# Is prostaglandin F2α administration at the beginning of a progesterone and estradiol-based treatment for FTAI an effective strategy in *Bos taurus* heifers?

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A B S T R A C T

The objective was to evaluate effects of prostaglandin (PG) F2α administration at the beginning of a progesterone and estradiol-based treatment period on ovarian response and pregnancy rate (P/AI) in *Bos taurus* beef heifers. Heifers were treated with 500 μg of cloprostenol administered: a) in two half-doses (250 μg) at the time of progesterone device insertion and removal (two-PG), or b) in a single dose at the time of device removal (one-PG). In the two-PG group, administration of PG at device insertion resulted in lesser serum progesterone concentrations during the 7-d treatment period ($P < 0.05$). Additionally, diameter of the follicle from which ovulation occurred was greater, and ovulation occurred earlier in the two-PG compared with one-PG group ($P < 0.05$). Fixed-time artificial insemination (FTAI) was performed in 3479 heifers with two times for FTAI (48 compared with 54 h from device removal). There was no effect on P/AI percentage for the PG treatment or the time of FTAI. The FTAI at 54 h resulted in a greater P/AI percentage in the one-PG than two-PG group (70.5%, 253/359 and 63.5%, 254/400, respectively; $P < 0.05$). There were no differences between PG treatments when FTAI was performed at 48 h after device removal. In conclusion, the administration of PG at the time of intravaginal progesterone device insertion results in lesser progesterone concentrations, and an increased size of the follicle from which ovulation occurs, and the time of ovulation is earlier after device removal. The pregnancy rate was not affected by the PG administration at the time of device insertion.

## 1. Introduction

The market for fixed-time artificial insemination (FTAI) programs is growing in the global cattle industry. Because this technology does not require estrous detection, is effective in estrous cycling and postpartum anestrous females, heifers and suckling cows, is easy to use for producers and practitioners, and is a low-cost and high impact technology, there is a greater use of FTAI by producers than what has traditionally occurred with use of estrous synchrony regimens. Progesterone and estradiol-based protocols are the preferred treatments for FTAI of beef cattle in South America (Bó et al., 2018). The expected pregnancy percentage per AI (P/AI) with use of these FTAI programs averages 50%, and new alternatives have been proposed to increase this outcome. One of the factors affecting fertility appears to be associated with size of the follicle from which ovulation occurs (Perry et al., 2007), probably associated with an increase in the ovulation rate (Sales et al., 2015), ovulation of a more functional oocyte (Dickinson et al., 2016), greater preovulatory

estradiol production (Jinks et al., 2012), and greater postovulatory luteal size and progesterone production (Wiltbank et al., 2012a). Different strategies have been utilized to increase pregnancy rate by inducing an increase in diameter of the follicle from which ovulation occurs, enhancing ovulation rate and improving luteal function after ovulation (Núñez-Olivera et al., 2014; Perry et al., 2014). Different strategies to stimulate preovulatory follicular development, therefore, should be undertaken when attempting to improve the effectiveness of FTAI protocols in enhancing pregnancy percentages.

With FTAI protocols, the super-physiological progesterone concentrations at intravaginal device insertion associated with intramuscular (i.m.) estradiol administration is required to ensure follicular regression and emergence of a new wave of follicular development (Adams et al., 2008; Bó et al., 2016). Super physiological progesterone concentrations for too many days after device insertion could suppress luteinizing hormone (LH) pulse frequency affecting dominant follicle development (Bergfeld et al., 1996; Ireland and Roche, 1982). In beef cattle, greater than optimal progesterone concentrations throughout treatment could negatively affect the ovulation rate, and therefore, pregnancy success may be negatively affected (Dadarwal et al., 2013; Martins et al., 2017). In *Bos indicus* beef heifers, lesser circulating progesterone concentrations have been achieved by inducing luteolysis with prostaglandin (PG) F2α administration at the beginning of a progesterone and estradiol-based synchronization treatment regimen. This approach resulted in a greater fertility than in heifers with relatively greater progesterone concentrations at the end of the treatment (Carvalho et al., 2008; Meneghetti et al., 2009; Peres et al., 2009). Similar findings have been reported in *Bos indicus* by *Bos taurus* crossbred heifers (Cutaia et al., 2007). Most practitioners have included this strategy in *Bos indicus* heifers. Due to the assumption that this approach to FTAI would have similar effects in *Bos taurus* heifers, this protocol was also incorporated for *Bos indicus* cattle (reviewed by Bó et al., 2016). Because differences in steroid sensitivity and metabolism have been proposed between *Bos indicus* and *Bos taurus* heifers (Carvalho et al., 2008, 2017; Sartori et al., 2016a, 2016b), the convenience of using treatments to bring about lesser progesterone concentrations with PG administration at the time of progesterone device insertion in FTAI treatment regimens of *Bos taurus* beef heifers has not been assessed for efficacy and requires further investigation.

