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Evaluation of the short and long estrus synchronization protocol in inseminated sheep with frozen semen

Evaluación del protocolo corto y largo de sincronización de celo en borregas inseminadas con semen congelado

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Abstract

The objective of the study was to evaluate the effect of short (5 days) and long (9 days) SC protocols in ewes inseminated with frozen semen on the diameter of the uterine horn and pregnancy rate. The experiment was carried out at the Carolina Experimental Center, belonging to the National University of the Altiplano, and 40 ewes between Corriedale and Criollas were used; in groups of 20 for each treatment, which were synchronized with intravaginal sponges based on Medroxyprogesterone acetate, for 9 days (long protocol) and 5 days (short protocol) and at the time of sponge removal, 350 IU of eCG were applied. To measure the diameter of the uterine horn, ultrasonography was performed at 36 h post sponge removal and at 56 h after cervical insemination with thawed semen and a subsequent ultrasonography at 35 days to confirm gestation. Quantitative data were analyzed using the statistical t-test for uterine horn diameter and Chi-square for gestation rate. The results for uterine horn diameter were 11.11±1.52 and 12.12±2.12 mm for the short and long synchronization protocol, respectively. Regarding the pregnancy rate, 21.05 % and 25 % were obtained for the short and long synchronization protocol, respectively. Therefore, we can conclude that the long progesterone treatment had better results than the short treatment both in uterine diameter and pregnancy rate, but this difference was not significant (p>0.05).

Resumen

El objetivo del trabajo fue evaluar el efecto de los protocolos corto (5 días) y largo (9 días) de SC en borregas inseminadas con semen congelado sobre el diámetro del cuerno uterino y tasa de preñez. El experimento se realizó en el Centro Experimental Carolina, perteneciente a la Universidad Nacional del Altiplano, y se utilizó 40 ovejas entre Corriedale y Criollas, en grupos de 20 para cada tratamiento, que fueron sincronizadas con esponjas intravaginales a base de Acetato de Medroxiprogesterona, durante 9 días (protocolo largo) y 5 días (protocolo corto) y al momento del retiro de la esponja se aplicó 350 UI de eCG. Para medir el diámetro del cuerno uterino se realizó la ecografía a las 36 h post retiro de la esponja y a las 56 h la inseminación cervical con semen descongelado y una posterior ecografía a los 35 días para confirmar la gestación. Los datos cuantitativos fueron analizados mediante la prueba estadística de "t" para diámetro de cuerno uterino y Ji cuadro para tasa de gestación. Los resultados para el diámetro de cuerno uterino fueron de 11.11±1.52 y 12.12±2.12 mm para el protocolo de sincronización corto y largo, respectivamente. Respecto a la tasa de preñez se obtuvo 21.05 % y 25 %, para el protocolo de sincronización corto y largo, respectivamente. Por lo que podemos concluir que el tratamiento largo de progesterona, tuvo mejores

Palabras clave:

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preñez,
semen descongelado.

resultados al tratamiento corto tanto en el diámetro de cuerno uterino y tasa de gestación, pero no fue significativo esta diferencia (p>0.05).

Introduction

Peru sheep sector has great potential, as it has a large number of animals, but unfortunately there is a negative growth rate (-3.5%) and therefore a decrease in production (1.17%)[1] therefore, it is necessary to establish improvements in the productive and reproductive indexes through livestock improvement programs in order to increase herd production, hence the promotion of the dissemination of semen for the production of semen for the production of sheep, the dissemination of semen of high genetic value in the ovine species Artificial insemination (AI) is an appropriate reproductive biotechnology to achieve this goal[2]

Estrus synchronization (ES) methods are a very useful tool in AI programs[2]. Several ES techniques have been developed to induce estrus in sheep[3]. Long-term progestogen treatments (12 to 14 days) are commonly applied in sheep[4-13], with a percentage of animals showing acceptable estrus and fertility rates[12], but with lower fertility than natural estrus[14-16]. This low fertility rate is due to different causes such as, deficiencies in detection and ES, slow follicular turnover that generates a persistent follicle[4,17,18] on the other hand the traditional synchronization system brings vaginal infections[15,19], with bacterial proliferation[20] has also been associated with accumulation of offensive vaginal mucus, bad odor[21], vaginal adhesions, vaginitis[22], which result in low fertility rates, after AI with fresh semen. Short protocols (5 to 7 days) with progestogenic devices[4,23] have been reported to be as effective as traditional protocols (12-14 days)[24].

