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## ESTROUS SYNCHRONIZATION PROTOCOLS FOR LAPAROSCOPIC ARTIFICIAL INSEMINATION IN OVIS ARIES, HUANTA, AYACUCHO

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### SUMMARY

Heat synchronization in sheep is traditionally based on the insertion of intravaginal devices with progesterone for 12-14 days, plus the administration of a dose of the hormone equine chorionic gonadotrophin (eCG) upon removal of the device. The objective is to develop estrous synchronization protocols in laparoscopic artificial insemination (IAL) in sheep, to determine the correct eCG dose and the optimal time of ALI. Two experiments were carried out, the first to determine the appropriate dose of eCG and the optimal moment of IAL in Creole sheep, which were synchronized using a vaginal sponge for 7, 12 and 14 days, corresponding to the T1, T3 and T2 treatments, respectively; on the day of sponge removal, eCG doses of 100, 150 and 200 IU were applied for T1, T2 and T3, respectively, and ultrasound evaluations were performed on the first and second day after sponge removal to evaluate the time of ovulation. The IAL was performed according to the optimal time of service with frozen semen straws from East Friesian and Dohne Merino males, evaluating the pregnancy rate at 45 days post-IAL; while the second experiment was to determine the device and the duration of fertility in IAL, placing the vaginal sponge for 7, 12 and 14 days. In the short 7-day treatments, prostaglandin was applied at the time of intravaginal device placement, evaluating the pregnancy rate at 45 days post-IAL, The interval of hours of the moment of IAL is different in the three treatments and is significant ( $p < 0.05$ ), in T1 the time of IAL

*was 52.50±0.55 hours, in Q2 it was 54.14±0.69 hours and in T3 it was 49.71±0.76 hours. The pregnancy rate was assessed at 45 days post-IAL with transrectal ultrasound, which determined the significant increase with the doses of eCG applied in each treatment ( $p<0.05$ ). In the return of heat after IAL. Ewes showed heat return of 66.7, 85.7 and 75% for T1, T2 and T3, respectively. Ultrasound results at 45 days post-IAL were 50, 57.1%, and 71.4% for T1, T2, and T3, respectively. It is concluded that, the higher the doses of eCG, the greater the estrus response and the shorter the heat interval after removal of the vaginal sponge.*

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**KEYWORDS:** Ovis Aries; Laparoscopy; Estrous Synchronization; eCG.

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## 1. INTRODUCTION

The sheep population in the department of Ayacucho was registered in 2012 for 616,910 heads, of which 7.73 correspond to the Corriedale breed; 3.03% to Hampshire Down; 1.02% to the Black Belly, 3.92% are sheep of other breeds, and 84.3% corresponds to Creole sheep, being necessary genetic improvement programs to reduce this high percentage and increase the number of purebred sheep, which produce better quality meat, wool and milk. On the other hand, some 50,935 sheep are registered in the province of Huanta, which represents 8.3% of the entire Ayacucho region, of which 96.5% are Creole sheep; 0.63% Corriedale breed, 0.43% Hampshire breed; 0.77% are Black Belly breeds, and 1.33% are of other breeds (INEI, 2012).

Artificial insemination (AI) was the first major biotechnology applied to improve the genetics of farm animals, and its implementation allows the rapid and massive dissemination of desirable characteristics of males with high productive potential (Gibbons et al., 2019); it allows a genetic improvement process to be carried out in the flock, in such a way that the efficiency of this process is closely related to the choice of protocols, since these allow post-induction heat presentation of between 60 and 100% of the ewes; as well as progesterone concentrations during superovulation protocols on follicular growth (Yotov et al., 2023; Sartori et al., 2023; Espinoza et al., 2020). Fixed-time AI versus AI with heat detection, avoids the use of vasectomized males ("cuts") for heat detection, the confinement and daily handling of females to separate ewes in heat and males, technology has contributed significantly to reducing stress in animals (Džermeikaitė et al., 2023) by minimizing the number of running of the bulls; as well as the control of the transmission of diseases between animals (Cojic & Morrell, 2023); by the way, superovulatory diets relieve stress in animals (Zhang et al., 2023); however, the success of an FTAI program depends, among other factors, on the use of an efficient heat synchronization protocol that guarantees a pregnancy percentage of between 60-70% (Sartori et al., 2023).

