Reproductive performance of crossbreed ewes subjected to estrus synchronization protocols in the Amazon

Desempenho reprodutivo de ovelhas mestiças submetidas à protocolos de sincronização de estro na Amazônia

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Paula Natalia Martins Rodrigues
Bacharel em Zootecnia pela Universidade Federal do Amazonas
Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus – AM
E-mail: paulanatalia18@gmail.com

Roseane Pinto Martins de Oliveira
Doutora em Ciência Animal pela Universidade Estadual do Norte Fluminense Darcy Ribeiro
Docente do curso de Zootecnia na Universidade Federal do Amazonas
Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200- Coroado I, Manaus - AM
E-mail: roseanepmoliveira@gmail.com

Marcia Lorena Monteiro da Silva
Bacharel em Zootecnia e Mestranda em Ciência Animal e Recursos Pesqueiros - PPGCARP pela Universidade Federal do Amazonas
Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus – AM
E-mail: marciamontteiro_@hotmail.com

Franklyn Ferreira de Oliveira
Médico Veterinário pela Universidade Federal Rural da Amazônia
Mestre em Ciências Ambiental pela Universidade Federal Fluminense
Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200-Coroado I, Manaus-AM
E-mail: franklynfeoli@hotmail.com

João Paulo Ferreira Rufino
Mestre em Ciência Animal e Doutor em Biotecnologia pela Universidade Federal do Amazonas
Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200- Coroado I, Manaus - AM
E-mail: joaopaulorufino@live.com

ABSTRACT

The objective of this study was to evaluate two protocols for estrus synchronization in crossbreed ewes in the Amazon region. Twenty crossbreed (Santa Inês x Dorper) ewes presenting ages between 2 and 3 years-old and average body score in 2.7 were used. The experimental design was completely randomized, where the treatments were constituted by two protocols for estrus synchronization (short and long) with ten animals each. Estrus occurrence data were described for each protocol. Data of estrus detection, pregnancy rate, live lambs at birth and spontaneous abortions were subjected to exact Fischer test. Data of prolificity, birth weight per lamb and twin births were firstly subjected to ANOVA and a

subsequent Tukey test. Results were considered significant at p≤0.05. Results were considered significant at p≤0.05. Both protocols presented great success rate to estrus occurrence, with all tested ewes manifesting the estrus. Comparing protocols, the short-term protocol presented better (p<0.05) results to pregnancy rate, prolificity, birth weight of lambs, and twin births. However, positive estrus, live lambs, and spontaneous abortions presented the same results (p>0.05). It was concluded that both protocols presented satisfactory results to estrus manifestation, live lambs, and spontaneous abortions. However, in Amazon environmental conditions, the short-term protocol provided better reproductive performance (pregnancy rate, prolificity, birth weight of lambs, and twin births).

Keywords: Ewes. Pregnancy. Progesterone. Reproduction. Amazon.

RESUMO

O objetivo deste estudo foi avaliar dois diferentes protocolos para sincronização do estro em ovelhas mestiças na região Amazônica. Foram utilizadas 20 ovelhas mestiças (Santa Inês x Dorper) com idade entre 2 e 3 anos e escore corporal médio em 2,7. O delineamento experimental foi inteiramente casualizado, onde os tratamentos foram constituídos por dois protocolos de sincronização do estro (curto e longo) com dez animais cada. Os dados de ocorrência do estro foram descritos para cada protocolo. Dados de detecção de estro, taxa de gravidez, cordeiros vivos ao nascimento e abortos espontâneos foram submetidos ao teste exato de Fischer. Os dados de prolificidade, peso ao nascer por cordeiro e partos gemelares foram submetidos à ANOVA e subsequentemente ao teste de Tukey. Os resultados foram considerados significativos em p≤0,05. Os resultados foram considerados significativos em p≤0,05. Ambos os protocolos apresentaram ótimo resultado para ocorrência de estro, com todas as ovelhas testadas manifestando o mesmo. Comparando os dois protocolos, o protocolo de curto prazo apresentou melhores resultados (p<0,05) para taxa de prenhez, prolificidade, peso ao nascer de cordeiros e incidência de parto gemelar. No entanto, a taxa de estro positivo, cordeiros vivos e abortos espontâneos apresentaram os mesmos resultados (p>0,05). Concluiu-se que ambos os protocolos apresentaram resultados satisfatórios para manifestação de estro e cordeiros vivos ao nascer. No entanto, nas condições ambientais da Amazônia, o protocolo de curto prazo proporcionou melhor desempenho reprodutivo.

