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Use Intensity of Progesterone Devices for Fixed-time Artificial Insemination in Gyr Crossbred Cows from the Peruvian Tropics

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ABSTRACT

Background: The implementation of routine fixed-time artificial insemination programs can generate significant waste and costs, which good management practices can help mitigate. The objective of the study was to assess the effects of second- and third-use progesterone devices on estrus characteristics and pregnancy rates in synchronized crossbred Gyr cows.

Methods: Ten cows were synchronized with first-use Bovine Intravaginal Progesterone Devices (DIB), nine with second-use DIB and ten with third-use DIB. All cows were ≥90 days postpartum, had an average milk production of 6.9±1.9 L/day and were aged 4-10 years. Estrus signs were recorded at insemination and pregnancies were confirmed at 60 days by ultrasonography.

Result: No significant associations were found between DIB use intensity and estrus detection patch response, mucus color, body condition score (CC), calvings, or breed purity ($p>0.05$). Body condition was associated with uterine turgor tone ($p<0.05$) and 100% of cows with CC 3.0 showed turgor tone 3. The highest pregnancy rate was found in second-use DIB (55.6%) and CC 2.8 (47.1%), though neither association was significant ($p>0.05$). DIBs may be used up to a second-use, as cervical mucosal stimulation and pregnancy rate remain unaffected.

Key words: Bovine intravaginal device, Cervical mucus, Estrus synchronization, Reuse, Tropical livestock.

INTRODUCTION

Cebu cattle, such as the Gyr breed and its crosses in subtropical and tropical zones, generally have low productive and reproductive indexes, with a longer calving interval, leading to higher costs compared to taurine cattle (Mellado *et al.,* 2011; Mungube *et al.,* 2019). Artificial insemination, especially Fixed-Time Artificial Insemination (IATF), can improve these indexes by using genetic material from elite breeders with adequate management (Chawala *et al.,* 2021).

IATF involves administering exogenous hormones and a progesterone intravaginal device (DIB) to perform artificial insemination without detecting heat signs (Oosthuizen *et al.,* 2020). In batches of reproductive-age females, on day zero, the presence of a DIB and estradiol benzoate can control follicular dynamics and development for estrus synchronization (Kaminski *et al.,* 2019; Abdelnaby and Abo El-Maaty, 2021). In a normal cycle, progesterone is produced in the corpus luteum but may be absent when initiating a synchronization protocol, making the application of DIBs necessary (Bisinotto *et al.,* 2015).

However, in the Peruvian tropics, limited access to genetic material, high hormone costs and distrust in the effectiveness of the technique hinder its adoption (Carrell *et al.,* 2021). Due to the high cost of hormonal inputs, DIB reuse has been studied in taurine cattle to improve economic viability. DIB reuse is common in IATF programs, helping reduce costs and waste, though there is uncertainty about the efficacy on estrus intensity and pregnancy rate in Gyr cows. The study aimed to determine the effect of the use intensity of DIB on estrus characteristics and pregnancy rate of Gyr crossbred cows synchronized for IATF in the Peruvian tropics.

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MATERIALS AND METHODS

Location

The study was carried out from July to September 2022 at the Agrarian Experimental Station "El Porvenir" of the Instituto Nacional de Innovación Agraria (INIA) in Juan Guerra, San Martín, Peru (Fig 1). The station is located between the coordinates 354872.00 m east latitude and 9271237.48 m north latitude, at 330 meters above sea level. The agroecological zone is classified as High Humid Forest with annual temperatures between 17 and 35°C and rainfall from 1000 to 1500 mm.

Ethical statement

All animal care procedures for experimental treatments were carried out according to ARRIVE 2.0 guidelines (https://arriveguidelines.org/arrive-guidelines/). The study was not submitted to an ethical approval committee because human-animal interaction was minimal during the gynecological evaluation, hormone application, artificial insemination and ultrasonography.

Animals and samples

The station's herd consist of 125 crossbred Gyr cows (Gyr ×Brown Swiss and Gyr×Holstein), with an average breed purity of 84±0.2%, in a semi-stabled production system. The cows aged 3 to 16 years, with 1 to 9 calvings and body condition (CC) between 2.0 and 4.0 points on a scale from 1.0 to 5.0, where CC 1.0 are cows with lower muscle composition, with protruding ribs and vertebrae and CC 5.0 are cows with higher muscle and obesity (Edmonson *et al.,* 1989; Kim *et al.,* 2023). A sample of 29 cows was selected according to the following criteria: ≥90 days postpartum, average milk production of 6.9±1.9 L/day; 2-6 calvings (average 3.3±1.2), aged 4-10 years (mean 7.3±1.3); and a CC from 2.5 to 3.0 (mean 2.8±0.1). The cows were subjected to a 9-hour grazing regime on *Brachiaria brizantha* pastures, a diet consisting of 72% rice by product, 20% corn, 6% soybean meal, 1% mineral salt and 1% common salt, with fresh water freely available. A gynecological evaluation was performed to rule out reproductive issues such as ovarian cysts, infections, or genital and uterine alterations (Mimoune *et al.,* 2021) and cyclic ovarian status was confirmed by ultrasonography with a 7.5 MHz linear array rectal transducer (DRAMINSKI iScan, Poland), as seen in Fig 2.

