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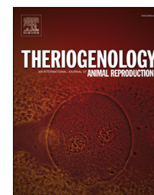

Effect of different hormonal combinations on follicular wave emergence and superovulatory response in sheep

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A B S T R A C T

The aim of the present study was to compare hormonal treatments to induce and synchronize follicular wave emergence to improve the results of superovulatory (SOV) treatments in ewes. In Experiment 1 ($n = 66$), ewes were treated with a progesterone intravaginal implant plus a $PGF_{2\alpha}$ analogue (group G_{P4}), or with the same treatment plus estradiol benzoate (G_{P4+EB}), a GnRH agonist ($G_{P4+GnRH}$), or both, estradiol benzoate and a GnRH agonist ($G_{P4+EB+GnRH}$) in a 2 × 2 factorial arrangement. Follicular wave emergence was determined by ultrasound. Follicular wave did not emerge during the studied period in 10 females (one from G_{P4}, six from G_{P4+EB} and three from $G_{P4+EB+GnRH}$). Follicular emergence was less synchronized ($P = 0.007$) when estradiol was administered (G_{P4+EB}: 103.6 ± 22.0 h), without any interaction with GnRH treatment ($G_{P4+EB+GnRH}$: 80.1 ± 21.4 h, $G_{P4+GnRH}$: 52.5 ± 8.7 h, G_{P4}: 56.6 ± 10.4 h). Estradiol administration delayed the moment of follicular emergence ($P = 0.007$) and the follicular wave emergence moment in which follicular dominance was achieved ($P = 0.009$), without interactions between estradiol and GnRH in the moment of follicular wave emergence or dominance. In Experiment 2 ($n = 22$), two SOV protocols were compared: the best treatment of Experiment 1 (G_{P4}) was used to synchronize follicular wave emergence, initiating the SOV treatment 2.5 days later; in the control treatment, SOV treatment started 80 h after a short-term protocol to synchronize ovulation ($G_{control}$). The number of corpora lutea (CL) and the evaluation of the collected embryos were performed six days after estrus. Blood samples were collected daily for plasma progesterone determination. Although the number of CL was similar in $G_{control}$ (7.1 ± 1.0) and G_{P4} (6.9 ± 5.1), the number of structures and viable embryos recovered were greater in $G_{control}$ ($P < 0.05$). The occurrence of luteal premature regression was significantly greater in G_{P4} (60%) than in $G_{control}$ (8.3%). The use of GnRH agonist alone did not improve synchronization of follicular wave emergence. When EB was used (alone or associated) follicular wave emergence was less synchronized. The SOV protocol proposed had a similar ovarian response; however, it resulted in less transferable embryos.

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1. Introduction

The manipulation of sheep reproduction allows increasing the productive results through the reproductive and productive efficiency of the herds, or by multiplying the animals with superior genotype. The sheep embryo transfer world industry is supported by *in vivo* embryo production, also known as multiple ovulation followed by embryo transfer (MOET) [1,2]. However, as there is a

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wide variability of results using the same techniques, MOET is possibly the most frustrating technology among the assisted reproduction biotechnologies. Even with the same stimulating protocol, results range from total success to total failure [3]. In this context, superovulation is the least predictable event and, thus, there is still great variability in the expected responses. It is well known that the efficiency of the superovulatory protocols vary in relation to the stage of follicular growth present in the ovary at the onset of FSH administration [4]. Although estrous synchronization facilitates and optimizes MOET results, the unpredictability of the moment in which follicular waves emerge is the main limiting factor to predict the response to superovulation [2].

The synchronization of the follicular wave before the beginning of the superovulatory treatment has improved MOET results in cattle [5,6]. In ewes, the administration of GnRH in any moment of the estrous cycle triggers a peak of LH that promotes the ovulation or the luteinization of the dominant follicles, emerging a new follicular wave two days later [7]. Indeed, studies determined that several synthetic forms of estrogens (E2) led to suppression of gonadotropins, followed then by the regression of the growing follicles, and the emergence of a new synchronized follicular wave in beef cows [8] and in anestrous ewes [9].

