



# Reproductive performance of Creole goats subjected to estrus synchronization protocols differing in duration and GnRH administration

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## ABSTRACT

Reproductive efficiency in goats is essential for improving productivity, with estrus synchronization and artificial insemination (AI) being strategic reproductive tools. Although post-AI GnRH administration has been proposed to improve luteal function and fertility, its effectiveness under different synchronization protocols in goats remains unclear. Therefore, this study evaluated the effect of GnRH administration five days after AI on the reproductive response of goats synchronized using short- and long-term protocols. A total of 39 Creole goats were distributed according to protocol type (short-term: n = 26; long-term: n = 13) and treatment (control: n = 18; GnRH: n = 21). In both groups, intravaginal sponges containing medroxyprogesterone acetate were inserted on Day 0. Artificial insemination was performed 64 h after device removal, and the depth of cervical semen deposition was recorded. Estrus expression was concentrated between 24 and 48 h after device removal in both protocols, with greater synchrony in the long-term protocol. No significant differences were observed in pregnancy rate between protocols (57.7 vs. 53.8%) or treatments (57.1 vs. 55.6%). Intermediate cervical semen deposition resulted in a greater pregnancy rate (69.2%) than superficial deposition (25.0%). Multiple correspondence analysis revealed associations between reproductive variability, insemination depth, and estrus expression patterns. In conclusion, neither post-AI GnRH administration nor protocol duration affected pregnancy rate. However, reproductive performance appeared to depend more on insemination-related factors than on hormonal treatments under field conditions in Creole goats subjected to cervical artificial insemination programs.

## 1. Introduction

Goat production represents an important livestock activity for small- and medium-scale producers in Peru, particularly in arid, semiarid, tropical, and coastal valley production systems, contributing to food security and income generation. Peru has a goat population of 1,749,263 animals, of which 9.2% are concentrated in the Lima region (MIDAGRI, 2025). However, despite this significant population, goat production systems in Peru still present limitations in their reproductive and health indicators, with variations among regions, production systems, and management and sanitary conditions (Tafur et al., 2024). In this context,

improving reproductive performance is essential to increase productivity and sustainability in these production systems. The use of reproductive biotechnologies such as artificial insemination (AI) has become an important tool for improving genetic progress, sanitary management, and productive efficiency in goat production systems (Baldassarre & Karatzas, 2004; Souza-Fabjan et al., 2023) through the dissemination of superior genetic material, which contributes to genetic progress and improves productive efficiency in goat production systems under adverse environmental conditions (Luo et al., 2019; Gore et al., 2021). In recent years, the use of this technique has increased considerably, including hormonal induction and estrus synchronization programs

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(Gonzalez-Bulnes et al., 2020), facilitating AI within a shorter period, even outside the natural breeding season in goats (Menchaca & Ungerfeld, 2018; Rehman et al., 2021). For example, the Peruvian State, through the Ministerio de Desarrollo de Agricultura y Riego, has been promoting the use of biotechnological tools such as artificial insemination with frozen semen and estrus synchronization in goats (MIDAGRI, 2025).

Despite the advantages of AI, pregnancy rates in goats remain highly variable, ranging from 35.3 to 54.8%, reflecting the influence of multiple factors associated with herd management and AI technique (Arrebola et al., 2012). In caprines, AI is limited by factors related to the anatomy of the reproductive tract, characterized by multiple cervical rings and tortuous folds, as well as by cervical mucus characteristics, which in goats may present greater viscosity and structural complexity than in other domestic species, affecting cervical sperm transport and AI efficiency (Fair et al., 2019; Srirangam et al., 2026). On the other hand, deeper semen deposition and estrus expression increase the probability of pregnancy in goats (Casali et al., 2017; Nascimento-Penido et al., 2018; Quintero-Elisea et al., 2025). Therefore, the implementation of hormonal programs aimed at optimizing the reproductive response of goats through exogenous hormone administration remains necessary.

Since the discovery of prostaglandin  $F_{2\alpha}$  (PGF $_{2\alpha}$ ) as a luteolytic agent in livestock, it has been widely used as a synchronization tool to shorten the estrus cycle and coordinate breeding (Riaz et al., 2011). Other hormones, such as PGF $_{2\alpha}$  provided in both single (Greyling & Van Niekerk, 1990) and double administrations at 10–11-day intervals, have been reported effective for estrus synchronization in goats (Khanum et al., 2006). Subsequently, synchronization protocols evolved from traditional long-term regimens based on progestogen administration for 11–14 days toward short-term protocols (5–7 days), which reduce costs and improve follicular dynamics, although reproductive responses remain variable (Menchaca & Rubianes, 2004). Carvalho-de-Paula et al. (2020) reported that short-term synchronization protocols using progesterone devices (~6 days), combined with equine chorionic gonadotropin (eCG) and PGF $_{2\alpha}$ , promote an adequate ovarian response and efficient follicular synchrony. In addition, administration of gonadotropin-releasing hormone (GnRH) during the post-AI period has been proposed as a strategy to promote the formation of accessory corpora lutea and increase progesterone concentrations (approximately 7.6–9.4 ng/mL), potentially improving the uterine environment for embryonic development and pregnancy maintenance (Hashem et al., 2015; Fernandez et al., 2018; Wittayarat et al., 2024). Therefore, GnRH was administered five days after AI because this period corresponds to early luteal development, when it may promote accessory corpus luteum formation and increase progesterone secretion. However, the reproductive response to this intervention may vary according to the synchronization protocol used, particularly between short- and long-term protocols, because differences in progesterone exposure may influence subsequent luteal responses and the formation of accessory corpora lutea after GnRH administration. Furthermore, information regarding the effectiveness of these strategies in Creole goats remains limited, despite the importance of Creole goats as a local zoogenetic resource adapted to challenging production environments. In this sense, the objective of this research was to evaluate the effect of GnRH administration five days after AI on the reproductive response of goats synchronized using short- and long-term protocols with Boer and Murciano-Granadina semen. We hypothesized that artificial insemination with a deposition of frozen semen at a greater depth could result in a higher pregnancy rate; in addition, a short protocol with GnRH administration could improve the pregnancy rate.