Palavras-chave: Ovelhas. Prenhez. Progesterona. Reprodução. Amazônia.

1 INTRODUCTION

Reproductive performance is one of the most important factors in sheep farming (Oliveira et al., 2014). The majority of ewe breeds differ in reproductive behavior according to season changes, latitude/longitude, length of the photoperiod among other factors. From this, several strategies have been used to control ovarian activity focusing on improving fertility (Cavalcanti et al., 2012), and avoid the anestrus, most common reproductive disorder in ewes that causes great economic losses to the farmers (Ezzat et al., 2016).

Estrus synchronization is an interesting tool for increasing the pregnancy rate in ewes through estrus manifestations (Moraes et al., 2002). Technically, the synchronization of estrus in ewes focuses on the manipulation of the estrus cycle (Zonturlu et al., 2011), the manipulation of either the luteal or the follicular phase. 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 et al., 2014). Applications of exogenous hormones for increased reproductive performance in domestic ewes usually focus on estrus synchronization (Najafi et al., 2014).

The most popular protocols for FTAI in ruminants are based in progesterone administration, which has been the default method for several years. In sheep, the administration of this hormone is performed by intravaginal route by using silicone devices (like CIDR) or polyurethane devices (sponges) historically placed during periods between 6 to 14 days associated with a dose of eCG at the end of the treatment. The technique is based on the inhibitory role of progestogens on the hypothalamus-pituitary axis, acting like an exogenous corpus luteum (Menchaca et al., 2017).

Previous studies reported that the improvement of estrus synchronization depends on more effective manipulation of the corpus luteum and follicular development (Martemucci and 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; Metodiev, 2015).

Hormonal treatment to control ovulation and reproduction is an important tool to obtaion the successful breeding and increasing the number of pregnant females (Dias et al., 2018), resulting in a short breeding period and a more uniform newborn crop (Husein and Kridli, 2003). Previous studies reported that the key element of methods for estrus synchronization in small ruminants to control of luteolysis and lifespan of the corpus luteum (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 et al., 2008). Thus, the objective of this study was to evaluate two protocols for estrus synchronization in crossbreed ewes in the Amazon region.

2 MATERIAL AND METHODS

The study was conducted in Manaus, AM (2° 38' 43.8" S 60° 02' 27.4" W). The experimental protocol was approved by the Animals Use Ethics Committee (protocol n. 012/2019) of Federal University of Amazonas (Manaus, AM), and that it was conducted according to the Brazilian animal protection standards in teaching and research

Twenty crossbreed (Santa Inês x Dorper) ewes presenting ages between 2 and 3 years-old and average body score in 2.7 (scales at 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 soya, 60 g corn, 64 g bran, 14 g of mineral/vitaminic supplement. All ewes had free access to salt stone and fresh water. At the time of analyses, environmental conditions presented an average temperature of 33.12±0.23 ºC, and average relative humidity of the air of 70.42±0.12%.

The experimental design was completely randomized in blocks, where the treatments were constituted by two protocols for estrus synchronization (short-term - up to 6 days; and long-term – up to 12 days) with ten animals (replicates) each. Ewes submitted to the short-term protocol were identified with red collars, being used a Progespon®-soaked vaginal sponge (60 mg of medroxyprogesterone acetate) inserted into ewes for six days. On the 4th day of the protocol, 100 mg of Sincrocio® (Sodic cloprostenol) and 350 to 400 IU of Novormon® (Equine Chorionic Gonadotropin) were intramuscularly injected in each ewe. On the 6th day, the sponge was removed to finish the protocol.