However, despite the numerous reports in the bovine species, there is scarce information on how to synchronize the follicular wave emergence in ewes with available pharmacological estradiol salts, and thus, applied superovulatory treatments in relation to this [2]. Thus, overall, advances in follicular wave synchronization and superovulation protocols that reduce the variation in ovulation and embryo recovery rates may contribute to an increase in the application of MOET in farm conditions. Thus, this study aimed to compare the effectiveness of four hormonal treatments to synchronize the emergence of the follicular waves based in GnRH and E2 agonists in Santa Inês sheep. A second aim was to compare the results of a traditional superovulatory treatment with a treatment initiated according to the protocol that provided the best synchronization of the follicular wave emergence.

2. Materials and methods

2.1. Ethics, experimental design, animals and facilities

The Ethical Committee for Animal Use of Universidade Federal Fluminense approved all the procedures performed in the present study (Protocol #452/13). Two consecutive studies were conducted at the Unidade de Pesquisa em Caprinos e Ovinos (Unipeco), located at Cachoeira de Macacu, Rio de Janeiro, Brazil (22°27′ S, 43°39′ W) from August of 2015 to April of 2016. Santa Inês is a sheep breed with a low degree of reproductive seasonality in this latitude [10]. In the first experiment, four pharmacological associations were applied in a 2 × 2 factorial arrangement to synchronize the emergence of follicular wave. The protocol that provided the best follicular wave synchronization was used to synchronize the emergence of the follicular wave before the beginning of a superovulatory treatment (SOV). The results were compared to a traditional SOV protocol developed by our research group [7].

Overall, 66 and 22 adult female Santa Inês ewes were used in experiments 1 and 2, respectively. The ewes were 1.5–3 years old, weighed 30–45 kg, and presented body condition score (BCS) of 2–3 (scale 1 to 5) [11]. All animals were free of any reproductive abnormality detectable by ultrasonography or clinical evaluation. All animals were allocated in collective pens, and fed with chopped grass (*Pennisetum purpureum*), sugar cane, and concentrate (12% protein and 70% TDN). Mineral salt and water were offered *ad libitum*. In the second experiment, rams (n = 3) of proved fertility were used to mate the embryo donors.

2.2. Experiment 1

2.2.1. Hormonal protocol

In a random day of the estrous cycle, an intravaginal silicone device impregnated with 0.33 mg progesterone (Eazi-Breed CIDR, Zoetis Indústria de Produtos Veterinários Ltda, São Paulo, Brazil) was inserted to all ewes. At the same moment (Day 0, D0), all ewes received 0.24 mg of cloprostenol sodium i.m (Estron, Agener, Brazil) to induce luteolysis in case a corpus luteum (CL) was present. Thereafter, the animals were randomly allocated into four groups in a 2 × 2 factorial arrangement, with two factors: the administration of E2 and the administration of a GnRH agonist, both administered on Day 0. Thus, groups were conformed as follows: 1) ewes that received no other hormonal treatment (G_{P4}, n = 15); 2) estradiol benzoate group (G_{P4+EB}, n = 18), conformed by animals that received 2.0 mg of estradiol benzoate (EB) i.m. (RIC-BE, Agener Union, São Paulo, Brazil); 3) GnRH agonist group ($G_{P4+GnRH}$, n = 16), whose females received 0.025 mg of lecilerin i.m. (Gestran Plus, Agener, Brazil); and finally, 4) a group in which both drugs were administered ($G_{P4+EB+GnRH}$, n = 17), that received 2.0 mg of EB i.m associated with 0.025 mg of GnRH i.m. The progesterone device stayed *in situ* for eight days or until a complete follicular wave (follicular emergence, selection, and dominance) was detected. A schematic representation of the experimental procedures is presented in Fig. 1.

2.2.2. Ovarian ultrasonographic evaluation

The ovaries were scanned with a B-Mode ultrasonography using a portable device (SonoScape, Modelo S6, Shenzhen, China) equipped with a 7.5 MHz transrectal transducer adapted for use in small ruminants. The examinations were performed every 24 h from D-3 to D0, every 12 h from D0 to D3, and every 24 h from D3 until the withdrawn of the progesterone devices. In each examination, the number, position, and diameter of the ovarian follicles were recorded and saved in individual computer folders.

