was observed on conception rate (G1 = 22.2%, G2 = 35.7%; P = 0.24), recipients utilization rate (G1 = 75.4%, G2 = 70.3%; P = 0.86), and CL diameter (G1 = 17.4 ± 3 mm, G2 = 18.1 ± 3.2 mm; P = 0.27). However, the P4 treatment (for re-synchronization) significantly increased the conception rate (G1 = 22.2%, G2 = 35.7%; P = 0.04), which was similar to that in a conventional TET protocol performed with a new device (38.8%). We conclude that reusable intravaginal P4 devices can help accelerate TET protocols, suggesting an alternative application method; furthermore, this protocol may help reduce waste production in assisted animal breeding.
Key words: Cattle. Embryos. Sustainable reproductive management.

Resumo

O objetivo deste estudo foi avaliar a reutilização de um dispositivo intravaginal de progesterona (P4) durante o diestro de vacas Nelore receptoras de embriões, a fim de melhorar a taxa de concepção, acelerar a ressincronização do ciclo estral de animais não prenhes e reduzir a quantidade de resíduos gerados por biotécnicas na produção animal. Foram realizados dois experimentos com 268 vacas Nelore multíparas. No experimento 1, todos os animais foram submetidos a um procedimento de transferência de embriões

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em tempo fixo (TETF), mas no momento da transferência embrionária foram estabelecidos dois grupos de tratamento: T1 - o tratamento controle (N = 132) e T2 - animais recebendo um dispositivo de segundo uso CIDR® por 12 dias (N = 136). O experimento 2 foi realizado em vacas que permaneceram vazias após o experimento 1 usando dois grupos: G1 - um grupo controle (N = 69) e vacas ressincronizadas (G2) que receberam um dispositivo P4 por 12 dias para a TETF (N = 74) No experimento 1, não foi observado efeito significativo do tratamento P4 na taxa de concepção (T1 = 37,9%, T2 = 39,7%; P = 0,50) e diâmetro do corpo lúteo (CL) (T1 = 17,5 ± 3,4 mm, T2 = 18,1 ± 3,4 mm; P = 0,61). No experimento 2, nenhum efeito significativo do tratamento foi observado na taxa de concepção (G1 = 22,2%, G2 = 35,7%; P = 0,24), taxa de utilização de receptoras (G1 = 75,4%, G2 = 70,3%; P = 0,86), e diâmetro do CL (G1 = 17,4 ± 3 mm, G2 = 18,1 ± 3,2 mm; P = 0,27). No entanto, o tratamento P4 (para ressincronização) aumentou significativamente a taxa de concepção (G1 = 22,2%, G2 = 35,7%; P = 0,04), semelhante ao de um protocolo TETF convencional realizado com dispositivo de primeiro uso (38,8%). Concluímos que dispositivos P4 intravaginais reutilizáveis podem ajudar a acelerar protocolos TETF, sugerindo um método de aplicação alternativo; além disso, este protocolo pode ajudar a reduzir a produção de resíduos nas biotécnicas de reprodução assistida.
Palavras-chave: Bovinos. Embriões. Manejo reprodutivo sustentável.

Introduction

Brazil is one of the largest meat producers in the world (ABIEC, 2018) and due to a globally increasing demand, production efficiency must be improved. Artificial reproduction biotechnologies such as timed artificial insemination (TAI) and *in-vitro* embryo production (IVEP) are potential methods to meet the growing demand (BARUSELLI, 2016; VIANA, 2018).

Regarding IVEP, little is known about strategies to decrease the number of required recipients and the interval between ovum pick-up (OPU) and drug use. Due to variations in conception rates and the minimum required OPU interval, a large number of recipients are currently required to achieve satisfactory results (NOGUEIRA et al., 2013).

Initial embryonic deaths in recipients are important for the efficiency of large-scale timed embryo transfer (TET) programs (BINELLI et al., 2009). Among the causes of gestational losses is insufficient capacity of the corpus luteum (CL) to secrete progesterone (P4) or inefficiency of the conceptus to produce specific biochemical signals that suppress prostaglandin synthesis (BINELLI et al., 2001). Supplementing cows with P4 when a CL is present at the time of embryo transfer can increase systemic P4 concentrations, which helps establish and maintain pregnancy (MANN; LAMMING, 2001). Therefore, methods that increase circulating P4 levels in embryo recipients may reduce embryonic losses associated with an insufficiently functioning CL. At the same time, this supplementation during a certain period may prepare and synchronize recipient for a second TET due to the inhibitory action of P4 on luteinizing hormone (LH) peaks (WEHRMAN et al., 1997).

