Research Article

Factors Affecting Pregnancy in Ewes Subjected to Estrus Synchronization and Artificial Insemination Under Field and High-Altitude Conditions

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Abstract | The objective of this study was to identify factors influencing pregnancy rates in artificial insemination (AI) programs for ewe lambs subjected to estrus synchronization protocols under field and high-altitude conditions. The study was conducted in the Llalli district, Melgar province, involving 120 ewe lambs aged 2 to 4 years with body condition scores between 2.5 and 3.5, all managed by local producers under field conditions. Two estrus synchronization protocols were applied using intravaginal progestogen sponges: 14 days (TI, n = 60) and 12 days (TII, n = 60). Upon sponge removal, 400 IU of equine chorionic gonadotropin (eCG) was administered intramuscularly, and AI was performed 56 hours later using fresh diluted semen from two proven fertile rams. Ultrasonographic evaluations were used to measure uterine horn diameter on the day of AI and to confirm pregnancy 35 days post-AI. The variables analyzed to identify factors affecting pregnancy included breed, body condition score (BCS), age, breeder (I or II), treatment group (TI or TII), time of AI, and uterine horn diameter. Logistic regression and machine learning analysis identified uterine horn diameter, ewe age, and synchronization protocol as significant predictors of pregnancy. Uterine horn diameter averaged 10.8 mm in TI and 10.1 mm in TII. Younger ewes had a higher pregnancy rate than older ones, and the pregnancy rate in TI (14-day protocol) was significantly higher at 78.3%, compared to 46.7% in TII (12-day protocol) ($P < 0.001$). In conclusion, under field and high-altitude conditions, key factors influencing pregnancy in ewe lambs subjected to estrus synchronization include uterine horn diameter, age, and the synchronization protocol used. The 14-day protocol yielded a significantly higher pregnancy rate than the 12-day protocol.

Keywords | Ewes, Insemination, Pregnancy, Progesterone, Semen, Uterine diameter

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INTRODUCTION

Peru has significant livestock potential and maintains a large sheep population. However, the country is currently experiencing a negative growth rate in both sheep (-3.5%) and goats (-0.1%). The Puno region holds the largest concentration of sheep, accounting for 21.9% of the national flock. This population decline has also led to a reduction in meat production, with an average annual decrease of -1.17% (Mazinani and Rude, 2020). To reverse this trend, improvements in the productive and reproductive performance of sheep are essential. Implementing genetic improvement programs that enhance herd and flock productivity through the dissemination of superior genetic material particularly via artificial insemination (AI) represents a promising reproductive biotechnology to achieve this goal (Gibbons, 1997).

Estrus synchronization methods are valuable tools in artificial insemination (AI) programs (Gibbons, 1997), and several techniques have been developed to induce estrus in ewes (Knights et al., 2001). Long-term progestogen (P4) treatments, typically lasting 12 to 14 days, are commonly used in small ruminants to induce and synchronize estrus. While these protocols result in a high percentage of animals exhibiting estrus, fertility rates tend to be lower compared to those observed with natural estrus cycles (Viñoles et al., 2004).

Short-term estrus synchronization protocols (5 to 7 days) using progestogen-based treatments during the reproductive season have been reported to be at least as effective as traditional long-term protocols (12–14 days) (Viñoles et al., 2004). Several studies have found that short-term treatments result in fertility rates comparable to those achieved with conventional synchronization methods (Martínez-Ros *et al*., 2018; Amer and Hazzaa, 2009; Ungerfeld and Rubianes, 2002; Knights et al., 2001). Long-term protocols also remain effective, as demonstrated in recent research conducted under high-altitude conditions (Quispe *et al*., 2021). Estrus synchronization in ewes is widely practiced through regional and local government programs, which are often accompanied by data collection. These data provide insight into the factors that influence pregnancy rates under real-world field conditions, especially among small and medium-scale producers.

Therefore, the objective of this study was to identify the key factors affecting pregnancy outcomes in artificial insemination (AI) programs for ewes subjected to estrus synchronization protocols under field and high-altitude conditions.