## 2. Materials and methods

### 2.1. Ethical approval

The materials and procedures used in this study were approved by

the Comité Institucional de Ética en la Investigación (CIEI) of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM) under approval number CIEI-N° 00053. The study was conducted in accordance with current national regulations for animal research and institutional guidelines for animal welfare.

### 2.2. Experimental location

The study was conducted at the Goat Production Center of the Estación Experimental Agraria Donoso of the Instituto Nacional de Innovación Agraria (INIA), located in the district and province of Hualal, Lima, Peru (Fig. 1). The experimental study was conducted from February to March 2025, during the breeding season. The center is located approximately at coordinates 255904.10 m east and 8725909.43 m north, at 181 m above sea level. The agroecological zone is classified as a subtropical coastal desert, characterized by annual temperatures ranging from 16 to 32°C and annual precipitation below 50 mm/year (MINAM, 2019; SENAMHI, 2026).

### 2.3. Animals and experimental design

The experimental population consisted of 39 Creole does that were sexually active, clinically healthy, and suitable for AI. All animals presented a body condition score ranging from 2.5 to 3.5 (scale 1–5), based on the classification of Very thin animal (Score 1), Skinny, lean animal (Score 2), Animal with good body condition (Score 3), Animal with excellent body condition (Score 4), Fat and smooth, overweighed animal (Score 5) (Vall et al., 2025). Boer and Murciano-Granadina bucks were used as semen donors during the experiment. Detailed distribution of animals according to synchronization protocol, hormonal treatment, buck donor breed, and semen deposition site is presented in Supplementary Table 1. Animals were managed under homogeneous conditions, with free access to water and a forage-based diet composed of green maize forage, chicory, and/or forage plantain, offered twice daily (07:00 and 16:00 h). In addition, each animal received 400 g/day of a concentrate formulated by the research team rather than a commercial feed. The formulation consisted of soybean whole meal (15.08%), soybean meal (23.39%), wheat bran (38.98%), ground maize (16.11%), maize gluten (4.63%), calcium carbonate (0.69%), salt (0.69%), and a standard premix (0.42%).

The study followed a  $2 \times 2$  factorial design consisting of female synchronization protocol type (short-term,  $n = 26$ ; long-term,  $n = 13$ ) and hormonal treatment (GnRH,  $n = 21$ ; saline solution,  $n = 18$ ). For this study, semen from two goats was used (1 Boer and 1 Murciano-Granadina). Owing to the field conditions and animal availability during the experimental period, the distribution of some categorical variables, including buck breed and semen deposition depth, was not completely homogeneous among experimental groups (Supplementary Table 1). The does were randomly assigned to the experimental groups. In addition, variables associated with the insemination process were recorded, including semen deposition depth (superficial, intermediate, or intrauterine), cervical mucus characteristics, and estrus expression.

### 2.4. Semen collection for artificial insemination

Semen samples were collected from Murciano-Granadina and Boer bucks using an artificial vagina maintained at 39–40°C, with a teaser doe. Samples were collected into graduated tubes, protected from light, and immediately transported to the laboratory at approximately 35°C within 5 min. Ejaculates were evaluated for volume and sperm concentration using an SDM 1 photometer (Minitüb, Germany). Sperm motility was evaluated using a computer-assisted sperm analysis (CASA) system (AndroVision®, Minitüb, Germany). Subsequently, semen samples were diluted 1:1 with the commercial extender OptiXcell® (IMV Technologies, France) and centrifuged at  $1500 \times g$  for 3 min to remove seminal plasma. The sperm pellet was resuspended and adjusted to a

