

Reproductive parameters of Santa Inês ewes submitted to short-term treatment with re-used progesterone devices

[Características reprodutivas de ovelhas Santa Inês que receberam dispositivos de progesterona reutilizados]

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ABSTRACT

The aim of this study was to verify the efficacy of reusing intravaginal progesterone (P4) devices on the reproductive parameters in Santa Inês ewes. Females received intravaginal P4 devices for their first, second or third use for five days plus 300 IU eCG IM and 5mg dinoprost laterovulvar 24h before device removal. Blood was collected at different moments. Transrectal ultrasonography was performed from device removal to ovulation. Part of the ewes were submitted to artificial insemination by laparoscopy (IAL – n=55) with fresh semen, whereas the rest were bred by fertile rams (n=41). On the initial 18 h, ewes that received devices for the first time showed higher P4 concentrations (5.1 ± 1.8 vs 3.5 ± 1.4 vs 2.4 ± 1.1 – $P < 0.05$). However, after the first 48h no difference was observed among all treatments and P4 supraluteal concentrations were detected in all ewes upon device removal. Estrous response, interval from device removal to estrus, rate of ovulating animals, number of ovulations, time from device removal to ovulation and average conception rates after IAL or natural mating were similar among all 3 groups. Intravaginal progesterone devices can be used up to three times without altering reproductive parameters in Santa Inês ewes.

Keywords: sheep, artificial insemination, CIDR, intravaginal progesterone insert, ovulation

RESUMO

Avaliou-se a eficácia da reutilização de dispositivos intravaginais de progesterona (P4) sobre características reprodutivas em ovelhas Santa Inês. As fêmeas receberam dispositivos intravaginais contendo P4 para o seu primeiro, segundo ou terceiro uso por cinco dias, associado a 300 UI eCG IM e 5mg dinoprost laterovulvar 24h antes da remoção dos dispositivos. Ultrassonografia transretal foi realizada da remoção dos dispositivos até a ovulação. Parte das ovelhas foi submetida à inseminação artificial laparoscópica (IAL – n=55) com sêmen a fresco, enquanto outra parte foi acasalada por machos férteis (n=41). Nas 18 h iniciais, as ovelhas que receberam dispositivos pela primeira vez apresentaram maior concentração de P4 (5.1 ± 1.8 vs 3.5 ± 1.4 vs 2.4 ± 1.1 – $P < 0.05$). Entretanto, após as primeiras 48h nenhuma diferença foi observada entre os tratamentos quanto as concentrações circulantes de progesterona. Foram encontradas concentrações de progesterona supraluteais em todas as ovelhas na retirada dos dispositivos. Estro, intervalo da retirada ao estro, taxa de animais que ovularam, número de ovulações, momento da retirada à ovulação e taxa média de concepção após IAL ou monta natural foram similares entre os tratamentos. Dispositivos intravaginais de progesterona podem ser usados até três vezes sem alterar as características reprodutivas de ovelhas Santa Inês.

Palavras-chave: ovino, inseminação artificial, CIDR, dispositivo intravaginal de progesterona, ovulação

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INTRODUCTION

World sheep production is undergoing important changes and in Latin-America. The research on ovine reproduction has improved both quantitatively and qualitatively since the end of the 1980's (Rubianes and Ungerfeld, 2002). The Brazilian territory consists of a wide variety of latitudes (5°16'N to 33°45'S), strongly or weakly influencing ewe reproduction. Studies have indicated that in the Southeastern region, Santa Inês ewes, a Brazilian tropical breed, shows estrus throughout the year, and are considered unseasonal (Sasa *et al.*, 2002; Coelho *et al.*, 2006), but this still does not have clear support.

Induction of synchronized estrus has often been used to optimize reproductive parameters and increase production. The most frequently used estrous synchronization protocols are based on the use of equine chorionic gonadotropin associated with progesterone or progestagen intravaginal devices (Amorim *et al.*, 2008). Regarding the duration of progestagen treatment, Viñoles *et al.* (2001) showed that traditional progestagen treatments (12–14 days) are associated with the ovulation of aged follicles and a decrease in subsequent fertility when compared to the short-term protocols (6 days). Since then, many papers were published using this method (Ungerfeld *et al.*, 2003; Dixon *et al.*, 2006).