2.2.3. End points

End points determined were: response to treatment (follicular wave emergence detected by ultrasound during eight days), wave emergence (retrospectively determined considering it as the moment at which a group of follicles started to grow and later one of them became the dominant follicle) and follicular dominance (when a follicle achieved at least 5 mm in diameter).

2.3. Experiment 2

2.3.1. Superovulatory treatment, estrus detection and mating

In this experiment, the animals were randomly allocated into two SOV groups: a group in which SOV was initiated in relation to follicular wave emergence with the treatment that provided the best synchronization in Experiment 1 (G_{P4}, n = 10) and a control group that was treated with a SOV protocol previously developed by our research group [7] ($G_{control}$, n = 12). Briefly, an intravaginal sponge impregnated with 60 mg medroxyprogesterone acetate (MAP, Progespon, Zoetis, São Paulo, Brazil) was inserted in $G_{control}$ ewes in a random day of estrous cycle, and maintained *in situ* for six days. One day before sponge removal, each ewe was administered with 300 IU equine chorionic gonadotropin (Novormon, Schering Plough, São Paulo, Brazil) and 0.24 mg cloprostenol sodium (Estron, Tecnopec, São Paulo, Brazil); 36 h after sponge removal, females received 0.025 mg of lecilerin i.m. (Gestran Plus, Tecnopec, São Paulo, Brazil). Superovulation treatment started 80 h after sponge removal. For SOV, 200 mg pFSH (Folltropin-V, Bioniche Animal Health, Ontario, Canada) were administered in six decreasing doses with a 12 h interval. A new sponge was inserted simultaneously

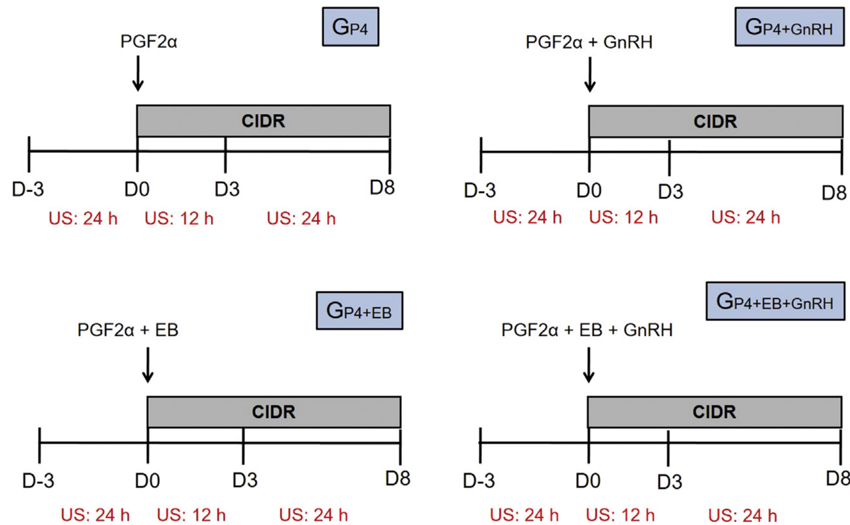

Fig. 1. Schematic representation of the experimental design in Experiment 1. Four experimental treatments were performed.
*CIDR: intravaginal silicone device impregnated with 0.33 mg progesterone; PGF2α: 0.24 mg of cloprostenol sodium i.m; EB: 2.0 mg of estradiol benzoate i.m.; GnRH: 0.025 mg of lecilerin i.m.; US: Ultrasound exams were conducted every 24 h, from D-3 to D0, every 12 h from D0 to D3, and every 24 h from D3 until the withdrawn of the progesterone devices.

with the first FSH dose, and it was withdrawn when the fifth dose was administered. Cloprostenol sodium (0.24 mg) was administered simultaneously with the sixth dose, and 24 h later the animals also received 0.025 mg of lecilerin. In G_{P4}, the follicular wave emergence was synchronized using the same protocol than in Experiment 1. At a random day of the estrous cycle, 0.24 mg of cloprostenol sodium was administered i.m. and an intravaginal implant of progesterone was inserted and kept for 4.5 days. The SOV treatment started according to the time observed in Experiment 1 of follicular wave onset, 56 h after implant insertion and was identical to that described above.