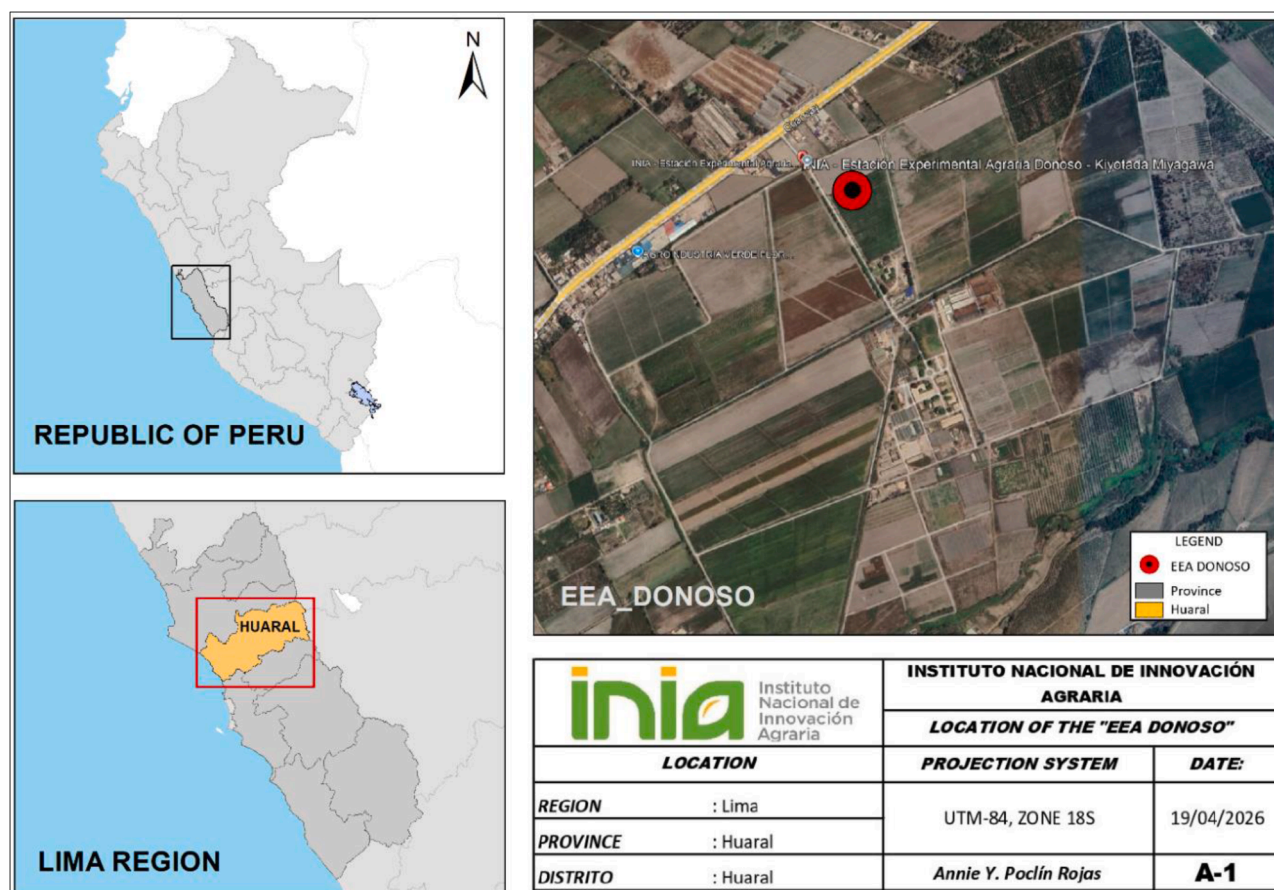


Fig. 1. Location of the Estación Experimental Agraria Donoso of INIA in Lima region, Peru (map generated using ArcGIS 10.8).

final concentration of  $100 \times 10^6$  spermatozoa/mL. Samples were equilibrated at room temperature for 30 min, loaded into 0.25-mL straws, and gradually cooled to 4°C for 6 h. For freezing, the protocol of Yodminkwan et al. (2016) was adapted, the straws were exposed to liquid nitrogen vapor at 5 cm above the liquid nitrogen surface for 10 min, then plunged into liquid nitrogen and stored at -196°C until use. Post-thaw sperm motility was assessed using a CASA computerized semen analysis system (AndroVision®, Minitüb, Germany), and only samples with total motility greater than 70% were used for artificial insemination.

## 2.5. Short- and long-term estrus synchronization protocols

All does received an intravaginal sponge containing 60 mg of medroxyprogesterone acetate (MAP; Sincrogest®, Laboratorios Ovejero S.A., León, Spain) on Day 0 (D0).

In the short-term protocol, animals were treated according to the regimen described by Carvalho-de-Paula et al. (2020). On Day 5 (D5), 250 IU of eCG (SERIGAN®, Laboratorios Ovejero S.A., León, Spain) and 30 µg of d-cloprostenol (Dextrogestol®, Von Franken, Argentina) were administered intramuscularly, and the MAP sponge was removed 24 h later (D6).

The long-term protocol was performed according to Murtaza et al. (2020). On Day 10 (D10), does received 200 IU of eCG and 30 µg of d-cloprostenol, and the MAP sponge was removed 48 h later (D12).

Subsequently, at 5 days post-AI, corresponding to Day 13 (D13) in the short-term protocol and Day 19 (D19) in the long-term protocol, does were randomly assigned to one of the following treatments: a control group receiving 1 mL of sterile 0.9% saline solution and a treated group receiving 25 µg of a GnRH analog (leirelin; TEC-Relin®, Agener União/Tecnopec, Brazil). The experimental synchronization protocols

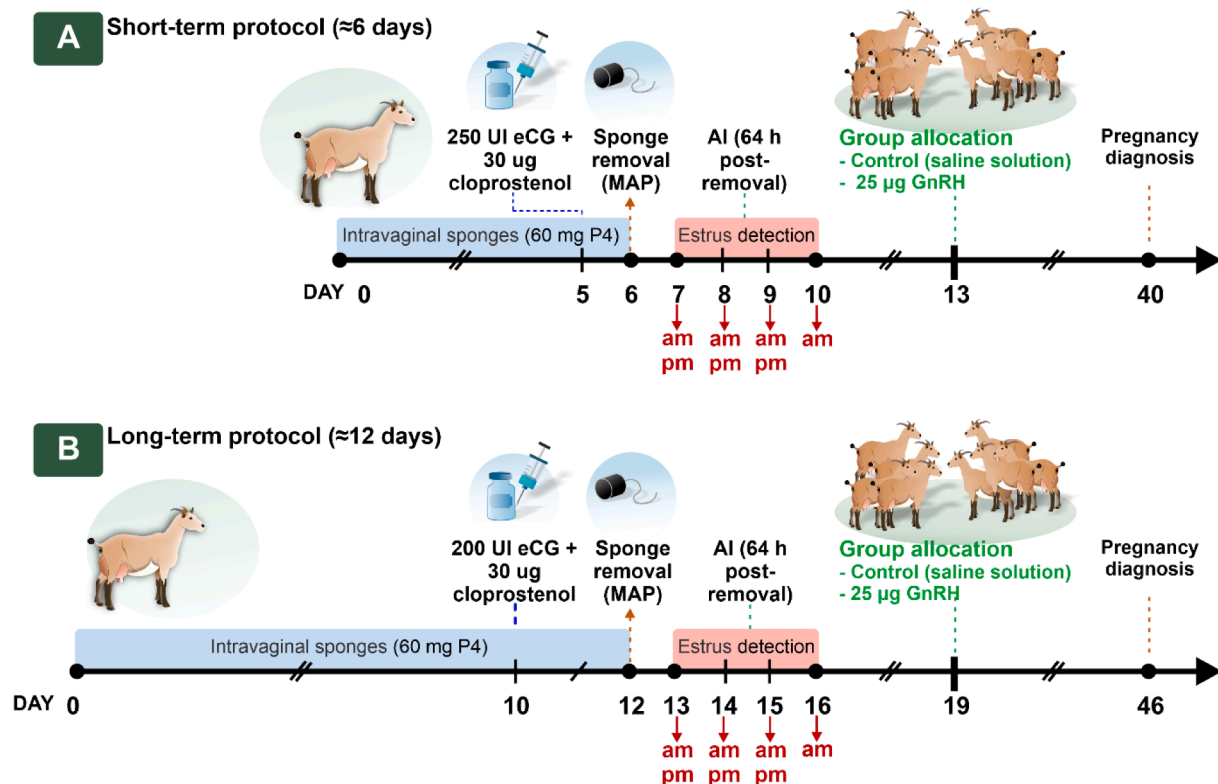
used in the present study are illustrated in Fig. 2.

## 2.6. Estrus detection and artificial insemination

Estrus detection (ED) was performed by direct observation twice daily (07:00 and 17:00 h). Each observation session lasted approximately 30 min before feeding, and females that accepted mounting were considered to be in estrus. The observation period began 15 h after removal of the intravaginal device, which was considered the first post-removal observation period (ED1 a.m.), and extended until the fourth day after device removal (ED4 a.m.). Monitoring was conducted from Day 7 to Day 10 am in the short-term protocol and from Day 13 to Day 16 in the long-term protocol.