MATERIALS AND METHODS

Study site and animals

The research was conducted in the communities of the Llalli District, Melgar Province, in the Puno Region of Peru, located at an altitude ranging from 3,500 to 4,100 meters above sea level. The study took place between May and August. A total of 120 non-pregnant ewes, approximately 2 to 4 years old, with a body condition score (BCS) between 2.5 and 3.5 (on a 1 to 5 scale), were used. The animals belonged to the Corriedale and Creole breeds, with an average body weight of 38 kg. Selected ewes were dewormed and, following a physical examination, were deemed clinically normal.

All animal handling procedures were conducted in accordance with protocols approved by the Animal Research Ethics Committee of the Universidad Nacional del Altiplano (Ethical Approval Certificate No. CEBA-FMVZ-PCA-2024-09).

Management and facilities

The ewes selected for artificial insemination (AI) were kept on natural, cultivated pastures for 7 hours per day; most of them spent the night in sheds. However, it should be noted that management was probably not uniform, as it depended on the environmental conditions provided by the owners.

MATERIALS AND METHODS

Heat synchronization

The ewes were treated with intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP) (Progespon®, Laboratorios Zoetis, Buenos Aires) for either 14 days (Treatment I: TI) or 12 days (Treatment II: TII). Upon sponge removal, 400 IU of equine chorionic gonadotropin (eCG) (Novormon® 5000, Laboratorios Zoetis, Buenos Aires) was administered intramuscularly. The injection site was disinfected with iodinated alcohol, following the protocols recommended by Espinoza *et al*. (2020) and Quispe *et al*. (2021).

Ultrasonographic evaluation

Ultrasound examinations were performed on the day of artificial insemination (AI) using a portable real-time B-mode ultrasound system (Draminski 4Vet®, Owocowa, Olsztyn, Poland) equipped with a 9.0 MHz transrectal linear transducer. Uterine horn diameter was measured using calipers positioned at the outer limits of the uterine horns, as shown in Figure 1A. Pregnancy diagnosis was conducted 35 days after AI using the same ultrasound equipment, as illustrated in Figure 1B.

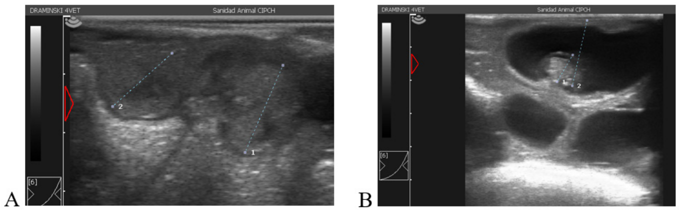

Figure 1: Ultrasonographic evaluation of the diameter of the uterine horn (a) and gestation at 35 days post-insemination (b).

Sperm collection and artificial insemination (AI)

Semen collection was performed with an artificial vagina (AV) as recommended by various authors (Aisen and Venturino, 2004; Evan and Maxwell, 1990) who indicate that the appropriate temperature was 42 °C for the thermal stimulus and air entering. The AV was responsible for mechanical stimulus. The semen collected from two breeders with similar mass motility characteristics were diluted according to the number of ewes to be inseminated with the commercial dilutor AndroMed ® (minitube, Germany) at a temperature of 37 ° C. The AI of the ewes was performed 56 hours after the removal of the sponges, after cleaning the perianal area and preparing the AI pipette by placing 0.1 ml of diluted semen per ewe at the entrance of the cervix.

Statistical analysis

The data were first subjected to descriptive statistical analysis, followed by tests for normality and homogeneity of variance. Depending on the distribution of the data, either parametric or non-parametric tests were used to compare the two treatment groups (14-day and 12-day protocols), with comparisons evaluated using the Student's *t*-test. A logistic regression analysis was performed with pregnancy status (pregnant vs. not pregnant) as the dependent dichotomous variable. The independent variables included uterine diameter, body condition score, treatment, breed, age, time of insemination, and semen donor. Additionally, a machine learning approach was applied, using pregnancy outcome as the dependent variable. This resulted in a decision tree model, as illustrated in Figure 2. A non-parametric chi-square test was used to assess statistical differences in pregnancy rates between the two treatment groups (TI and TII). All statistical analyses were conducted using the RStudio statistical software.