Experimental design

The study followed a complete randomized design with three treatments. Twenty-nine cows were randomly distributed in three groups by DIB usage, where 10 cows were synchronized with first-use DIB, 9 with second-use DIB and 10 with third-use DIB. The groups did not show significant differences ($p<0.01$) according to milk production, calvings, age, breed purity and body condition. DIBs for reuse were cleaned with water to eliminate organic residues, disinfected in water with 3 mL/L iodine solution (Vanodine, Zoetis, Argentina), dried in a clean, alcohol-disinfected area and stored in sealed polyethylene bags at room temperature. Before treatments, all cows were supplemented with vitamins A, D and E and minerals including selenium, potassium, phosphorus and zinc (Molefe and Mwanza, 2020).

Synchronization of estrus and IATF

For the IATF protocol, estrus synchronization involved applying a progesterone DIB and exogenous hormones intramuscularly, as detailed in Fig 3. On day 0, vitamins,

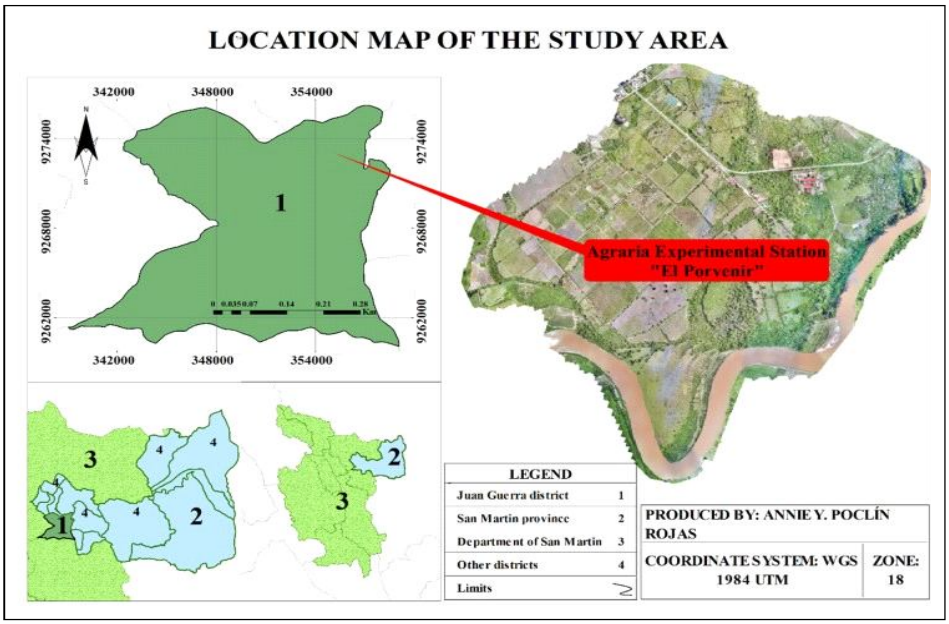
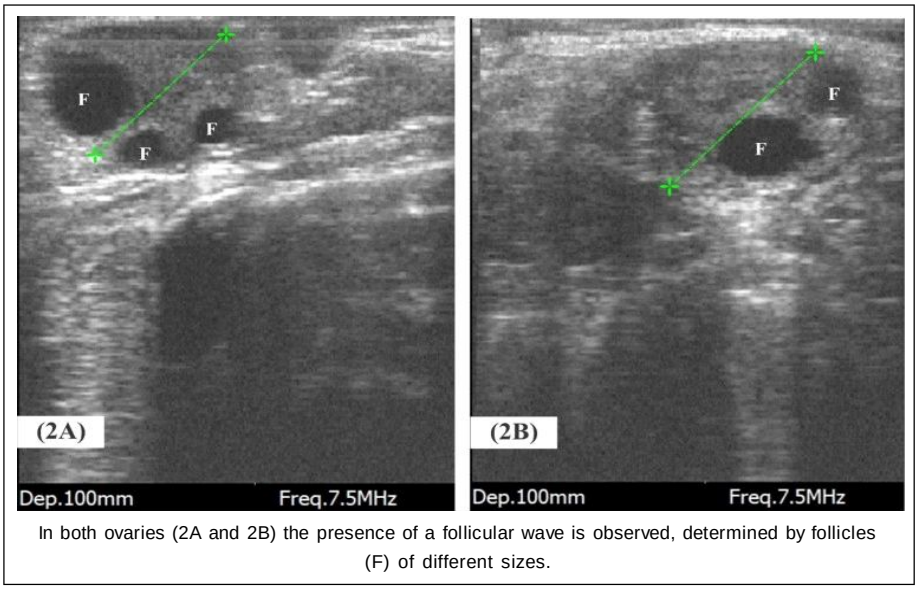


