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Effect of different protocols for estrus synchronization on reproductive performance of Santa Inês ewes under Amazon environmental conditions

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ABSTRACT. The objective of this study was to evaluate the effect of different hormonal protocols on the reproductive performance of Santa Inês ewes in Amazon environmental conditions. Twenty-two Santa Inês ewes between 3 and 4 years-old were distributed in a randomized block experimental design, where the treatments consisted of two protocols for estrus synchronization (short and long) with eleven animals each. Data on the occurrence of estrus were described for each protocol. Data of estrus, pregnancy, and prolificity were firstly subjected to ANOVA and a subsequent Tukey's test. Results were considered significant at $p \leq 0.05$. The short-term protocol presented an interesting successful rate, where above 70% ewes tested had estrus. The long-term protocol also achieved a high successful rate, where above 80% ewes tested had estrus. However, comparing the protocols, the long-term protocol presented better results of positive estrus and pregnancy rates in ewes. Thus, it can be concluded that both protocols presented satisfactory results regarding estrus manifestation, and prolificity (lambs produced per ewe). However, under Amazon environmental conditions, the long-term protocol presented better results regarding positive manifestation of estrus and pregnancy rate.

Keywords: ewes; hormones; pregnancy; progesterone; reproduction.

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Introduction

Reproduction is important for animal production, in order to maintain the production system continuation. It is reported that not less than 60% local farm profits come from selling lambs, this commodity is influenced by the lambing rate, lambing interval and the reproductive efficiency in general (Pedrosa, Santana Junior, Oliveira, Eler, & Ferraz 2010). The majority of ewe breeds differ in reproductive behavior depending on season changes, latitude/longitude, the length of the photoperiod and other factors. From this, several strategies have been used to control ovarian activity focusing on improving fertility of small ruminants (Cavalcanti, Brandão, Nogueira, & Fonseca, 2012), and prevent anestrus, the most common reproductive disorder of ewes, which causes great economic losses to the farmers due to lower fecundity (Ezzat, Ahmed, Elabdeen, & Sabry, 2016).

Estrus synchronization or induction is an interesting tool for increasing the pregnancy rate in ewes. Modern ewe husbandry has improved the efficiency of extensive production and controlled the reproductive process for intensive production. Basically, the synchronization of estrus in ewes focuses on the manipulation of the estrus cycle (Zonturlu, Özyurtlu, & Kaçar, 2011), the manipulation of either the luteal or the follicular phase of the estrus cycle. In this sense, hormonal treatment to control ovulation and reproduction is an interesting alternative for successful breeding and increasing the number of pregnant females (Abdalla, Farrag, Hashem, Khalil, & Abdel-Fattah, 2014). Applications of exogenous hormones for increased reproductive performance in domestic ewes usually focus on estrus synchronization (Najafi, Cedden, & Maleki, 2014).

It has been reported in the literature that the improvement of estrus synchronization depends on more effective manipulation of the corpus luteum and follicular development. The ovarian follicular dynamics has been described as wave-like, with follicular dominance during the estrus cycle of the ewe, as well as during anestrus in determining the efficiency of synchronization (Martemucci & D'Alessandro, 2010). In ewes, the opportunity for control is greater during the luteal phase, which is of longer duration and more responsive to manipulation. Strategies can be employed to extend the luteal phase by supplying exogenous progesterone or to shorten this phase by prematurely regressing existing corpora lutea (Wildeus, 2000). A second opportunity in small ruminants is the propensity of many breeds to carry and raise multiple offspring, which can often be controlled by adjustments in dosage levels and nutritional manipulations as part of the estrus synchronization regimen (Metodiev, 2015).

In general, protocols based on the use of a progesterone source are associated with prostaglandin and eCG (Abecia, Forcada, & Gonzalez-Bulnes, 2011). In the literature, there is a tendency of decreasing the progestogen maintenance period (Beilby, Grupen, Thomson, Maxwell, & Evans, 2009), which was traditionally used for 12 to 14 days, because longer treatment periods can cause lower fertility by disrupting follicular development (Abecia, Forcada, & Gonzalez-Bulnes, 2012; Shabankareh, Seyedhashemi, Torki, Kelidari, & Abdolmohammadi, 2012).