One way to decrease costs in assisted reproduction programs is the re-use of progesterone devices which represent the highest individual cost. Controlled internal drug releasing (CIDR) is an intravaginal device that sustains sufficient concentrations of progesterone in plasma to block LH surges and prevent ovulation (Savio *et al.*, 1993) and due its physical characteristics, the CIDR device can be washed and reused. It has been reported that the conception rate decreases in relation to the time that CIDRs have been previously used in ewes (Smith *et al.*, 1991; Ungerfeld and Rubianes, 1999). However, if its reutilization efficiency could be proved, it would be a valuable management tool for farmers. For cattle, it is well known that these devices release progesterone (P4) for at least 15 days (MacMillan *et al.*, 1991) and if this could be reproduced for ewes, when working with short-

term protocols, it would mean that they might potentially be used up to three times.

The knowledge of ovarian follicular development and of the ewe's endocrinology could be essential to determine key points supporting field uses. Thus, the objectives of this study were to investigate Santa Inês seasonality, to characterize estrous response and its associated parameters, and to analyze ovulatory dynamics and the progesterone profile of ewes receiving new or reused intravaginal progesterone devices in a short-term treatment.

MATERIALS AND METHODS

This study was carried out in Cachoeira de Macacu, RJ, Brazil (latitude 22°55'67" S and longitude 42°65'86" W). The average altitude was 57m with Cwa climate, according to Köppen classification (dry winter and humid summer) with an average annual temperature from 18.0 to 23.0°C and rainfall from 2.000 to 2.600mm³.

Experiment 1 was conducted during October and November (spring; anestrus season) of the first year in order to identify the ovulatory and progesterone profiles in this breed. In the beginning of the study ewes were examined through transrectal ultrasonography (Aloka SSD 500®, Tokyo, Japan) equipped with a 5.0 MHz linear-array transducer to determine pregnancy or the existence of abnormalities of the reproductive tract. A total of 35 ewes were used (24 Santa Inês and 11 Santa Inês – Dorper crossbred) weighing on average of 44.6±8.2kg and BCS of 3.2±0.4.

Ewes were equally assigned according to breed, body weight and body condition score (BCS; 1-5 scale; Suiter, 1994) into three treatments. Females received intravaginal progesterone devices (0.33g progesterone; Eazi-Breed CIDR-G®, Pfizer Animal Health, São Paulo, Brazil) for their first (T_{1x}; n=12), second (T_{2x}; n=12) or third uses (T_{3x}; n=11). Carboxymethylcellulose gel was used on the applicator device. In all treatments, progesterone devices were maintained for five days. On the day before its removal, 300 IU eCG (Novormon 5000®, Syntex, Buenos Aires Argentina) IM and 5mg dinoprost (Lutalyse®, Pfizer Animal Health, São

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Paulo, Brazil) IM were administered. These devices had been previously used for 5 days (once-used) or 10 days (used twice for 5 days) prior to this study. Immediately after device removal, all devices were carefully washed thoroughly with water, with emphasis on removing mucus and debris. After this procedure, they were allowed to air-dry and placed in empty bags for storage at 5°C. The sheep herd used in this study was free of leptospirosis, brucellosis, toxoplasmosis, neosporosis and Maedi-Visna.

Animals were free to graze on *Brachiaria decumbens* during the day and were kept inside a barn at night, when they received *Pennisetum purpureum*, *Saccharum officinarum* and concentrates. Drinking water and mineralized salt (Purinafós®, PURINA, Paulínea, Brazil) were offered *ad libitum*.

Ovarian ultrasonography was performed in 30 ewes (10 per treatment) to determine the time, number of ovulations, and diameter of preovulatory follicles. Thus, ultrasonography examinations were performed every 12 h after device removal until ovulation by the same operator. A 5 MHz transducer (Aloka SSD 500®, Tokyo, Japan) was adapted to a PVC tube with a tape so that it could be manipulated externally into the rectum.

The animals were maintained in a standing position inside a contention pen. Fecal pellets were removed by hand and carboxymethylcellulose gel (20 mL) administered into the rectum with a syringe. The number, diameter and position of ovarian follicles ≥ 3 mm were recorded. The procedure to locate the ovaries was the same as described by Ginther and Kot (1994). The day of ovulation was defined as the day when the largest follicle, identified before, was not present anymore. The preovulatory follicle diameter was considered the last measurement obtained before ovulation.