Ewes submitted to the long-term protocol were identified with beige collars, being used a Progespon®-soaked vaginal sponge (60 mg of medroxyprogesterone acetate) inserted into ewes for 12 days. On the 8th day of the protocol, 100 mg of Sincrocio® (Sodic cloprostenol) and 350 to 400 IU of Novormon® (Equine Chorionic Gonadotropin) was intramuscularly injected in each ewe. On the 12th day, the sponge was removed to finish the protocol.

After sponge's removal in both groups, the ewes were organized according to the treatments and submitted 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 was grouped into five distribution periods (<24, 24 to 36, >36 to 48, >48 to 60, and >60 to 72 hours after sponge removal) using the vasectomized rams. After estrus confirmation, ewes were subjected to rams with attested fertility. The efficiency of the tested protocols was determined from positive estrus rate (%), pregnancy rate (%), prolificity (lambs per ewe), live lambs (%), spontaneous abortions (%), birth weight per lamb (kg), and twin births (births per ewe).

Estrus occurrence data were described for each protocol. Data of estrus detection, pregnancy rate, live lambs at birth and spontaneous abortions were analyzed using the GLM procedure of SAS (Statistical Analysis System, v. 9.2) and estimates of treatments were

subjected to exact Fischer test. Results were considered significant at $p \leq 0.05$. Data of prolificity, birth weight per lamb and twin births were analyzed using the GLM procedure of SAS (Statistical Analysis System, v. 9.2) and estimates of treatments were firstly subjected to ANOVA and a subsequent Tukey test. Results were considered significant at $p \leq 0.05$.

3 RESULTS AND DISCUSSION

The short-term protocol presented a great success rate, where all tested ewes presented estrus' occurrence and fast response (Table 1). Previous studies reported that several hormonal treatments have been used to synchronize estrus in small ruminants, where short-term protocols presented successfully in inducing and synchronizing estrus during both breeding and non-breeding seasons (Neves et al., 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 finished (Menchaca et al, 2017).

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

Periods	Ewes (n)	(%)
< 24 hours	4	40
24 to 36 hours	3	30
36 to 48 hours	3	30
48 to 60 hours	0	0
60 to 72 hours	0	0
Without estrus	0	0
Total occurrence	Ewes (n)	(%)
Ewes with estrus	10	100.00
Ewes without estrus	0	0

Own authorship table

There are also other problems linked with 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. Previous studies pointed out that the use of short-term protocols is directly related with the utilization of high dosages of hormones in a short-term period during the estrus induction protocol aiming to improve the reproductive performance (Amer and Hazzaa, 2009; Menchaca et al., 2017). In this sense, we may associate this fact with the fast response obtained in this study using short-term protocols.

On the other hand, the use of long-term protocol also presented a great success rate in this study, where above all tested ewes presented the occurrence of estrus (Table 2). The use of this protocol did not cause a fast response but caused a prolonged effect along the exposure period of ewes to hormones. Previous studies reported that usual long-term protocols used intravaginal sponges inserted over periods of 9 to 19 days together with the injection of hormones, being particularly used for the out-of-breeding season. Progesterone blocks FSH and LH secretion by suppressing the hypothalamus and also indirectly the pituitary anterior lobe and temporarily stops the end part of follicular development to the preovulatory stage. This suppression disappears with the removal of the sponges and estrus behavior are observed along with follicular development (Koyuncu et al., 2016). The results of this study indicated that up to 72 hours after sponge removal may be detected a relative rate of estrus occurrence, extending this limit for identification of estrus occurrence from 24 to 72 hours. Long-term progesterone estrus synchronization protocols can affect follicular dynamics and fertility of ewes. Initially, an increase in follicular renewal may occur. However, a low luteal effect may happen and decrease the speed of follicular renewal (Takada et al., 2012).