Hormonal treatment to control ovulation and reproduction is a prerequisite for successful breeding and increasing the number of pregnant females (Dias et al., 2018), resulting in a short breeding period and more uniform flock (Husein & Kridli, 2003). Previous studies reported that the key element of methods for estrus synchronization in small ruminants is to control luteolysis and the corpus luteum lifespan (Cavalcanti et al., 2012). Progesterone can prevent ovulation during the period in which spontaneous luteolysis may occur in animals whose dominant follicles are not responsive to GnRH injection. However, there are not standardized protocols and doses, and a variety of synchronization protocols and product combinations have been described (Titi, Kridli, & Alnimer, 2008).

Thus, the objective of this study was to evaluate two different protocols for estrus synchronization in Santa Inês ewes in the Amazon region.

Material and methods

The study was conducted in Manaus, State of Amazon (2° 38' 43.8" S 60° 02' 27.4" W). The experimental protocol was approved by the Ethics Committee on Animal Experimentation of the Federal University of Amazonas (Manaus, AM), and conducted according to the Brazilian animal welfare standards in teaching and research

Twenty-two Santa Inês ewes with ages between 3 and 4 years-old and average body score of 2.7 (scales from 1 to 5) were used. All ewes were examined and clinically considered as healthy. Ewes were managed in a free-range system, where levels of nutrition remained equal and without changes as each ram was daily fed by using 65% *Brachiaria humidicula* cv. Dyctioneira (1.3 kg) and 35% commercial concentrate (400 g) consisting of 250 g barley, 36 g soybean, 60 g corn, 64 g wheat bran, and 14 g supplement. All ewes had free access to salt stone and fresh water. At the time of analyses, environmental conditions presented an average temperature of 34.23±0.12°C, and average relative humidity of the air of 71.24±0.13%.

The experimental design was completely randomized, where the treatments consisted of two protocols for estrus synchronization (short and long) with eleven animals each. Ewes subjected to the short-term protocol were identified with red collars, being used a 60 mg Progespon®-soaked vaginal sponge inserted into ewes for six days. On the 4^{th} day of the protocol, 100 mg Sincrocio® and 350 - 400 IU Novormon® was intramuscularly injected in each ewe. On the 6^{th} day, the sponge was removed to finish the protocol.

Ewes subjected to the long-term protocol were identified with beige collars, being used a 60 mg Progespon®-soaked vaginal sponge inserted into ewes for 12 days. On the 8^{th} day of the protocol, 100 mg Sincrocio® and 350 - 400 IU Novormon® was intramuscularly injected in each ewe. On the 12^{th} day, the sponge was removed to finish the protocol.

After removing the sponges, in both groups, the ewes were organized according to the treatments and exposed to vasectomized rams in a 1:8 ratio. Rams were greased in the pectoral region using oily mixture and pigment to detect covered ewes and a possible estrus.

Estrus occurrence were grouped into three distribution periods (<30, 30 to 54, and 55 to 72 hours after sponge removal) using the vasectomized rams. After estrus confirmation, ewes were exposed to rams with proven fertility. The efficiency of the tested protocols was determined from positive estrus rate (%), pregnancy rate (%), and prolificity (lambs per ewe). Data on the occurrence of estrus were described for each protocol. Data of estrus, pregnancy, and prolificity were analyzed using the GLM procedure of Statistical Analysis System (SAS, 2008) and estimates of treatments were firstly tested by ANOVA and a subsequent Tukey's test. Results were considered significant at $p \leqslant 0.05$.

Results and discussion

The short-term protocol presented an interesting successful rate, where above 70% of ewes tested had estrus. The literature reported that several hormonal treatments have been used to synchronize estrus in small ruminants, in which long-term protocols the most usual. Short-term protocols also have been reported to be successful in inducing and synchronizing estrus during both the breeding and non-breeding seasons (Neves, Ramos, & Silva, 2010; Taher, 2014). However, there are problems associated with controlled breeding such as the limitation of the time and degree of estrus response. Thus, if a method can predetermine the time from withdrawal of protocol to onset of estrus, the need for estrus detection could be reduced or even eliminated (Menchaca, Santos Neto, & Cuadro, 2017).