Fig 1: Location map of the agrarian experimental station "El Porvenir", San Martín, Peru.

minerals, 2 mg estradiol benzoate (BE) (Estradiol Benzoate Von Franken, Von Franken, Argentina) and a DIB with 1.2 g of Progesterone (P4) (Disposint 1200, León Pharma, Argentina) were administered. On day 9, the DIB was removed, mucus color recorded and 150 mg of prostaglandin (PGF2α) (D+Tenol, León Pharma, Argentina), 400 IU of equine chorionic gonadotropin (eCG) (Novormon, Zoetis, Argentina) and 1 mg of estradiol cypionate (EC) (Estracip 100, León Pharma, Argentina) were applied and an estrus detection patch was placed on the rump (Kamar Heatmount, KAMAR, Inc., USA) (Stevenson and Atanasov, 2022). Insemination occurred on day 11, 48-52 hours after DIB removal (Pessoa *et al.,* 2019), where the percentage of estrus detection patch response with ≥50% for active patch (Franco *et al.,* 2018), color of cervical mucus and uterine tone by rectal palpation (Lech *et al.,* 2022), were recorded. Ovulation generally followed 70 hours after DIB removal and 30-32 h after the onset of estrus (days 12-13) (Peralta-Torres *et al.,* 2010). Sixty days after IATF, pregnancy was confirmed by ultrasonography. In pregnant cows, the presence of the fetus with a white tone (echogenic), amniotic fluid with a black tone (anechogenic), surrounded by the amniotic membrane and in the ovary, a corpus luteum, was recognized. In non-pregnant cows, the uterine horn has a circular appearance with a white hue, developing follicles



In both ovaries (2A and 2B) the presence of a follicular wave is observed, determined by follicles (F) of different sizes.

Fig 2: Presence of ovarian structures in cyclic cows by ultrasonography.

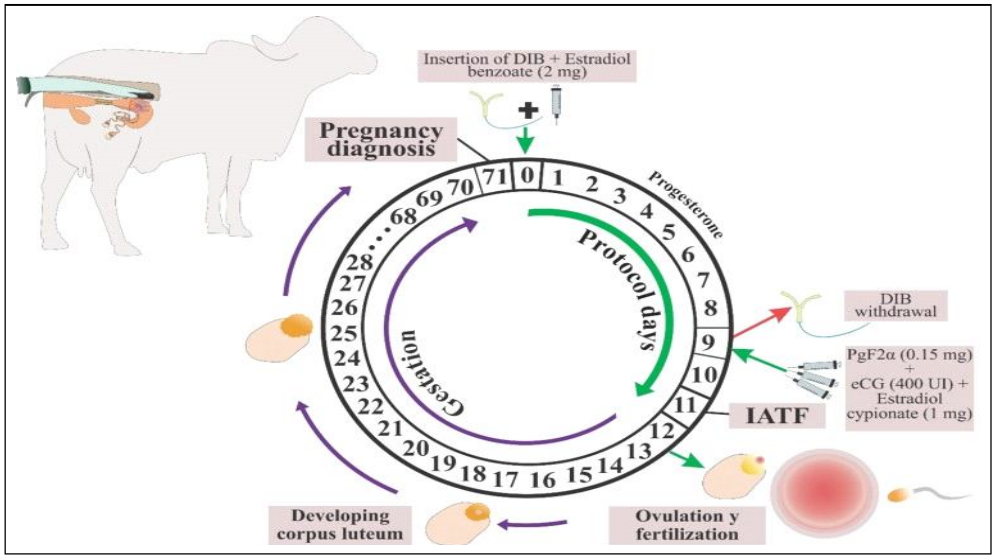


Fig 3: Fixed-time artificial insemination protocol from day 0 to day 11 and gestation diagnosis by ultrasonography, 60 days after artificial insemination.

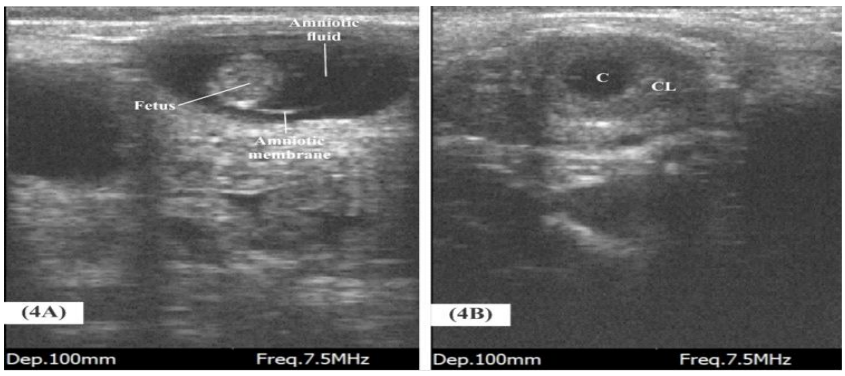
and cavitary corpus luteum, as detailed in Fig 3 and Fig 4 (Curran *et al.,* 1986; Matsui and Miyamoto, 2009; Romano *et al.,* 2006; Szenci, 2021).

