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Estrus synchronization protocols in Blackbelly sheep in tropical region: prostaglandin and male effect

Protocolos de sincronização de estro em ovinos Barriga Negra em região tropical: prostaglandina e efeito macho

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ABSTRACT: Estrus synchronization protocols may include reproductive hormones or management practices, with differences mainly in relation to costs, labor, number of managements, and time until estrus manifestation. This study aimed to evaluate estrus synchronization protocols in sheep using prostaglandin or male effect in a tropical climatic region. Three estrus synchronization protocols were evaluated in 60 adult Blackbelly sheep, including 1) 150 µg intramuscular prostaglandin D0 and D7; 2) 30 µg vulvar submucosal prostaglandin D0 and D7; 3) male effect: ram introduction. After the last administration of prostaglandin (intramuscular or vulvar) or introduction of the male (male effect), a ram with ink in the chest was introduced, and the females were monitored for signs of estrus every 24 h. Data were tabulated and subjected to descriptive statistical analysis. Within 48 h, 70–80% females that received prostaglandin, regardless of the administration route, showed estrus. Both protocols with prostaglandins manifested estrus in 100% ewes within 72 h. In the male effect group, 50% ewes showed estrus within 48 h. The other 50% manifested estrus after 72 h, with 30% ewes between 96–144 h. In conclusion, regardless of the synchronization protocol, all ewes manifested estrus within six days. The male effect showed a higher interval in synchronization up to 144 h. Protocols with both intramuscular and vulvar submucosal prostaglandin administration resulted in estrus manifestation in all sheep within 72 h. Vulvar submucosal administration of a reduced dose is laborious and reduces hormone cost.

KEYWORDS: breeding; heat; hormones; luteolytic; mating.

RESUMO: Protocolos de sincronização de estro incluem uso de hormônios ou práticas de manejo, apresentando diferenças em relação a custos, mão de obra, número de manejos e tempo até manifestação de estro. Esse estudo objetiva avaliar protocolos de sincronização de estro em ovinos, utilizando prostaglandina ou efeito macho, em região de clima tropical. Foram avaliados 3 protocolos, utilizando 60 ovelhas adultas, raça Barriga Negra, incluindo 1) Prostaglandina intramuscular 150µg no D0 e D7; 2) Prostaglandina submucosa vulvar 30µg no D0 e D7; 3) Efeito macho: introdução do reprodutor. Após última administração de prostaglandina ou introdução do reprodutor (efeito macho), foi inserido um carneiro marcado com tinta e as fêmeas monitoradas para manifestação de estro a cada 24h. Dados foram tabulados e submetidos a estatística descritiva. Em até 48h, 70-80% das fêmeas que receberam prostaglandina, independente da via, apresentaram estro. Ambos protocolos com prostaglandina resultaram em estro em 100% das ovelhas em até 72h. No grupo efeito macho, 50% das ovelhas apresentou estro em até 48h. Os outros 50% após 72h, sendo que 30% apresentou após 96h, entre 96-144h. Em conclusão, independente do protocolo de sincronização, todas ovelhas apresentaram manifestação de estro em até 6 dias. O efeito macho apresentou maior intervalo na sincronização, em até 144h. Os protocolos com prostaglandina, tanto por via intramuscular como por submucosa vulvar, resultaram na manifestação de estro em todas ovelhas em até 72h. A administração de dose reduzida na submucosa vulvar é mais laboriosa e apresenta redução na dose e, consequentemente, nos custos com hormônio.

PALAVRAS-CHAVE: cio; estação de monta; hormônios; luteolítico; monta.

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INTRODUCTION

In tropical climate regions, sheep have continuous estrous cycles throughout the year and are considered continuous polyestrous. The estrous cycle is manipulated to increase the reproductive efficiency of animal production systems. Estrus synchronization allows time for mating programming, reduces birth intervals, concentrates births, and homogenizes lamb weight (Fonseca, 2003).

Knowledge of endocrine dynamics in the estrous cycle has allowed the development of hormonal protocols, isolated or associated with management techniques, to improve the control of estrus manifestation and ovulation, including the male effect and prostaglandin hormone use.

The male effect is based on keeping ewes away from rams for at least 30 days and includes visual, olfactory, and auditory separation (Thimonier *et al.*, 2000). The introduction of rams into the female herd stimulates GnRH/LH release, resulting in estrus synchronization. Thus, the male effect is a sustainable and efficient technique for determining estrus onset and ovulation in sheep (Thimonier *et al.*, 2000).