Before AI, the vulvar region of each female in estrus was cleaned, and a lubricated vaginal speculum was inserted. With the aid of a light source (flashlight), cervical opening was evaluated. In addition, the presence and characteristics of cervical mucus were recorded and classified according to appearance as follows: 0 = absence of mucus (absence of estrus); 1 = crystalline mucus; 2 = crystalline-striated mucus; 3 = striated mucus; 4 = striated mucus with abundant discharge; and 5 = caseous mucus, according to the classification described by Fonseca et al. (2017a).

At the time of AI, semen straws were thawed at 37°C for 30 s, dried with absorbent paper, cut at the sealed end, and loaded into the AI gun (Kang et al., 2025). Subsequently, the cervix was immobilized using EMBRAPA® forceps, and the AI gun was introduced intravaginally until reaching the cervix. The site of semen deposition was recorded according to penetration depth of the AI gun through the cervix and classified as follows: superficial cervical (1–3 cm), when the applicator partially penetrated the cervical canal and reached the first or second cervical ring; intermediate cervical (3–4 cm), when the applicator



**Fig. 2.** Estrus synchronization and hormonal treatment protocols used in goats. (A) Short-term protocol (~6 days) and (B) long-term protocol (~12 days). MAP, medroxyprogesterone acetate; eCG, equine chorionic gonadotropin; AI, artificial insemination; GnRH, gonadotropin-releasing hormone; am, ante meridiem; pm, post meridiem. The figure was created using CorelDRAW 2021.

advanced to the third cervical ring; and intrauterine ( $\geq 5$  cm), when the applicator passed through all cervical rings, allowing semen deposition directly into the uterus, according to [Fonseca et al. \(2017b\)](#). AI was performed 64 h after intravaginal device removal in both groups.

## 2.7. Pregnancy diagnosis

Pregnancy diagnosis was performed 32 days after AI by transrectal ultrasonography using a portable ultrasound scanner (S6 model; BMV Technology Co., Ltd., Beijing, China) equipped with a 7.5 MHz transducer. Pregnancy was confirmed by the visualization of intrauterine fluid, embryonic vesicles, and/or an embryo ([Lazim & Al-Watar, 2025](#); [Sultana et al., 2025](#)). Does without ultrasonographic evidence of pregnancy were classified as non-pregnant. In addition, reproductive outcomes were classified according to pregnancy maintenance and number of offspring born as non-pregnant, abortion (pregnancy loss), single kidding (one offspring), twin kidding (two offspring), or triplet kidding (three offspring).

## 2.8. Statistical analysis

Data analysis was performed using R software version 4.5.1 (2025-06-13 UCRT) with the packages dplyr, tidyr, ggplot2, vcd, FactoMineR, and factoextra. Initially, a descriptive analysis was conducted by calculating absolute frequencies and percentages for all categorical variables included in the study. Subsequently, associations between the independent variables—protocol type, treatment, buck breed (Boer and Murciano-granadina), insemination depth, cervical mucus characteristics, and estrus expression—and the dependent variable, pregnancy status, were evaluated using Pearson's Chi-square test, considering a significance level of  $P < 0.05$ . For each association, the magnitude of the effect was estimated using Cramér's V and reported with its corresponding 95% confidence interval. A post-hoc power analysis was also

performed for each Chi-square test using the observed effect size, sample size, degrees of freedom, and a significance level of  $\alpha = 0.05$ . Because post-hoc power is directly related to the observed P-value, statistical interpretation was primarily based on effect sizes and their confidence intervals rather than on statistical significance or achieved power alone. Given the exploratory nature of the study and the unequal distribution of the categorical variables presented in Supplementary Table 1, interpretation focused on patterns of association rather than on independent causal effects. Finally, Multiple Correspondence Analysis (MCA) was applied to identify structural association patterns among the categorical variables. The interpretation of the MCA was based on the percentage of inertia explained by the dimensions, category contributions, and squared cosine values ( $\cos^2$ ), which were used to evaluate the quality of representation of each category in the factorial space.

## 3. Results

### 3.1. Estrus expression and cervical mucus characteristics according to synchronization protocol

Estrus expression was low during ED1 am in both protocols ( $\chi^2 = 0.406$ ,  $p = 0.524$ ), with values of 3.85% (1/26) in the short-term protocol and 15.38% (2/13) in the long-term protocol, increasing progressively in both groups. The long-term protocol showed greater proportions of estrus expression than the short-term protocol during ED1 pm; however, this difference was not significant ( $\chi^2 = 0.000$ ,  $p = 1.000$ ; 61.54 vs. 57.69%) and ED2 am ( $\chi^2 = 0.222$ ,  $p = 0.638$ ; 92.31 vs. 80.77%). From ED3 onward, estrus frequency decreased in both protocols ( $< 31\%$ ), with no significant differences between groups (ED3 am:  $\chi^2 = 0.000$ ,  $p = 1.000$ ; ED3 pm:  $\chi^2 = 0.000$ ,  $p = 1.000$ ; ED4 am:  $\chi^2 = 0.000$ ,  $p = 1.000$ ), and at least one doe remaining in estrus until ED4 am. Regarding cervical mucus characteristics, the short-term protocol showed a greater proportion of striated mucus (38.46% [10/26]),

whereas the long-term protocol showed predominance of crystalline-striated mucus (61.54% [8/13]). No significant association was observed between synchronization protocol and cervical mucus characteristics ( $\chi^2 = 4.958$ ,  $p = 0.292$ ; Cramér's  $V = 0.357$ ; Table 1).

### 3.2. Effects of synchronization protocol, hormonal treatment, buck breed, and semen deposition site on pregnancy rate in goats

Pregnancy was confirmed in 22 of the 39 does (56.4%; 95% CI: 41.0–70.7%). Pregnancy rates were similar between the short- and long-term synchronization protocols (57.7% and 53.8%, respectively) and between the GnRH and saline treatments (57.1% and 55.6%, respectively). The corresponding effect sizes were negligible for synchronization protocol (Cramér's  $V = 0.037$ , 95% CI: 0.000–0.321; achieved power = 5.6%) and treatment (Cramér's  $V = 0.016$ , 95% CI: 0.000–0.244; achieved power = 5.1%). Does inseminated with semen from Murciano-Granadina bucks showed a numerically greater pregnancy rate than those inseminated with Boer semen (65.0% vs. 47.4%), although the observed effect was small and imprecise (Cramér's  $V = 0.178$ , 95% CI: 0.000–0.492; achieved power = 19.9%; Tables 2 and 3).