The most commonly used assisted reproduction techniques in sheep are heat synchronization, AI and embryo transfer. Traditional heat synchronization protocols use two doses of prostaglandin F2 alpha (PGF) with an interval of 7-14 days between both applications (Hölper et al., 2023; Boudaud et al., 2023) or employ progesterone-impregnated intravaginal devices or progestin-containing intravaginal sponges for 12-14 days in conjunction with intramuscular administration of equine chorionic gonadotrophin

(eCG) at variable doses and timing (Eldomany et al., 2023; Daham et al., 2023). In the province of Huanta, Ayacucho region, it is evident that reproductive biotechnologies such as laparoscopic artificial insemination (LAI) have not been used as real alternatives for genetic improvement, therefore, animals of high genetic value have not multiplied; while the use of reproductive biotechnologies in sheep has allowed genetic improvement in the medium term in other regions of Peru (Céspedes, 2018). Working to establish a mating synchronization protocol in Creole sheep in the province of Huanta would be an alternative genetic improvement program in the medium term for the benefit of producers, since it would allow multiplying animals of high genetic value; which is why the objective of this research is to develop estrous synchronization protocols for artificial insemination in sheep in the province of Huanta, Ayacucho region, to determine the correct dose of eCG, the optimal time of artificial insemination, the sponge device and the duration time on fertility.

## 2. MATERIALS AND METHODS

### Location

The research was carried out in the peasant community of Pampalca, district of Huanta, province of Huamanga, Peru, whose latitude and longitude coordinates are -74.0722373, -12.7576911, respectively.

### Experimental animals

20 Creole sheep, between 2 and 3 years of age, 1 to 3 births, body condition of 1.5 to 2.5 (on a scale of 1 to 5) were used, and a clinical-gynecological evaluation was performed to rule out anomalies or reproductive disorders.

During the experimental period, the ewes of each treatment remained in free grazing, and supplemented *ad libitum* with *Avena sativa* hay.

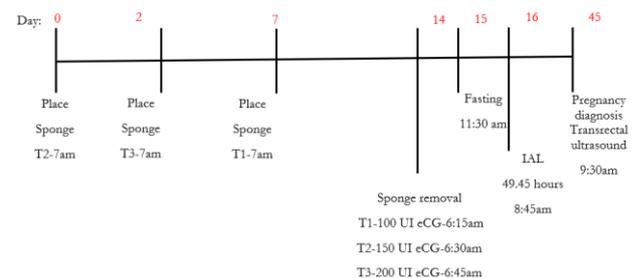


Figure 1: Protocols for synchronizing heat with progestogen in different treatments for Creole sheep.

### Heat Synchronization Protocol

All ewes were synchronized with intravaginal sponges impregnated with medroxyprogesterone

acetate -MPA (MPA 60 mg/sponge) for 7, 12 and 14 days, corresponding to T1, T3 and T2 treatments, respectively. When the sponge was removed, the animals received according to the T1, T2 and T3 treatments with application of 100, 150 and 200 IU doses of eCG (Novormon) intramuscularly.

**Heat Detection and Laparoscopic Artificial Insemination**

For the detection of heat and ILO, ultrasound evaluations of the ewes were performed on the first and second day after removal of the sponge to evaluate the time at which ovulation occurs. The IAL to females was performed according to the optimal time of service in sheep with 0.5 ml frozen semen straws from East Friesian and Dohne Merino males (Figure 1).

**Non-Return Rate and Gestation**

Cases of heat return were monitored between days 12 and 14 post-insemination with rams with aprons, making the diagnosis of pregnancy at 45 days of service by transrectal ultrasonography equipped with a 5 MHz linear transducer complemented with a 15 cm PVC (device penetration) tube to give rigidity to the transducer.

**Statistical Analysis**

The variables evaluated were: post-induction heat, heat return, pregnancy rate and birth rate, expressed as a percentage, which were analyzed using the chi-square test. For both tests, a significance level of 5% was established. All analyses were performed using RStudio and SPSS statistical software.

**3. RESULTS**

The appropriate doses of eCG for ovulation induction in Creole sheep and the determination of the device and duration time on fertility in IAL are shown in Tables 1 and 2, respectively.

**Table 1: Reproductive performance of eCG-treated Creole ewes associated with different heat synchronization protocols.**

	S1	S2	S3
	E-7D 100 UI (n=6)	E-14D 150 UI (n=7)	E-12D 200 IU (n=7)
IAL Time (hours)	52.50 ± 0.55 (p < 0.05)	54.14 ± 0.69 (p < 0.05)	49.71 ± 0.76 (p < 0.05)
Pregnancy rate (%)	50.0	57.1	71.4
Birth rate (%)	16.7	42.7	28.6

E-7D: 7-Day Sponge, E-12D: 12-Day Sponge, E-14D: 14-Day Sponge.  
Significance at 0.05

Table 1 shows that the interval of hours at the time of IAL is different in the three treatments and is significant (p<0.05), in T1 the time of IAL was 52.50±0.55 hours, in T2 it was 54.14±0.69 hours and T3 was 49.71±0.76 hours. The pregnancy rate was assessed at 45 days post-IAL with transrectal ultrasound, which determined the significant increase with the applied doses of eCG in each treatment (p<0.05). The birth rate decreased with each treatment, probably due to external environmental factors or the handling the animal received during gestation.