Prostaglandin is a luteolytic hormone that induces corpus luteum lysis, shortens diestrus phase, and stimulates a new estrous cycle (Herrera *et al.*, 1990). Different prostaglandin administration routes have already been tested in large ruminants for reducing the dose while maintaining efficient estrus synchronization (Alvarez et al., 1991; Chauhan *et al.*, 1986). In sheep and goats, reduced prostaglandin dose associated with laterovulvar administration at intervals between 7–10 days (two doses) is efficient for estrus synchronization (Bahari *et al.*, 2023; Chagas *et al.*, 1994; Esteves *et al.*, 2013; Siqueira *et al.*, 2012).

Prostaglandin administration into the vulvar submucosa reaches the ovaries without reaching the systemic circulation, due to anatomical particularities of ruminants, with an intimate relationship between the vulvar, ovarian, and uterine vessels (Alvarez, 1991; Horta *et al.*, 1986).

In this context, this study aimed to evaluate estrus synchronization protocols in Blackbelly sheep using prostaglandins and male effect in a tropical climate region, aiming to optimize reproductive practices and reduce costs.

MATERIAL AND METHODS

This study used animals as the experimental models and was approved by the Ethics Committee on the Use of Animals of the Universidade Federal de Roraima (CEUA-UFRR) under registration 002/2021.

Three estrus synchronization protocols were evaluated in sheep allocated in a tropical climate region (2°49'10" N, 60°40'17" W) in Boa Vista, Roraima, Brazil. We included 60 Blackbelly sheep, 2–6-year-old, classified as adult, with body score 3–3.5 (scale 1–5), considered healthy after general and specific clinical examination of the reproductive system, and not pregnant.

The females were divided into three treatment groups: 1) intramuscular prostaglandin (PG-IM): 150 µg prostaglandin was administered intramuscularly on D0 and D7 (n=20); 2) vulvar submucosal prostaglandin (PG-VS): 30 µg prostaglandin was administered in vulvar submucosa on D0 and D7 (n=20); 3) male effect: introduction of the ram after a 60-day total separation, without any visual, olfactory, and auditory contact between males and females (n=20).

In protocols with prostaglandin (cloprostenol, Cioson®), a ram was introduced on D7 (on the last day of prostaglandin application, regardless route) and maintained for five consecutive days. In the male effect group, rams were introduced to the ewes and maintained for seven consecutive days. Paint was applied daily to the rams' chests to identify the ewes that allowed mating and consequently showed estrus. The females were evaluated every 24 h (Figure 1).

One ram for every 10 ewes was used, with the females kept in the same flock from the beginning to the end of the experiment. Each flock was housed in a semi-intensive feeding system. During the day, the animals had access to the rotated paddock areas with *Brachiaria humidicola* and *Panicum maximum* grass. At night, the animals were confined to sheepfolds. Water was provided *ad libitum*.

Data were subjected to descriptive statistics using the Excel® 2016 computer program.

RESULTS AND DISCUSSIONS

All ewes subjected to protocols with prostaglandins or male effects showed estrus within six days (Table 1).

All sheep that received prostaglandin, regardless of the dose and route, showed estrus within 72 h after the last hormone dose administration.

In the PG-VS and PG-IM groups, 70% and 80% ewes, respectively, showed estrus within 48 h.

The results of this study support that the reduction in dose (30 µg), with a change in the administration route (vulvar submucosa), efficiently concentrated estrus manifestation in ewes within 72 h. Animals were located and adapted to a tropical climate, considered annually polyestrous; that is, synchronization with prostaglandin could be used throughout the year, since one of the prerequisites for the use of this hormone is the presence of a functional corpus luteum. Furthermore, prostaglandin administration is not a prerequisite for the absence of direct or indirect auditory, visual, and olfactory contact between rams and ewes, in contrast to the male effect.

Corroborating our findings on the efficiency of estrus synchronization with dose reduction and vulvar submucosal administration, Chagas *et al.* (1994) reported that the vulvar submucosal administration of a full dose (0.20 mg) and half dose (0.10 mg) tiaprost resulted in 86.6% and 85.7% estrus concentrations, respectively, within up to 24 h. ESTEVES *et al.* (2013) reported that latero-vulvar administration of 30 µg d-cloprostenol in dairy goats 10 days apart (D0 and D10) was efficient for estrus synchronization. Siqueira *et al.* (2012) reported efficient estrus synchronization in Toggenburg

goats with two applications of 22.5 mg synthetic prostaglandin (d-cloprostenol) at 10 days interval. The authors also reported that the presence of a corpus luteum responsive to prostaglandins at the time of application determined the percentage of animals capable of expressing estrus (Siqueira *et al.*, 2012).

Bahari *et al.* (2023) reported that estrus length and estrous sign expression in goats following intravulvar submucosal prostaglandin injections were comparable to those following intrasmuscular injections. The authors considered intravulvar administration to be straightforward and administered in goats just as quickly as the intramuscular route, allowing for the cost-effective and economical use of prostaglandins in goats.

