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EVALUATION OF PREGNANCY RATE THROUGH THE APPLICATION OF RECOMBINANT AND TRADITIONAL Ecg IN A FIXED-TIME ARTIFICIAL INSEMINATION (FTAI) PROTOCOL IN CHAROLAIS COWS

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RESUMEN

Fixed-time artificial insemination (FTAI) is a reproductive biotechnology that enables the manipulation of the estrous cycle in cattle, improving genetic production efficiency and reducing calving intervals. This study aimed to evaluate the pregnancy rate in Charolais cows synchronized using a 5-day Co-Synch protocol, with the application of recombinant and traditional eCG. Twenty Charolais cows were randomly divided into two groups. All animals received a modified Co-Synch protocol: on day 0, 0.01 mg of GnRH (IM) was administered, and a 1.32 g intravaginal progesterone device was inserted for 5 days. Upon removal, 0.6 mL of cloprostenol (Estrumate®) and either 105 IU recombinant eCG (1.5 mL Folirec®) or 0.55 mL traditional eCG (Novormon 500®) were administered. Seventy-two hours later, FTAI was performed in cows showing estrus; those without signs received a second dose of GnRH before insemination. All cows had adequate body condition and functional ovarian structures. The group treated with recombinant eCG showed 45% estrus expression and a 70% pregnancy rate, compared to 35% and 30%, respectively, in the traditional eCG group. The total cost was slightly higher with recombinant eCG (USD 1214.10 vs. 1193.40), but the cost per pregnancy was considerably lower. In conclusion, recombinant eCG in FTAI protocols proved more efficient both reproductively and economically, offering a viable alternative to improve pregnancy rates in beef cattle.

Keywords: *FTAI, 5-day Co-Synch, eCG, estrus, follicle, pregnancy rate*

INTRODUCTION

Globally, the selection of superior animals has prioritized functional traits to increase productivity indices, which has negatively affected reproductive physiology, reducing conception rates in cattle herds. This phenomenon has been observed since the implementation of biotechnologies such as artificial insemination and the study of the bovine estrous cycle (Giraldo, 2019).

Artficial Insemination and Fixed-Time Artificial Insemination: A Tool for Genetic and Reproductive Improvement.

Artificial insemination (AI) is a key reproductive biotechnology that allows the dissemination of high-value genetic traits by introducing fresh or cryopreserved semen into the female's reproductive tract during the optimal fertilization period (Müller et al., 2020). Its main advantages include:

- *Genetic improvement:* Greater use of sires with proven genetic value.
- *Reproductive efficiency:* A single ejaculate can be used to inseminate multiple females.
- *Sanitary control:* Reduced risk of transmission of venereal diseases.
- *Ease of herd management:* Eliminates the need to maintain breeding males.
- *Production system optimization:* Supports the use of zootechnical records and facilitates the implementation of synchronization and crossbreeding programs.

However, AI presents certain limitations, such as the need for accurate estrus detection, investment in specialized equipment (e.g., dewars and thawing systems), and technically trained personnel (Sanín et al., 2023).

A significant evolution of this technique is fixed-time artificial insemination (FTAI), which uses hormonal protocols to control and synchronize the estrous cycle of females, inducing ovulation through neuroendocrine and gonadal interventions (Yánez et al., 2018). This biotechnology offers substantial advantages in three key areas:

Improvements in reproductive indices:

- Reduction in calving intervals and open days.
- Increased proportion of early-pregnant females.
- Greater control over calving, concentrating it within defined periods (Randi et al., 2021).

Optimization of reproductive management:

- Eliminates the need for estrus detection.
- Reduces livestock handling time.
- Shortens postpartum anestrus, especially useful in animals with silent heats.
- Allows for mass insemination of females in a short time frame (Bo et al., 2019).

Productive homogenization and efficiency:

- Increased kilograms of weaned calves per cycle.
- Production of more uniform offspring, facilitating early weaning.
- Improved utilization of forage resources.

The combination of artificial insemination (AI) with estrus and ovulation synchronization protocols has shown significant improvements in productive parameters such as birth weight, weaning weight, ease of calving, as well as meat and milk production (Wild et al., 2022). These biotechnologies represent fundamental tools for genetic improvement and the sustainability of cattle production.

Use of Estradiol in Reproductive Protocols

In South America, the use of estradiol salts combined with progesterone has shown high effectiveness in estrous cycle synchronization by inducing a prolonged proestrus that enhances ovarian activity and estrus expression, thus increasing pregnancy rates (Marizancén & Pimentel, 2017). However, its use has been restricted in regions such as the United States and the European Union due to concerns about environmental impact and potential negative effects on consumer health (Dias et al., 2020).