The main objective of the present study was to evaluate the effect of PGF2α administered at the beginning of a treatment period with an intravaginal progesterone device and estradiol-based treatment on serum progesterone concentrations, preovulatory follicular growth and fertility in *Bos taurus* heifers. In addition, it was determined if pregnancy percentage was affected by timing of insemination when there was early PG administration using the progesterone-based protocol.

## 2. Materials and methods

### 2.1. Animals and management

Two experiments were conducted during the spring beef cattle breeding season in the Southern Hemisphere (October-January, 33 °S, Uruguay) in Hereford-Angus crossbred heifers. All heifers were examined using ultrasonography to determine the presence or absence of a corpus luteum (CL; estrous cycling or anestrous heifers, respectively) at the beginning of the treatment period. In each experiment, heifers were all maintained in the same paddock, grazing rangeland pasture produced from crystalline–based soil and had unrestricted access to water and mineral supplement. All experimental procedures involving animals including injections, intravaginal device insertion, blood collection, and ovarian ultrasonographic examinations were approved by the Internal Animal Care Committee of Fundación IRAUy and were conducted in accordance with the guidelines of the National Council of Animal Care (CNEA) of Uruguay.

### 2.2. Experiment 1

The aim of this experiment was to evaluate the effect of PGF2α administration at the beginning of a progesterone and estradiol-based treatment period on serum progesterone concentrations, preovulatory follicle development, diameter of follicle from which ovulation occurred, timing of ovulation and serum estradiol-17β concentrations. This study was conducted using 2-year-old Hereford heifers ($n = 25$) with a body weight of 299.2 ± 5.0 kg (mean ± SEM) and body condition score (BCS) of 4.5 ± 0.1 (scale 1 to 8; 1 = emaciated and 8 = obese; (Vizcarra and Wettemann, 1996)). The females were selected 14 d before the experiment from a herd of 150 heifers based on the presence of a CL, and 25 heifers were pre-estrous synchronized using one dose of PGF2α (500 µg of cloprostenol sodium, Ciclase DL, Zoetis, Buenos Aires, Argentina) with the aim that all heifers be in the stage of the estrous cycle at 7 to 12 d after the time of ovulation (Day 0 of the experiment). On Day 0, the presence of CL was confirmed using ultrasonography and the heifers were treated with an intravaginal progesterone releasing device (0.5 g, DIB 0.5, Zoetis) that was in place for 7 d and 2 mg of estradiol benzoate (Gonadiol, Zoetis) was administered i.m. at the time of device insertion. The heifers were randomly allocated to one of two experimental groups to receive 500 µg of cloprostenol sodium in two half-doses (250 µg each) administered at the time of progesterone intravaginal device insertion and removal (two-PG group; $n = 13$), or in a single dose administered at device removal which was considered to be the control group (one-PG group, $n = 12$). All the heifers were treated i.m. with one dose of 0.5 mg estradiol cypionate (Cipiosyn, Zoetis) and 300 IU of equine chorionic gonadotropin (eCG; Novormon, Zoetis) at the time of device removal (Day 7).

### 2.3. Ultrasonic examinations

Ovarian examinations using ultrasonography were performed daily utilizing a B–Mode ultrasonic device with a linear–array transducer (7.5 MHz, WED–9618 V Well.D, Shenzhen, China). Examinations were conducted by the same operator every 24 h from

the day of device insertion until removal and every 12 h from device removal to the time ovulation was detected to have occurred or until 144 h later, whichever occurred first. All follicles greater than 3 mm in diameter were measured using an internal electronic caliper, video recorded and registered on an individual ovarian map for each heifer. Two images of the largest follicle were measured at its maximum diameters (width and height), and the average was considered for analyses. Ovulation was considered to have occurred when there was disappearance of the largest follicle between two consecutives ultrasonic examinations and confirmed 12 h later.