In contrast, semen deposition site showed a moderate observed association with pregnancy outcome (Cramér's  $V = 0.330$ , 95% CI: 0.000–0.615;  $P = 0.120$ ), although the achieved power was only 43.7%. Pregnancy rates were 25.0% for superficial cervical deposition, 69.2% for intermediate cervical deposition, and 61.1% for uterine deposition. Compared with superficial deposition, the odds of pregnancy were higher for intermediate cervical deposition (OR = 6.75, 95% CI: 0.93–49.23) and uterine deposition (OR = 4.71, 95% CI: 0.73–30.28). However, neither pairwise comparison remained statistically significant after Holm adjustment. The wide confidence intervals and low statistical power indicate considerable uncertainty; therefore, these findings should be interpreted as exploratory (Table 4).

### 3.3. Effects of synchronization protocol, hormonal treatment, and semen deposition site on kidding type in goats

The short-term protocol showed a greater proportion of twin kidding (23.08%), whereas the long-term protocol showed a more uniform distribution of kidding types. Regarding hormonal treatment, the GnRH group showed a greater frequency of single kidding (19.05%) and triplet

**Table 1**

Distribution of estrus response (%) and cervical mucus characteristics (%) in goats during ED1–ED4 after sponge removal according to synchronization protocol.

| Variable <sup>a</sup>    | Estrus Synchronization Protocol <sup>b</sup> |          |       | $\chi^2$ | $p$ -value | Cramér's $V$ |
|--------------------------|----------------------------------------------|----------|-------|----------|------------|--------------|
|                          | Short (%)                                    | Long (%) |       |          |            |              |
| Estrus response          |                                              |          |       |          |            |              |
| Estrus response D1 am    | 3.85                                         | 15.38    | 0.406 | 0.524    | 0.204      |              |
| Estrus response D1 pm    | 57.69                                        | 61.54    | 0.000 | 1.000    | 0.037      |              |
| Estrus response D2 am    | 80.77                                        | 92.31    | 0.222 | 0.638    | 0.151      |              |
| Estrus response D2 pm    | 73.08                                        | 92.31    | 0.963 | 0.326    | 0.225      |              |
| Estrus response D3 am    | 26.92                                        | 30.77    | 0.000 | 1.000    | 0.040      |              |
| Estrus response D3 pm    | 11.54                                        | 15.38    | 0.000 | 1.000    | 0.054      |              |
| Estrus response D4 am    | 3.85                                         | 7.69     | 0.000 | 1.000    | 0.082      |              |
| Cervical mucus           |                                              |          |       |          |            |              |
| 0–no discharge           | 7.69                                         | 7.69     | 4.958 | 0.292    | 0.357      |              |
| Crystalline (1)          | 23.08                                        | 7.69     | 4.958 | 0.292    | 0.357      |              |
| Crystalline/striated (2) | 26.92                                        | 61.54    | 4.958 | 0.292    | 0.357      |              |
| Striated (3)             | 38.46                                        | 23.08    | 4.958 | 0.292    | 0.357      |              |
| Striated/caseous (4)     | 3.85                                         | 0.00     | 4.958 | 0.292    | 0.357      |              |

<sup>a</sup> Variables were evaluated per goat.

<sup>b</sup> Short-term synchronization protocol ( $n = 26$ ), and long-term synchronization protocol ( $n = 13$ ).

D, day; am, ante meridiem; pm, post meridiem;  $\chi^2$ , chi-square test; Cramér's  $V$ , effect size measure of association.

**Table 2**

Pregnancy rate and 95% confidence interval according to estrus synchronization protocol, hormonal treatment, buck donor breed, and semen deposition site in goats.

| Factor                   | Category                       | n  | Pregnant, n | Pregnancy rate, % (95% CI) |
|--------------------------|--------------------------------|----|-------------|----------------------------|
| Synchronization protocol | Short-term                     | 26 | 15          | 57.7 (38.9–74.5)           |
|                          | Long-term                      | 13 | 7           | 53.8 (29.1–76.8)           |
| Hormonal treatment       | GnRH                           | 21 | 12          | 57.1 (36.5–75.5)           |
|                          | Saline solution (control)      | 18 | 10          | 55.6 (33.7–75.4)           |
| Buck donor breed         | Boer                           | 19 | 9           | 47.4 (27.3–68.3)           |
|                          | Murciano-Granadina             | 20 | 13          | 65.0 (43.3–81.9)           |
| Semen deposition site    | 1–3 cm (superficial cervical)  | 8  | 2           | 25.0 (7.1–59.1)            |
|                          | 3–4 cm (intermediate cervical) | 13 | 9           | 69.2 (42.4–87.3)           |
|                          | ≥5 cm (uterine)                | 18 | 11          | 61.1 (38.6–79.7)           |

Confidence intervals for pregnancy proportions were calculated using the Wilson method. AI = artificial insemination.

kidding (14.29%), whereas the control group showed a greater proportion of twin kidding (33.33%). Regarding semen deposition site, intrauterine semen deposition resulted in a greater proportion of twin kidding (38.89%), whereas intermediate cervical deposition showed greater frequencies of single kidding (30.77%). Pregnancy loss was observed in both synchronization protocols and treatments, with greater frequency in does subjected to intermediate cervical semen deposition (23.08%). However, kidding outcomes were not significantly associated with synchronization protocol ( $\chi^2 = 0.379$ ,  $p = 0.984$ ; Cramér's  $V = 0.328$ ), hormonal treatment ( $\chi^2 = 5.729$ ,  $p = 0.220$ ; Cramér's  $V = 0.383$ ), or semen deposition depth ( $\chi^2 = 13.692$ ,  $p = 0.090$ ; Cramér's  $V = 0.419$ ), although the latter showed a moderate effect size suggesting a potentially relevant association (Tables 5 and 6).