There are also other problems related to the period of the sponge remaining inside the vagina due to the variations in progesterone concentration during the protocol used (Holtz, 2005; Sidi et al., 2016), besides other hormones. Some years ago, researchers put into practice the short-term protocols, which consist of only 5-7 days of exposure to progesterone aiming to use hormonal peaks during the first days of the estrus induction protocol. This treatment is associated with one dose of 200 to 400 IU eCG to induce ovulation and one luteolytic dose of PGF2α at sponge removal (Amer & Hazzaa, 2009; Menchaca et al., 2017). The results of this study indicated that due to these hormonal peaks that occur in short-term period, the estrus occurrence in ewes subjected to short-term protocols presented a regularity, where in all periods evaluated, they presented a regular rate of estrus occurrence.

Table 1. Occurrence of estrus in ewes subjected to short-term protocol along 72 hours.

Periods	Ewes (n)	(%)
< 30 hours	3	27.27
30 to 54 hours	2	18.19
55 to 72 hours	3	27.27
Without estrus	3	27.27
Total occurrence	Ewes (n)	(%)
Ewes with estrus	8	72.73
Ewes without estrus	3	27.27

On the other hand, the long-term protocol also presented a high successful rate in this study, where above 80% ewes tested had estrus (Table 2). The long period also provided the possibility to observe a linear decrease in estrus occurrence at the final of the protocol management. Previous studies reported that usual long-term protocols used intravaginal sponges inserted over periods of 9 to 19 days together with injection of hormones, being particularly used for out-of-breeding season. It is important to mention that the hormones were injected at the time of sponge removal or 48 hours prior to sponge removal, where females usually exhibit estrus within 24 to 48 hours after sponge removal (Wildeus, 2000). Progesterone blocks FSH and LH secretion by suppressing the hypothalamus and also indirectly the pituitary anterior lobe and temporarily stops follicular development. This suppression disappears with the removal of sponges, and estrus behaviors are observed along with follicular development (Koyuncu & Öziş Altinçekiç, 2016). The results of this study indicated that up to 54 hours after sponge removal may be detected a relative rate of estrus occurrence, extending this limit for identification of estrus occurrence from 48 to 54 hours. Long-term progestagen estrus synchronization protocols can affect follicular dynamics and fertility of ewes. Initially, a supraluteal effect is expected, which means that an increase in follicular renewal may occur. In the end, however, a subluteal effect may occur and decreases the speed of follicular renewal (Takada et al., 2012).

According to researchers, the controlled internal drug release devices (CIDR's) should remain in the vagina between 10 and 14 days aiming to confirm the length of the luteal phase of the natural estrus cycle (Hosseinipanah et al., 2014). Administration of hormones, such as progesterone or its analogues (progestagens) and prostaglandins modify the luteal phase of the cycle, whilst melatonin acts through changes in the perception of photoperiod and the annual pattern of reproduction (Abecia et al., 2012). Thus, follicular development during the estrus cycle may be controlled with hormone manipulation (Olivera-Muzante, Fierro, López & Gil, 2011)

Table 2. Occurrence of estrus in ewes subjected to long-term protocol along 72 hours.

Periods	Ewes (n)	(%)
< 30 hours	3	54.54
30 to 54 hours	6	27.27
55 to 72 hours	0	0.00
Without estrus	2	18.19
Total occurrence	Ewes (n)	(%)
Ewes with estrus	9	81.81
Ewes without estrus	2	18.19

However, when compared both protocols (Table 3), the long-term protocol presented better results regarding positive estrus and pregnancy rates of ewes. The results of different treatment protocols are variable between different studies, breeds and husbandry systems. The results of out-of-season breeding season that are induced with (3-67%) or without (0-28%) the use of exogenous hormones have a significant variability (De, Kumar, Balaganur, Gulyani, & Naqvi, 2016). Physiologically, the estrus cycle is a series of hormonal cascades that change the morphology of the female reproductive system to prepare for pregnancy (Fatet, Pellicer-Rubio & Leboeuf, 2011). At the commercial level, synchronization of estrus allows to control and shorten lambing and kidding, with subsequent synchronization of weaning of young animals for slaughter (Abecia et al., 2012). Regarding the duration of progestagen treatment, previous studies reported 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 (Pinna et al., 2012). The mechanism is that progesterone is beneficial for pregnancy maintenance and tocolysis, and FSH promote the ovulation of animals (Wei *et al.*, 2015).