In both groups, estrus was recorded twice-daily beginning 12 h after the last pFSH dose. Sexual behavior was observed locating the ewe into a small pen with a fertile ram. It was considered that the ewe came into estrus when she accepted to be mated by the ram (estrous onset was the time from device removal to the mean time between the last moment in which the ewe was not in estrus and the first moment in which she accepted the mount). The ewe and the ram were separated after one mating. Estrus detection and mating continued twice daily until the end of estrus, i.e., when the ewe no longer accepted to be mounted by the ram.

2.3.2. *Number of corpora lutea and embryo collection*

The ovulation rate was determined six days after the beginning of estrus by laparoscopic observations, as described by Bruno-Galarraga et al. [12]. Briefly, the animals were deprived from food and water for 24 h and 12 h before the procedure, respectively, and then sedated with acepromazine maleate i.v (0.1 mg/kg, Acepran 1%, Vetnil, Louveira, São Paulo, Brazil), diazepam i.v. (0.3 mg/kg, Uni-Diazepax, União Química, São Paulo, Brazil), and morphine i.m. (0,4 mg/kg, Dimorf, Cristália, São Paulo, Brazil). General anesthesia was induced with ketamine hydrochloride i.v. (6 mg/kg, Cetamin, Syntec, São Paulo, Brazil) and propofol i.v. (1 mg/kg, Provive, Claris, São Paulo, Brazil). Anesthesia was maintained with isoflurane (Isoforine, Cristália, São Paulo, Brazil). The laparoscopic procedure was performed using a 5 mm 30° endoscope (Karl Storz, Tuttlingen, Germany) to visualize the ovaries and a babcock atraumatic forceps (33533BL Karl Storz) was used to grasp and manipulate the ovaries. The entire ovarian surface was observed for the presence of CL, which were classified according to their color as vascularized (red), or regressed (white). Other ovarian structures such as anovulatory follicles or cysts (follicular or luteal) were also recorded.

If ewes had > 3CL, embryos were surgically collected immediately after CL count according to Lima et al. [13]. Briefly, after uterus exposure, a Foley nº 8 catheter (Embramac, São Paulo, Brazil) was inserted into the uterine lumen at the lower portion of the uterine horn and the balloon was inflated to fix the catheter in position. At the upper portion of the uterine horn (near the utero-tubal junction), an 18 G catheter (BD, New Jersey, USA) was inserted into the uterine lumen. A syringe was connected to an 18 G catheter and warmed (37 °C) buffered phosphate solution was injected into uterine lumen and recovered by the Foley catheter at the lower portion of the uterine horn and stored in 100 × 20 mm Petri dishes. Each uterine horn was flushed separately with 40 mL of buffered phosphate solution. The flushing media was collected in Petri dishes observed under stereoscope microscope (20 ×) to identify the number and type of all the structures recovered. Embryos were transferred to TQC Holding Plus (Nutricell, São Paulo, Brazil) and evaluated (50 × magnify) to classify them according to their development stage.

2.3.3. *Ovarian ultrasonographic exam*

Ovaries were evaluated by B-Mode ultrasonography using the same equipment described in Experiment 1. The ultrasound observations were performed every 24 h from D-3 to D0 (in order to determine the presence of CL) and from D0 to the beginning of SOV protocol (to monitor follicular population). At the beginning of SOV treatment, ultrasound exams were conducted every 12 h (to determine the follicular population and time of ovulation). After the end of SOV treatment and the last ovulation, exams were performed every 24 h until embryo collection. In each exam, the number, position, and diameter of the ovarian follicles were recorded and stored in individual files. It was assumed that ovulation occurred when a dominant follicle was no longer viewed. The time of ovulation was considered as the average period between the last exam at which the preovulatory follicle was observed and the first exam at which it was no longer observed.