Blood was sampled (n=11 or n=12 per treatment) through jugular venipuncture using vacuolated 5mL tubes (Becton & Dickinson Vacutainer® Systems, Americana, São Paulo, Brazil) seven days before the insertion of the progesterone device (D_{-168h}), at the time of device insertion (D₀), every 6 h after device insertion during the initial 24 h (D_{6h}, D_{12h}, D_{18h},

D_{24h}) and then every 24 h (D_{48h}, D_{72h}, D_{96h}, D_{120h}, D_{144h}, D_{168h}) for seven days. Tubes were immediately placed and maintained on ice until transported to the laboratory, when they were centrifuged at 1000 X g for 15 min and plasma was aliquoted and stored at -20°C until hormone analyses. Plasma P4 concentration was determined through the use of solid phase radioimmunoassay (RIA), using a commercial kit (Coat-a-Count® progesterone kit, DPC, Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and inter-assay coefficient of variation was 8.8% and 9.7%, respectively.

The second experiment was done in June (winter) and October (spring) of the second year, both considered anestrous season to aim for the detection of estrous behavior parameters and fertility rate. Animals were also evaluated in the beginning of this experiment. A total of 103 ewes were used (69 Santa Inês and 34 Santa Inês – Dorper crossbred) having an average weight of 41.9±9.1 kg and BCS of 3.3±0.5.

Ram ejaculates were collected using an artificial vagina and evaluated through the standard routine criteria i.e., mass movement activity, sperm concentration and live and dead sperm counts according to CBRA (Manual..., 1998). Two fertile Dorper and four fertile Santa Inês rams ageing 3-5 years were used.

Seven animals had to be removed from the experiment (See Results). Fifty-five ewes were submitted to artificial insemination by laparoscopy (Karl Storz Endoscopes GmbH & Co., Germany) with fresh semen diluted in Tris-Fructose (Evans and Maxwell, 1987) down to a concentration of 100×10^6 mobile sperms per 0.25mL straw. The procedure was performed using a 5-mm laparoscope (Karl Storz Endoscopes GmbH & Co., Germany). Semen deposition was done in the greater curvature of each uterine horn, with a 45° needle angulation, using equal parts of the straw contents on each side. One ewe from each treatment was inseminated consecutively until the last one. Thus, ewes from all treatments were inseminated with no time difference among them, varying from 50.7 to 55.2 h from device withdrawal – an average of 52.4 h for all treatments.

The remaining females (n=41) were bred with the same rams. They were taken to the male pens, in late afternoon, where they remained until the following day. This procedure began on the day of device removal and was repeated for two days. Males were marked with a solution containing soybean oil and colored powdered dye (Pó xadrez[®], Lanxess, Porto Feliz, São Paulo, Brazil) to allow the identification of bred females. Pregnancy rate was detected 45 days after AI or mating by ultrasonography.

Parameters related to estrous behavior were recorded only from natural mated ewes. The variables evaluated were: estrous response(%) – number of ewes in estrus/number of treated ewes x100; interval to estrus (hours) – interval from device removal to first mounting acceptance; estrous duration – interval from the first to last mounting acceptance (hours); interval from device removal to AI (hours); interval from device removal to ovulation (hours); ovulating animals(%) – number of animals which ovulated/number of animals evaluated through ultrasonography x 100; number of ovulations – average number of ovulations per ewe ovulated; largest and second

largest follicle diameter (mm); lost devices (%): number of devices lost per treatment; plasma P4 concentration (ng/mL); conception rate – number of pregnant ewes/number of ewes bred by natural mating or laparoscopic artificial insemination.

Statistical analysis was performed for a 95% confidence interval. Parametric variables were submitted to one-way analysis of variance and compared through the Tukey test. Non-parametric variables were analyzed with the use of the chi-square test (Ayres *et al.*, 2000). Data were analyzed using SAEG (Ribeiro Júnior, 2001).

RESULTS

In experiment I there was no difference or interaction ($P>0.05$) of ewe's breed (Santa Inês and Santa Inês–Dorper crossbred ewes) in relation to the studied traits. The ovulating animals, number of ovulations, largest and second largest follicle diameters and the interval from device removal to ovulation were similar ($P>0.05$) for all three treatments (Table 1).

Table 1. Follicle dynamics from ewes submitted to estrous synchronization receiving new or reused progesterone devices for five days (mean \pm SD)

Variable	Number of uses of the device		
	New	Once-used	Twice-used
Ovulating animals (%)	77.8 (7/9)	90.0 (9/10)	100.0 (8/8)
Number of ovulations	1.4 \pm 0.6	1.7 \pm 0.7	1.4 \pm 0.5
Interval to ovulation (h)	55.1 \pm 4.9	55.8 \pm 4.4	54.2 \pm 5.2
Largest follicle diameter (mm)	5.7 \pm 0.6	6.6 \pm 1.2	6.2 \pm 1.1
Second largest follicle diameter (mm)	5.6 \pm 0.8	5.7 \pm 1.0	6.1 \pm 1.0

No differences detected between treatments ($P>0.05$). () Number of animals.