### 3.4. Multiple correspondence analysis (MCA)

MCA showed that the first two dimensions explained 28.7% of the total variability (Dimension 1: 17.3%; Dimension 2: 11.4%), accounting for a large proportion of the observed multivariate structure. In the overall biplot, the “Pregnant” category was closed to intrauterine and intermediate semen deposition and with twin kidding, whereas the “Non-pregnant” category was located more distant and close to superficial semen deposition and non-kidding outcomes (Fig. 3.E). In addition, extreme reproductive events and late estrus manifestations were distributed in distinct regions of the multivariate space. Analysis according to insemination depth showed greater dispersion in intrauterine deposition compared with superficial deposition (Fig. 3.A). In contrast, synchronization protocols showed high overlap, without evident structural separation between groups (Fig. 3.B).

Breed analysis showed differences in dispersion, although without evident separation between groups (Fig. 3.C), with greater variability observed in the Murciano-Granadina breed and a more concentrated distribution in the Boer breed. Regarding treatment, the GnRH group showed greater dispersion, whereas the control group tended to cluster around the origin; however, both groups showed broad overlap within the multivariate space (Fig. 3.D). Category analysis suggested the presence of three main clusters (Fig. 3.E): a central cluster related to intrauterine/intermediate semen deposition, intermediate cervical mucus, and pregnant status; a second cluster associated with superficial semen deposition, reduced mucus, and non-pregnant status; and a third cluster associated with extreme reproductive events and late estrus

**Table 3**

Association tests, Cramér's V effect sizes with 95% confidence intervals, and post-hoc power for pregnancy status in goats.

| Factor                   | $\chi^2$ | df | P-value | Cramér's V | 95% CI      | Post hoc power (%) |
|--------------------------|----------|----|---------|------------|-------------|--------------------|
| Synchronization protocol | 0.000    | 1  | 1.000   | 0.037      | 0.000–0.321 | 5.6                |
| Hormonal treatment       | 0.000    | 1  | 1.000   | 0.016      | 0.000–0.244 | 5.1                |
| Buck donor breed         | 0.619    | 1  | 0.431   | 0.178      | 0.000–0.492 | 19.9               |
| Semen deposition site    | 4.241    | 2  | 0.120   | 0.330      | 0.000–0.615 | 43.7               |

Post-hoc power was calculated from the observed Cramér's V, sample size, degrees of freedom, and  $\alpha = 0.05$ . Confidence intervals for Cramér's V were obtained using the noncentral chi-square distribution.

**Table 4**

Pairwise comparisons of pregnancy odds among semen deposition sites in goats.

| Comparison                   | OR   | 95% CI     | Fisher P | Holm-adjusted P |
|------------------------------|------|------------|----------|-----------------|
| Intermediate vs. superficial | 6.75 | 0.93–49.23 | 0.080    | 0.241           |
| Uterine vs. superficial      | 4.71 | 0.73–30.28 | 0.202    | 0.403           |
| Uterine vs. intermediate     | 0.70 | 0.15–3.17  | 0.718    | 0.718           |

Odds ratios greater than 1 indicate higher pregnancy odds for the first category listed. Pairwise P-values were obtained using Fisher's exact test and adjusted using the Holm method.

manifestations.

#### 4. Discussion

The results of the present study indicate that synchronization protocol duration, whether short- or long-term, did not affect estrus expression or pregnancy rate. Although the long-term protocol tended to concentrate the estrus response within the first 24–48 h after sponge removal, this greater synchrony did not translate into improved reproductive performance. This finding is partially consistent with the report of [Menchaca and Rubianes \(2004\)](#), who described that short-term progestogen treatments (5–7 days) can achieve pregnancy rates close to 60–65% when AI is performed approximately 54 h after device removal, supporting the effectiveness of short-term synchronization protocols in small ruminants. Similarly, [Gonzalez-Bulnes et al. \(2020\)](#) reported that estrus synchronization does not necessarily result in greater fertility rates.

Exogenous progesterone administration mimics the luteal phase, regulating LH secretion and follicular growth ([Hansel & Convey, 1983](#)). Following device removal, the rapid decline in progesterone concentrations induces estrus onset, generally within the first 24–48 h ([Wheaton et al., 1993](#)). In short-term protocols, this effect is complemented by the luteolytic action of PGF<sub>2</sub> $\alpha$ , promoting corpus luteum regression and synchronization of the estrus cycle ([McCracken et al., 1972](#); [Ungerfeld & Rubianes, 1999](#)). Although these protocols have been proposed to reduce the effects associated with prolonged exposure to progestogens ([Menchaca & Rubianes, 2004](#)), such physiological differences were not reflected in pregnancy rate in the present study. Furthermore, the greater concentration of estrus events between ED1 pm and ED2 am, followed by a decline from ED3 onward, reflects the expected dynamics after device removal and individual endocrine variation ([Fonseca et al., 2019](#)), although variability associated with

field conditions and individual animal responses may also have contributed to the observed estrus patterns.

Regarding post-AI GnRH administration, no evident effect on pregnancy rate was observed in the present study. Although GnRH administration may induce ovulation of residual follicles, improve ovulatory synchrony in goats (91.7–100%), and promote the formation of functional luteal structures ([Cosentino et al., 2020](#)), these physiological responses may not necessarily result in improved reproductive performance under field conditions. [Fernandez et al. \(2018\)](#) reported that GnRH administration during the early post-AI period ( $\approx$ Day 4) did not significantly increase progesterone concentrations, probably due to insufficient luteal stimulation. Similarly, [Hashem et al. \(2015\)](#) observed that administration around Day 7 after mating may increase progesterone concentrations ( $\approx$ 7.6–9.4 ng/mL) and promote the formation of accessory corpora lutea (0.27–0.50), without increasing conception rate, although pregnancy losses were reduced. In this context, the limited reproductive response observed in the present study may reflect the inherent variability among synchronized does and the multifactorial nature of reproductive performance under field conditions. Likewise, [Wittayarat et al. \(2024\)](#) reported that the effects of GnRH on embryonic survival and reproductive performance depend on the physiological condition of the animals and the synchronization protocol used.