2.3.4. *Blood samples and plasma progesterone concentrations*

Blood samples were collected daily in all animals from the insertion of the first intravaginal sponge until the embryo collection. Samples were collected by jugular venipuncture using 4 mL

tubes containing EDTA with vacuum system, centrifuged at 5 °C and 1000 *g* for 15 min, and plasma was then separated and stored at −20 °C until analysis. Plasma progesterone (P4) concentration was determined by radioimmunoassay using commercial kits (MP Biomedicals, LLC, Diagnostics Division, Orangeburg, NY, USA). Sensitivity and intra-assay coefficient of variation were 0.05 ng/mL and 9%, respectively. All data were within maximum and minimum points of the curve.

2.3.5. End points

End points determined were: estrous response (number of ewes in estrus/number of treated ewes × 100); time of estrous onset; estrous length (interval from the first to last mounting); ewes that responded to SOV protocol (ewes that had > 3 CL at laparoscopy); interval from device removal to first ovulation; interval from onset of estrus to first ovulation; ovulation rate (number of CL in ewes that ovulated); number of viable, degenerated and unfertilized structures after embryo collection; rates of embryo recovery (embryos recovered/total CL counted at laparoscopy x 100), number of ewes with premature luteal regression (PLR) by laparoscopy (number of ewes with CL that regressed/total CL counted at laparoscopy x 100) and PLR by P4 concentrations (number of ewes with P4 < 1 ng/mL three days before embryo collection).

2.3.6. Statistical analysis

In both Experiments, the outcome variables were tested for normality using the Liliefors test. In Experiment 1, dispersion of the onset of the follicular wave and of the time to achieve dominance were compared with Barttlet' test. In this experiment, variables normally distributed were compared with a 2 × 2 Analysis of Variance (ANOVA) including in the model the treatments (administration of estradiol and GnRH), period of the year of the treatments, presence of CL and parity, as well as the interaction between estradiol and GnRH as main factors. Differences between means were compared by Tukey's test. Nonparametric variables were compared by the Kruskal Wallis test followed by Dunn's test. In Experiment 2, variables normally distributed were compared by ANOVA and the non-parametric variables were compared by the Kruskal Wallis test. The frequencies were compared with the Fisher exact test. The presence of CL at the beginning of the study was included in the model, and as it was not significant, was later removed. In both Experiments, alpha was considered as 5%.

3. Results

3.1. Experiment 1

The presence of an active CL at the beginning of the treatment did not affect any of the variables evaluated; regarding the non-responsive ewes, six had and four did not have a CL. Follicular emergence and achievement of follicular dominance were less synchronized when estradiol was administered ($P = 0.007$ and $P = 0.014$) without any interaction with GnRH treatment (Table 1). Estradiol administration delayed the moment of follicular emergence ($P = 0.009$) and the moment in which follicular dominance was achieved ($P = 0.014$) (Table 1). There were no interactions between estradiol and GnRH in the time needed for follicular wave emergence or dominance (Table 1).

3.2. Experiment 2

At the beginning of the study, 90.9% (20/22) of the animals had a CL. There were no differences in sexual behavior or ovulatory parameters between both groups (Table 2). Follicular population observed either at the sponge insertion (first sponge in $G_{control}$) between $G_{control}$ (7.8 ± 2.6) and G_{P4} (6.0 ± 1.6) or at the moment of administering the first pFSH dose (8.2 ± 2.6 vs. 8.9 ± 2.3 for $G_{control}$ and G_{P4}, respectively) were similar between groups. At the beginning of the SOV treatment, the presence of a dominant follicle (≥5 mm in diameter) was observed in only one $G_{control}$ ewe (8.3%) and in two G_{P4} ewes (20.0%). The presence of ovarian cyst was observed in five (41.7%) $G_{control}$ and five G_{P4} (50.0%) animals at the day of laparoscopy and embryo collection.

Embryo collection was not done in two G_{P4} and three $G_{control}$ ewes due to the low response (<3 CL). Ovulation rate was similar between groups; however, the recovery rate was greater in $G_{control}$ than in G_{P4} ($P < 0.01$; Table 3). Consequently, the number of structures and viable embryos recovered was greater in $G_{control}$ than G_{P4} (Table 3). It is noteworthy that PLR was observed laparoscopically in one $G_{control}$ and in six G_{P4} ewes ($P < 0.05$). The occurrence of this phenomenon was later confirmed by plasma progesterone concentrations and, interestingly, only five G_{P4} animals presented low P4 concentrations ($P < 0.05$; Table 3).