Three types of products that deliver P4 are used in synchronization protocols: subcutaneous implants, injectable solutions, and intravaginal devices which can be single- or multi-dose (reusable). Multi-dose devices can be reused three to four times which increases per-treatment profitability (MENEGHETTI et al., 2009) and decreases waste production (MAZZA et al., 2014).

In cattle, supplementation with P4 may improve conception rates, and if cows do not conceive it may improve re-synchronization for a subsequent embryo transfer. Therefore, using reusable devices in TET programs can reduce the required time and cost of this technique and decrease waste production.

The objective of this study was to assess the effect of a reusable intravaginal P4 device on conception rates and re-synchronization in Nelore cows and to examine potential economic and environmental benefits of this method.

Material and Methods

This study was conducted at the School Farm of the University Center of Maringa - Unicesumar, state of Paraná (23 ° 25'S, 51º57'W and altitude of 550 meters), from January to December 2017.

A total of 396 multiparous Nelore cows with a body condition score of 2.5-3.0 (on a scale from 1 to 5; according to Mantovani et al., 2003), an average weight of 400 kg, and aged 4 to 10 years were used approximately 50 days postpartum. The animals were kept on a *Brachiaria brizantha cv MG-5* pasture, received mineral supplementation, had access to water *ad libitum*, and were subjected to the facility's standard sanitary and hygiene management. Two experiments were conducted as follows.

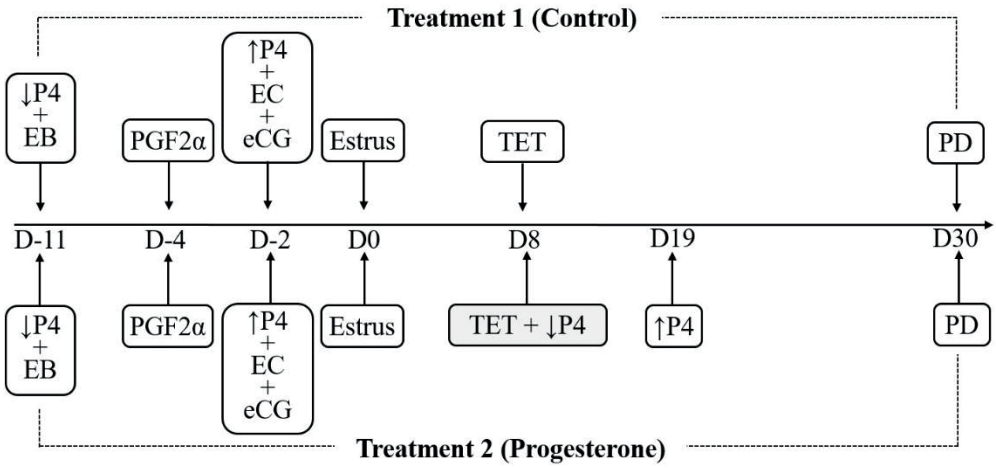
Experiment 1: Effects of reusing intravaginal P4 devices after embryo transfer on conception rates

All animals were subjected to a TET protocol, and at the time of embryo transferembryo transfer two treatment groups were established: T1 - a control group (N = 132) and T2 - animals receiving a second-use CIDR® device for 12 days (N = 136).

In T1, the recipients received a first-use intravaginal P4 device (CIDR®, Zoetis, Sao Paulo, SP) plus an intramuscular injection of 2.0 mg estradiol benzoate (BE; Gonadiol®**;** Zoetis, Sao Paulo, SP) on day -11. On day -4, 12.5 mg of the prostaglandin analog dinoprost (Lutalyse®**;** Zoetis, São Paulo, SP) was administered; on day -2, the devices were removed and the animals were intramuscularly injected with 1.0 mg estradiol cypionate (ECP®; Zoetis, Sao Paulo, SP) and 300 IU equine chorionic gonadotropin (Novormon®**;** Zoetis, Sao Paulo, SP). Day 0 was the estimated day of ovulation, and TET was performed on day 8 after identification and measurement of the CL diameter by transrectal ultrasound (Mindray DP-2500 ™, 7.5 MHz).