At the reproductive tract level, variations in cervical mucus characteristics were observed among treatments, with a tendency toward more favorable patterns in one of the protocols (short-term: greater proportion of striated mucus, 38.46%; long-term: predominance of crystalline-striated mucus, 61.54%). The cervical mucus classification used in this study, based on standardized visual criteria, allows differentiation of physiological states associated with the reproductive environment. According to [Fonseca et al. \(2017a\)](#), striated-caseous mucus patterns predominate at ovulation (72.2%), followed by crystalline-striated mucus (22.2%). In addition, greater follicular diameters were observed in does presenting crystalline, crystalline-striated, or striated mucus ( $6.7 \pm 1.4$  mm,  $7.2 \pm 1.1$  mm, and  $7.3 \pm 1.3$  mm, respectively) compared with those showing striated-caseous ( $5.3 \pm 1.4$  mm) or caseous mucus ( $4.5 \pm$

**Table 6**

Chi-square association analysis and Cramér's V effect sizes for kidding categories according to synchronization protocol, hormonal treatment, and semen deposition depth in goats.

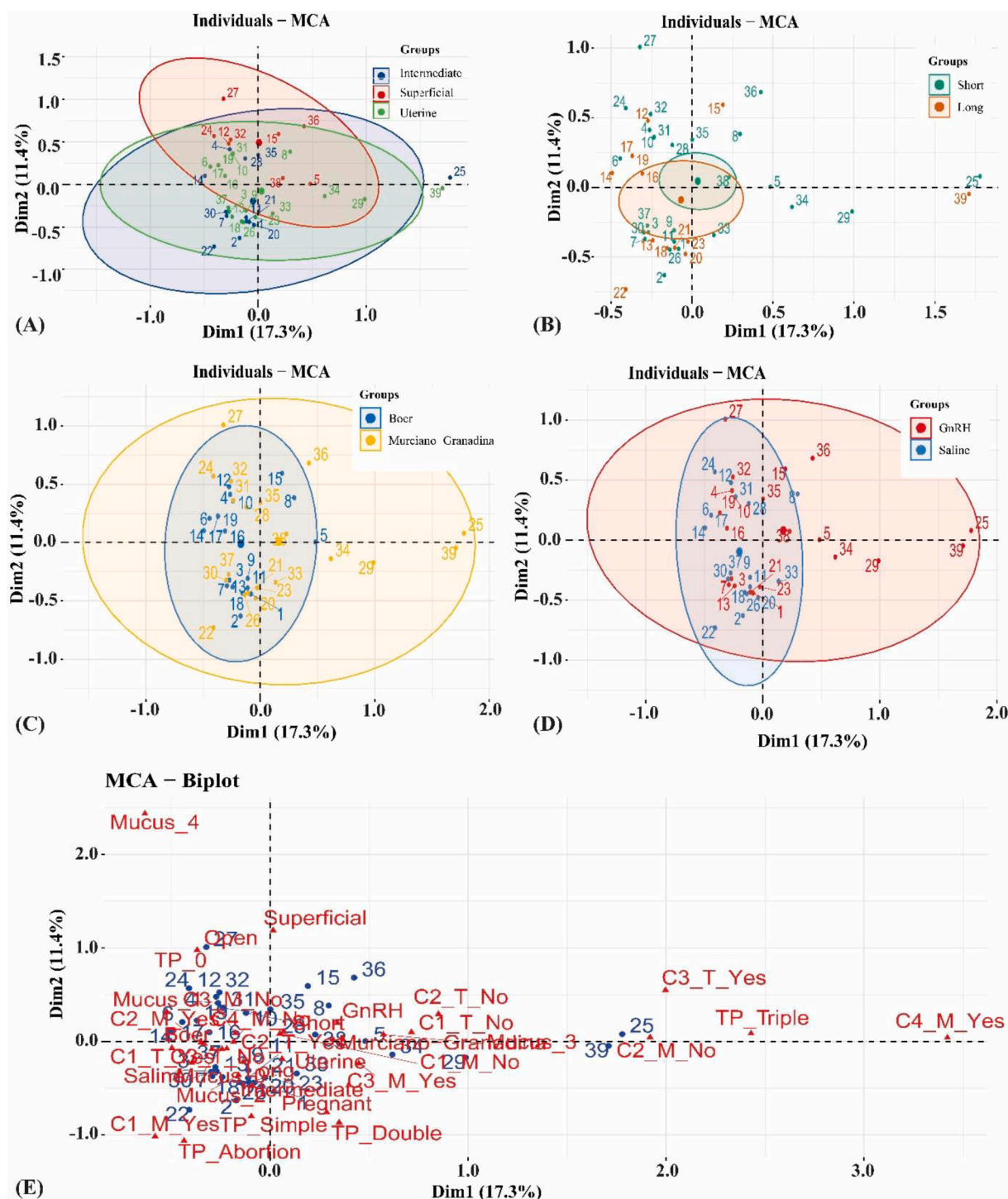
| Factor                   | $\chi^2$ | p-value | Cramér's V |
|--------------------------|----------|---------|------------|
| Synchronization protocol | 0.379    | 0.984   | 0.328      |
| Hormonal treatment       | 5.729    | 0.220   | 0.383      |
| Semen deposition depth   | 13.692   | 0.090   | 0.419      |

**Table 5**

Distribution of kidding outcomes (%) according to estrus synchronization protocol, hormonal treatment, and semen deposition site in goats.

| Variable <sup>a</sup> | Estrus Synchronization Protocol |       | Tratamiento |                                 | Local of semen deposition     |                                |                |
|-----------------------|---------------------------------|-------|-------------|---------------------------------|-------------------------------|--------------------------------|----------------|
|                       | Short                           | Long  | GnRH        | Saline solution (control group) | 1–3 cm (Superficial cervical) | 3–4 cm (Intermediate cervical) | 5 cm (Uterine) |
| Abortion (%)          | 11.54                           | 15.38 | 14.29       | 11.11                           | 0.00                          | 23.08                          | 11.11          |
| Single (%)            | 15.38                           | 15.38 | 19.05       | 11.11                           | 12.50                         | 30.77                          | 5.56           |
| Twin (%)              | 23.08                           | 15.38 | 9.52        | 33.33                           | 0.00                          | 7.69                           | 38.89          |
| Triple (%)            | 7.69                            | 7.69  | 14.29       | 0.00                            | 12.50                         | 7.69                           | 5.56           |

<sup>a</sup> Variables were evaluated per female.



