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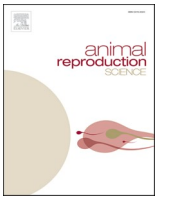

Evaluation of minimally invasive estrus synchronization protocols in brown brocket deer (*Subulo gouazoubira*)

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ABSTRACT

This study aimed to evaluate minimally invasive protocols for estrus synchronization in the brown brocket deer (*Subulo gouazoubira*). Females were submitted to Latin square design, in different treatments. All females received 0.25 mg of estradiol benzoate on the first day of treatment, concomitant with one of the following sources of progesterone: (1) DIP: an intravaginal progesterone releasing device for eight days, (2) MGA1x: once a day (in the morning) oral dose of 1 mg melengestrol acetate for eight days, (3) MGA2x: twice a day (morning and afternoon) oral doses of 0.5 mg of MGA for eight days, (4) P4LA: a single i.m. administration of 75 mg of long-acting progesterone (P4LA). Eight days after the beginning of each treatment, females received an i.m. administration of 265 μg of prostaglandin ($PGF_{2\alpha}$; cloprostenol). Treatment efficacy was evaluated by manifestation of behavioral estrus after treatment and concentration of fecal progesterone metabolites (FPM). The time to onset of estrus in treatment P4LA was significantly longer (180 ± 38.9 h) compared to DIP (63 ± 6.6 h), MGA1x (53 ± 14.4 h) and MGA2x (41 ± 10.1 h) ($P = 0.008$). According to individual baseline FPM and FPM concentration during the days after estrus, the corpus luteum formation was suggested in all females which responded to the treatments (93.75 %). Low synchrony, longer interval between $PGF_{2\alpha}$ administration and onset of estrus suggest that the P4LA dose (75 mg) is too high and not effective for *S. gouazoubira*. DIP, MGA 1x and MGA 2x, were effective in estrus synchronization.

1. Introduction

The progressive decrease in the Neotropical deer population has been occurring at a much higher rate than speciation due to environmental destruction, forest fragmentation, and poaching, which has increased homozygosity and inbreeding in isolated and fragmented populations (Sontakke, 2018; Comizzoli, Holt, 2019; Almond et al., 2020). It causes loss genetic variability and, consequently, accelerate extinction processes (Duarte, 2005). In this scenario, the development of reproductive biotechnologies for wildlife

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species is suitable for the genetic management of their populations; besides, it contributes to the germplasm banks for future conservation efforts (Roldan et al., 2006; Herrick, 2019; Rola et al., 2021).

The brown brocket deer (*Subulo gouazoubira*) (Bernegossi et al., 2022) is the most common deer species in Brazil and is classified as "least concern" by the IUCN Red List (Black-Decima and Vogliotti, 2016). It, therefore, has been used as an experimental model for reproductive biotechniques, aiming to clarify limiting reproductive aspects in Neotropical deer and contributing to their conservation (Zanetti et al., 2010). It is classified as polyestrous nonseasonal breeder with a mean estrous cycle duration of 26.9 ± 1.7 days and a mean behavioral estrus duration of 2.3 ± 0.2 days (Pereira et al., 2006). Controlling the precise timing for estrus and ovulation is essential for the success of some reproductive biotechnologies (Asher et al., 1992). Besides, estrus synchronization allows predetermining a specific interval for its manifestation, saving technician's time and effort in its detection (Hodges, 1996).

Several estrus synchronization protocols tested in deer are adaptations of protocols already used for domestic ruminants. The success rate, however, relies upon a solid understanding of basic reproductive physiology, knowledge of species-specific differences, commercially available hormones, and avoiding stress during hormonal treatment (Sontakke, 2018; Comizzoli and Holt, 2019). Intravaginal progesterone releasing device (DIP) is successfully used for estrus synchronization in several seasonal deer species from temperate regions (Asher et al., 1992; Morrow et al., 1992; Hosack et al., 1999). While for Neotropical deer species, few reports are found in the literature (Zanetti et al., 2010; Cursino et al., 2014; Galindo et al., 2015). DIP application, however, requires physical or chemical restraint (Morrow et al., 2009), which can cause stress in animals. Deer have a high susceptibility to stress, which might cause an increase in adrenal progesterone concentration and interfere with the physiological response to the hormonal treatment (Landaeta-Hernández et al., 2002; Monfort et al., 1990).