The treatment schemes of T2 and T1 were identical until day 0, and on the day of embryo transfer (day 8), the cows received the second-use intravaginal P4 devices which were removed on day 19 (Figure 1).

Figure 1. Treatments 1 and 2 of experiment 1 on progesterone (P4) supplementation. In both treatments, an intravaginal P4 device was inserted (↓) and 2 mg estradiol benzoate (EB) was administered on day -11; on day -4, 12.5 mg dinoprost (PGF2α) was administered; the P4 device was removed (↑) on day -2 and 1.0 mg estradiol cypionate (EC) plus 300 IU equine chorionic gonadotropin (eCG) was administered; day 0 was the estimated day of estrus; on day 8, and timed embryo transfer (TET) was performed. Animal of treatment group 2 received a second-use intravaginal P4 device at the time of the TET. Pregnancy diagnosis (PD) was performed on day 30.



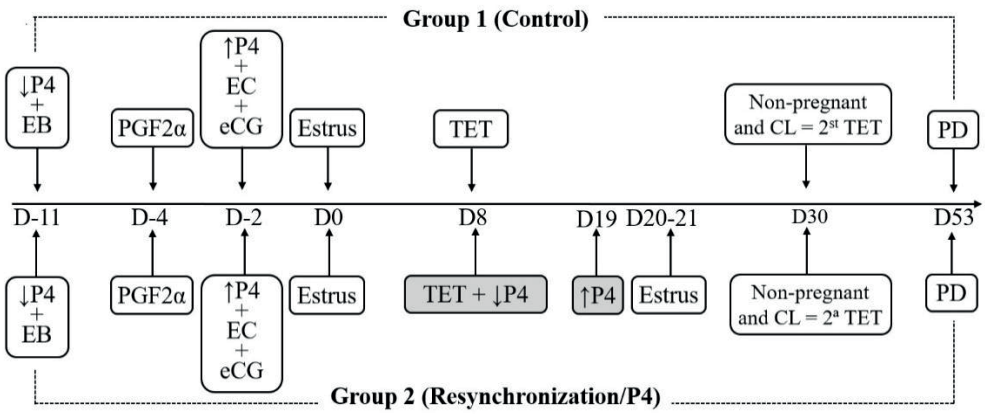
Pregnancy diagnosis was performed by transrectal ultrasound (Mindray DP-2500 ™, 7.5 MHz) on day 30, i.e., on the 22nd day after embryo transfer. All transfers were performed by one individual technician.

Experiment 2: Estrus cycle re-synchronization using reusable intravaginal P4 devices in non-pregnant recipients

Experiment 2 was performed using the non-pregnant recipients of experiment 1 to assess the use of reusable intravaginal P4 devices from day 8 to day 19 to perform a second embryo transfer on day 30.

Treatments the pattern applied in experiment 1, and the control group (G1) non-pregnant recipients from treatment 1 (N = 69) with a CL (N = 52) received a new embryo on day 30. Treatment group 2 (G2) for estrus cycle re-synchronization comprised non-pregnant recipients from treatment 2 (N = 74) with a CL (N = 52) received a new embryo on day 30 (Figure 2). Conception rates of the first and second TET were compared with respect to treatment groups. CL diagnosis and measurement were performed as in experiment 1.

Figure 2. Estrus cycle re-synchronization of recipients in groups 1 and 2 of experiment 2. The control group were not pregnant on day 30 and showed a corpus luteum (CL). Group 2 (re-synchronization) comprised non-pregnant animals and with a CL after treatment 2 of experiment 1 that received a reusable intravaginal progesterone (P4) device.



Pregnancy diagnosis was performed by transrectal ultrasonography on day 53, i.e., 23 days after the second TET. On the same day, the CL diameter was measured and categorized (08.1-14.0 mm, 14.1-17.0 mm, 17.1-20.0 mm, and 20.1-28.7 mm), and the ovarian side (right or left) was recorded.