**Fig. 3.** Multiple correspondence analysis (MCA) of reproductive variables in goats subjected to short- and long-term synchronization protocols. MCA according to semen deposition depth during artificial insemination (AI) (A); synchronization protocol (B); buck donor breed (C); hormonal treatment (D); and overall MCA biplot showing the relationships among variable categories and individuals (E). In panel E, red labels represent variable categories and their spatial associations within the multivariate analysis. Variable categories are represented using abbreviated labels. Abbreviations: C1–C4 = estrus detection periods after sponge removal; M = morning observation; T = afternoon observation; Yes = presence; No = absence; TP = reproductive outcome; Mucus = cervical mucus category; Superficial, Intermediate, and Uterine = semen deposition depth during AI.

1.1 mm). However, in the present study these variations were not reflected in differences in pregnancy rate, suggesting that although cervical mucus reflects the endocrine environment, its influence on reproductive success may depend on additional components, such as insemination efficiency.

In contrast, semen deposition showed relevant trends, with greater

pregnancy rates observed when insemination was performed at the intermediate cervical or intrauterine level compared with superficial deposition. This tendency is consistent with the findings of [Casali et al. \(2017\)](#), who reported that deeper semen penetration facilitates sperm transport toward the upper reproductive tract. In that study, pregnancy rate was significantly greater when semen deposition exceeded 4 cm

within the cervix (60%) compared with more superficial deposition sites (35.1%), highlighting the impact of insemination technique on reproductive efficiency. However, the authors also reported that this level of penetration was achieved in only a limited proportion of does (28.8%), emphasizing the anatomical constraints of the cervix and their implications for reproductive outcomes. Likewise, the complex anatomy of the caprine cervix, characterized by multiple rings and tortuous folds, may limit the progression of the insemination instrument and consequently affect procedural efficiency (Fair et al., 2019; Sringam et al., 2026). Although the differences observed in the present study were not statistically significant, the moderate effect size associated with semen deposition depth suggests a relevant trend that should be interpreted considering the unequal representation among deposition categories. Additionally, the variation observed according to buck donor breed may be related to differences in reproductive potential under different production conditions (Luo et al., 2019; Gore et al., 2021).

Finally, multivariate analysis suggested that the reproductive response in goats was more closely related to functional components associated with insemination technique and estrus dynamics than to the hormonal protocol itself. In particular, the association between pregnancy status and intrauterine or intermediate cervical semen deposition highlights the importance of insemination technique, which was consistent with the results of the present study, where intermediate cervical deposition achieved greater pregnancy rates (69.2%) compared with superficial deposition (25.0%). This behavior agrees with the findings of Casali et al. (2017), who reported greater pregnancy rates when semen deposition exceeded 4 cm within the cervix (60.0%) than when semen was deposited more superficially within the cervical canal (35.1%) and even greater values with intrauterine insemination (~50.2%). This pattern is also consistent with the report of Arrebola et al. (2012), who indicated that fertility in artificial insemination programs depends on multiple variables with different relative contributions. Although the multivariate associations should be interpreted cautiously considering the distribution of some categorical variables, the consistency of the observed trends reinforces the importance of insemination-related factors in reproductive success.

These conditions reflect common husbandry constraints under field settings, including limited buck availability and individual anatomical variation that may compromise artificial insemination efficiency. Interpretation should also consider the influence of reproductive seasonality, regulated by photoperiod, which may reduce ovarian activity under subtropical conditions (Nogueira et al., 2015; Hameed et al., 2020), as well as the repeated use of eCG, which has been associated with antibody formation and alterations in follicular dynamics (de Andrade et al., 2021). Overall, the results suggest that variability in reproductive performance was more closely related to functional components of the reproductive process, particularly insemination depth and estrus dynamics, than to the hormonal treatments or synchronization protocols applied. Although the present study provides relevant information regarding the reproductive response of Creole goats subjected to different synchronization protocols and contributes to improving fixed-time artificial insemination strategies under practical field conditions, additional studies integrating endocrine, ovarian, and luteal function variables may contribute to a better understanding of the mechanisms associated with post-AI GnRH administration.

## 5. Limitations

This study should be interpreted considering its relatively small and unbalanced sample, the use of only one buck per breed, and the influence of individual cervical anatomy and field conditions on semen deposition depth. Furthermore, the study was conducted at a single experimental station and did not include hormonal, ovarian, or luteal measurements. These factors may have reduced statistical power and limited the generalizability of the findings. Nevertheless, the results provide reasonably convincing evidence under the conditions evaluated

and support the main interpretation of the study. Further research with larger, more balanced populations, multiple bucks per breed, and broader reproductive assessments is needed to confirm these findings and clarify the underlying mechanisms.

## 6. Conclusions

In the present study, neither post-AI GnRH administration nor synchronization protocol duration was associated with pregnancy rate in Creole goats, with similar values between GnRH and saline-treated does (57.1% vs. 55.6%) and between short- and long-term protocols (57.7% vs. 53.8%). In contrast, pregnancy rates tended to be higher following intermediate cervical (69.2%) and uterine semen deposition (61.1%) than superficial cervical deposition (25.0%). However, these findings should be interpreted cautiously considering the limited statistical power. Overall, semen deposition depth appeared to be more closely associated with reproductive performance than the hormonal treatment or synchronization protocol under field conditions.

## Ethics declaration

This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This study was approved by the Comité Institucional de Ética en la Investigación de la Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas. (Approval No. CIEI-N° 00053).

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## CRediT authorship contribution statement

**Annie Yoselin Poclín-Rojas:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emmanuel Alexander Sessarago:** Writing – review & editing, Validation, Supervision, Methodology. **Miriam Benites-Muñoz:** Writing – original draft, Visualization, Investigation, Data curation. **Josselin Rivas:** Validation, Resources, Investigation. **Rensson Homero Celiz-Ygnacio:** Validation, Resources, Investigation. **José Américo Saucedo-Uriarte:** Writing – review & editing, Methodology, Formal analysis. **René Pinazo-Herencia:** Writing – review & editing, Validation, Supervision. **Juancarlos Alejandro Cruz-Luis:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data Availability

Supplementary data file associated with this article can be sent upon

reasonable request.

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