Statistical analysis

Qualitative variables were analyzed with contingency tables and a Chi-square test ($p<0.05$) to determine associations between estrus synchronization treatments, grouping variables and response variables. All tests were performed in SPSS v.26.

RESULTS AND DISCUSSION

The relative frequency of mucus color on the DIB was determined according to use intensity at removal in Gyr crossbred cows, where no significant association was



(4A) Positive pregnancy with an echogenic fetus (white tone), anechogenic amniotic fluid (black tone) and surrounding amniotic membrane. (4B) Negative pregnancy with an ovary with gray-toned corpus luteum (CL) and a cavity (C) filled with anechogenic fluid.

Fig 4: Gynecological evaluation at 60 days post-insemination.

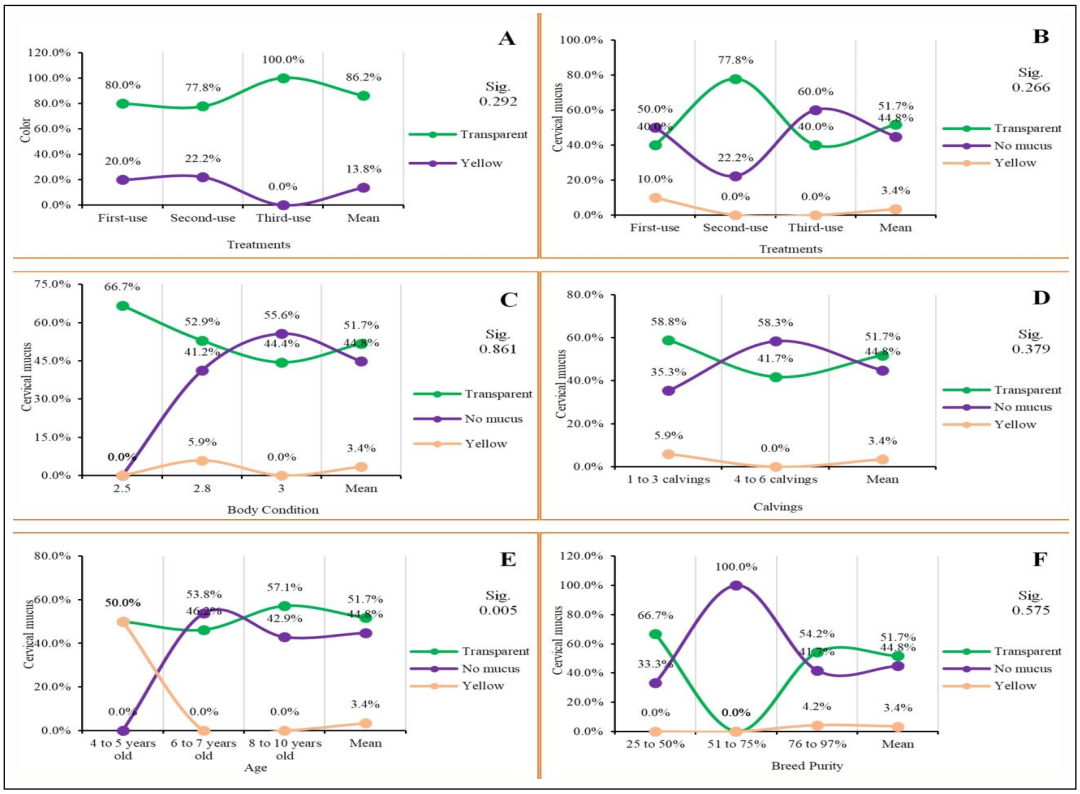


Fig 5: Relative frequency of mucus color on DIB upon removal, according to use intensity. (A); Presence and mucus color during insemination, according to DIB use intensity (B); Body condition (C); Calvings (D); Age; (E) and Breed purity; (F) in crossbred Gyr cows.

found (p>0.05). In the third-use DIB, 100% of transparent mucus was observed, compared to 80.0% and 77.8% in first-use and second-use DIB, respectively (Fig 5A).

