

Communication

[Comunicação]

**Use of human intravaginal tampon embedded with natural progesterone induces synchronous estrus in Santa Inês ewes**

[Uso de tampão intravaginal humano embebido com progesterona humana para indução e sincronização de estro em ovelhas Santa Inês]

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The production of Santa Inês sheep in Brazil is very important to farmers because they are rustic, adapted to most part of gastrointestinal parasites and have good maternal ability (Sousa *et al.*, 2003; Amarante *et al.*, 2004). To maintain these characteristics, the animals are selected, and reproductive biotechnologies are applied to induce estrus with progestagens throughout the year, due to their seasonality. Different strategies of estrus induction and synchronization are used worldwide (Balaro *et al.*, 2017) but, traditionally, intravaginal progestagen devices are often applied. One of the disadvantages of hormonal treatment is the cost itself. Currently, there are three different commercial brands in Brazil and overall, the cost of those devices is relatively high. Alternatives must be proposed in order to make efficient treatments, without adversely affecting the reproductive efficiency. Therefore, the aim of this study was to evaluate the efficiency of three protocols of synchronous estrus induction during the transition period in Santa Inês ewes.

This study was approved by Embrapa Dairy Cattle Animal Care Committee (2512100516) and it was conducted under the principles of Brazilian Society of Laboratory Animal Science. The experiment was carried out on February, during the transition season in Coronel Pacheco (21°35' S and 43°15' W), Minas Gerais, Brazil.

All ewes were kept in an intensive system and fed with corn silage, chopped *Pennisetum purpureum*, *Cynodon* spp. hay. Furthermore, a balanced concentrate supplement was given according to their demand, as well as mineralized salt (Caprinofós<sup>®</sup> Tortuga, São Paulo, Brazil) and drinking water were available *ad libitum*.

A total of 24 pluriparous ewes was used. Ewes were equally allocated into three treatments according to their parity, body weight (BW) and body condition score (BCS, range 1 to 5): CONTROL, OB 200 and OB 400. Ewes in the CONTROL group (n= 8; 57.6±12.0kg, BCS 3.3±0.8) received a progesterone releasing device containing 330g progesterone (CIDR<sup>®</sup>, Pfizer Animal Health, São Paulo, Brazil). Ewes in the other two groups received intravaginal Mini tampon (o.b.<sup>®</sup>, Johnson & Johnson, São José dos Campos, Brazil) embedded with 200mg natural human progesterone (Evocanil<sup>®</sup>; Zodiac Produtos Farmacêuticos S/A, Pindamonhangaba, Brazil) (OB 200, n= 8; 58.5±13.8kg, BCS 3.4±0.5) or 400mg progesterone (OB 400, n=8; 56.6±13.5kg, BCS 3.3±0.6). All progesterone devices were maintained *in situ* for 6d. At 24h before device removal, 37.5µg d-cloprostenol (Prolise<sup>®</sup>; ARSA S.R.L., Buenos Aires, Argentina) laterovulvar and 300IU eCG (Novormon 5000<sup>®</sup>; Coopers, São Paulo, Brazil) i.m. were administered.

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Blood was sampled by jugular puncture into heparinized vacuolated tubes always in the morning (0600 to 0800h) previously to feeding on Days 0 (D0) (immediately before device insertion), D0+6h (6h after), daily from D1 to D6 (progesterone removal) and 24h after (D7). After collection, the tubes were kept at 5 C until centrifugation at 1,500×g for 15min. The plasma was then aspirated and stored at -20 C until analyzed for progesterone (P4) concentrations. The time spent from blood collection to plasma aspiration time did not exceed two hours. Plasma P4 concentration (ng/mL) was determined through the use of the solid phase radioimmunoassay (RIA), using a commercial kit (Beckman Coulter; Immunotech, Marseille, France) at the Hormonal Dosage Laboratory, Animal Reproduction Unit, Fluminense Federal University. The assay sensitivity and intra-assay coefficients of variation were 0.05ng/mL and 12%. In addition, all data were within the maximum and minimum points of the curve.

After device removal, estrus was monitored with the use of fertile rams twice a day (0700 and 1900h) and females were considered to be in estrus when allowed to be mounted. Ewes displaying signs of estrus were mated by fertile Santa Inês rams (n= 4) at the onset of estrus and every 12h if they were still in estrus. Rams were

used for approximately equal numbers of ewes from each treatment, with a ram:ewe ratio about ≤ 1:6. Approximately 30 days after mating, conception rate was diagnosed by a B-mode transrectal ultrasonographic scanner (KX2000G Vet®, Kaixin, Xuzhou, China) with multifrequential (4.5 to 7.5MHz) transducer adapted with a plastic rigid tube for use in small ruminants.

