

Luteolytic failure as the cause of low efficiency in synchronization with prostaglandins in cows under tropical grazing

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ABSTRACT

Objective: To determine the reason for the low response to the exclusive use of prostaglandin in synchronization programs in cows under tropical grazing compared with the use of progesterone (CIDR).

Design/Methodology: Thirty-five cows with CL were randomly distributed in two groups. The first group (GPG; n=23) was synchronized using two doses of PGF2 α (25 mg of Dinoprost[®]) with a 12-day interval. The second group (GCIDR; n=12) was synchronized with an intravaginal device (1.9 g of progesterone, 2 mg of estradiol benzoate, and 50 mg of progesterone); a PGF2 α (pm) dose was applied on day 7, before removing the CIDR (am) on day 8. CL regression, luteolytic failure, progesterone concentration, and CL size were determined. Data was subjected to a normality test, followed by the Mann-Whitney U test or independent Student's t-test and chi-square test.

Results: Only 82.6% (19 out of 23) of the GPG cows that received the second dose of prostaglandins have a functional CL (<1 ng mL⁻¹ of progesterone). The CL recorded a regression only in 43.5% of the cows in GPG vs. 91.7% in GCIDR (P=0.0001). In addition, GPG cows showed a luteolytic failure of 39.1% and an asynchrony of 17.4%.

Conclusions: The low effectiveness of prostaglandin on the synchrony and regression of the CL (luteolytic failure) in cows fed under tropical grazing can be attributed to the low efficiency of the synchronization programs.

Keywords: regression of the corpus luteum, luteolytic pattern, luteolytic efficiency.

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INTRODUCTION

For decades, as a result of its luteolytic action, prostaglandin was used in estrous and ovulation synchronization programs (Córdova-izquierdo *et al.*, 2011; Colazo *et al.*, 2017).



However, the results reported in sheep show variability: Arroyo-Ledesma *et al.* (2015) reported a 100% estrous response, while Meilán and Ungerfield (2014) and Ungerfield (2011) reported a 90% response. Synchronization is reported in only 70% of the female bovines (Giordano *et al.*, 2013; Liu *et al.*, 2018), as well as an estrous response that ranges from 87% (Mérola *et al.*, 2012; Bover *et al.*, 2019) to 64% (Gioso *et al.*, 2005). However; a failure in the effectiveness of prostaglandin on the CL regression is reported in 62.7% of sheeps (Hernández-Cerón *et al.*, 2001). CL regression is attributed to the action of prostaglandins, ending diestrous and triggering proestrous (3 and 2 days for cattle and sheep, respectively) (Atuesta and Diaza, 2011). This results would enable an estrous response the following day, basing its action within 72 h in sheep (Thimonier 1981; Ávila-Castillo *et al.*, 2019) and 96 h in cattle (Córdova *et al.*, 1983; Liu *et al.*, 2017, 2018). In published reports, the period considered is greater than the effect attributed to the action of prostaglandin. There are few exclusive studies about the effectiveness of prostaglandin in CL regression in cattle; a mere 51.6% efficiency has been reported in embryo transfer programs —*i.e.*, a synchrony failure in 48.4% of recipient cows (Baruselli *et al.*, 2000). This phenomemon can be attributed to inefficiency on the lysis of the corpus luteum (Hernández-Cerón *et al.*, 2001), which would allow females with luteolytic failure to have a natural/normal estrous cycle which may coincide with the assessment periods. The lack of evidence about the luteolytic failure of prostaglandin in synchronization protocols in cattle merits further research about the efficiency of the prostaglandin application to lyse the corpus luteum in a period no longer than 4 days (response period). The effectiveness of prostaglandin on the CL regression in cows synchronized with 2 single doses was assessed for that purpose.

MATERIAL AND METHODS

The study was performed in Villahermosa, Tabasco, in the tropical region of southeastern Mexico (18° 20' N, 17° 78' S, 92° 95' E, and 93° 15' W), which has a warm-humid-dry climate with a maximum temperature of 43.5 °C and a minimum temperature of 10.5 °C (SMN, 2010).