Thus, low-invasive protocols for estrus synchronization in deer species would mean its greater applicability in zoos, rescue, and conservation centers. The work of animal keepers could be facilitated and risks of animal handling, for both keeper and deer, reduced. Hence, the melengestrol acetate (MGA), a highly palatable oral progestin that does not need any type of restraint for its application, appears as an alternative for deer estrus synchronization protocols. A preliminary study in *S. gouazoubira* with the dose suggested for sheep (0.25 mg/day) resulted in a low estrus response after treatment (Orjuela, 2016), similar to the dose indicated for cattle (0.5 mg/day) (Tanaka et al., 2020). The dose of 1 mg/day, nonetheless, promoted a suppressive effect on behavioral estrus (Tanaka et al., 2020), as observed in other deer species (Raphael et al., 2003; Patton et al., 2007). On the other hand, another progesterone source studied for estrus synchronization protocols in ruminants (cows) is the long-acting progesterone (Rocha et al., 2011; Campos et al., 2016), which can be administered in a single i.m. administration, although not yet reported in deer.

A reliable method to monitor ovarian functionality is measuring cyclic fluctuations in progesterone concentrations, suggesting the presence of a functional corpus luteum (Thompson et al., 1998). Moreover, to avoid physical or chemical restraint during biological sampling due to deer stress response, non-invasive monitoring methods appear to be the best choice (Monfort, 2002). Fecal hormone analysis has been described in several deer species (Monfort et al., 1990; Knox et al., 1992; Garrot et al., 1998; Morrow and Monfort, 1998; Schwarzenberger, 2007; Christofoletti et al., 2010; Krepschi et al., 2013; Polegato et al., 2018; Abrahão et al., 2021; Toledo et al., 2023). Nevertheless, in contrast to blood samples that express the immediate concentration of the hormone, the feces of ruminants provide a pool of hormone metabolites concentration, reflecting a delay of 12–24 h (Thompson et al., 1998; Christofoletti et al., 2010; Morrow and Monfort, 1998).

The present study aimed to evaluate minimally invasive protocols for estrus synchronization in *Subulo gouazoubira* regarding the onset and duration of estrus, as well as measuring concentrations of fecal progesterone metabolites (FPM) that point out the occurrence of ovulation.

2. Materials and methods

The present study was approved by the Ethics Committee on Animal Use (CEUA) of the School of Agricultural and Veterinarian Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil (protocol number 012090/19). This is in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA).

2.1. Animals

Four adult females (Female A: unknown age, 21.4 kg; Female B: 8 years old, 19.8 kg; Female C: 5 years old, 14.8 kg; Female D: 2 years old, 14.2 kg) and one adult male (fertile, 8 years old, 19.6 kg) of *S. gouazoubira* were used in this study. All animals belong to the Deer Research and Conservation Center (NUPECCE), FCAV-UNESP and were housed in individual stalls (4.0 m × 4.0 m), preserving olfactory and auditory contact with conspecific, and exposed to natural light. The daily diet consisted of pelleted ration diet (Equitech® - Presence, Paulínia, São Paulo, Brazil) and perennial soybean (*Neonotonia wightii*), mulberry branches (*Morus alba*) and ramie branches (*Boehmeria nivea*), provided according to their availability in the field. Water was available ad libitum.

2.2. Treatments

Four treatments were randomly distributed in Latin square experimental design. Four females of the present study were submitted to four different treatments, performed in four different periods (I, II, III, and IV), with an interval of 50 days between them.

In all treatments, all females received an i.m. administration of 0.25 mg (0.25 mL) of estradiol benzoate (EB) (Sincrodiol®; Ourofino Saúde Animal Ltda., Cravinhos, São Paulo, Brazil) on the first day of treatment and a source of progesterone, as described below. An i.m. dose of 265 μg (1 mL) of cloprostenol ($PGF_{2\alpha}$) (Ciosin® - MSD Saúde Animal, São Paulo, Brazil) was administered eight

days after the beginning of treatment. Intramuscular applications were carried out in the containment box suitable for *S. gouazoubira*. Diagram of all treatments is shown in Fig. 1.