### 2.4. Hormonal determinations

Blood samples were collected daily from the beginning of the treatment until 96 h after progesterone device removal from the jugular vein into 10-mL tubes. All samples were centrifuged 1 h after collection at 1000*g* for 20 min, and serum was stored at –20 °C until assayed in the *Laboratorio de Endocrinología y Metabolismo Animal, Facultad de Veterinaria*, Montevideo, Uruguay. Both serum progesterone and estradiol-17β concentrations were determined using a radioimmunoassay (RIA) in direct solid phase and liquid phase, respectively, following the recommendations of each commercially available specific kit (DIAsource ImmunoAssays S.A., Louvain-la-Neuve, Belgium). Serum progesterone concentrations were determined from device insertion until 24 h after device removal, and serum estradiol-17β concentrations were determined from device removal until the time of detection that ovulation had occurred. Circulating progesterone concentrations were determined in four assays; the sensitivity was 0.262 ng/mL, and the intra-assay coefficients of variation (CVs) for quality Control 1 (0.4 ng/mL) and quality Control 2 (3.0 ng/mL) were 10.2% and 7.4%, respectively. The inter-assay CVs for the same quality controls were 10.8% and 8.7%, respectively. Circulating concentrations of estradiol-17β were determined after using the ether extraction method utilizing a double antibody RIA. This extraction protocol is routinely used in this laboratory and has been previously reported (Meikle et al., 1997). Samples were analyzed using a duplicate method in one assay, and the sensitivity was 3.5 pg/mL and the intra-assay CVs for quality Control 1 (88 pg/mL) and quality Control 2 (288 pg/mL) were 8.0% and 3.6%, respectively.

### 2.5. Experiment 2

The aim of this experiment was to evaluate the effect of PGF2α administration at the beginning of progesterone and estradiol-based treatment on pregnancy rate in *Bos taurus* beef heifers. The study was conducted in 11 replicates on different farms located in Uruguay. A total of 3479, predominantly Hereford-Angus crossbred heifers, with a BCS of 4.5 ± 0.1 (scale 1–8) were used; 2690 of the heifers were 2-years of age (10 replicates), and 789 were 14-months of age (one replicate). In each replicate, the presence or absence of the CL was confirmed in each heifer using ultrasonography at the beginning of the treatment (Day 0). All herds enrolled in the study had similar management as that described in Experiment 1 in rangeland conditions with *ad libitum* access to water, salt and mineral mixture. The heifers were treated using the same progesterone- and estradiol-based treatment regimen described for Experiment 1. Prostaglandin F2α (500 μg of cloprostenol sodium, Zoetis) was administered in two half doses (250 μg each) at the time of insertion and removal of progesterone intravaginal device (two-PG group) or in a single dose at the time of device removal (one-PG group). Estradiol cypionate and eCG were administered on Day 7 at the time of device removal as described for Experiment 1. Heifers were randomly assigned to be inseminated in the morning or in the afternoon of Day 9 (*i.e.*, at 48 or at 54 h after device removal) in a 2 × 2 factorial design. Thus, the heifers were treated with PGF2α at the time of device insertion and removal with FTAI occurring at 48 (*n* = 852) or 54 (*n* = 896) h after device removal or there was treatment with a single dose of PGF2α at the time of device removal with FTAI occurring at 48 (*n* = 835) or 54 (*n* = 896) h after device removal. Fixed-time AI was performed by two technicians using frozen semen of one or two bulls per replicate, which were assigned equally to each experimental group. Pregnancy diagnosis was determined using ultrasonography 30–35 d after insemination.