Animals used for the experiment

Thirty-five cows (3-4 years old) fed with humidicola grass (*Brachiaria brizantha*) and an average body condition of 3.2 ± 0.49 points (scale: 0 to 5) (Edmonson *et al.*, 1989) with at least one corpus luteum (CL) were randomly distributed as follows: the first group (GPG; n=23) was subjected to a synchronization protocol with two luteolytic doses of PGF2 α (25 mg of Dinoprost, Lutalyse[®] by Zoetis), with a 12-day interval between doses (Selk *et al.*, 1988); and the second group (GCIDR; n=12) was subjected to a synchronization protocol consisting of an intravaginal device inserted on day 0 (1.9 g of progesterone, CIDR[®] by Zoetis), plus 2 mg of estradiol benzoate (IM; Sincrodiol[®] by Ourofino), and 50 mg of IM progesterone (Progesvit A-E[®] by Brovel), followed by a 25-mg dose of PGF2 α (Lutalyse[®] by Zoetis) administered on day 7, and the removal of the CIDR on day 8 (Baruselli *et al.*, 2011). Both groups were provided with clean, fresh water on a daily basis.

Variables

Luteal dynamics: A Mindray DP-10 Vet ultrasound with a 7.5-MHz intracavitary real-time, linear array transducer was used to perform an ultrasonography, in order to determine the diameter of CL from the second PGF2 α dose on day 0, 2, 4, and 7. The diameter of CL was determined with the equation described by Sartori *et al.* (2004):

$$D = \frac{L + A}{2}$$

D =Diameter of CL (mm), L =length of CL (mm), A =width of CL (mm).

Blood progesterone concentration (P4)

Progesterone was determined 0, 1, 2, 5 and 7 days after the second PGF2 α dose or the removal of the CIDR using blood samples obtained by venipuncture, using BD Vacutainer[®] tubes and needles with 6 ml of anticoagulant (80-100 IU of heparin) which were then refrigerated at 4 °C. Subsequently, they were centrifuged (3000 x g for 10 min at room temperature) and stored at -20° C before they were analyzed. The solid-phase enzyme-linked immunosorbent assay (ELISA) technique was used for this determination, based on the principle of competitive binding described by Siregar *et al.* (2017), using a DGR[®] EIA 1561 commercial kit (GmbH, Germany) and a 450 \pm 10 nm calibrated microplate reader.

Data analysis

Data was analyzed in 2 phases. The first compared the efficiency of two synchronization protocols (prostaglandins, GPG; n=23 *vs.* Progesterone, GCIDR, n=12) in cows fed with grazing. The second determined if the cause of the low efficiency of prostaglandin (GPG) on CL regression in bovines is similar to the cause reported in sheep by Hernández-Cerón *et al.* (2001). The diameter of CL (according to the equation described by Sartori *et al.*, 2004) was determined based on the luteal dynamics of GPG, while the functionality of the corpus luteum was determined according to the blood progesterone concentration. Less than 1.0 ng mL⁻¹ was considered non-functional CL and a higher concentration was considered functional CL (Ribeiro *et al.*, 2012). Therefore, a luteolytic failure was determined, when the CL remained functional throughout the assessed process (Callejas *et al.*, 2003; Ribeiro *et al.*, 2012; Liu *et al.*, 2017). Structural lysis was determined based on a statistical decrease in diameter of the CL of up to 9 mm (Balara *et al.*, 2017).