The variables determined were: estrous response (number of females in estrus/number of females induced×100), interval to estrus (h) (interval from device removal to first time ewe stood to be mounted), duration of estrus (interval from the first to last acceptance of mounting), conception rate (number of pregnant females/number of females exposed×100), plasma progesterone concentration (P4; ng/mL). Statistical analysis was performed using all tests with P< 0.05 considered significant. Parametric variables were submitted to one way analysis of variance (ANOVA) and compared by Tukey test by the SAEG program (System for Statistical Analysis). Non-parametric variables were analyzed by the use of the chi-square test. The results are described as mean ± SEM.

Reproductive end points are listed in Table 1. Estrus response rate was overall 87.5% (21/24).

Table 1. Reproductive parameters of Santa Inês ewes after induction of synchronous estrus with 6 days progesterone treatments\* plus cloprostenol and eCG administration 24h before device removal (mean ± SEM or %)

Parameter	Treatment		
	Control	OB 200	OB 400
Estrous response (%)	100.0 (8/8)	62.5 (5/8)	100.0 (8/8)
Interval to estrus (h)	46.3 ± 3.9 <sup>a</sup>	26.4 ± 4.5 <sup>b</sup>	31.2 ± 5.8 <sup>a,b</sup>
Duration of estrus (h)	39.0 ± 3.8	36.0 ± 7.6	44.4 ± 5.7
Conception rate (%)	50.0 (4/8)	50.0 (4/8)	50.0 (4/8)

<sup>a,b</sup>Means with different superscripts within rows differed (Tukey test; P< 0.05). ( )Number of animals. \*Treatments: CONTROL (CIDR330mg), OB 200 and OB 400 (o.b. tampon embedded with 200 or 400mg progesterone).

In sheep, it is well-known that plasma progesterone concentration (P4) increases from basal levels after ovulation, reaches the plateau during diestrus and becomes progressive lower reaching levels inferior to 1ng/mL during proestrus (Quirke *et al.*, 1979). At this time, this lower level of P4 (<1ng/mL), also called as sub-luteal level (Minton *et al.*, 1991; Channa *et al.*, 2017) implies in decreasing the negative feedback of progesterone on LH pulse frequency. As a consequence, final follicular development

was possible culmination in estrus behaviour and ovulation (Ginther *et al.*, 2006). In the present study it was observed P4 under 1ng/mL (Figure 1).

A total of fourteen ewes (14/24) had supraluteal P4 (>1ng/mL) on Day 0. P4 superior to 1ng/mL at the moment of device removal were detected in all females in Control group, and in five ewes in OB 400 group. All the ewes in OB 200 group, had subluteal P4 (<1ng/mL) at the moment of

device removal. P4 was similar ( $P > 0.05$ ) in all treatments throughout the experimental period, except at the day of device removal, when Control had greater ( $P < 0.05$ ) P4 ( $2.5 \pm 0.3 \text{ ng/mL}$ ) than OB 200 ( $0.6 \pm 0.1 \text{ ng/mL}$ ) and OB 400

( $1.2 \pm 0.4 \text{ ng/mL}$ ). On the following day (Day 7) P4 decreased to subluteal concentrations in all ewes of the OB 200 group, and in seven ewes of the Control and OB 400 group of treatments.