**Table 2: Reproductive efficiency of Creole ewes subjected to heat synchronization and IAL, according to the synchronization protocol (T1-7days, T3-12days and T2-14days).**

	T1		T2		T3		p-value
	E-7D 100 UI		E-14D 150 UI		E-12D 200 UI		
	N	%	N	%	N	%	
<b>Heat return</b>							
No	4	66.7	6	85.7	5	75.0	0.705
Yes	2	33.3	1	14.3	2	28.6	
<b>Ultrasound (45 days)</b>							
Positive	3	50.0	4	57.1	5	71.4	0.608
Negative	1	16.7	2	28.6	0	0.0	
Empty	2	33.3	1	14.3	2	28.6	
<b>External runoff (Perneo 90 days)</b>							
Abortion	2	33.3	1	14.3	3	42.9	
Empty	3	50.0	3	42.9	2	28.6	
Pregnant	1	16.7	3	42.9	2	28.6	0.587
<b>Born</b>							
Yes	1	16.7	3	42.9	2	28.6	0.587
No	5	83.3	4	57.1	5	71.4	

E-7D: 7-Day Vaginal Sponge, E-12D: 12-Day Vaginal Sponge, and E-14D: 14-Day Vaginal Sponge  
Significance at >0.05

Table 2 shows that in the return of heat after IA. The ewes in T1 showed 2 ewes at heat return, but no significant heat non-return rate of 66.7% compared to T2 ewes which was 85.7% and T3 which was 75% with (p>0.05). Ultrasound results at 45 days post-IAL were positive, T1 50%, T2 57.1%, and T3 71.4% with a significance of (p>0.05).

In the diagnosis of pregnancy by external ballot at 90 days with the perneous method, pregnant ewes were observed in T1 was 16.7%, T2 was 42.9% and T3 was 28.6% were statistically similar, showing that all estrus synchronization protocols give similar results. The birth rate in T1 was 16.7%, T2 was 24.9 and T3 was 28.6%, as well as similar between synchronization protocols evaluated with a significance of (p>0.05).

**4. DISCUSSION**

The interval of hours at the time of IAL if there are statistical differences (p < 0.05), in T1 the time of IAL was 52.50±0.55 hours, in T2 it was 54.14±0.69 hours

and T3 was  $49.71 \pm 0.76$  hours, in contrast to Martínez et al. (2006) who reported in relation to the interval in hours, from the withdrawal of the CIDR to the presentation of synchronized estrus there were no statistical differences ( $p > 0.05$ ) between treatments, being  $44 \pm 24.2$ ,  $43.7 \pm 17.3$  and  $39.8 \pm 08.3$  h for T1, T2 and T3, respectively; likewise, Arbués et al. (2018) and Gardón et al. (2015) worked with heat synchronization protocols in Merino sheep, obtaining a shorter interval to the post-removal heat of the sponge in sheep that received 300IU of eCG of 43.8 hours and 450 IU eCG of 44.3 hours.

The pregnancy rate was evaluated at 45 days post-IAL with transrectal ultrasound, finding a significant increase with the doses of eCG applied in each treatment ( $p < 0.05$ ); while López et al. (2021) working with biotypical fur animals and with intravaginal sponges, achieved a pregnancy rate of 71.4% in treated groups of 400 IU of eCG compared to sheep treated with 100 and 250 IU of eCG. Labra (2021) reports sheep treated with 200, 300 and 400 IU eCG, for T1 70%. T2 and T3 80 % for both cases, with no significant differences ( $p > 0.05$ ).

Likewise, with similar results, Quispe (2022) worked for seven days with 24 ewes, separated into 12 gilts and 12 multiparous sheep treated with 350IU of eCG, and obtained a pregnancy rate of 50% and 58.33%, respectively; however, Canaza (2017) reports a 60% pregnancy rate, similar to that reported by Catalano et al. (2007). who found a 54.14% pregnancy rate with the MAP and eCG protocol, in agreement with Mellisho et al. (2006) who reported a 54.7% pregnancy rate of Black Belly sheep managed in a stabled breeding and artificially inseminated with the frozen semen laparoscopic technique.