Treatment DIP (n = 4): each female received one intravaginal progesterone releasing device with 0.33 g of progesterone (CIDR-type T; Controlled Internal Drug Release, Zoetis, Campinas, São Paulo, Brazil) which remained for eight days. The device was inserted into the vaginal canal with the aid of a specific applicator after manual physical restraint of each female.

Treatment MGA1x (n = 4): once a day (in the morning) oral dose of 1 mg of melengestrol acetate (MGA®, Zoetis, Campinas, São Paulo, Brazil) (MGA) mixed with mashed banana for eight days.

Treatment MGA2x (n = 4): twice a day oral doses of MGA (0.5 mg in the morning and 0.5 mg in the afternoon) mixed with mashed banana for eight days.

Treatment P4LA (n = 4): a single i.m. administration of 75 mg (0.5 mL) of long-acting progesterone (Sincrogest injetável®, Ourofino Saúde Animal Ltda., Cravinhos, São Paulo, Brazil) on the first day of treatment.

2.3. Estrus detection and behavioral data

Behavioral estrus was identified by allowing each female in contact with an adult male. The female that remained static while the male performed mating attempts (standing estrus) was considered in estrus (Krepschi et al., 2013). Estrus detection was performed twice a day (morning and afternoon) for one month before the beginning of each synchronization protocol (data from the last natural behavioral estrus) until the end of the experiment. We maintained this management in case of inefficiency of the exogenous progesterone to inhibiting the estrus manifestation during treatments. After the first estrus detection, monitoring was performed every four hours until the last estrus detection to precisely determines the estrus duration.

2.4. Evaluation of estrus synchronization

Interval $PGF_{2\alpha}$ - estrus was considered as the interval between the administration of cloprostenol (MSD Saúde Animal) and the onset of behavioral estrus. Synchrony of each treatment was calculated considering the interval between the first and the last female to exhibit estrous behavior.

2.5. Fecal sampling, progesterone extraction and enzyme immunoassays (EIA)

Fecal samples were collected daily (at 06:00 am), from the day following the last day of behavioral estrus (D0) until nine days later. The fecal samples were frozen (−20 °C) within 20 min of collection until FPM analysis was performed. All samples were oven dried at 56 °C for 72 h (FANEM LTDA, Guarulhos, São Paulo, Brazil), then manually pulverized with a rubber mallet hammer and all solid material (e.g., dietary fiber) were removed (Yamauchi et al., 1997; Hamasaki et al., 2001). Samples FPM were extracted according to the method described by Graham et. (2001) (Graham et al., 2001). A proportion of the resulting powder (0.5 g) was extracted with 5 mL of 80 % methanol. The mixture was vortexed for 30 s at high speed, then was subjected to a mechanical shaker (Mod. AP22®, Phoenix Ltda., Araraquara, Brazil) for 12 h. After centrifugation at 400× *g* for 20 min, the supernatant was transferred into a clean tube. Aliquots of the supernatant were diluted 1:32 or 1:64 (during estrous behavior period) to 1:128 or 1:256 (a few days after behavioral estrus) for EIA analysis (Multiskan MS, Labsystem, Helsinki, Finland). The concentrations were determined using the CL425

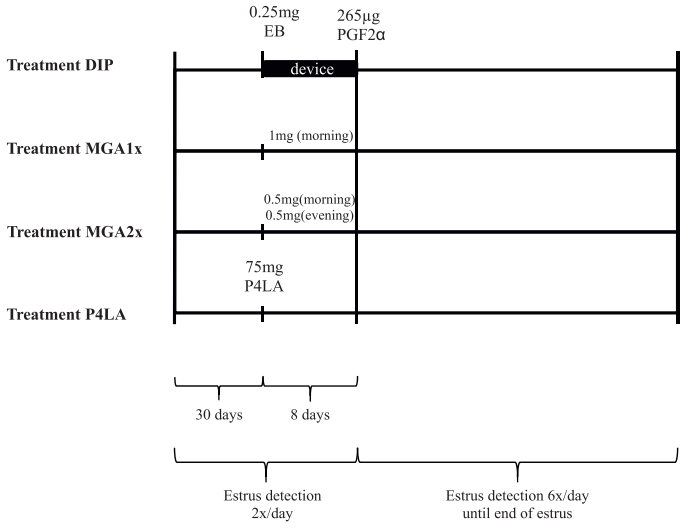

Fig. 1. Diagram of the experimental model of the four treatments in females of *Subulo gouzoubira*. EB: estradiol benzoate; $PGF_{2\alpha}$: sodium cloprostenol; DIP: Intravaginal progesterone releasing device; MGA: Melengestrol acetate; P4LA: long-acting progesterone.