In this sense, Ungerfeld and Rubianes (1999) reported that short-term treatment (5-6 d) with different progestagen devices during the non-breeding season was as effective as long-term treatment to induce estrus and the subsequent fertility, corroborating the results of this study. Intra-vaginal devices containing different types of progestagens, maintained during 6-14 days associated with or without eCG and ProstaglandinF2α (PGF2α) combinations have been usually used for these long-term protocols. As a result of the estrus synchronization protocols, a high percentage of ewes had estrus (Ustuner, Gunay & Ustuner, 2007). According to researchers, CIDR should remain in the vagina between 10 to 14 days that it confirmed with the length of the luteal phase of the natural estrus cycle (Hosseinipanah et al., 2014). Furthermore, Martin, Oldham, and Lindsay (1981) and Wani et al. (2017) reported that the "ram effect" causes secretion of GnRH and then LH, FSH and ovulation, helping in the induction of estrus. It is important to mention that breeder rams with confirmed fertility were used in this study, which may had been contributed to good results of pregnancy.

Mechanca et al. (2017), also comparing the short-term protocol (6 days) versus the traditional long-term protocol (14 days), reported a significantly greater pregnancy rate in short-term protocol than the long traditional protocol (43.5% vs. 37.8%, respectively; $p < 0.05$). In another study using ewes with fixed time artificial insemination (FTAI) and fresh semen by cervical route, in which the females were treated for 6 vs. 14 days with intravaginal devices of second use (in both cases previously used for 6 days), the same authors reported that pregnancy rate was also greater with the short-term protocol (41.2% vs. 29.1%, respectively; $p < 0.05$). These results in line with previous studies obtained with the short-term protocol associated with FTAI in ewes, and overall, this information add more evidence to the concept that as progesterone levels decrease by using intravaginal devices during long periods, negative conditions that predispose to lower fertility are promoted (Candappa & Bartlewski, 2011).

However, this study did not use FTAI, presenting another perspective of analysis, where the ram effect and the natural breeding should be considered. Other studies also indicated that there are concerns with short-term protocols related to inconsistency in estrus response, increased interval to estrus, problems in pregnancy maintenance and prolificity. In these protocols, estrus cannot be precisely predicted, and the interval from CIDR removal to estrus may range from 60 to 108 h (Jackson, Neville, Mercadante, Waters, &

Lamb, 2014). In this sense, long-term synchronization protocols have proven to result in shorter intervals from CIDR removal to estrus when compared with short-term protocols, presenting a most reliable response to pregnancy and prolificity results. Even indicating the need of a longer time to stimulate hormonal functions of ewes, the long-term protocol may provide more security to manage estrus synchronization and reproduction of ewes (Vilariño, Rubianes, & Menchaca, 2011; Jackson et al., 2014).

Conception in small ruminants subjected to estrus synchronization protocols is a major concern in production. If animals are successfully synchronized but fail to conceive after breeding, there is no benefit in subjecting females to synchronization protocols (Cetin, Sagcan, Gungor, Ozyurtlu & Uslu, 2009). Apparently, the use of short-term CIDR protocols does not appear to have a negative effect on fertility during the natural breeding period. However, these may present problems in pregnancy rates and conception processes (Vilariño et al., 2011; Jackson et al., 2014). In turn, the use of long-term CIDR protocols does not cause adverse fertility results and pregnancy maintenance problems, further to present better results than short-term protocols in pregnant ewes and lambs produced. Ozyurtlu, Kucukaslan, & Cetin (2010) corroborate these affirmations reporting a conception rate above 50% in ewes subjected to long-term protocols than short-term protocols during a seasonal anestrus.

Table 3. Reproductive performance of Santa Inês ewes subjected to different protocols of estrus synchronization.

Variables	Short-term protocol	Long-term protocol	p-value	CV, %
Positive estrus, %	72.73[b]	81.81[a]	0.01*	3.53
Pregnancy rate, %	27.00[b]	81.00[a]	0.01*	7.89
Prolificity, %	2.00	2.11	0.07[ns]	10.23

CV – Coefficient of Variation. * Significant effect (p < 0.01). ns – non-significant.

Conclusion

Thus, it can be concluded that both protocols presented satisfactory results regarding estrus manifestation, and prolificity (lambs produced per ewe). However, under Amazon environmental conditions, the long-term protocol presented better results regarding positive manifestation of estrus and pregnancy rate.

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