### 2.6. Statistical analysis

Statistical analyses were performed using InfoStat software (Di Rienzo et al., 2018). Data were assessed for normality and homogeneity of variance using histograms, q-q plots, and formal statistical tests as part of the univariate procedure. Follicular development, serum progesterone and estradiol-17β concentrations were analyzed using generalized linear mixed models (GLMMs), including the treatments and time as fixed effects with the interactions, and the animal as a random effect. The type of variance-covariance structures was chosen depending on the magnitude of the Akaike information criterion (AIC) for models conducted when there was heterogeneous compound symmetry, unstructured, autoregressive, spatial power, and first-order ante-dependence. The model with the least AIC was selected. The ovulation rate (heifers ovulated/treated) was analyzed using the Fisher's exact test. In Experiment 2, the pregnancy rate was analyzed using GLMMs, including the effect of PGF2α treatments (two treatments), the insemination times (48 h and 54 h), the presence/absence of CL (*i.e.*, estrous cycling/anestrus), and the interactions as fixed effects. While the replicate, animal identification, technician for insemination, bull and semen batch were included as random effects. Because the effect of the splitting of PG treatment may be evident only in estrous cycling heifers, data were analyzed with inclusion of all females and including only those females bearing a CL. The 2-year-old and 14-month-old heifers were analyzed separately. Data are presented as mean ± SEM and the significance level was considered to be *P* = 0.05.
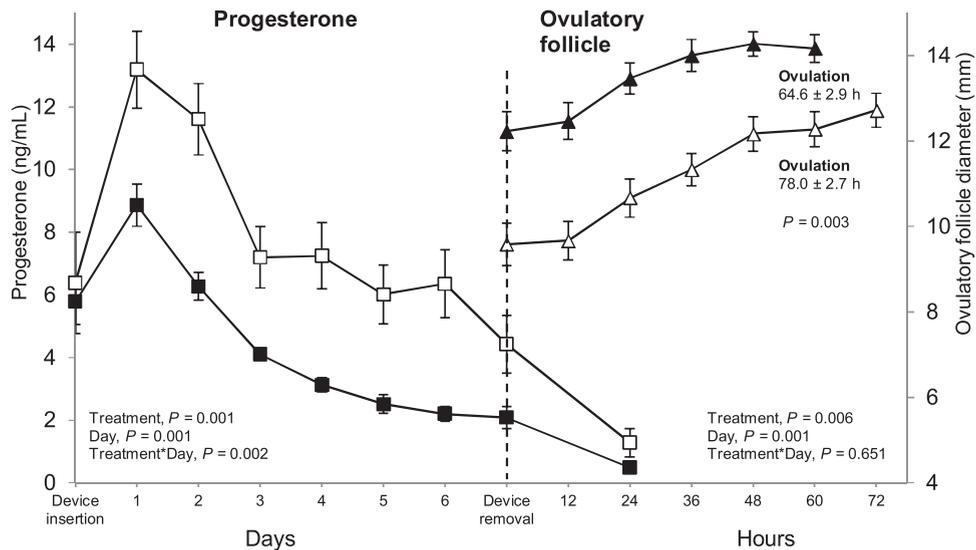
**Fig. 1.** Mean ( ± SEM) serum progesterone concentrations (left panel) and ovulatory follicle development (right panel) after prostaglandin (PG) F2α administration in two half doses at device insertion and removal (filled squares/triangles) or in a single dose at device removal (open squares/ triangles), with a progesterone and estradiol-based treatment regimen for FTAI being imposed in estrous cycling beef heifers (*Bos taurus*).

## 3. Results

### 3.1. Experiment 1

The treatment with PGF2α at the time of insertion of the progesterone intravaginal device resulted in lesser serum progesterone concentrations throughout the 7-day intravaginal treatment period than the concentrations of the control group (4.2 ± 0.3 compared with 8.0 ± 1.0 ng/mL; $P < 0.01$; Fig. 1). The two-PG treatment group had a larger preovulatory follicular diameter at the time of progesterone device removal (12.2 ± 0.5 mm) compared with females that were treated with a single dose of PG at the time of device removal (9.6 ± 0.5 mm; $P < 0.01$; Fig. 1). In addition, the heifers that were treated the two-PG administrations had a larger follicular diameter at the time of the last ultrasonic assessment before ovulation was detected to have occurred (14.4 ± 0.4 mm compared with 12.8 ± 0.4 mm; $P < 0.05$; Table 1). There were no differences in the mean of estradiol-17β concentrations from the time of device removal until the time ovulation was detected to have occurred between the experimental treatments ($P = 0.44$; Fig. 2). Heifers in the two-PG group had a lesser time interval from the time of device removal to the time of detection that ovulation had occurred than those heifers treated only at the time of device removal (Table 1). Ovulation rate did not differ for the two treatment groups, and the data for results are shown in Table 1, and distribution of ovulation is depicted in Fig. 2.