antibody (California University; Davis, CA, USA) for progesterone (P4) and progestins. This antibody was chosen due to their great amount of cross-reactivity with the metabolites excreted in *S. gouazoubira* feces — 5α- and 5β-pregnanes (Polegato, 2004). According to Brown et al. (2004), validation of FPM dosages was conducted by comparison between the standard curve and the curve obtained by the pool of fecal extracts prepared by serial dilution ($y = -0.0215x + 2.5153$, $r^2 = 0.98$). Also, validation of FPM dosages was performed according to physiological relevance of the results associated to different days of the reproductive response. Inter-assay coefficients of variation for two separate controls were 4.4 % ($n = 23$ plates, 72 % of binding) and 9.5 % ($n = 23$ plates, 30 % of binding). Intra-assay coefficients were < 10 %. All fecal data are expressed on a dry-weight basis.

2.6. Statistical analysis

Interval $PGF_{2\alpha}$ – estrus and time of estrus duration were plotted in a Latin square. The effects of animal (row), period (column) and treatment were obtained using Analysis of Variance (ANOVA). The comparison between the means of each effect was obtained by the Tukey test. Baseline FPM concentrations were defined for each hind separately by taking the least FPM concentration for each treatment, similar to the data analysis described by Thompson et al. (1998). A mean and standard deviation (SD) were calculated for these resulting concentrations. Concentrations above the criterion value (mean ± 2 SD) were considered indicative of the luteal phase. To match cycles and calculate daily hormonal means (S.E.M.), D0 of each cycle was defined as the day following the last day of behavioral estrus. Female B was removed from statistical analysis in treatment 2 and Female C was removed from statistical analysis in treatment 3. All analyzes were performed using the R Software (R Core Team, 2020), adopting a significance level equal to 5%.

3. Results

The induced behavioral estrus was observed in all females (4/4) in treatments DIP, MGA2x, and P4LA. In treatment MGA1x only 75 % of females (3/4) displayed behavioral signs of estrus. Female B did not display behavioral signs of estrus or a reliable FPM profile after treatment MGA1x, therefore, she was removed from the statistical analysis. Another particularity occurred with female C, which had a "silent estrus" in treatment MGA2x, verified by FPM profile. Consequently, she was excluded of the statistical analysis in the variables of interval $PGF_{2\alpha}$ - estrus and duration of estrus (Fig. 2). FPM concentration of Female C, however, increased progressively after the end of estrus, reaching concentrations above the FPM baseline, suggesting subsequent ovulation in treatment MGA2x (Fig. 2). Interval between the administration of $PGF_{2\alpha}$ and onset of estrus was significantly higher in treatment P4LA when compared to DIP, MGA1x, and MGA2x ($P = 0.008$; Table 1). Synchrony, the interval between the first and last female displaying estrus after the end of treatment, was shorter in treatment DIP and treatment MGA2x (Table 1).

No difference was observed in duration of estrus between treatments (Table 1). Nonetheless, a significant difference between