A total of eight animals lost their devices, of which 3/12 (25.0%), 2/12 (16.7%) and 3/11 (27.3%) for T_{1x}, T_{2x}, and T_{3x}, respectively. Blood samples of these eight animals were included until the day before their devices were lost. Plasma P4 concentration of animals according to the time of blood collection is listed in Tab. 2. A total of 48.5% (16/33) of the ewes had P4 concentration superior to 1ng/mL compared to 51.5% (17/33) of them that had it below 1ng/mL in the beginning of the experiment, regardless of the treatment received (Table 2).

In experiment 2 reproductive traits of ewes after natural mating or laparoscopic artificial insemination respective to treatments of estrous induction are listed in Table 3. In this second experiment, four animals lost their devices and were removed; 2/34 (5.9%), 0/35 (0.0%), and 2/34 (5.9%) for T_{1x}, T_{2x}, and T_{3x}, respectively. Three other animals became ill and were also removed from the experiment.

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Table 2. Plasma progesterone concentration (ng/mL) from ewes submitted to estrous induction receiving new or reused progesterone devices for five days (mean±SD)

Hours	Number of uses of the device		
	New	Once-used	Twice-used
D _{-168h}	1.2 ± 1.5a (12)	1.9±2.0a(11)	1.6±1.4a(11)
D ₀	2.2 ± 1.8a (12)	1.6±1.3a(11)	1.0±1.0a(11)
D _{6h}	7.5 ± 3.4a (12)	3.4±1.8b(11)	2.5±1.1b(11)
D _{12h}	5.8 ± 2.5a (12)	3.0±1.2b(11)	2.3±0.8b(11)
D _{18h}	5.1 ± 1.8a (12)	3.5±1.4b(11)	2.4±1.1b(11)
D _{24h}	5.7 ± 2.1a (11)	4.1±1.9a,b(11)	3.1±1.6b(9)
D _{48h}	5.5 ± 2.0a (10)	4.4±1.2a,b(10)	3.4±1.3b(8)
D _{72h}	5.1 ± 1.1a (9)	4.5±1.5a(10)	3.8±2.0a(8)
D _{96h}	4.3 ± 0.9a (9)	3.9±0.9a(10)	3.4±1.8a(8)
D _{120h}	3.7 ± 2.5a (9)	3.7±2.7a(9)	2.9±1.3a(8)
D _{144h}	1.3 ± 2.2a (9)	1.1±1.4a(9)	1.7±1.6a(8)
D _{168h}	0.8 ± 1.6a (9)	1.3±1.6a(9)	0.2±0.2a(8)

Means with different superscripts within rows differed (Tukey, P<0.05). () Number of animals.

Table 3. Reproductive traits from ewes submitted to estrous induction receiving new or reused progesterone devices for five days (mean±SD) and natural mounted (NM) or laparoscopic artificial inseminated (IAL)

Variable	Number of uses of the device		
	New	Once-used	Twice-used
Estrous response (%)	92.9 (13/14)	92.9 (13/14)	100.0 (13/13)
Interval to estrus (h)	46.0±9.8(13)	46.1±14.1(13)	36.8±15.6(13)
Duration of estrus (h)	52.3±1.1(13)	52.5±1.2(13)	52.4±1.0(13)
Conception rate after IAL (%)	23.5 (4/17)	20.0 (4/20)	33.3 (6/18)
Conception rate after NM (%)	78.6 (11/14)	42.9 (6/14)	61.5 (8/13)
Total conception rate (%)	48.4 (15/31)	29.4 (10/34)	45.2 (14/31)

No differences detected between treatments (P>0.05). () Number of animals.

DISCUSSION

Average plasma P4 concentrations measured before the onset of the experiment were >1ng/mL in all treatments. These concentrations could suggest a regular cycle of Santa Inês ewes in the Brazilian Southeast region in the spring (anestrous season). However, when evaluating ewes separately, more than half of them had P4 <1ng/mL in the beginning of the experiment, which suggests that many females were in seasonal anestrus. On the first 18h after device insertion, ewes receiving new inserts showed higher (P<0.01) plasmatic progesterone concentrations than the other groups. It is already known that sublethal concentration elevates LH pulse frequency leading to a prolonged growth of ovulatory follicles, which may compromise fertility, as reported in cattle (Mihm *et al.*, 1994). However, after the initial 48 h no difference was observed (P>0.05) among all three treatments and all ewes showed

supraluteal P4 during the period of device insertion. This pattern of increase to a maximum and then decline in a similar way is seen by Satterfield (2004) and by Husein and Ababneh (2008) in Awassi ewes, when they reported a rapid increase two days after device insertion, from 0.2 to 5.0ng/mL and a gradual decrease to 1.7ng/mL on the day of its removal.