### 3.2. Experiment 2

Pregnancy rates of the 2-year-old and 14-month-old heifers are shown in Table 2. In 2-year-old heifers, overall pregnancy rate regardless estrous cycling or anestrus status, was similar for heifers of two-PG and one-PG treatment group (64.3%, 872/1,356; and 66.5%, 887/1,334, respectively; $P = 0.23$). In 14-month-old heifers, there was a statistical tendency between the two-PG and one-PG treatment group for pregnancy percentage (48.5%, 190/392; and 54.7%, 217/397, respectively; $P = 0.08$). The pregnancy rate was greater in the heifers that had a CL at the beginning of the treatment period than those heifers without a CL (67.1%, 989/1474 compared with 63.3%, 770/1216, respectively; $P < 0.05$), and there was no difference in 14-month-old heifers with and without a

**Table 1**
Effect of the administration of prostaglandin (PG) F2α in two half doses at device insertion and removal (two-PG treatment) or in a single dose at progesterone intravaginal device removal (one-PG group) with a progesterone- and estradiol-based treatment regimen being imposed for FTAI in beef heifers (mean ± SEM).

|  | Serum progesterone concentrations* (ng/mL) | Follicular diameter at device removal (mm) | Follicular diameter at ovulation (mm) | Follicular growth rate** (mm/d) | Ovulation rate (%) | Interval to ovulation** (h) |
| --- | --- | --- | --- | --- | --- | --- |
| One-PG treatment | 8.0 ± 1.0 | 9.6 ± 0.5 | 12.8 ± 0.4 | 0.9 ± 0.2 | 83.3% (10/12) | 78.0 ± 2.7 |
| Two-PG treatment | 4.2 ± 0.3 | 12.2 ± 0.5 | 14.4 ± 0.4 | 0.8 ± 0.1 | 100% (13/13) | 64.6 ± 2.9 |
| *P*-value | *0.001* | *0.001* | *0.015* | *0.498* | *0.425* | *0.003* |

\* Mean ( ± SEM) during 7-day intravaginal progesterone treatment.
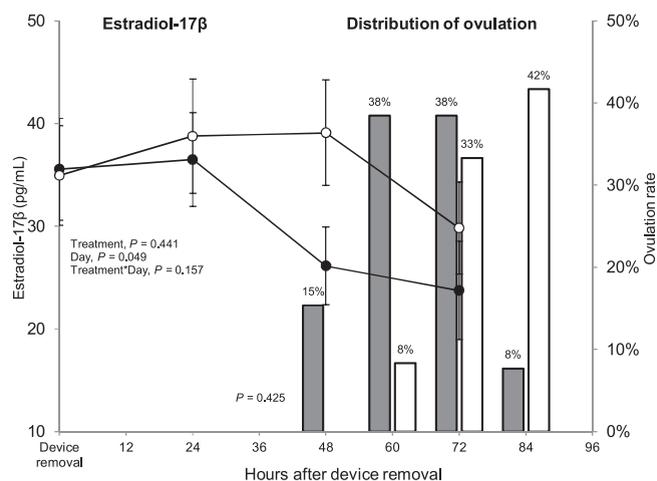\*\* From device removal to ovulation.

**Fig. 2.** Mean ( ± SEM) serum estradiol-17β concentrations (left panel) and ovulation rate (ovulated heifers/treated heifers; right panel) after prostaglandin (PG) F2α administration in two half doses at device insertion and removal (filled points/bars), or in a single dose at device removal (open points/bars), with a progesterone and estradiol-based treatment regimen for FTAI being imposed in estrous cycling beef heifers (*Bos taurus*).

CL at the time of device insertion (50.0%, 193/386 compared 53.1%, 214/403, respectively, $P = 0.38$). Regarding those heifers that had a CL present at the time of device insertion, pregnancy rate was not affected by the split PG treatment ($P = 0.33$). A tendency ($P = 0.07$) was found for the interaction between treatments and insemination times for 2-years-old estrous cycling heifers. Estrous cycling heifers that were FTAI at 54 h after device removal had a lesser P/AI percentage in the two-PG group than the one-PG group ($P < 0.05$; Table 2). For those heifers that did not have a CL at device insertion, there were no differences in pregnancy rate or insemination times between treatment groups ($P = 0.19$).

## 4. Discussion

The results of this study indicate that in estrous cycling *Bos taurus* beef heifers the ovarian responses during the progesterone and estradiol-based treatment are affected by PGF2α administration at the time of progesterone device insertion. Administration of PG at the time of device insertion resulted in lesser progesterone concentrations throughout the period of intravaginal device treatment, increased the diameter of the ovulatory follicle and an earlier time when ovulation occurred relative to the time of progesterone device removal. The pregnancy rate was not affected by the PG administration at the time of device insertion, and consequently there was a lesser fertility when FTAI was delayed to 54 h after device removal.