No significant association was found between cervical mucus and DIB use intensity, CC, calvings, age and breed purity (p>0.05). During insemination, 77.8% of transparent cervical mucus was observed in second-use DIB; mucus absence in 50.0%, 22.2% and 60.0% in cows treated with first-use, second-use and third-use DIB, respectively (Fig 5B). Cows with CC 2.5 had 66.7% of transparent mucus, whereas cows with CC 2.8 and 41.2% and 55.6% mucus absence, respectively (Fig 5C). A 58.8% of transparent mucus was found in cows with 1-3 calvings; mucus absence in 35.3% and 58.3% of cows with 1-3 and 4-6 calvings, respectively (Fig 5D). For age, 57.1% of transparent mucus was found in cows aged 8-10 years, mucus absence in 53.8% and 42.9% in cows aged 6-8 and 8-10 years, respectively (p<0.01) (Fig 5E). Regarding breed purity, 66.7% of transparent mucus was found in cows with 25 to 50% purity; mucus absence was found in 33.3%, 100.0% and 41.7% of cows with 25 to 50%, 51 to 75% and 76 to 97% purity (Fig 5F).

The estrus detection patch showed effectiveness across all DIB use intensities; however, no significant associations were found in DIB use intensity, CC, calvings, age, or breed purity (p>0.05). Total patch discoloration (100%) was recorded in 100% of cows with first-use DIB, 66.7% with second-use and 80.0% with third-use (Fig 6A). Among cows with CC 3.0, 100.0% showed total discoloration (100), 76.5% for CC 2.8 and 66.7% for CC 2.5 (Fig 6B). Total discoloration patches were found in 76.5% and 91.7% of cows with 1-3 calvings and 4-6 calvings, respectively (Fig 6C). Full patch discoloration frequency was observed in 100% of cows aged 4-5 years (Fig 6D). Active patch were most frequent observed in cows with 51-75% breed purity, while lower frequency were noted in cows with 25-50% and 76-100% purity (Fig 6E).

During artificial insemination, 72.4% of cows showed uterine turgor at tone 3, with no significant associations found with DIB reuse, calvings, age, or breed purity (p>0.05). Uterine tone 3 was registered in 80.0% of cows treated with first-use and third-use DIB and 55.6% with second-use DIB (Fig 7A). Uterine tone 3 was higher in cows with 4-6