4. Discussion

In the first experiment, we demonstrated that the use of P4 alone was enough to synchronize follicular wave emergence in ewes, and that the addition of E2 or GnRH is not needed to improve the synchronization of its' emergence. Moreover, the administration of E2 delayed the emergence, probably as it prevents the emergence while it is acting [14]. However, in the second experiment, the SOV treatment associated to the emergence of the synchronized follicular wave ended in poorer recovery rates of viable embryos than the control treatment. Therefore, this treatment at least as applied in this study cannot be recommended for practical use in MOET application.

In the first experiment, regardless of the hormone used, follicular wave emergence was successfully induced in 84.4% (56/66) of the animals. The longer interval provoked by estrogen administration until follicular wave emergence coincides with previous reports in cattle in which EB delayed the occurrence of the FSH peak and consequently the emergence of the follicular wave [15]. Similar to what was observed in the present study, follicular wave

Table 1
Presence of corpus luteum (CL) at the beggining of treament, response and ovarian status at the emergence and dominance of first follicular wave after four hormonal treatments in Santa Inês ewes.

Parameter	G_{P4}	G_{P4+EB}	$G_{P4+GnRH}$	$G_{P4+EB+GnRH}$	EB	GnRH	EB[a]GnRH
Presence of CL (%)	53.3 (8/15)	55.5 (10/18)	50.0 (8/16)	58.8 (10/17)	ns	ns	ns
Response to treatment (%)	93.3 (14/15)	66.7 (12/18)	100.0 (16/16)	82.3 (14/17)	ns	ns	ns
Wave emergence (h)[a]	56.6 ± 10.4	103.6 ± 22.0	52.5 ± 8.7	80.1 ± 21.4	0.009	ns	ns
Dominance (h)[a]	91.7 ± 13.5	148.4 ± 25.7	86.3 ± 11.3	131.1 ± 25.5	0.014	ns	ns

Treatments: Progesterone device (0.3 g) and 0.24 mg cloprostenol associated with: 1) 1 mg estradiol benzoate (G_{P4+EB}), 2) 0.025 mg GnRH ($G_{P4+GnRH}$), 3) both estradiol benzoate and GnRH at the same dosage ($G_{P4+EB+GnRH}$) or no other treatment (G_{P4}).

[a] Data were more dispersed in treatments with than without EB ($P = 0.007$ and 0.014 respectively).

Table 2
Sexual behavior and ovulatory parameters in Santa Inês ewes after two treatments of estrus syncronization and superovulation (Mean ± SEM).

	Gcontrol (n = 12)	GP4 (n = 10)
	Mean	Mean
Estrous response (%)	100.0	100.00
Estrous onset (h)	31.2 ± 2.4	25.2 ± 1.2
Estrus duration (h)	40.8 ± 5.5	54.0 ± 2.0
Ewes that responded to SOV protocol (%)	66.7 (8/12)	80.0 (8/10)
Interval from device removal to ovulation (h)	47.0 ± 3.1	40.8 ± 2.2
Interval from estrus to ovulation (h)	13.2 ± 2.2	15.6 ± 2.4
Ovulation rate (nb of CL)	6.9 ± 5.1	7.1 ± 1.0

Treatments: Progesterone device (0.3 g) and 0.24 mg cloprostenol (GP4) or the same treatment (Gcontrol) previously described by Balaro et al. (2016) for estrus synchonization. In both, 200 mg FSH in decreasing doses were applied.
$P > 0.05$.

emergence in cattle occurred 3–5 days after estrogen treatment [5,15]. Also in anestrous ewes, it was observed that although follicular wave emergence was synchronized, it was delayed three days when 17β-estradiol was administered [9]. In this sense, it should be considered that 17β-estradiol is metabolized faster than EB, and thus, the use of EB delays more the emergence. This may also provoke a greater dispersion of the follicular wave emergence as the time of metabolization may be more dispersed between different animals. Although the application of 17β-estradiol is possibly effective, this product is not easily available in many markets, so it will not be later applied in practice. Treatment with a GnRH agonist alone was not more efficient to synchronize follicular wave emergence than control treatment. It remains to be studied if this is because the maximum response is achieved only with P4 treatments or it may be used without previous synchronization of the ovarian activity induced by P4.