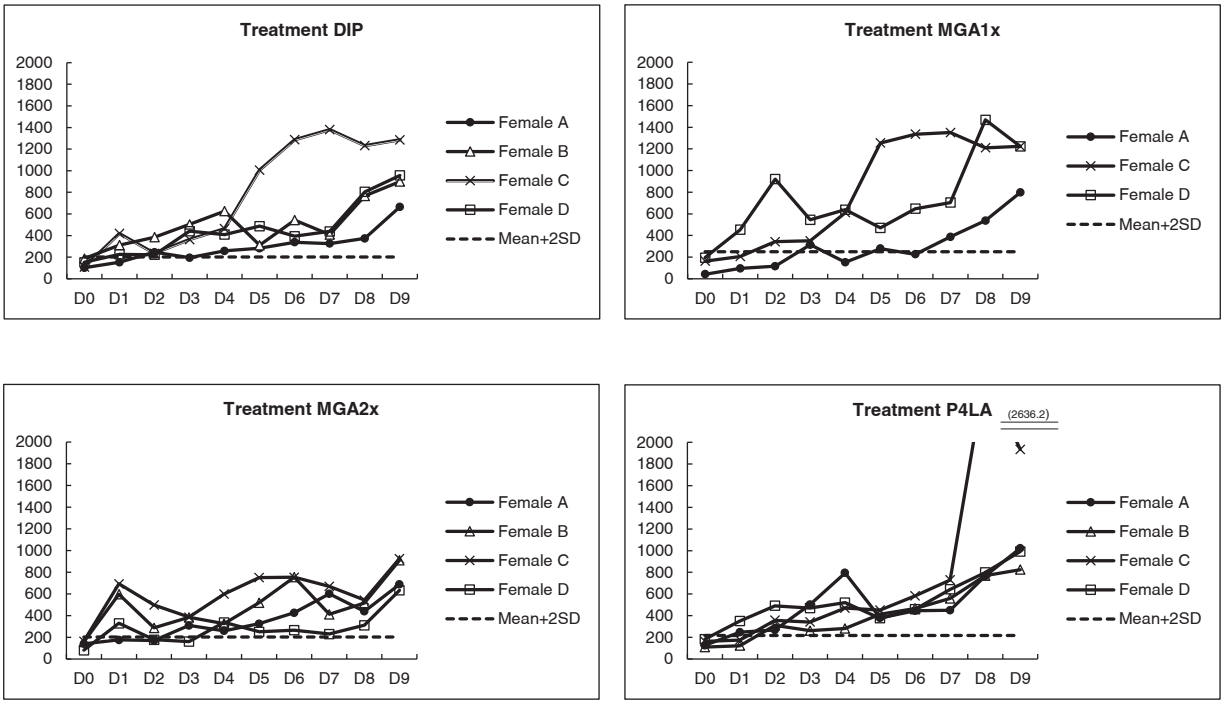

Fig. 2. Fecal progesterone metabolites concentration of four females of *Subulo gouazoubira* A, B, C, and D, from one day after the last day of estrus behavior (D0) up to nine days later (D9) Treatment DIP, Treatment MGA1x, Treatment MGA2x, and Treatment P4LA, respectively. Dashed line: FPM baseline concentration. Concentrations above this limit suggest corpus luteum formation.

females (line effect) on duration of estrus ($P = 0.001$; Table 2) was observed. Regarding duration of estrus, periods (column effect) I and II of study were significantly longer compared to periods III and IV ($P = 0.05$; Table 3).

The increasing FPM concentration per female in each treatment could be observed after the last day of behavioral estrus, with concentrations above the FPM baseline (Mean ± 2 SD). The FPM profiles characterization of females A, B, C, and D are shown in Fig. 2. Mean FPM concentration on D0 was 137.6 ± 19.8 ng/g for treatment DIP, 133.3 ± 46.8 ng/g for MGA1x, 136.3 ± 19.0 ng/g for MGA2x, and 145.6 ± 16.7 ng/g for P4LA. Mean FPM concentrations on D9 was 952.0 ± 127.9 ng/g for treatment DIP, 1078.5 ± 144.6 ng/g for MGA1x, 789.5 ± 75.2 ng/g for MGA2x, and 1193.1 ± 250.7 ng/g for P4LA.

4. Discussion

To the best of our knowledge, this is the first study comparing different doses of MGA, long-acting progesterone, and an intravaginal progesterone releasing device all in the same study for a deer species. Our results pointed out that offering the daily dose of MGA split twice promoted better synchrony of estrus among the females, similar to the DIP control group. On the other hand, the use of P4LA was not effective in estrus synchronization for *S. gouazoubira*.