It has been reported that the efficacy of reused devices to promote estrus and fertility rates in ewes after a first use was lower when compared to the use of a new one (Smith *et al.*, 1991; Ungerfeld and Rubianes, 1999; Ungerfeld, 2009). However, in the present study no difference was detected in any evaluated reproductive parameter when using new, once or twice-used devices in short-term protocols. Reused device liberates P4 in sufficient concentrations to allow an adequate blockade of LH, maintaining steroid levels well enough to assure good quality of ovulated oocytes and not

promoting any differences among treatments. Therefore, this phenomenon was possibly also responsible for the results obtained in the ovulation parameters.

In the present study, the use of intravaginal progesterone devices up to three times did not affect ($P>0.05$) the rate of ovulating animals (a total of 88.9%, 24/27), similar to the 90% also obtained in Santa Inês ewes receiving a short-term treatment with progestagen (Cavalcanti *et al.*, 2006). These data indicate that short-term protocols with progesterone, eCG and prostaglandin are sufficient to induce estrus and ovulation in ewes. The average number of ovulations we verified (1.5 ± 0.6) are similar to those reported in Western White Face ewes (Bartlewski *et al.*, 1999). The largest follicle diameters obtained is in accordance to Ginther *et al.* (1995), Bartlewski *et al.* (1999) and Evans (2003), who reported that ewe's preovulatory follicles reach maximum diameter of 5 to 7 mm. The interval from device removal to ovulation was not different among treatments. The present results were different from those previously reported by Cline *et al.* (2001), about 75.6h but similar to Cavalcanti *et al.* (2006), who obtained a time interval of 58.7h also with Santa Inês. All these traits associated to ovulation were comparable to those in the literature when using new implants.

Estrous response was on average 95.2%, superior to that observed by Knights *et al.* (2001), which was 75%. Furthermore, Ungerfeld (2009) working with Corriedale ewes receiving devices for 5 days, reported that estrus was affected by the use of previously used progesterone devices (30.2% for 18 days vs. 39.4% for 12 days evidencing a different pathway possibly due to breed). In the present study, the average conception rate after natural mating was 61%. Thus, these results are in accordance to other studies; 53% were reported in anestrus ewes after the use of progesterone devices for 5 days (Knights *et al.*, 2001) or 58% were detected in ewes receiving a progestagen for 6 days associated to eCG (Viñoles *et al.*, 2001), both also after natural mating. Conversely, average conception rates obtained after laparoscopic artificial insemination (IAL) with fresh semen was lower (25.4%) when

compared to authors using similar protocols. Luther *et al.* (2007) synchronized estrus with norgestomet and obtained a pregnancy rate of 70% with frozen semen. The same authors used fluorogestone acetate and verified a pregnancy rate after IAL of 66.7%.

The unsatisfactory result obtained in ewes in this treatment might have been caused by IAL average time (52.4 h after device removal), which was shown to be more effective when using frozen semen (Luther *et al.*, 2007). In the present study, the interval from device removal to IAL was very close to ovulation time (55 h). Thus, the capacity of fresh spermatozoa to perform adequate fertilization of oocytes was not possible. Indeed, Evans and Maxwell (1987) suggested that when using frozen semen intra-uterine AI should be performed between 60 h and 66 h after device removal.

In the first experiment the use of carboxymethylcellulose gel on the applicator device might have caused the increased rate of lost devices. Colazo *et al.* (2004) reported that CIDR loss rate tended to be higher in heifers receiving a used CIDR than in those receiving new ones. However, no significant difference was found in the number of lost devices between ewes receiving new or used ones. Finally, although progesterone devices have been successfully used in the present study, the experimental animals were free of most common infectious disease that can be vectored by iatrogenic form. Thus, it should be emphasized that the possibility of intravaginal device reuses may provoke unwanted sanitary risks. In this way, autoclaving the devices proved to be an alternative to assure sterilization without loss of efficiency in the induction of synchronized estrus in goats (Souza *et al.*, 2009).

CONCLUSIONS

The results of this study indicate that intravaginal progesterone devices may be used up to three times in sheep herds free of reproductive diseases, without altering the ewe's reproductive performance in estrus induction-synchronization programs. This procedure allows producers to supply meat products throughout the year.

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