Vulvar submucosal administration requires a skilled and trained professional and is a slightly longer and less practical procedure than intramuscular administration. None of the ewes showed inflammatory reactions at the application site.

In sheep, the corpus luteum is sensitive to the luteolytic effect of prostaglandins approximately on the third day after ovulation (Menchaca *et al.*, 2004). The first protocol with prostaglandin lasted approximately 9–10 days; however, an

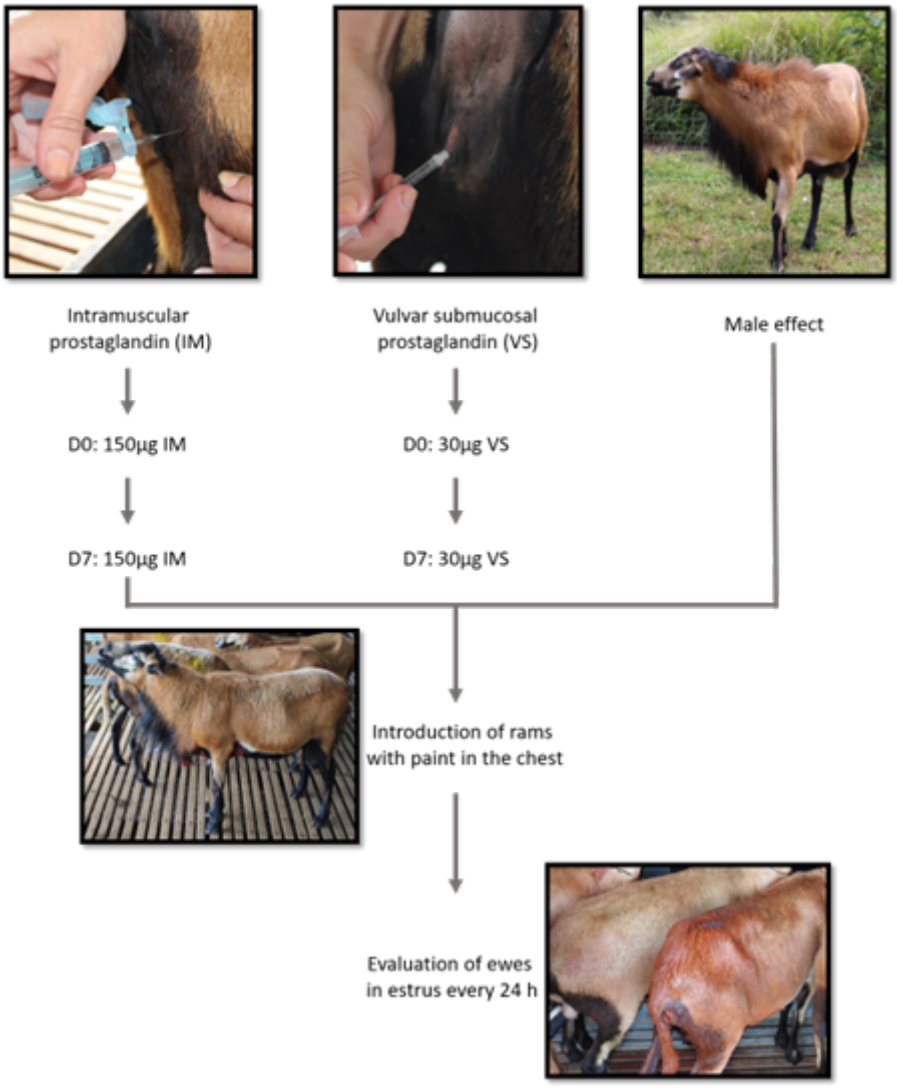

Figure 1. Schematic representation of estrus synchronization protocols, including intramuscular prostaglandin, vulvar submucosal prostaglandin, and male effect in Blackbelly sheep located in a tropical region.

Table 1. Frequency (%) of Blackbelly ewes showing estrus after the introduction of rams, according to estrus synchronization protocols, with intramuscular prostaglandin, vulvar submucosal prostaglandin, and male effect.

Protocol	N	0–24 h	24–48 h	48–72 h	72–96 h	96–144 h
Intramuscular prostaglandin	20	30	50	20	0	0
Vulvar submucosal prostaglandin	20	30	40	30	0	0
Male effect	20	30	20	0	20	30

interval of 7 days is currently recommended because at the time of the second dose, a large portion of sheep will have a functional corpus luteum and consequently be sensitive to the luteolytic effect (Menchaca *et al.*, 2004).