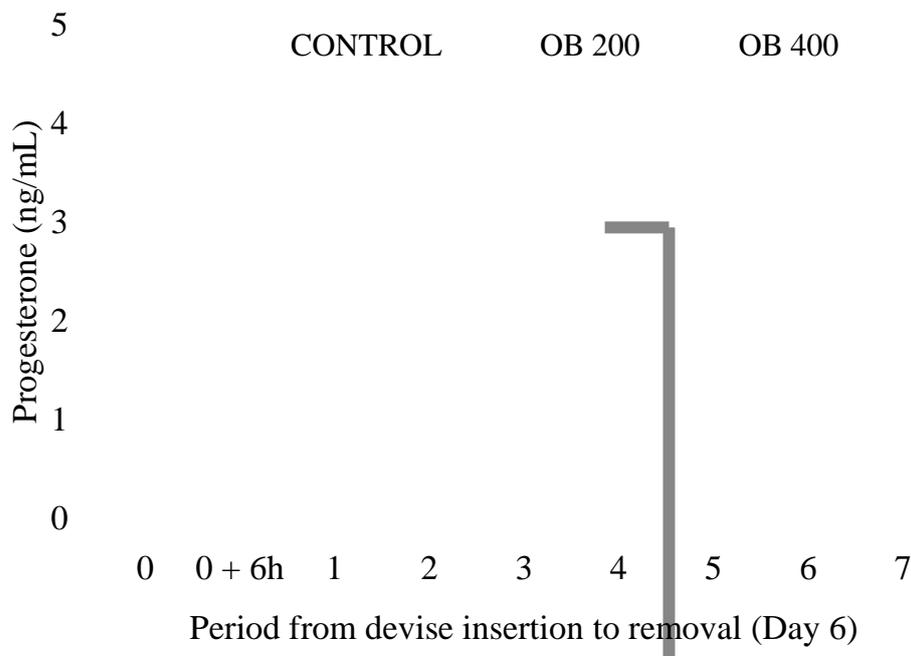


Figure 1. Plasma progesterone concentration in Santa Inês ewes after receiving intravaginal commercial progesterone device (CIDR 330mg progesterone; Control) or human intravaginal tampon embedded with either 200mg (OB 200) or 400mg (OB 400) human progesterone for six days (D0-D6). ( - ) denotes the limit between subluteal and luteal levels of progesterone.

According to Figure 1, OB 200 ewes showed in average P4 near to limit considered as luteal until Day 4 after device insertion. Interestingly, on Day 5 (24h prior to device removal), all OB 200 ewes showed P4 inferior to 1ng/mL. It could mean that this level of P4 was not capable to inhibit LH efficiently. Thus, possibly this was the reason for shorter interval to estrus in this group. In addition, three ewes from OB 400 group showed P4 inferior to 1ng/mL at device removal. This fact might be involved in intermediary interval to estrus observed in this group.

In the present study, we compared the outcomes of using a human intravaginal tampon embedded with either 200 or 400mg progesterone with the CONTROL group, a commercial progesterone device commonly used for estrus synchronization in sheep. All protocols studied had a satisfactory estrus response and conception

rate after the treatments. Although the rates of estrus response and conception were statistically similar, the animals in the OB 200 group had a lower (numerically) response compared to the other two groups. Ewes from OB 400 group showed interval to estrus (31h) similar to CONTROL and also to those previously observed in the breed using estrous induction protocols using 60mg MAP sponges for six days 32h (Cavalcanti *et al.*, 2012).

In conclusion, results of the present study suggest that different intravaginal devices and with different dose of progesterone can be successfully applied for estrus induction in Santa Inês ewes, without adversely affecting reproductive efficiency.

*Keywords: progestagen; transition period; estrus induction; conception rate*

## RESUMO

Este estudo avaliou a eficiência de três protocolos de indução de estro sincronizado em ovelhas da raça Santa Inês. Vinte e quatro ovelhas adultas foram equitativamente distribuídas em três grupos, de acordo com ordem de parto, peso corporal (kg) e escore da condição corporal. As ovelhas receberam implante vaginal de progesterona natural por seis dias mais 37,5µg de d-cloprostenol laterovulvar e 300UI de eCG i.m., 24 horas antes da remoção do dispositivo. Ovelhas controle receberam CIDR330mg de progesterona, e as demais receberam dispositivo absorvente intravaginal humano, tamanho mini, embebido com 200 (OB200) ou 400mg (OB400) de progesterona. Coletas de sangue foram feitas nos momentos D0 (antes da inserção dos dispositivos), D0+6h e diariamente, até um dia após retirada do dispositivo (D7). A progesterona (ng/mL) foi semelhante ( $P>0,05$ ) em todos os tratamentos ao longo do período experimental, exceto no dia da remoção do dispositivo, quando as ovelhas controle ( $2,5\pm 0,3$ ) tiveram progesterona superior ( $P<0,05$ ) às ovelhas OB200 ( $0,6\pm 0,1$ ) e OB400 ( $1,2\pm 0,4$ ). O percentual de animais em estro (100,0, 62,5 e 100,0%) foi semelhante ( $P>0,05$ ) e o intervalo para o estro ( $46,3\pm 3,9^a$ ,  $26,4\pm 4,5^b$  e  $31,2\pm 5,8^{a,b}$ ) foi diferente ( $P<0,05$ ) entre os animais dos grupos controle, OB200 e OB400, respectivamente. A taxa de concepção das ovelhas foi de 50,0% (4/8) para cada grupo ( $P>0,05$ ). Dispositivos humanos e fonte de progesterona podem ser usados para induzir o estro sincronizado em ovelhas Santa Inês.

Palavras-chave: progestágeno, período de transição, indução de estro, taxa de concepção

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