Alternative Protocols without Estradiol

In response to estradiol use restrictions, hormonal schemes based on GnRH and PGF2α, such as the Ovsynch protocol and its Co-Synch variant, have been developed, both including progesterone via intravaginal devices (CIDR). These protocols have achieved pregnancy rates of up to 50%, demonstrating their effectiveness as sustainable alternatives (Marqués et al., 2022).

Specifically, the modified 5-day Co-Synch protocol, which includes GnRH, CIDR for five days, PGF2α, and eCG, followed by a second GnRH dose in animals without signs of estrus, has shown promising results in beef breeds, achieving efficient synchronization with less dependence on estradiol (Salgado et al., 2021).

Use of Prostaglandins (PGF2α)

PGF2α plays a crucial role in FTAI protocols by inducing luteolysis, which restarts the estrous cycle. Its effectiveness depends on whether the animals are cycling, leading to specific protocols such as:

- Double application of PGF2α with a 10–14 day interval, where insemination is performed after estrus detection or after the second dose in animals that did not show signs (Sanz et al., 2019).
- Rectal palpation plus PGF2α, identifying functional corpora lutea to synchronize estrus within a 2–3 day window (Sanz et al., 2019).

Optimization with Complementary Hormones: The Case of eCG

Equine chorionic gonadotropin (eCG) has been incorporated into protocols to improve ovarian response, especially in cows with low basal ovarian activity, anestrus, or low metabolic stress. Its action, similar to LH and FSH, stimulates the growth of dominant follicles and enhances corpus luteum functionality, increasing progesterone production, which favors embryonic implantation and improves pregnancy rates (Ortíz et al., 2017).

Moreover, combinations of eCG with hCG or estradiol have achieved pregnancy rates close to 60%, especially in beef cattle, by optimizing oocyte quality and hormonal activity during follicular growth (Tschopp et al., 2022).

Based on this analysis, the objective of this study was to evaluate the pregnancy rate through the application of recombinant and traditional eCG in an FTAI protocol in Charolais cows.

MATERIALS AND METHODS

This research was conducted at the San Vicente farm, located in the Selva Alegre area, in the Pastaza canton of the Puyo parish, belonging to the province of Pastaza.

Study Material

The study was carried out with a total of 20 cows with similar body condition and reproductive physiological status.

Experimental Design

A Completely Randomized Design (CRD) was applied with two experimental treatments:

Table 1. Factors under study

Treatments	Convinations	Descriptio
T1	A*B1	Co-Synch + eCG tradicional (*Novormon 500®*)
T2	A*B2	Co-Synch + eCG recombinante (*Folirec®*)

Each group included 10 animals. The comparison of means was performed using Duncan's test at a 5% significance level, employing SPSS software version 25.

Situational Assessment and Animal Selection

Animal selection was carried out by considering age, weight, body condition, and presence of calf at foot, ensuring homogeneity between the groups. The management system was extensive, with cows tethered and calves at foot.

Pre-Synchronization Ultrasound Evaluation

Reproductive status was assessed using transrectal ultrasonography with a 6.5 MHz linear probe. Animals were classified as follows:

- **Cyclic**: Presence of corpus luteum and/or follicles ≥10 mm.
- **Anestrous**: Absence of functional ovarian structures.

All cows were classified as cyclic.

Applied Hormonal Protocol

A modified 5-day Co-Synch protocol was used:

- **Day 0**: Administration of 0.01 mg of GnRH (IM) + insertion of an intravaginal device containing 1.32 g of progesterone (CIDR®).
- **Day 5**: Removal of the device + administration of 0.6 mL of PGF2α (cloprostenol, Estrumate®) and eCG according to treatment:
    - **T1**: Traditional eCG (0.55 mL, Novormon 500®)
    - **T2**: Recombinant eCG (105 IU, 1.5 mL, Folirec®)
- **72 hours later**:
    - Cows showing estrus: direct FTAI.
    - Cows not showing estrus: FTAI + second dose of 0.01 mg GnRH (IM).

Artificial Insemination

Conventional cryopreserved semen was used. Straws (0.5 mL) were thawed in distilled water at 35–37 °C for one minute and deposited into the uterus using an insemination gun fitted with a catheter and sanitary sheath.

Pregnancy Diagnosis

Pregnancy diagnosis was performed 60 days after FTAI via transrectal ultrasonography, confirming pregnancy by the presence of a viable fetus, fetal membranes, and a functional corpus luteum.

Evaluated Variables

- **Body condition score (1–5)**: Visual scale, ideal = 3.
- **Weight**: Estimated using bovine measuring tape (kg).
- **Age**: Recorded in years.
- **Presence of calf at foot**: Direct observation.
- **Ovarian dimensions (length/width)**: Measured via ultrasound (cm).
- **Preovulatory follicular diameter**: Assessed via ultrasound (mm).
- **Estrus intensity**: Recorded through clinical signs.
- **Pregnancy rate (PR)**: Calculated using the formula:

$$PR(\%) = \left(\frac{\text{Number of pregnant cows}}{\text{Total number of synchronized cows}} \right) \times 100$$

Economic Analysis

Total treatment costs (USD) were recorded, and the cost per pregnancy was calculated as an indicator of economic efficiency.