The resulting data were subjected to a Kolmogorov-Smirnov normality test, followed by a statistical test ($P \leq 0.012$). Subsequently, they were analyzed using non-parametric tests (Mann-Whitney U Test) or, when appropriate, they were subjected to a Student's t-test for independent groups. The proportion of cows showing lysis of the corpus luteum was submitted to a chi-square (χ^2) statistical test. Data were described as the arithmetic mean \pm standard error of the arithmetic mean, using the SYSTAT statistical package version 13 (Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Efficiency of synchronization programs on CL lysis

In both groups, the assessment began at the end of the synchronization program (GPG, n=23 and GCIDR, n=12); all the cows showed at least one CL. The percentage of cows showing CL regression from day 1 to day 7 is higher in cows with GCIDR than the efficiency of GPG cows ($p \leq 0.04$, Table 1). However, blood progesterone concentration did not differ between the two groups on day 0, 1, and 2 ($p \geq 0.09$; Table 1). Meanwhile, on day 4 and 7, the progesterone concentration was lower in cows with GCIDR *vs.* GPG ($p \leq 0.005$). Furthermore, the size of CL was similar in cows with GCIDR *vs.* GPG on day 0, 2, and 4 ($p \geq 0.21$), while on day 7 the size of CL was greater in cows with GPG ($p = 0.006$). This demonstrates the low efficiency of a prostaglandin dose on day 4 in 56.5% and on day 7 in 43.5% of the specimens.

Effectiveness of prostaglandin on the regression of corpus luteum

The presence of at least one CL was observed in 100% of the cows, following the application of the second prostaglandin dose; however, only 82.6% (19 out of 23) recorded a functional CL ($> 1 \text{ ng mL}^{-1}$ progesterone; $P = 0.002$) after the second dose, while 17.4% of the cows recorded the presence of a non-functional CL. Nevertheless, they had a functional CL ($> 1 \text{ ng mL}^{-1}$ progesterone) on day 4 (Table 2).

On day 4, a low efficiency in prostaglandin synchronization was observed in 56.5% of the cows with GPG (CL regression in 43.5%), reaching 43.5% on day 7. This inefficiency is attributed to two factors: the failure to synchronize the second prostaglandin dose in 17.4% of the cows that had a CL regression of the CL on day 0; and a luteolytic failure in 39.1% and 26.1% of the cows subjected to prostaglandin synchronization on day 4 and day 7, respectively. Therefore, in the following GPG analysis, cows with a functional CL

Table 1. Efficacy of synchronization on luteolysis in cows fed under tropical grazing (GCIDR=with CIDR and GPG=synchronization with two doses of prostaglandins).

| | Days later of the 2 nd dose of prostaglandins. | | | | |
|---------------------------------------|---|----------|----------|----------|---------|
| | 0 | 1 | 2 | 4 | 7 |
| Proportion of cows with luteolysis, % | | | | | |
| GCIDR, n=12 | 0 | 66.7 | 75 | 91.7 | 91.7 |
| GPG, n=23 | 0 | 13 | 39.1 | 43.5* | 56.5 |
| P ¹ | --- | 0.002 | 0.039 | 0.008 | 0.04 |
| Progesterone concentration, ng/ml | | | | | |
| GCIDR | 4.5±5.5 | 2.2±2.4 | 1.0±0.9 | 0.57±0.4 | 0.6±0.3 |
| GPG | 3.8±3.6 | 2.2±1.8 | 2.2±2.2 | 2.2±2.2 | 4.0±6.6 |
| P ¹ | 0.27 | 0.39 | 0.088 | 0.004 | 0.005 |
| Corpus luteum size, mm | | | | | |
| | 0 | 2 | 4 | 7 | |
| GCIDR | 20.9±3.4 | 11.2±3.3 | 9.3±4.2 | 7.9±2.0 | |
| GPG | 19.9±4.9 | 12.8±4.5 | 14.5±5.1 | 13.3±5.6 | |
| P ¹ | 0.47 | 0.21 | 0.31 | 0.006 | |

Table 2. Size of CL and proportion of cows with functional CL (with luteolytic failure or CL regression) or with non-functional CL.