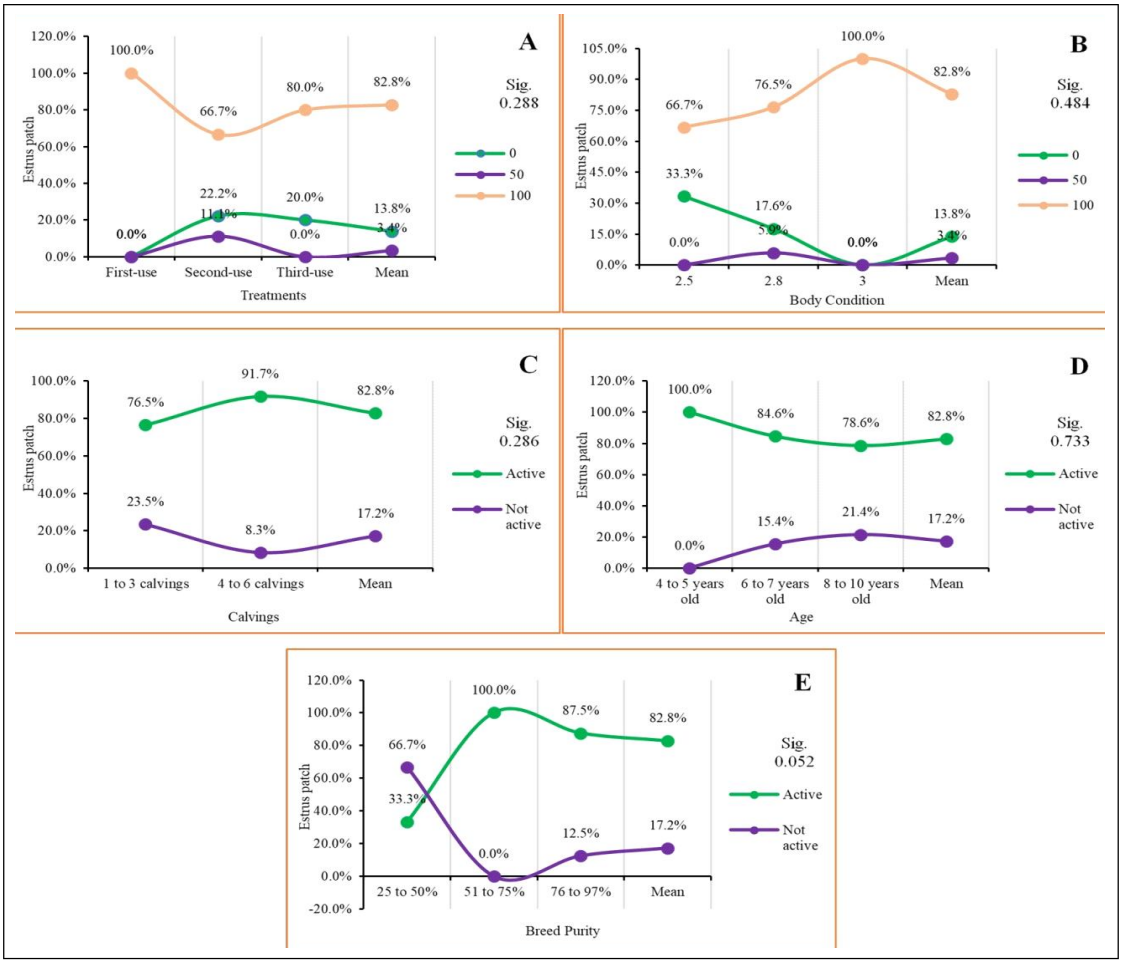


Fig 6: Frequency of estrus detection patch discoloration, according to use intensity of DIB (A), body condition (B), calvings (C), age (D) and breed purity (E), during the insemination of crossbred Gyr cows.

calvings (75.0%) than in cows with 1 to 3 calvings (29.4%) (Fig 7C). Cows aged 6-7 years exhibited a higher frequency of tone 3 (76.9%) than other groups (Fig 7D). Breed purity also influenced uterine tone 3 frequency, with 79.2% in cows of 76-97% purity, while tone 2 was more frequent in cows of 25-50% purity (Fig 7E). A significant association ($p<0.05$) was found according to CC, where 100% of cows with CC 3.0 showed uterine tone 3, while 33.3% of cows with CC 2.5 and 64.7% with CC 2.8 also showed tone 3 (Fig 7B).

The overall pregnancy rate was 41.1%, with no significant association with CC, calvings, age, or breed purity ($p>0.05$). Pregnancy rates were 40.0% in first-use DIB and 30.0% in third-use DIB, while cows with second-use DIB showed 55.6% (Fig 8A). According to CC, cows with CC 2.8 achieved 47.1%, CC 3.0 reached 44.4% and no pregnancies in cows with CC 2.5 (Fig 8B). Cows with 1-3 calvings showed the highest percentage of non-pregnancy (64.7%) (Fig 8C). Cows aged 6-7 years and 8-10 years achieved higher pregnancy rates (38.5% and 50%, respectively) (Fig 8D). Cows with 51-75% breed purity had the highest pregnancy rate (50.0%) and cows with 76-97% breed purity had a pregnancy rate of 41.7% (Fig 8E).

Bovine intravaginal devices (DIB) of progesterone can be reused and the residual progesterone in subsequent uses of the DIB may induce estrus synchronization with similar gestation rates as with first-time use. Cervical mucus discharge, detected during estrus, indicates sexual receptivity and correlates with fertility in Zebu cows (Ramírez-Iglesia *et al.,* 2007; Diaz-Quevedo *et al.,* 2023). Clear mucus contributes to the survival and transport of sperm to the fertilization site. Mucus absence during estrus can negatively impact herd bio-stimulation due to impaired pheromone-based communication (Sankar *et al.,* 2004; Tsigliani *et al.,* 2001).

However, reusing DIBs may lead to microbial contamination. To evaluate this, the study inspected cervical discharge color upon DIB removal. No significant correlation was found between DIB usage frequency and discharge color, which was generally clear or yellow. Interestingly, yellow discharge was observed in 20.0% and 22.2% of cows with first-use and second-use DIB,