In-vitro embryo production (IVEP)

The ovum pick-up (OPU) procedure was performed using an ultrasound device (Aloka SSD-500r) equipped with a 5-mHz micro-convex transducer (UST 974-5) and fitted to an aspiration apparatus specific to the bovine reproductive system. A 20-gauge needle was connected to a 50 mL Falcon tube via an aspiration system (Cook VBOA 18L). Negative pressure was produced using a Cook V-MAR 5000 pump and was adjusted to 38-45 mmHg with a flow rate of 12 mL medium/minute. Oocytes were aspirated using a solution containing 2.0% fetal bovine serum (Nutricell®), 25 IU sodium heparin/mL (Liquemine®), and 98.0% phosphate-buffered saline (Nutricell®).

Low epidural anesthesia was performed using 5 mL lidocaine (2%; Lidovet®, Bravet, Rio de Janeiro, Brazil) to prevent discomfort and peristaltic movements. Then, the transducer was inserted to the bottom of the vaginal fornix and was positioned by transrectal manipulation. The ovary was positioned for good visualization of the follicles. Aspirated follicles were positioned at the puncture line visualized by ultrasound until all suitable follicles were aspirated from both ovaries.

Oocytes were quantified and classified as either viable or non-viable. Viability was considered according to the presence of cumulus and homogeneity of the ooplasm, and oocytes were considered non-viable when they were naked or picnotic, heterogeneous, and showed apoptotic vesicles (LONERGAN et al., 2011).

Maturation was performed using TCM 199 containing Earle's salts, glutamine, and $NaHCO_3$ and supplemented with 10% fetal bovine serum, pyruvate (22 µg/mL), gentamicin (50 µg/mL), FSH (0.5 µg/mL), LH (50 µg/mL), and estradiol (1 µg/mL) at 39 °C and 5% CO_2 in air with maximum humidity for 22 to 24 hours. The oocytes were placed in 90 µL droplets of maturation medium and were covered using mineral oil.

In-vitro fertilization (IVF) was performed in 100 µL TALP medium supplemented with heparin (10 µg/mL), pyruvate (22 µL/mL), gentamicin (50 µg/mL), bovine serum albumin (no fatty acids), and PHE solution (2 µM penicillin, 1 µM hypotaurine, and 0.25 µM epinephrine). For IVF, frozen semen of one individual Wagyu bull was thawed in a water bath at 35 °C. To select mobile sperm cells and remove dilution medium and seminal plasma, Percoll gradient centrifugation (90 and 45%) was performed for 20 minutes, using a concentration of 1×10^6 sperm/mL. Oocytes were transferred to the droplets (20 oocytes per drop), where they were kept for 15-18 hours at 39 °C and in a gaseous atmosphere with 5% CO_2.

After fertilization, the zygotes were cultured in vitro in synthetic oviduct fluid supplemented with SFB on a granulosa cell monolayer. Cultivation was carried out for 15 hours post-insemination in an incubator and a gaseous atmosphere with 20% CO_2 and maximum humidity.

After 48 hours, the cleavage rate was assessed, and the culture medium was renewed. At this point, two-, four-, and eight-cell embryos were observed. Seven days after fertilization, the embryos were evaluated, placed in 0.25-mL straws and were transferred to the recipients. All media were prepared in-house.

Statistical analyses

The effect of P4 treatments on CL diameters was tested by fitting a generalized linear model using the treatment group as a fixed effect and all other variable as covariates. When a significant effect was found, means were compared using a Tukey's test. Differences in conception rates and treatment groups (control and P4) were analyzed by fitting a binary logistic regression model using the group as a fixed effect and the other variables as covariates. In both model types, all potential effector variables and possible interactions were considered. For descriptive analyses, quantitative data are shown as means and standard deviation (SD), and qualitative data are shown as percentages. All statistical analyses were performed using Minitab® statistical software, version 18.1. Statistical significance is reported at $p \leq 0.05$.

Results and Discussion

In experiment 1, no significant effect of the P4 device on the conception rate was observed (Table 1).

Previous studies reported similar results - Lonergan (2011) tested the insertion of new P4 devices (CIDR®) in two simultaneous experiments. In the first experiment, the animals were received

the devices after TAI from day 3 to day 6 (N = 64), and in the second experiment, the devices were used from day 4 to day 8 (N = 64). The author found an increase in serum P4 concentration one day after insertion of the device, and an increase in the embryonic survival rate during the administration period was observed in both groups; however, after implant removal, no difference in embryonic survival rates was observed.