Regarding the treatment DIP, all females displayed behavioral signs of estrus within a range of 49 – 81 h after the end of treatment, similar to that observed by Zanetti et al., 2010 (52 – 88 h) (Zanetti et al., 2010), with a short-term protocol (8 days). Moreover, Duarte and Garcia (1995) obtained a shorter period to onset of estrus after the end of treatment (48 – 72 h) using intravaginal medroxyprogesterone devices in a long-term protocol (14 days). Both studies also performed in *Subulo gouazoubira*. Short-term protocols can lead to a longer interval regarding the onset of estrus, as previously described in some deer species (Morrow et al., 1995; Flint et al., 1997). This interval could be associated to residues of progesterone concentration in adipose tissue after device removal (Wheaton et al., 1993) and with the follicular wave stage at the end of treatment (Roche et al., 1999). Nonetheless, short-term protocols are often used to avoid persistent growth of dominant follicle caused by long-term protocols (12 – 14 days) (Menchaca et al., 2017), which may affect the fertility rate, as observed in *Cervus elaphus* (Fennessy et al., 1990) and *Dama dama* (Morrow et al., 1995).

A daily dose of 1 mg of MGA offered once a day in estrus synchronization protocol suggests the effectiveness of this synthetic progestin in preventing behavioral estrus (Tanaka et al., 2020). Even so, a wider range of interval $PGF_{2\alpha}$ – estrus (synchrony of 60 h) was observed by Tanaka et al. (2020) compared to our study (synchrony of 48 h). Although MGA was offered to animals individually to ensure uniform consumption and avoid losses, the absorption and metabolism of this oral progestin could imply variation in circulating concentrations (Perry et al., 2005). Besides, MGA can be stored in adipose tissue and be released in different rates after finishing treatment among females, what would explain the wide range to onset of estrus (Neff, 1983; Martínez-Álvarez et al., 2007). Thus, metabolism of MGA may be related to particular factors such as age, diet, amount of food ingested, interval time between ingestions and functioning of the gastrointestinal tract (Stevens and Hume, 2004; Pereira and Polegato, 2010). Regarding this treatment, only Female B did not display behavioral signs of estrus after offering a dose of 1 mg of MGA in the morning for eight days, suggesting no

Table 1
Interval between prostaglandin administration and onset of estrus, synchrony, duration of estrus and fecal progesterone metabolites concentration on D0 and D9 of four females of *Subulo gouazoubira* in Treatment DIP (Intravaginal progesterone releasing device for eight days), Treatment MGA1x (MGA once daily for eight days), Treatment MGA2x (MGA twice daily) and Treatment P4LA (Long-acting progesterone in the first day of protocol synchronization).

Treatment	Female	Cycle day*	Time to onset on estrus (h)	Synchrony (h)	Estrus duration (h)	[P4] (ng/g feces)[1]	[P4] (ng/g feces)[2]
DIP	A	18	49	32	32	102.1	665.8
	B	13	61		40	186.6	899.7
	C	16	61		31	108.9	1286.1
	D	14	81		52	152.8	956.3
	Mean ± SEM		63 ± 6.6[b]		38.8 ± 4.9[a]	137.6 ± 19.8	952.0 ± 127.9
MGA1x	A	8	33	48	40	41.5	789.3
	B	3					
	C	13	45		24	163.3	1223.6
	D	12	81		68	195.1	1222.6
	Mean ± SEM		53 ± 14.4[b]		44 ± 12.9[a]	133.3 ± 46.8	1078.5 ± 144.6
MGA2x	A	16	33	32	36	141.0	687.8
	B	24	61		35	158.5	910.4
	C	17				164.3	926.0
	D	14	29		48	81.3	633.6
	Mean ± SEM		41 ± 10.1[b]		39.7 ± 4.2[a]	136.3 ± 19.0	789.5 ± 75.2
P4LA	A	21	285	188	56	126.1	1022.0
	B	30	165		32	110.0	824.8
	C	20	173		32	163.4	1934.0
	D	14	97		64	182.7	991.7
	Mean ± SEM		180 ± 38.9[a]		46 ± 8.3[a]	145.6 ± 16.7	1193.1 ± 250.7

Different letters within each variable indicate a significant difference ($P < 0.05$).
[a]One day following the last day of behavioral estrus (D0).
[b]Ten days following the last day of behavioral estrus (D9).
*Cycle day of each female was determined considering the date of the last natural behavioral estrus before the start of each treatment (day 0 = first day of behavioral estrus).

Table 2
Mean ± standard error of the females (A, B, C and D) in the variables PGF2α interval - estrus (hours) and duration of estrus (hours).