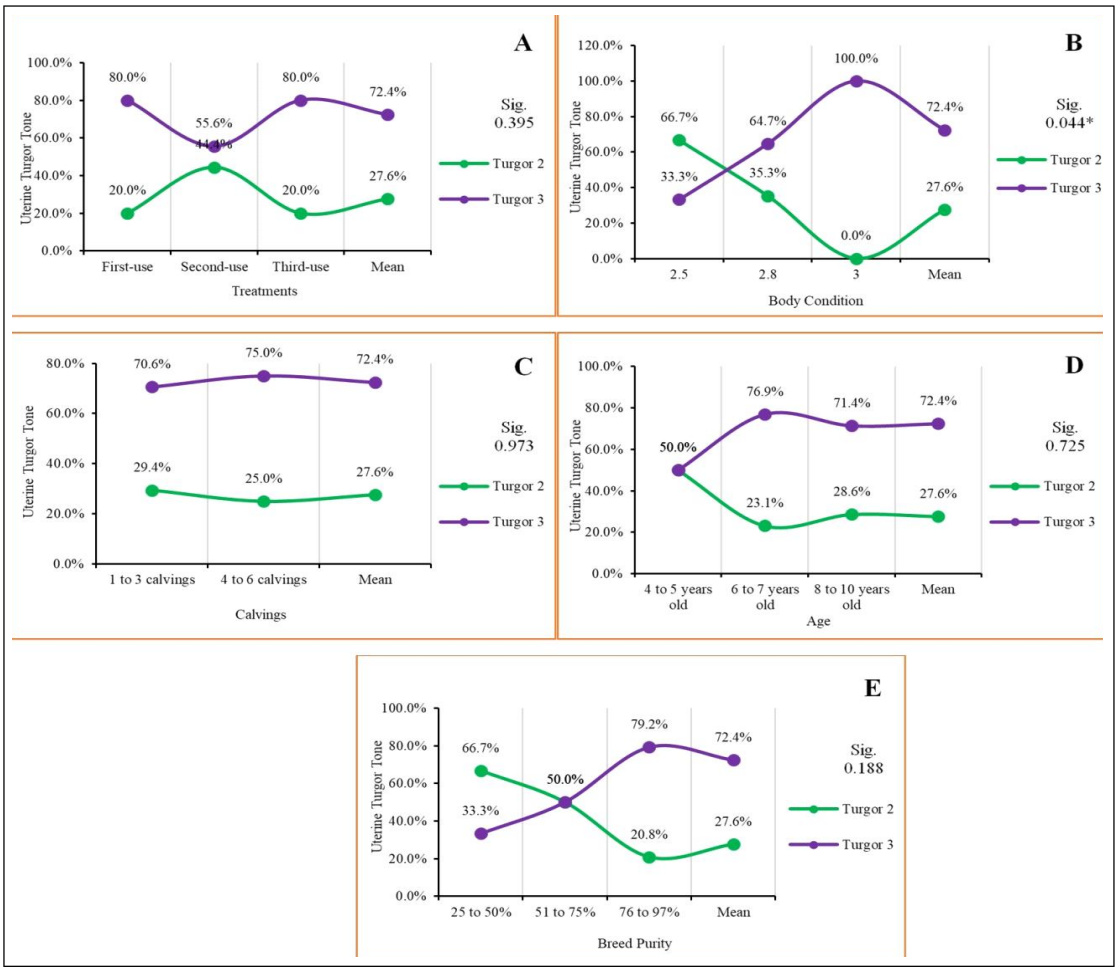


Fig 7: Relative frequency of uterine turgor tone, according to DIB use intensity (A), body condition (B), calvings (C), age (D) and breed purity of animals (E), during the insemination of crossbred Gyr cows.

respectively. Generally, estrus synchronization studies find clear/cloudy discharge in 65% of cases and red/brown in 2% (Chenault *et al.,* 2003), with non-clear discharge linked to infectious processes (Bhat *et al.,* 2015), potentially due to DIB insertion.

Estradiol is the dominant hormone during estrus, causing cervical mucus secretion, genital edema and uterine turgor, which are important signs for timing insemination (Schmitz *et al.,* 2017). This study revealed that 72.4% showed uterine turgor in tone 3, although it was not associated to DIB use intensity, calvings, age, or breed purity. The strong correlation between uterine tone and CC may be attributed to a higher ovarian and estrous response in cows with better nutritional status (Pereira *et al.,* 2018).

This study showed that DIB reuse impacts on estrus traits. The copious cervical mucus, secreted under estrogenic influence from endocervix cells (El-Banna and Hafez, 1972), determines estrus presence among cyclic cows. Clean mucus discharge during artificial insemination improves pregnancy rates (Diaz-Quevedo *et al.,* 2023), enhancing sperm survival and transport (Tsigliani *et al.,* 2001; Lim *et al.,* 2014). In contrast, cows without vaginal mucus discharge or with contaminated discharge containing urine, blood, or purulence have lower pregnancy rates (Mahmoudzadeh *et al.,* 2001). However, estrus detection patches aid in identifying cows in estrus without mucus discharge. The patches placed on the rump of synchronized cows change color when cows accept mounting from other cows, indicating sexual receptivity and helping determine the best moment for insemination (Ramirez-Iglesia, 2012). Estrus detection patches detected more estrus in cows with first-use DIBs, similar to the 100% reported by Manokaran *et al.* (2023) in synchronized Kangayam cows and Shinde *et al.* (2021) in Gyr cows, while third-use DIBs promoted a higher frequency of clear mucus discharge.

The pregnancy rate was lower than the 50% reported by Shinde *et al.* (2021) in Gyr and Naikoo *et al.* (2016) in Kankrej cows; however, in this study, it was slightly higher in cows treated with second-use DIB, but not significantly. According to Simões *et al.* (2018), progesterone concentration in synchronized cows can reach 1.5 ng/mL