Table 1. Conception rate of cows that received intravaginal progesterone devices at the time of timed embryo transfer and of the control group.

Animals (N)	396		
Utilization rate (N)	67.7% (268)		
	Groups		
	Control - T1	**Progesterone - T2**	**P-value**
Recipients with CL (N)	132	136	
Conception rate (n)	37.9% (50)	39.7% (54)	0.50

Machado et al. (2013) used Nelore cattle and observed no effect of one or two P4 implants (Norgestomet) from day 12 to day 21 after TAI. Similarly, Pugliesi et al. (2014) tested the effect of 150 mg of long-acting injectable P4 administered on day 4 after TAI using a treatment group (N = 390) and a control group(N = 393), and no significant effect on pregnancy rates was observed.

Sampaio (2013) tested the effect of 600 mg of injectable P4 three days after TAI in order to improve pregnancy rates, however, no significant effect was observed. Sala et al. (2014) found that in Nelore cows, P4 supplementation over a period of 16 days from 5 days after TAI did not affect pregnancy rates. Similarly, Cavichiolli et al. (2016) found no difference in pregnancy rates in postpartum Nelore cows after insertion of P4 devices (CIDR®) from day 7 to day 21 after TAI.

Considering the conception rate in relation to the CL diameter at the time of TET, no effect of P4 supplementation was observed in the present study (Table 2).

Table 2. Conception rates according to the size category of the corpus luteum diameter (CL) at the time of embryo transfer in progesterone-treated Nelore cows and in controls.

CL diameter (mm)	**N**	**Conception rate (%)**	
		Control group	**Progesterone treatment**
From 08.1 to 14.0	31	25.0	46.7
From 14.1 to 17.0	86	36.7	38.4
From 17.1 to 20.0	86	41.0	40.4
From 20.1 to 28.7	65	43.3	37.1

P4 is a hormone produced by the CL and is crucial to maintain pregnancy. The initial objective of this study was to artificially increase P4 concentrations in cows with insufficient P4 production; however, this objective was not achieved. Pessoa et al. (2014) related CL diameters at the time of TET to

pregnancy rates, using grade I (≥ 16 mm), grade II (12 to < 16 mm), and grade III (≤ 12 mm), and found no significant difference between groups.

In contrast, Pugliesi et al. (2014) found that animals injected with P4 four days after TAI and with smaller CLs showed higher pregnancy rates. Therefore, the authors suggested that injectable P4 treatments during anestrus at an early stage of the procedure may help increase success rates.

Regarding experiment 2, no significant difference in CL diameters was observed on the day of TET and on the day of pregnancy diagnosis, and no effect of the treatment on conception rates was found (Table 3). Thus P4 supplementation during diestrus did not affect the estrus cycle in animals that received embryos but did not remain pregnant.

According to a previous study, in cyclic females that did not become pregnant after estrus synchronization, re-synchronization using P4 may induce estrus between days 17 and 24 (MACHADO et al., 2013). In contrast, Stevenson et al. (2003) observed no difference in the occurrence of estrus between 18 and 26 days after artificial insemination when animals were administered P4 and supplemented or not with estradiol cypionate between 13 and 20 days after artificial insemination. Thus, it is possible to explain the similarity in the utilization rate, which it is based on the presence of a CL at the time of pregnancy diagnosis, making the fit recipients at the time of the second TET.

Table 3. Number of non-pregnant recipients on the day of the first pregnancy diagnosis (PD), corpus luteum (CL) diameter on the day of the embryo transfer (ET) and on the day of pregnancy diagnosis, and utilization and conception rates of Nelore cows fitted with intravaginal progesterone (P4) devices and of controls.