| Second dose, day 0 | | Effect of prostaglandins | | | |
|-------------------------------|----------------|--------------------------|----------|----------|----------|
| | | 1 | 2 | 4 | 7* |
| CL- Functional, % 82.6 | Failure | 69.5 | 43.5 | 39.1 | 26.1 |
| | luteolysis | 13 | 39.1 | 43.5 | 56.5 |
| | P ¹ | 0.000 | 0.732 | 0.732 | 0.016 |
| CL- Non-Functional, % 17.4 | Functional | 13 | 4.4 | 0 | 0 |
| | Non-Functional | 4.4 | 13.0 | 17.4 | 17.4 |
| Corpus luteum size, mm | | | | | |
| CL-Functional | Failure | 18.3±4.3 | 17.1±3.5 | 17.9±3.8 | 15.8±6.0 |
| | luteolysis | 23.6±2.8 | 8.9±1.7 | 6.8±5.2 | 6.8±5.5 |
| | P ¹ | 0.005 | 0.000 | 0.000 | 0.03 |
| CL- Non-Functional | | 11.1±1.4 | 13.0±0.4 | 15.5±4.3 | 15.9±5.5 |

after the application of the second prostaglandin dose (cows that underwent a regression of the CL *vs.* failure of CL regression) were compared with the evolution of cows with a non-functional CL after the application of the second prostaglandin dose. The proportion of cows with CL regression was similar to those showing a luteolytic failure on days 2 and 4 ($p=0.7$); meanwhile, a failure decrease was recorded on day 7 ($p=0.016$) (Table 2).

The progesterone concentration at day 0 was statistically similar between cows with CL lysis and cows with luteolytic failure ($p=0.5$) (Table 3), while the progesterone concentration at day 1, 2, 4, and 7 was higher in cows with luteolytic failure ($p\leq 0.03$) (Table 3). However, the size of the CL was lower in cows with luteolytic failure throughout the assessed period ($p=0.005$) (Table 2). The CL size was greater in cows that manifested luteolytic failure than in cows that suffered CL regression ($p\leq 0.03$) (Table 2). Therefore, the blood progesterone concentration did not differ in cows with luteal failure or lysis ($p=0.51$) (Table 3), while the progesterone concentration was higher in cows with luteal failure from day 1 to day 7 than in with cows that suffered CL regression ($p\leq 0.03$). Likewise, the size of the CL and the progesterone concentration in cows with non-functional CL increased from day 1 to day 7 after the application of the second prostaglandin dose (Table 3).

According to these results, 100% of the cows have at least one corpus luteum at the first and second luteolytic dose of prostaglandin. This potential synchronization is supported

Table 3. Blood progesterone concentration in GPG cows with functional CL (with luteolytic failure or lysis of CL) or with non-functional CL.

| Second dose, day 0 | | Effect of prostaglandins | | | | |
|--------------------|----------------|--------------------------|---------|---------|---------|----------|
| | | 0 | 1 | 2 | 4 | 7* |
| CL-Functional | Failure | 4.2±4.2 | 3.4±2.1 | 4.2±2.9 | 3.9±2.6 | 5.6±6.9 |
| | luteolysis | 4.8±3.5 | 1.5±1.2 | 0.7±0.2 | 0.7±0.5 | 0.9±0.8 |
| | P ¹ | 0.51 | 0.03 | 0.001 | 0.001 | 0.027 |
| CL- Non-Functional | | 0.6±0.1 | 1.2±0.9 | 1.3±0.5 | 2.1±1.0 | 8.3±11.2 |