RESULTS AND DISCUSSION

Table 1 shows the logistic regression to determine the factors that affect gestation in ewes of different ages inseminated with diluted fresh semen.

The factors that directly affected or had statistical differences were the age of the ewes, particularly between 2- and 4-years old ewes. Another variable with statistical difference was the uterine diameter. Ewes that presented with a larger diameter had greater success in gestation. The other variables that were not significant were: body condition, type of synchronization, breed of inseminated ewes, reproductive and non-reproductive season and sperm used (comparing only two males). The variables that were significant are the diameter of the uterine horn which was on average for TI of 10.8 ± 0.06 mm and 10.1 ± 0.04 for TII. TI had a higher pregnancy rate probably due to a higher concentration of estrogens that produce edema at the wall of the uterine horns, which in turn generates the turgor that the ultrasound shows as a structure with a larger diameter (Tibary *et al*., 2005). The age of the female at AI was also statistically significant as reported

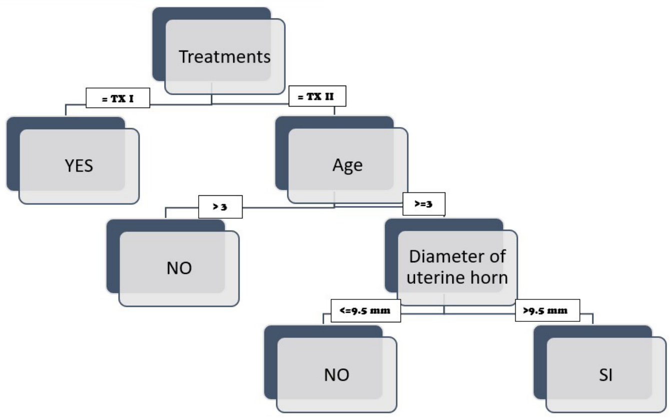

Figure 2: Decision tree with the most important variables in relation to pregnancy.

Table 1: Logistic regression analysis of factors affecting pregnancy in ewes.

Predictor	Estimator	EE	Z	P
Constant	-14.3894	8.774	-1.64	0.101
Uterine diameter	1.4713	0.886	1.6615	0.047
DC	-0.3584	1,086	-0.3301	0.741
Treatment				
II – I	-0.1742	0.802	-0.2171	0.828
Race				
Corriedale-creole	-0.0771	0.808	-0.0954	0.924
Age				
3 – 2	0.775	0.617	1.2561	0.209
4 – 2	1,771	0.624	2.8362	0.005
AI				
R – NR	-0.8115	0.641	-1.2661	0.205
Male				
II – I	0.2264	0.59	0.3838	0.701

DC: body condition; I = first treatment and II = second treatment; R: breeding season, NR = non-breeding season; in males, I = first breeder and II = second breeder.

by other researchers (Aisen, 2004; Anel *et al*., 2005) who indicate that fertility decreases between 2 to 3% per year in different breeds of sheep. Others indicate that after 1.5 years of age the parturition rate decreases by 1.74% per year. These logistic regression analyses are important because they allow the identification of independent variables that influence pregnancy success. These same analyses have been widely used in other species such as cattle and camelids in embryo transfer programs (Peixoto *et al*., 2007; Perez *et al*., 2019).

Figure 2 shows a decision tree created using machine learning, which allows us to assess which factors most affect pregnancy.