Martinez-Ros et al.[5], Amer & Hazzaa[6], Ungerfeld & Rubianes[7], Knights et al.[3], mention that short synchronization treatment has similar fertility to traditional ES, while Almadaly et al.[25], Viñoles et al.[4], Farfán et al.[9] Özyurtlu et al.[14] Vilariño et al.[13] Sareminejad et al.[26] report higher fertility results than the traditional synchronization treatment (5 to 7 days) could be an alternative to the traditional use of long synchronization, this would help to be more flexible in the field and thus avoid the problems described above.

In view of this dilemma, we intend to evaluate the short and long ES protocol in ewe lambs inseminated with frozen semen to determine which protocol is more efficient in terms of uterine diameter and pregnancy rate.

Materials and methods

Location and animals of the experiment. The experiment was carried out at the Experimental Center "Carolina", belonging to the Faculty of Veterinary Medicine and Animal Husbandry of the National University of the Altiplano, located in the District of Puno and province of Puno - Peru, around the coordinates 15 52 11 south latitude and 70 24 32 west longitude, at an altitude of 3995 meters above sea level. A total of 40 sheep were used (20 adult Corriedale ewes and 20 adult Criollo ewes) (Table 1), multipras taken at random from the flock of sheep, with a body condition of 2.5 (scale 1 to 5) that were ultrasounded to rule out pregnancy, clinically healthy,

with an extensive breeding system, kept before and during the experiment in grazing conditions in natural pastures without supplementation. The experiment was carried out between April and May.

Table 1 Distribution of sheep according to treatment

Corriedale sheep		Criollo sheep	
20		20	
*TL P4	**TC P4	*TL P4	**TC P4
10	10	10	10

*LT P4: Long treatment with progesterone.
** ST P4: Short Treatment with progesterone.

Synchronization of estrus. Bighorn sheep were given intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (MAP, Progespon Syntex S.A., Argentina)[27] on a random day of the estrous cycle (Day 0), for a total of 5 days for the short progesterone protocol[5,28] and 9 days for the long progesterone protocol[17,29]. On Day 5 and 9 of the treatments, the P4 sponges were removed and 350 IU of equine chorionic gonadotropin (eCG, Novormon Syntex S.A., Argentina) was applied intramuscularly to each ewe[7,15].

Ultrasonographic evaluation and artificial insemination. Ultrasonographic examinations for the measurement of uterine horn diameter (UHD) were performed at 36 h post application of eCG and gestation diagnosis at 35 days' post AI, in both cases a real time B-mode ultrasound scanner (Esoate Pie Medical, Tringa Linear VET) was used. The ewe lambs were inseminated at 56 h post application of eCG, using frozen semen in 0.25 mL straws, which had a sperm concentration of 300 x 10^6 after thawing at 35°C for 30 sec. A subjective evaluation of seminal motility was made, which was 45% on average[8], due to the fact that they belonged to the same breeder.

Statistical analysis. The data were subjected to descriptive statistics (mean, standard deviation, and coefficient of variability), comparing two groups (short and long treatment) and evaluating their normality and homoscedasticity, the UHD were subjected to t-test, while the pregnancy rate obtained in proportions was subjected to the non-parametric chi-square test, both tests were evaluated with a reliability of 95%, all data were evaluated in the R 3.4.1. program with its RCmdr (R Core Team, 2020) extension.

Results

Table 2 describes the statistics for the UHD in Corriedale and Criollo ewe lambs synchronized with the short and long synchronization protocol, observing similar means in relation to the type of protocol and breed (p=0.1206 and 0.6197 respectively), which indicates that the synchronization period does not influence the variation of the UHD nor the breed of ewe. A total of 5 sponges were lost (4 for the long protocol and 1 for the short protocol).

Table 2 Diameter of uterine horn in ewe lambs

Factors	Levels	No	Mean±D.S (mm)	CV (%)	Sign.
Synchronization period	Corto	19	11.11±1.52	13.68	0.1206
	Largo	16	12.12±2.12	17.49	
Sheep breed	Corriedale	19	11.11±1.63	14.36	0.6197
	Criollo	16	11.87±2.17	19.08	

Table 3 shows the pregnancy rate in ewe lambs according to synchronization period and breed, the proportion of pregnant ewes synchronized for 5 days was 21.05 % and in ewes synchronized for 9 days was 25 % (p=0.7817), in this sense there would be no dependence between the duration period of the

estrus synchronization protocol (short and long) in relation to the higher or lower pregnancy rate.