	Females	Mean ± SEM
Interval PGF2α - estrus (h)	A	100.0 ± 61.8[a]
	B	95.7 ± 34.7[a]
	C	93.0 ± 40.3[a]
	D	72.0 ± 14.8[a]
Duration of estrus (h)	A	41.0 ± 5.3[b]
	B	33.5 ± 1.5[bc]
	C	29.0 ± 2.5[c]
	D	58.0 ± 4.8[a]

Different letters within each variable indicate a significant difference in the Tukey test ($P < 0.05$).

Table 3
Mean ± standard error of the experimental periods (I, II, III and IV) in the variables PGF2α interval - estrus (hours) and duration of estrus (hours).

	Periods	Mean ± SEM
Interval PGF2α - estrus (h)	I	122.0 ± 54.5[a]
	II	63.7 ± 18.5[a]
	III	83.7 ± 45.0[a]
	IV	81.0 ± 29.8[a]
Duration of estrus (h)	I	47.5 ± 8.8[a]
	II	52.0 ± 12.0[a]
	III	37.3 ± 5.3[b]
	IV	36.0 ± 5.9[b]

Different letters within each variable indicate a significant difference in the Tukey test ($P < 0.05$).

response to the treatment.

According to Windorski et al. (2008), offering the daily dose of 1 mg of MGA split into two doses (morning and afternoon), could provide a lower variation of time to onset of estrus in ewes, resulting in a higher and constant concentration of progesterone in blood (Kojima et al., 2000). Our results indicate, a lower variation among females in treatment MGA2x (29 – 61 h) and, consequently, a better synchrony (32 h) when compared to treatment MGA1x. Nevertheless, no statistical difference was observed between treatments, probably due the low number of animals. After finishing treatment MGA2x, Female C did not allow the male's approach or mating, but it showed vaginal hyperemia and mucous secretion. Besides that, a progressive increasing of FPM concentration after the expected period for the manifestation of estrus was observed, suggesting a potential ovulation and subsequent formation of the corpus luteum. Hence, it was speculated that a silent estrus had occurred.

Regarding treatment P4LA, although few studies have evaluated P4LA in protocols of estrus synchronization, Campos et al. (2016) reported its effectiveness in synchronizing cows at the dose of 150 mg. This dose resulted in similar fertility rate to the device group (P4LA: 48.9 %, 22/45 and CIDR: 60.0 %, 27/45). Besides, Souza et al. (2012) observed that dose administered to beef cows is directly related to plasma P4 concentrations and duration of treatment after administration. The manufacturer dose for estrus synchronization, in bovine species, using P4LA (Ourofino Saúde Animal Ltda) is 150 mg. Nevertheless, the dose of 75 mg is the most used for estrus resynchronization in cows and estrus synchronization in heifers (Pugliesi et al., 2019; Vieira et al., 2021).

To the best of our knowledge, this is the first study using P4LA in estrus synchronization protocols for deer species; nevertheless, it should be noted that the pharmacodynamics of this exogenous progesterone is not yet fully understood. In the present study, the long time to onset of estrus after $PGF_{2\alpha}$ administration suggests that the dose of 75 mg of P4LA was too high for *S. gouazoubira*. We suggest that due to the progesterone storage in adipose tissue, described in several studies (Wheaton et al., 1993; Perry et al., 2005), the body weight ratio may be associated with the rate of steroid clearance in blood at different levels among animals (Windorski et al., 2008; Hamudikuwanda et al., 1996). It was also observed an association between female body condition with time to onset of estrus after single application of P4LA. Female A had higher body weight (21.4 kg) among females in the study and, consequently, showed the longest interval between administration of $PGF_{2\alpha}$ and estrus (288 h) for this treatment. Similarly, Female D has lower body weight (14.2 kg) and had the shorter interval $PGF_{2\alpha}$ and estrus for this treatment. Despite these considerations, data from the last day of estrus and FPM concentrations, it can be suggested that female A did not ovulate due to the presence of P4LA in the blood. According to Rodrigues et al. (2011), a considerable portion of P4 can be stored in adipose tissue and released into the blood, as body fat is mobilized in response to the negative energy balance in early postpartum in cows.

Although the use of P4LA was not effective in the present study, it should be noted that a single i.m. administration of P4LA, low animal handling and, consequently, low labor reflect its great applicability and relevance in reproductive biotechnologies for deer species. Notwithstanding, despite the limited number of animals, robust results were found with the use of P4LA. Thus, the relationship between body weight and P4LA metabolism in deer species should be further investigated and, in addition, species-specific dose adjustment should be considered.