Considering its form of action, prostaglandin is recommended for use during the reproductive season, which requires cycling in females. During the breeding season, using protocols with prostaglandin associated with progestin, SANTOS; BARCELOS (2012) reported that 75% and 25% sheep manifested estrus within 72 and after 96 h, respectively, after removal of the progestin-based ear implant (norgestomet, Crestar®). During the non-breeding season, ROSA *et al.* (2021), using an intravaginal progesterone device and equine chorionic gonadotropin, reported that 44.4%, 33.3%, and 22.2% females showed estrus between 12–24, 24–36, and 48–60 h, respectively, after device removal.

Elapsed time and number of ovulations after prostaglandin administration are variable, as depend on the follicular wave status. The best ovarian response can be expected when females are treated at the beginning and end of the luteal phase, with rapid estrus onset and more ovulating animals (Uribe-Velásquez *et al.*, 2011). Ovulation generally occurs between 24 and 36 h after estrus in sheep with a natural cycle and those treated with prostaglandin analogs (Uribe-Velásquez *et al.*, 2010).

Reduced prostaglandin doses via the vulvar submucosal route can induce luteolysis in a manner similar to intramuscular administration (Ono *et al.*, 1982; Chauhan *et al.*, 1986; Chagas *et al.*, 1994; Esteves *et al.*, 2013; Siqueira *et al.*, 2012). Chohan (1998) reported no significant difference in the period between treatment and estrus onset manifestation in female buffaloes treated with 500 µg intramuscular prostaglandin (95 h) or 125 µg via the vulvar submucosa, ipsilateral to the corpus luteum (96 h), in the day 11 of a group of buffaloes with previously synchronized estrus. Chacur *et al.* (2010) reported that 500, 250, 125 µg IM or SV cloprostenol can be administered for estrus synchronization in cattle, with similar pregnancy rates in cyclic Nelore cows.

The conventional dose of intramuscular prostaglandin in sheep is approximately 150 µg. The main hypothesis was that the dose could be reduced to 1/5, resulting in the vulvar submucosal application of 30 µg prostaglandin per dose. Consequently, it would reduce the cost by 80% when comparing the conventional dose (150 µg = 100%) with 1/5 of the dose (30 µg = 20%). For illustration purposes, if the conventional prostaglandin dose was 1 mL, the 1/5 dose was 0.2 mL. If 100 mL hormone costs $400, 1 mL would cost $4 and 0.2 mL would cost $0.80. In other words, changing the administration route could directly reduce the cost per dose by 80%, and according to our results, it presents the same efficiency in estrus synchronization in sheep, with all ewes in estrus within 72 h.

As an alternative to hormone use, the estrous cycle can be manipulated by sexual biostimulation, called the male effect, which can modulate GnRH/LH secretion (Thimonier *et al.*, 2000).

In this study, half of the ewes in the male effect group showed estrus within 48 h after ram introduction, and the other half showed estrus after 72 h (Table 1). Approximately 30% ewes showed estrus after 96 h (96–144 h), making it necessary to maintain the rams in the flock for three additional days. The main advantages of this method are the absence of hormones, cost, and fewer procedures (only one, to introduce the sheep into the flock, when compared to two processes for prostaglandin administration). However, a greater amplitude in estrus manifestation was observed in addition to the requirement of keeping the rams geographically far from the ewes, without direct or indirect auditory, visual, or olfactory contact, in the period prior to synchronization.

The quality and intensity of the responses to the male effect are associated with environmental, social, and physiological factors, and the results of previous studies are divergent. Oldham *et al.* (1979) reported that most sheep ovulate between 30–72 h, while Knight (1983) reported intervals between 50–65 h. Martin *et al.* (1986) reported that the introduction of rams into a flock of anovulatory sheep increased the frequency of LH pulses, with a pre-ovulatory peak at 36 h, resulting in ovulation within 2–4 days. Perkins and Fitzgerald (1994) found that 95% sheep ovulated 24 days after the initial exposure.

According to Boly *et al.* (2000), the results achieved with the male effect were similar to those obtained with hormonal treatments. Furthermore, they have the advantages of zero cost and the absence of hormonal residues. In this study, we observed that only 50% ewes showed estrus within 48 h after introducing the rams, and the rest showed estrus within 6 days, resulting in a low estrus concentration. However, this practice can be useful on farms where the breeding system is not intensive, when they can wait for a longer period for estrus to manifest, or when the hormone use is restricted.

CONCLUSIONS

Protocols with prostaglandin administration or male effects caused estrus synchronization within six days in Blackbelly ewes located in a tropical climate region.

The male effect does not involve medication costs; however, the disadvantage is the requirement of animal separation and was less efficient in concentrating estrus onset within six days.

Both protocols with intramuscular and vulvar submucosal prostaglandin administration resulted in estrus manifestation in all ewes within 72 h.

Although vulvar submucosal administration is more laborious than intramuscular administration, it is less expensive.

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