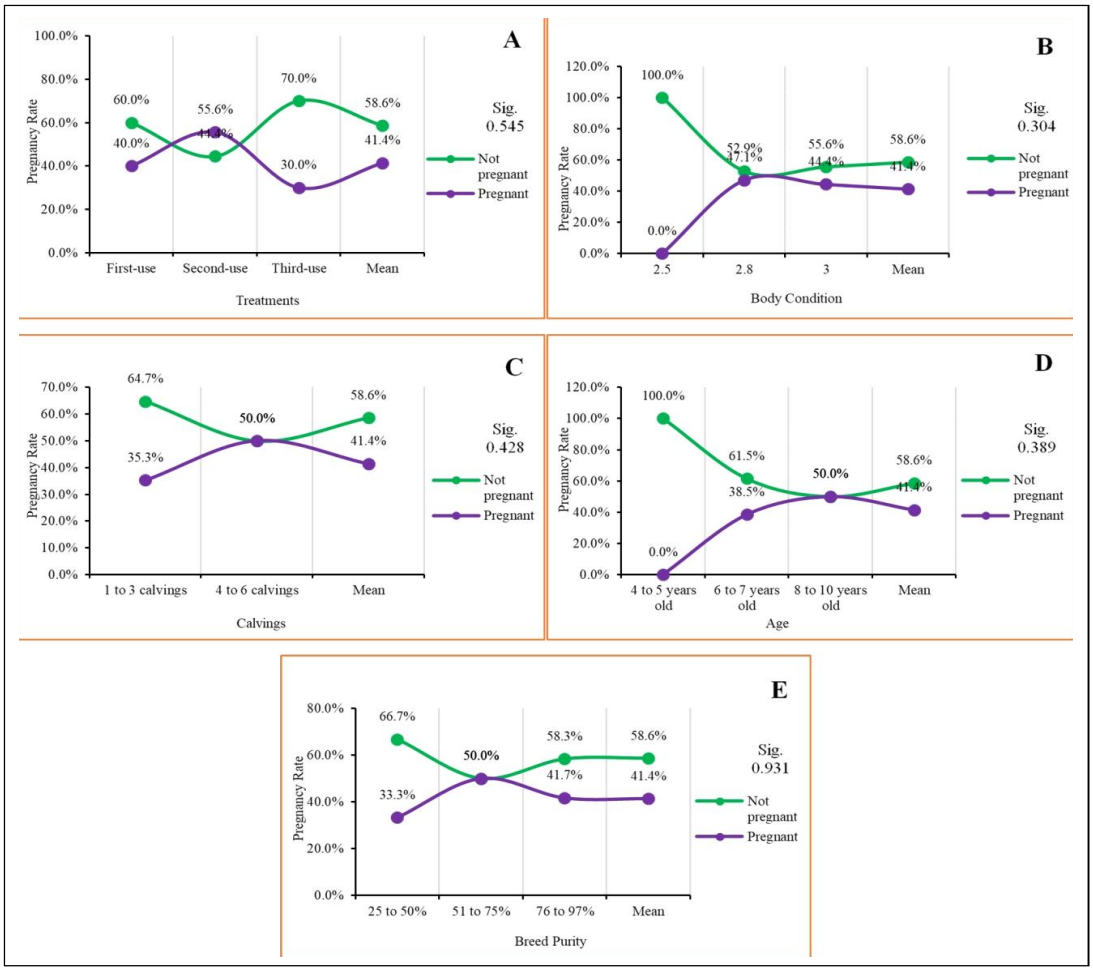


Fig 8: Pregnancy rate according to DIB use intensity (A), body condition (B), calvings (C), age (D) and breed purity (E), in crossbred Gyr cows from the Peruvian tropics.

up to 164 hours post-treatment. This suggests that residual progesterone concentration in reused 1.2 g DIBs can synchronize ovulation by influencing the ovarian cycle. Romero (2012) suggests that second-use DIBs may contribute to up to 40% of pregnancy outcomes. Pereira *et al.* (2018) report decreasing pregnancy rates with increased DIB use: 60.0% with first-use, 51.7% with second-use, 41.9% with third-use, showing a proportional decrease relative to DIB usage intensity, although the IATF protocol remained relatively effective.

Under tropical conditions, CC affects pregnancy rates in cows subjected to IATF with reused DIBs. Cows with CC of 2.75 to 4.0 achieved a 69.75% pregnancy rate, while those with CC of 2.0 to 2.5 achieved only 32.98% (Pereira *et al.,* 2018). No pregnancies were registered in cows with CC of 2.5. Low nutritional status is associated with lower success rates in artificial insemination (Pinedo *et al.,* 2022).

CONCLUSION

Bovine intravaginal devices of progesterone (DIB) remain effective for up to a second use, with high rates of cervical mucus presence and pregnancy. Estrus detection was higher with first-use DIBs, while third-use DIBs showed more transparent mucus discharge. Estrus patches showed complete discoloration in cows with first-use DIBs, though not significant affected according to CC, calvings, age, or breed purity. Uterine turgor tone correlated with CC, where better nutritional status enhanced tone-3 uterine turgor during estrus.

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Disclaimers

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Informed consent

All animal care procedures for experimental treatments were carried out according to Law 30407 on animal protection and welfare of the Peruvian government and ARRIVE 2.0 guidelines (https://arriveguidelines.org/arrive-guidelines/) for animal care during trials.

Conflict of interest

Authors declare no conflict of interests for this article.

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