In the second experiment, we compared a SOV protocol starting 2.5 days after progesterone and PGF2α analogue treatment (G_{P4}) with a SOV protocol based on "Day 0 protocol" previously described in sheep ($G_{control}$) [7]. The great advantage in the use of G_{P4}, is the shorter time spent from the first day of treatment to embryo collection. The number of animals that responded (~73%) and the ovarian responses (~7 CL) to hormonal treatment were similar between treatments, demonstrating that both were effective stimulating the follicular development and ovulation. These numbers are similar to those previously published by our group [13] and other groups using the same breed [16].

Although the ovarian response was similar, the recovery rate and thus the number of viable embryos collected with the control treatment was greater than with the new tested treatment. The lower recovery rate and number of embryos recovered in treatment associated with follicular wave onset were probably related with the high incidence of animals with PLR, a problem frequently observed in small ruminant submitted to SOV treatment [17]. In superovulated ewes, the incidence of PLR may reach even 75% of the treated animals (reviewed by Oliveira [18]). The occurrence of PLR can be related to high estradiol concentrations caused by the presence of anovulatory persistent follicles (reviewed by Rodriguez et al. [19]). However, the occurrence of these persistent follicles (cysts) at the time of embryo collection was similar between groups. This high plasmatic estradiol concentration triggers the events that leads to synthesis and release of PGF2α [17,20]. This high estradiol concentrations also promote an increased motility in the female tract, and thus, an earlier migration of the oocytes/embryos through it, with the negative consequences provoked by the inadequate environment they reach with an immature status. Although not significantly different, in the current experiment, estrus duration in G_{P4} was approximately 14 h longer than in $G_{control}$ ewes. This possibly implied that these ewes reached greater estrogen concentrations in the first group, triggering the PLR and, consequently, affecting the embryo recovery rate.

It is important to highlight that the source of progesterone used in each treatment was different: CIDR (progesterone for G_{P4}) and MAP (progestogen analogue for $G_{control}$). It is known that the analogue is more bioactive than natural progesterone [21]. Perhaps, this may have affected the negative feedback, blocking more efficiently the LH peak. It was demonstrated that the lower progesterone concentration observed in ewes with PLR negatively affects embryo quality [22], as well as embryo migration through uterine tube [23]. Similarly, the embryo recovery rate was significantly lower in ewes pretreated with CIDRs in comparison with MAP-treated animals [24].

Table 3
Embryo collection data in Santa Inês ewes after two treatments* of estrus syncronization and superovulation (Mean ± SEM).

	Gcontrol (n = 12)	GP4 (n = 10)
	Mean	Mean
Structures recovered (n)	4.8 ± 1.4[a]	0.8 ± 0.3[b]
Viable embryos (n)	3.5 ± 1.1[a]	0.3 ± 0.2[b]
Degenerated structures	0.8 ± 0.5	0.1 ± 0.1
Unfertilized oocytes	0.2 ± 0.2	0.3 ± 0.2
Recovery rate	75.6%[a]	8.1%[b]
PLRR** by laparoscopy	8%[a] (1/12)	60%[b] (6/10)
PLRR** by progesterone	8%[a] (1/12)	50%[b] (5/10)

*Treatments: Progesterone device (0.3 g) and 0.24 mg cloprostenol (G_{P4}) or the same treatment ($G_{control}$) previously described by Balaro et al. (2016) for estrus synchonization. In both, 200 mg FSH in decreasing doses were applied.
** Premature luteal regression rate (PLRR) classified according to their color in laparoscopy or by progesterone concentrations.
a,b: means within a row with different letters are different ($P < 0.05$).

5. Conclusions

The use of P4 devices associated with cloprostenol seems to be appropriate to synchronize follicular emergency in Santa Inês ewes without any benefit of adding GnRH agonists or EB. Although the SOV treatment associated to the emergence of the synchronized follicular wave promoted overall reasonable ovarian response, it adversely affected the number of embryos, possibly due to high PLR.

Acknowledgments

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