The evaluation of this technique allows us to indicate that TI generated a greater number of pregnancies. While TII was successful, age of the ewes influenced pregnancy rates. The majority of ewes older than 3 years do not get pregnant, while ewes equal to or less than 3 years old got pregnant as long as the diameter of the uterine horn was greater than 9.5 mm (in this study 10, 10.5, 11 and 11.5 mm). As mentioned previously these results could be the result of a greater production of estrogens with larger uterine horn size and the LH peak that produces ovulation (Aisen, E, 2004). Embryo transfer programs in cattle have also shown that younger animals have greater fertility than older animals (Pérez Durand *et al*., 2022).

Figure 3 shows a comparison of pregnancy rates according to the synchronization protocol. The ewes with the highest pregnancy rates were those exposed to the progesterone sponge for 14 days.

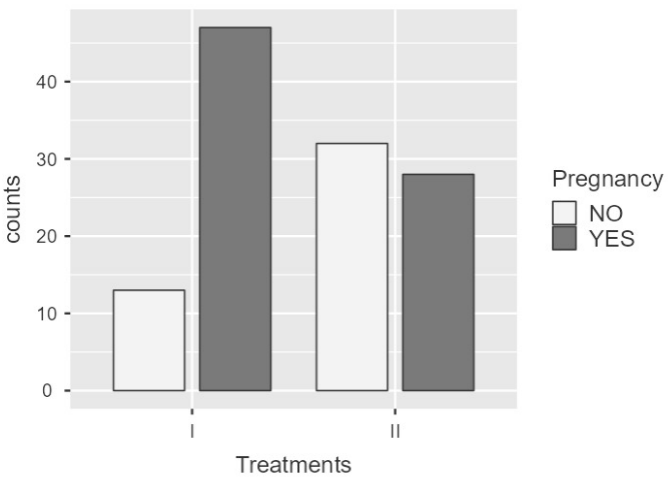

Figure 3: Comparison of pregnancy percentage in relation to the treatment used.

The pregnancy percentages for TI were 78.3% and 46.7% for TII. Statistical evaluation showed a high statistical difference ($p < 0.001$). The 14-day exposure to progesterone generated a better fertility response in Corriedale and Criolla females under high altitude conditions both in reproductive and non-reproductive season, all under field conditions with small and medium-sized producers. The TI pregnancy percentage is similar to that reported by other authors who synchronized heat with long protocols and AI with fresh semen similar to that of the present study (Espinoza *et al*., 2020; Molina *et al*., 2022). However, the TII percentage was lower than reported by these authors, probably because fewer days of Progesterone exposure produces less control of the follicular wave, which leads to poor synchronization of both the wave and ovulation (Viñoles, 2011). The percentages obtained are higher than those reported by other authors who report 21 and 25% pregnancy (Quispe *et al*., 2021). This difference is due to the fact that in the present study, diluted fresh semen was used, which guarantees good semen quality with motility percentages higher than 70%. The study by Quispe *et al*. (2021) indicates that AI was carried out with well-concentrated frozen semen via the cervical route. Sperm subjected to refrigeration and cryopreservation procedures are altered in various organelles such as mitochondria, flagella and, most importantly, at the plasma membrane level (Perez-Durand *et al*., 2020; Yeste, 2016). In general, the percentages obtained in the present study are acceptable for field conditions with small and medium-sized producers.

CONCLUSION

The factors that directly influence pregnancy outcomes in ewes subjected to estrus synchronization protocols under field and high-altitude conditions are uterine diameter, ewe age, and the synchronization protocol used. Notably, the 14-day protocol resulted in a significantly higher pregnancy rate compared to the 12-day protocol.

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NOVELTY STATEMENT

This study provides the first evidence under field and high-altitude conditions that uterine horn diameter, ewe age, and synchronization protocol are key predictors of pregnancy in ewe lambs subjected to AI. Unlike previous studies conducted under controlled environments, our findings highlight the practical relevance of a 14-day intravaginal progestogen sponge protocol, which significantly improves pregnancy outcomes compared to the commonly used 12-day protocol. Moreover, the integration of machine learning with conventional logistic regression offers a novel analytical approach to identify reproductive success factors in small ruminants.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

Generative AI and AI-assisted technology statement

No generative AI or AI-assisted technology was used in the preparation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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