Table 3 Pregnancy rate in ewe lambs

Factors	Levels	No	No of fertile	% of fertile
Synchronization period	Corto	19	4	21.05
	Largo	16	4	25

(P > 0.7817)

Discussion

The results indicate the presence of significant UHD in both treatments, which may be due to the application of eCG that reduces the atresia of preovulatory follicles, thus generating an increase in the estrogen concentration, manifesting estrus and ovulation in the ewe lambs[6,30-32] since we applied a dose of 350 IU, which influenced the estrogen concentration. However, a slight difference is observed between the UHD being slightly superior the long treatment in relation to the short one, this characteristic could be measured because the uterine wall has a more heterogeneous ecotexture (due to the increase of vascularization and the edema of the horns) than in estrus and during estrus, it is normally possible to differentiate the endometrial folds and the myometrium, as well as the small accumulations of fluid in the uterine lumen[33], this difference is due to the fact that during the long treatment a greater follicular growth could be observed[4], which is characterized by a greater estrogen production that leads to a slight increase in uterine diameter due to the increase in endometrial development and endometrial glands, in addition to edema due to greater vascularization compared to the short treatment[3,6,9]. In addition, Teixeira de Sousa et al.[34] reported in heifers that there is an increase in UHD due to the effect of estrogen, favoring pregnancy.

The comparison of UHD in relation to breed there is no statistical difference being for Creole sheep (11.87±2.17 mm) in comparison to Corriedale sheep (11.35±1. 63 mm) which would indicate that Creole and Corriedale ewes had similar responses to the synchronization protocols of the present study so estrogens influenced uterine diameter even though there are genetic or racial factors[30] that could have influenced since the Creole breed is more prolific than the Corriedale, which probably caused greater sensitivity to the synchronization protocol and therefore a greater UHD. Studies of UHD diameter due to the effect of the synchronization protocol are scarce, so this study could lay the foundation for further studies on the subject, and would also help to identify indicative parameters that are useful for discarding sheep or as an indicator of the presence of estrogen.

Gestation rates (Table 3) are similar to those reported by Gibbons & Cueto[10] and Muñoz et al.[11] for AI with frozen semen via cervical with a single insemination, while Perez et al.[8] had a gestation rate of 68% for their double insemination with thawed semen[11]. They also agree with Knights et al.[3], Ungerfeld & Rubia nes[7] and Martinez-Ros et al.[5], in reporting slightly higher gestation rates for long (12 days) than short (5 days) protocols; while Koyuncu et al[24], Da Silva et al.[12] and Amer & Hazzaa[6] had much higher gestation rates for the long protocol (14 or 12 days) with respect to the short protocol (6 or 7 days); but they do

not agree with Vilariño et al.[13], Farfán et al.[9], Özyurtlu et al.[14], Sareminejad et al.[26] nor with Verdol jak et al.[23] who reported a higher pregnancy rate for the short protocol (6 or 7 days) with respect to the long protocol (14 or 12 days). Our gestation results for the short treatment of 21. 05 % and for the long treatment of 25 % were low in comparison with other authors due to several factors such as the use of natural mating[3,5-7,12,25], balanced feeding[6,12], application of cloprostenol[12], application of eCG at higher doses[3,5,7,12], male effect[12], difference between breeds; while in our work we used thawed semen and it is known that semen freezing decreases the gestation rate[35,36], to this is added the nutritional deficiency typical of the time of year[6]. The difference in our results due to the effect of the type of protocol could be due to the fact that eCG (350 IU) was applied at the end of the two treatments, which could have caused the formation of follicular cysts in the short treatment[4], while in the long protocol it could have counteracted the fertility problems[4,17,37,38].

In addition, the long protocol had this improvement possibly due to the lack of use of prostaglandin in the short protocol[19,39] to eliminate the functional corpus luteum since it could affect its effectiveness5 in the productive period; finally, it should be considered that our long treatment was only 9 days, so it did not exceed the half-life of the corpus luteum (11 to 12 days)[17], favoring follicular exchange, which could generate oocytes in good conditions[40,41]. Therefore, we can conclude that the long synchronization protocol had a higher UHD and gestation rate than the short synchronization protocol, but not significantly. Nevertheless, from an economic and management point of view, it is possible to replace the traditional P4 regimen (12-14 days) with a short synchronization protocol (5-7 days).

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Conflicts of interest

The authors declare that this research was carried out at the Universidad Nacional del Altiplano Puno and that there is no conflict of interest between the authors of this article.

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Ethical considerations

The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry of the Universidad Nacional del Altiplano and the guidelines established by this Committee were followed.

Authors' contribution to the article

Manrique Quispe Yan Pierr, wrote the manuscript and the experimental part of the research. *Perez Guerra Uri Harold Uri*, carried out the experimental design and writing of the manuscript. *Malaga Apaza Julio*, revised and wrote the manuscript. *Ayma Flores Wilbur Ruben*, performed the experimental part of the research and writing of the manuscript. *Cardenas Oscar*, performed the experimental design and statistical analysis. *Perez Durand Manuel Guido*, did the writing and final revision of the manuscript.

Limitations in the research

There were no limitations for the development of the research.

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