by several studies (25 mg, Moreno *et al.*, 1986; Bó *et al.*, 2004; Montiel-Palacios *et al.*, 2011). However, progesterone profiles determine that 74.1% of the cows are synchronized (functional CL at the second dose), which matches the results of Liu *et al.* (2018), who reported that only 69.6% of the cows become synchronized with the use of prostaglandins; meanwhile, Giordano *et al.* (2013) mentions that 70 to 80% of the cows become synchronized. In contrast, our results indicate that only 35.7% of the subjected cows showed luteolysis within the 4-day period attributed to the action of exogenous prostaglandin (Córdova *et al.*, 1983; Liu *et al.*, 2017; 2018); however, 7.1% showed lysis before the second prostaglandin dose, with a functional CL after the application, as indicated by Olivera (2007) and 14.3% showed a CL regression after the action period of the exogenous prostaglandin (day 7). Our results differ from some studies in bovines, including Liu *et al.* (2017) and Ribeiro *et al.* (2012), who show that a standard prostaglandin dose causes CL lysis in 60% of the cows. However, a 35.7% (Hernández-Cerón *et al.* 2001) to 50% (Granados-Villareal *et al.* 2017) and 42.8% (Álvarez-Reyna 1994) effectiveness of prostaglandin is reported in sheep. This phenomenon is attributed to the high percentage of luteolytic failures recorded in females. Therefore, these studies do not report the percentage of females which had a luteolytic failure since the first prostaglandin dose. Based on our results, 28.6% of the cows do not have functional CL at the second dose, perhaps as a consequence of a possible luteolytic failure of prostaglandin since the first application.

These results —obtained from a high luteolytic failure— are attributed to various causes, such as age, functionality, and size of the CL at the time of prostaglandin action (Stevenson *et al.*, 1984; Moreno *et al.*, 1986; Berroa-Pinzón 1988). For example, Oliveira *et al.* (2007) mention that the sensitivity of the CL to the action of prostaglandin in cattle starts at day 5 of maturation, while Menchaca and Rubianes (2004) report a sensitivity at day 3 after ovulation in sheep. In our study, synchronization with the first prostaglandin dose suggests that the CL had a minimum age of 7 days (3 to 4 days after the start of the estrous and ovulation of the first dose, plus 8 to 9 days following the development of the CL). However, we observed that 28.6% of the cows did not have CL functionality at the time of the second dose, which could indicate CL lysis before this dose; therefore, luteolytic failure in some cows from the first dose of prostaglandin can be inferred, as has been shown in some studies on luteolytic failure in the second application of prostaglandin.

Prostaglandin sensitivity is related to size and progesterone concentrations (Spell 2001; Sartori *et al.*, 2002). In our study, cows that suffered luteolysis showed a larger CL (23.6 mm *vs.* 17.9 mm in cows with luteolytic failure); however, no differences in progesterone concentration (3.8 mL^{-1} and 2.6 mL^{-1}) were recorded between cows that suffered lysis and those that had luteolytic failure. This result is different from the findings of Granados-Villareal *et al.* (2017), who pointed out that a high progesterone concentration prior to prostaglandins results in a higher proportion of females with luteolytic failure.

In this study, 91.9% of the cows subjected to synchronization with a vaginal device and prostaglandins synchronized at the onset of the estrous and ovulation in a 4-day period, while in the protocol with two prostaglandin doses only 71.4% of the cows became synchronized with the first dose of prostaglandin and only 50% are synchronized with the second dose after a 7-day period. These results can be attributed to an effect in

progesterone release by CIDR, which improves the sincronization response as indicated by Beard and Lamming (1994). In a long progesterone period, it increases estrogen receptors; consequently, they increase the oxytocin receptors that, along with exogenous prostaglandin, induce endogenous prostaglandin on day 7 after CIDR insertion, which improves the efficiency on CL regression in GCIDR synchronized cows. These results demonstrate that the low pregnancy rate observed in synchronization protocols with two prostaglandin doses is caused by the luteolytic failure of prostaglandin in the application of the first and second doses. For example, Riveiro *et al.* (2012) report a 28% pregnancy rate using prostaglandin and artificial insemination; in contrast, the CIDR and prostaglandin protocols have been shown to achieve a pregnancy rate up to 81% (Hernández *et al.*, 2008).

CONCLUSIONS

The low efficiency of prostaglandin in the synchronization programs can be attributed to the high proportion of cows that do not synchronize and the high proportion of cows with luteolytic failure. This factor may be the cause of the low pregnancy rate in prostaglandin synchronization programs reported by some studies. Likewise, synchronization with progesterone is more effective than the use of two prostaglandin doses applied with a difference of 12 days.

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