Variables	Groups		P-value
	G1/Control	G2/Resynchronization	
Non-pregnant recipients at first PD (N)	69	74	
CL diameter on the day of ET (mm)	17.4 ± 3.0	18.1 ± 3.2	0.27
Utilization rate (n)	75.4% (52)	70.3% (52)	0.865
CL diameter on the day of PD (mm)	16.2 ± 2.4	16.5 ± 3.2	0.74
Conception rate % (n/N)	22.2% (10/45)	35.7% (15/42)	0.24%

No effect of P4 supplementation on CL diameter on the day of embryo transfer and on day of pregnancy diagnosis was observed (Table 3). We hypothesized that prolonged use of the device (13 days) associated with CL-derived P4 would produce a persistent follicle resulting in a new larger CL. Mion et al. (2017) found a positive correlation of follicular diameter at the time of TAI and CL volume after ovulation. However, Mantovani (2003) observed that P4 implants used during diestrus elicit a follicular turn-over process and prevents the formation of a persistent follicle due to inhibitory and non-regulatory effects of LH peaks, which may also explain the results of the present study.

After embryo transfer to non-pregnant females, no significant effect of P4 supplementation during diestrus on the conception rate of treated animals (35.7%) compared to control group (22.2%) (Table 3). However, females that received P4 for re-synchronization at the time of TET showed conception rates similar to those observed after the first TET in experiment 1 (35.7% and 38.8%, respectively; Table 4).

Table 4. Utilization and conception rates from Nelore cows submitted or not to resynchronization with intravaginal progesterone (P4) device at the time of timed embryo transfer (TET).

Variables	Utilization rate (n/N)	Conception rate (n)*
First TET	67.7 (268/396)	38.8% (104) [a]
Re-synchronization using P4	70.3 (52/74)	35.7% (15) [ab]
Control	75.4 (52/69)	22.2% (10) [b]
p-value	0.32	0.04

*Different superscript letters in a column indicate statistical significance.

The initial hypothesis was that P4 supplementation via an intravaginal device can synchronize estrus cycles in non-pregnant cows. It was assumed that the CL observed at the time of pregnancy diagnosis would be compatible with the age of the embryo to be transferred, which may explain the results obtained in this study.

Machado et al. (2013) found no effect of the permanence of reused implants (Norgestomet/ Crestar®) used from day 12 to day 21 after the first TAI on the conception rate from a second artificial insemination. Similarly, Stevenson et al. (2003) and Colazo et al. (2007) used P4 implants from day 13 to day 20 after TAI and found no effects on conception rates. However, regarding the results of the present study, re-synchronization using P4 during diestrus is considered beneficial, as the produced results were similar to those of conventional TET protocols (Table 4).

Apart from productivity, also economic and environmental issues must be considered in animal breeding. The reuse of intravaginal devices has been shown to increase profitability and may help reduce waste production. Roversi (2013) suggested that in less densely populated rural areas, conventional waste disposal services are sometimes not available, leading to inappropriate disposal methods. Reusing various products can be a method to increase environmental sustainability in rural areas, as it extends its useful life and, at the same time, reduces environmental pollution (MAZZA et al., 2014). Roversi (2013) also states that it is necessary to invest in environmental education, in order to reduce consumption and value the reuse of solid waste in rural areas.

Furthermore, this method may help to reduce the time needed in conventional TET protocols and use shorter transfer intervals (42 days), thereby maximizing animal use, reducing the number of recipients, and increasing conception rates.

In commercial IVEP programs, pregnancy diagnoses are performed 23 days after ET, and in non-pregnant animals a new synchronization protocol should be initiated at this point; however, the results of the present study show that it is possible to transfer embryos to non-pregnant cows which show a CL and which received P4 during diestrus. Thus, this transfer can be performed at the time of pregnancy diagnosis, and it is not necessary to induce a subsequent hormone treatment. Taken together, P4 supplementation with reusable devices during diestrus appears to be a promising method to increase profitability and sustainability.

Conclusion

Reusing intravaginal P4 devices in cows during diestrus did not affect the conception rate in Nelore embryo recipients. This strategy of re-synchronization did not affect CL diameters, utilization rates, or conception rates. We conclude that this method may help increase the productivity of established TET protocols and reduce their environmental impact.

Conflict of Interests

The authors declare no conflicts of interest.

Approval of the Bioethics and Biosafety Committee

All procedures involving animals were performed in accordance with the regulations approved by the Institutional Committee for Ethics in the Use of Animal Production (CEUA-UNICESUMAR) of the Maringá University Center, Brazil, Protocol 009/2017.

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