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

Periods	Ewes (n)	(%)
< 24 hours	0	0
24 to 36 hours	3	30
36 to 48 hours	3	30
48 to 60 hours	2	20
60 to 72 hours	2	20
Without estrus	0	0
Total occurrence	Ewes (n)	(%)
Ewes with estrus	10	100.00
Ewes without estrus	0	0

Own authorship table

Comparing both protocols, the short-term protocol presented better ($p<0.05$) results on pregnancy rate, prolificity (Table 3), birth weight of lambs and twin births (Table 4). However, estrus detection, live lambs at birth, and spontaneous abortions presented the same result between the treatments (Table 3). In this sense, Ungerfeld and Rubianes (1999) reported that short-term treatment (5-6 d) with different progesterone devices during the non-breeding season was as effective as the long-term treatment to induce estrus and the subsequent fertility, corroborating with results of this study. Intra-vaginal devices containing different types of progesterone, 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 present estrus (Ustuner et al., 2007).

Table 3. Estrus detection, pregnancy rate, live lambs at birth and spontaneous abortions of crossbreed ewes submitted to different protocols of estrus synchronization in Amazon environmental conditions.

Variables	Short-term protocol		Long-term protocol		p-value
Estrus detection	n	%	n	%	
Positive	10	100.00	10	100.00	0.05**
Negative	0	0.00	0	0.00	
Pregnancy rate	n	%	n	%	
Positive	8	80.00	5	50.00	0.01*
Negative	2	20.00	5	50.00	
Live lambs at birth	n	%	N	%	
Positive	10	100.00	10	100.00	0.05**
Negative	0	0.00	0	0.00	
Spontaneous abortions	n	%	N	%	
Positive	0	0.00	0	0.00	0.05**
Negative	10	100.00	10	100.00	

CV – Coefficient of Variation. * Significant effect (p<0.01). ** Significant effect (p<0.05).
Own authorship table

Table 4. Prolificity, birth weight per lamb and twin births of crossbreed ewes submitted to different protocols of estrus synchronization in Amazon environmental conditions.

Variables	Short-term protocol	Long-term protocol	p-value	CV, %
Prolificity, lambs per ewe	2.12[a]	2.00[b]	0.05**	10.50
Birth weight per lamb, kg	6.50[a]	5.50[b]	0.01*	3.52
Twin births, lambs	3.00[a]	1.00[b]	0.03**	4.52

CV – Coefficient of Variation. * Significant effect (p<0.01). ** Significant effect (p<0.05).
Own authorship table

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 to subjecting females to synchronization protocols (Cetin et al., 2009). From the results of this study, it was observed that the use of short-term protocols did not caused a negative effect on fertility, pregnancy, and birth of lambs (Vilariño et al., 2011; Jackson et al., 2014).

Physiologically, the estrus cycle is a series of hormonal cascades that change the morphology of the female reproductive system to prepare for pregnancy (Fatet et al., 2011). In commercial level, the synchronization of estrus allows control and shortening of lambing and kidding, with subsequent synchronization of weaning of young animals for slaughter (Abecia et al., 2012). Regarding the duration of progesterone treatment, the traditional progesterone 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 promotes the ovulation of animals (Wei et al., 2015).

Menchaca 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; $p<0.05$).

However, the present study did not use FTAI, which provide another perspective to this analysis, with the “ram effect” and the natural breeding being considered. Previous studies also reported concerns related the short-term protocols and an 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 et al., 2014). However, in this study, short-term synchronization protocols use has proven to result in shorter intervals from sponge removal to estrus when compared with long-term protocols, presenting a most reliable response to pregnancy and prolificity results. Even the long-term protocol aparently indicating more security to manage estrus synchronization and reproduction of ewes due to the time of exposure of the ewes to CIDR (Vilariño et al., 2011; Jackson et al., 2014), the use of short-term protocol caused a good effect on reproductive performance in a lower time than the other protocol.

5 CONCLUSIONS

It was concluded that both protocols presented satisfactory results to estrus manifestation, live lambs, and spontaneous abortions. However, in Amazon environmental

conditions, the short-term protocol provided better reproductive performance (pregnancy rate, prolificity, birth weight of lambs, and twin births).

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