Menchaca et al. (2017), when conducting trials with 1750 multiparous ewes that received intrauterine insemination, the pregnancy rates found were 43.5 and 37.8%, with short-term protocol and traditional long protocol, respectively ( $p < 0.05$ ). In another experiment with 922 ewes that underwent cervical TAI with fresh semen, pregnancy success was also higher with the short-term protocol than with the traditional long protocol, using second-use intravaginal devices, obtaining pregnancy rates of 41.2 and 29.1%, respectively ( $p < 0.05$ ).

The birth rate decreased in each treatment, probably due to external environmental factors or the management that the animal received during gestation, in contrast to what was reported by Hermadi et al. (2023), who assert that eCG favors follicular growth and estrogen secretion, ensures the chances of oestrus and ovulation occurring in a higher percentage in animals, increasing the

possibility of pregnancy; however, it is important to take into consideration that doses of recombinant eCG increase the pregnancy rate in cows (Villarraza et al., 2021). On the other hand, the synchronization of heat of short periods such as 5 to 7 days improves the fertility rate, due to the drop in exogenous progesterone levels; although the advantage of using natural progesterone-releasing intravaginal devices (CIDR) in short periods, such as six days, is recognized, as it reduces the time of hormone exposure in sheep, without reducing reproductive variables (López et al., 2023).

In the heat return after IAL, some ewes in T1, T2 and T3 returned to heat, but not significantly in heat return at T1 was 66.7% compared to T2 ewes which was 85.7% and T3 was 75% with ( $p > 0.05$ ), indicating that they could have become pregnant; these results are consistent with values obtained by Espinoza et al. (2022) who found similar results with a greater number of animals in each treatment, such that, in T1 the rate of no heat return was 81.25% compared to T2 ewes that obtained 87.25% ( $p > 0.05$ ); although short-term CIDR treatments of 5 days without eCG administration are equally effective in inducing estrus behavior, preovulatory LH discharge and ovulation in sheep as with classic protocols based on 14-day treatments plus eCG in CIDR withdrawal; however, the application of a 5-day protocol without eCG for fixed-time artificial insemination would be adapted to a later ovulation time (Uriol et al., 2019).

No statistical differences were found between the three heat synchronization protocols ( $p > 0.05$ ), with a lower percentage of heat return in the three heat synchronization protocols

The results of the ultrasound at 45 days post-IAL were positive in T1 was 50%, in T2 it was 57.1% and T3 was 71.4% with a significance level of ( $p > 0.05$ ). In the diagnosis of pregnancy by external balloting with the perneous method at 90 days, 16.7%, 42.9% and 28.6% of pregnancy were detected for the T1, T2 and T3 treatments, respectively, being statistically similar, showing that both heat synchronization protocols give similar results. Espinoza et al. (2022) reported that the pregnancy rate detected by transrectal ultrasound at 41 days post-IATF was 81.25% and 87.50% for T1 and T2 treatments, respectively, and for the diagnosis of pregnancy by ballot (T1: 81.25; T2; 86.67%) also reported that they were statistically similar. Likewise, Mango (2015) obtained pregnancy percentages of 42.1% (8/19) and 61.1% (11/18) with 300 and 600 IU of eCG, respectively, similar to the percentages obtained in this research. The pregnancy percentages obtained in this research can be considered moderate, taking into

account the sample size in each protocol; likewise, in the external ballot of the perneo method at 90 days post-ALI, the pregnancy rate with the ultrasound diagnosis was much lower, due to the presence of abortions due to a deficit in animal feed and other uncontrollable factors.

The birth rate was 16.7%, 24.9 and 28.6% for the T1, T2 and T3 treatments, respectively, as well as similar between heat synchronization protocols evaluated with a significance of ( $p>0.05$ ).

## 5. CONCLUSIONS

The doses of eCG in the induction of ovulation in Creole ewes is given in the proportion of eCG doses that are given to the animal and the heat

synchronization time, the ewes that received higher doses of eCG will present a greater heat response than those that received lower doses, it is also related between the eCG dose and the heat synchronization time.

The optimal time for IAL is given according to the response to heat and the doses of eCG applied, that is, the higher the doses of eCG, the greater the heat response and the shorter the heat interval after the removal of the sponge.

The device and duration of heat synchronization with the application of eCG in short protocols such as 7 days achieve similar results as when applying the heat synchronization protocol with the application of eCG of 12 or 14 days in Creole sheep.

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