Duration of estrus in treatments DIP, MGA2x and P4LA was similar to that described by Zanetti et al. (2010), with a duration of 24 –

52 h in synchronized females of *S. gouazoubira* and to the duration of natural estrus (12 – 54 h) described by Santos et al. (2001). These intervals, however, are shorter when compared to treatment MGA1x, with an estrus duration of 24 – 68 h, similar to that observed by Pereira et al. (2006) for the duration of natural estrus in the same species (23 – 80 h).

Periods of study I and II (winter season) had longer estrus duration compared to periods III and IV (spring season). Although *S. gouazoubira* does not have reproductive seasonality, it is known that environmental variations, nutrition, management, and humidity can affect reproductive physiology in Neotropical deer (Pereira and Polegato, 2010; Bubenik et al., 1991). In cows, heat stress is described to promote a short duration of estrus (Gwazdauskas et al., 1981; Gwazdauskas, 1985), suggesting that the increase in adrenocorticotrophic hormone would negatively influence the estrogen/P4 ratio, decreasing the expression of estrus (Landaeta-Hernández et al., 2002). According to the average temperatures during the study periods, it can be suggested that period I (Min: 12.3 °C; Max: 26.5 °C) and period II (Min: 13.8 °C; Max: 29.4 °C) presented a more pleasant natural climatic conditions than Periods III (Min: 22.4 ºC; Max: 37.9 ºC) and Period IV (Min: 19.3 ºC; Max: 31.5 ºC) (INMET, 2021). In addition to the temperature/stress factor, studies report the influence of genotype, age, and reproductive status on estrus duration in cows (Landaeta-Hernández et al., 2002). This might also explain the difference in estrus duration observed between periods in *S. gouazoubira* females in the present study ($P < 0.05$).

FPM concentrations were measured on D9 after the end of behavioral estrus in the attempt to observe a progressive increase that might suggest subsequent ovulation. Periods of behavioral estrus were associated with nadirs in FPM concentrations, as also observed by Pereira et al. (2006) and Zanetti et al. (2010). A progressive increase in FPM concentration, with concentrations above the FPM baseline, could be observed over the days after estrus, suggesting corpus luteum formation regarding all treatments (15/15). Pereira et al. (2006) observed that the mean of FPM concentrations during the luteal phase (357.3 ± 22.0 ng/g) in the natural estrous cycle of *S. gouazoubira* can reach three times the mean of the concentrations of the interluteal phase (91.3 ± 9.6 ng/g). Although the present study did not assess the entire estrous cycle period, it is possible to observe that, in treatment DIP the FPM mean concentration on D9 (ten days after behavioral estrus) was seven-fold higher than the FPM mean concentration on D0 (one day after the last day of behavioral estrus). In treatment MGA1x, the FPM mean concentration on D9 was eight-fold higher than the FPM mean concentration on D0. In treatment MGA2x, the FPM mean concentration on D9 was almost six-fold higher than the FPM mean concentration on D0. Finally, in treatment P4LA, the FPM mean concentration on D9 was eight-fold higher than the FPM mean concentration on D0.

Although treatment DIP showed high efficacy and high synchrony among females, treatment MGA2x proved to be better applicable in deer species achieving similar results. Besides, it obtained the shortest time to onset of estrus after treatment and avoided stress caused by intravaginal devices, despite its application being more labor intensive.

Due to the limited number of animals, the experimental design and the adoption of a minimally invasive protocol, it was decided not to perform ultrasound or laparoscopy to visualize the corpus luteum. The high susceptibility to stress would result in the same animals having to undergo successive anesthesias in a short period of time. Likewise, surgical procedures were unfeasible in our experimental conditions.

5. Conclusion

Treatments DIP, MGA1x and MGA2x were effective in synchronize estrus in *Subulo gouazoubira*, with corpus luteum formation evaluated by indirect fecal progesterone metabolites concentration assessment. On the other hand, the dose of 75 mg of P4LA used in treatment P4LA seemed to be too high and not effective for the species, as observed in the long time to onset of estrus and low synchrony between the females.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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