Statistical Analysis

Statistical analysis was performed using SPSS version 25 to observe differences between the proposed treatments.

RESULTS AND DISCUSSION

Initial Reproductive Status and Response to Synchronization

Before initiating the synchronization protocol, the 20 selected cows showed adequate body condition (average BCS of 3.0), no visible clinical abnormalities, and functional integrity of the reproductive tract confirmed by transrectal ultrasonography. The ultrasound evaluation allowed for the classification of all animals as cyclic, with active ovarian structures (follicles ≥10 mm or presence of corpus luteum), which is consistent with the findings of Ortíz et al. (2017), who stated that the effectiveness of hormonal synchronization significantly improves in animals with basal ovarian activity.

After applying the modified 5-day Co-Synch protocol, the group treated with recombinant eCG (Folirec®) showed higher estrus expression (45%) compared to the traditional eCG group (Novormon 500®), which registered only 35%. Although this difference was not statistically significant ($p > 0.05$), it aligns with the findings of Salgado et al. (2021), who demonstrated that recombinant eCG can induce more precise and homogeneous stimulation of follicular dynamics, especially in beef breeds.

Preovulatory Follicular Diameter

Transrectal ultrasonography was used to measure the dominant follicle prior to insemination, revealing an average diameter of 13.2 ± 1.1 mm in the recombinant eCG group and 11.8 ± 1.3 mm in the traditional eCG group. This difference, though moderate, is physiologically relevant, as a larger follicular diameter has been associated with more predictable ovulation and greater oocyte competence (Ortíz et al., 2017; Tschopp et al., 2022). This finding supports the use of recombinant eCG as an effective follicular growth stimulator in FTAI protocols.

Pregnancy Rate

Pregnancy diagnosis performed 60 days after insemination revealed significant differences between the two treatments. The group treated with recombinant eCG achieved a pregnancy rate of 70%, while the traditional eCG group reached only 30% ($p < 0.05$). These results are in line with those reported by Marqués et al. (2022) and Salgado et al. (2021), who demonstrated that protocols based on GnRH and $PGF_2\alpha$ complemented with eCG yield higher conception rates than traditional schemes, even without the use of exogenous estrogens.

The positive effect of recombinant eCG can be explained by its greater structural stability and more specific action on FSH and LH receptors, promoting the development of functional dominant follicles and corpora lutea with enhanced progesterone-producing capacity (Ortíz et al., 2017;

Tschopp et al., 2022). This creates a more favorable uterine environment for embryo implantation and pregnancy maintenance.

Economic Evaluation

The economic analysis revealed that the treatment with recombinant eCG (T2) had a total cost of USD $1214.10, while the traditional eCG treatment (T1) cost $1193.40. However, when these values were related to the number of pregnant cows, it was evident that the cost per pregnancy was considerably lower in the T2 group, confirming its superior reproductive and economic efficiency. This result is particularly relevant for beef production systems aiming to optimize investment per pregnant unit without compromising animal health or relying on restricted hormones such as estradiol (Dias et al., 2020).

Table 2. Comparative reproductive and economic outcomes

Variable	T1: Traditional eC	T2: Recombinant eCG
Treated cows	10	10
Cows in heat (%)	35% (n=7)	45% (n=9)
Preovulatory follicle diameter (mm)	11.8 ± 1.3	13.2 ± 1.1
Pregnant cows (n)	3	7
Pregnancy rate (%)	**30%**	**70%**
Total treatment cost (USD)	$1193.40	$1214.10
Cost per pregnancy (USD/approx.)	$397.80	$173.44

The findings of this study support the use of recombinant eCG as an effective and safe tool in FTAI protocols for beef breeds such as Charolais. The combination of a 5-day Co-Synch protocol with this hormone significantly improved pregnancy rates and the economic efficiency of the treatment, without requiring the inclusion of estradiol salts. This biotechnological approach aligns with international trends seeking sustainable and efficient alternatives for bovine reproduction (Bo et al., 2019; Wild et al., 2022; Marqués et al., 2022).

CONCLUSIONS

The application of recombinant eCG in a 5-day Co-Synch protocol significantly improved the pregnancy rate in Charolais cows (70%) compared to traditional eCG (30%), suggesting greater effectiveness in stimulating follicular dynamics and overall reproductive efficiency.

Despite a slightly higher investment cost, the recombinant eCG treatment proved more cost-effective by achieving a lower cost per pregnancy, establishing itself as an efficient and sustainable biotechnological alternative for fixed-time artificial insemination (FTAI) programs in beef cattle systems.

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