The positive effect of relatively lesser progesterone concentrations on the growth of the dominant follicle has been clearly documented (Dadarwal et al., 2013; Sirois and Fortune, 1990; Utt et al., 2003), an effect that is mediated by an increase in the frequency of LH pulses (Ginther et al., 2001, 2013a; Ireland and Roche, 1982). Relatively greater progesterone concentrations decrease the frequency of LH pulses (Bergfeld et al., 1996; Ginther and Beg, 2012) and, consequently, suppress follicular growth and results in smaller follicles (Adams et al., 2008; Stock and Fortune, 1993) from which there is sometimes a failure of ovulation to occur. If ovulation from small follicles occurs, there is a lesser fertility, probably due to: a) the development of a smaller CL and subsequently relatively lesser progesterone concentrations than when there is ovulation for relatively larger follicles (Crowe, 2008; Wiltbank et al., 2012b), b) less oocyte developmental competence (Abreu et al., 2018; Lequarre et al., 2005) or c) lesser preovulatory estradiol concentrations that negatively affect uterine programming (Binelli et al., 2017; Bó et al., 2016). The optimal progesterone concentrations for having an endocrine milieu and physiological conditions that results in maximal pregnancy in cattle with use of FTAI protocols has not been clearly established, and the current study contributes novel information in this regard. In the present study, circulating progesterone concentrations in heifers treated with PGF2α at the time of device insertion and removal were less throughout the device treatment period than in heifers treated with a single PG dose at device removal. This effect promoted preovulatory follicular growth with a greater diameter of the follicle from which ovulation occurred at device removal and at the time of ovulation. Thus, the results of the current study indicate that in estrous cycling *Bos taurus* heifers with the use of this strategy, there was an effective maintenance of optimal progesterone concentrations for promotion preovulatory follicular growth.

In general, the larger the dominant follicle at the end of treatment for FTAI, the lesser the interval to the onset of estrus and ovulation (Adams et al., 2008; Ginther et al., 2013b; Savio et al., 1993). Ovulatory follicle size and its physiological maturity are implicated in contributing to the establishment and maintenance of pregnancy (Lamb et al., 2001). In the present study, the interval from device removal to ovulation was less in heifers that received the split PG treatment. The convenience with use of this treatment regimen is controversial, because the benefit of increased ovulatory size is negated by the earlier time of ovulation as compared to when this split PG treatment regimen is not imposed, which is associated with lesser endogenous estradiol during the preovulatory period (Binelli et al., 2014, 2009) and uterine programming (de La Mata et al., 2018; Sponchiado et al., 2017). Because the interval from device removal to ovulation appears to affect fertility (Bridges et al., 2008), novel strategies to prolong proestrus length have

**Table 2**

Pregnancy rate obtained after prostaglandin (PG) F2α administered in two-half doses at device insertion and removal (two-PG treatment) or in a single dose at progesterone intravaginal device removal (one-PG treatment) with a progesterone and estradiol-based treatment regimen being imposed for FTAI; 2-year-old heifer data in the upper panel and 14-month-old heifer data are in the lower panel.

| | Two-PG treatment | | | One-PG treatment | | | P-value | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | FTAI 48 h | FTAI 54 h | Overall | FTAI 48 h | FTAI 54 h | Overall | PG treatment | Time of FTAI | PG x FTAI |
| 2-year heifers | | | | | | | | | |
| With CL (n = 1474) | 68.4% (249/364)[ab] | 63.5% (254/400)[a] | 65.8% (503/764) | 66.4% (233/351)[ab] | 70.5% (253/359)[b] | 68.5% (486/710) | 0.31 | 0.90 | 0.07 |
| Without CL (n = 1216) | 63.1% (183/290) | 61.6% (186/302) | 62.3% (369/592) | 65.3% (190/291) | 63.4% (211/333) | 64.3% (401/624) | 0.48 | 0.53 | 0.93 |
| 14-month heifers | | | | | | | | | |
| With CL (n = 386) | 44.9% (44/98) | 47.7% (42/88) | 46.2% (86/186) | 57.0% (57/100) | 50.0% (50/100) | 53.5% (107/200) | 0.16 | 0.68 | 0.33 |
| Without CL (n = 403) | 45.0% (45/100) | 55.7% (59/106) | 50.5% (104/206) | 54.8% (51/93) | 56.7% (59/104) | 55.8% (110/197) | 0.28 | 0.21 | 0.38 |

Simple effects are compared only when an interaction was a P-value < 0.1; For this case in the same row, a compared with b differs (P < 0.05).

been proposed and implemented with some success (Bridges et al., 2010; De La Mata et al., 2018). Thus, this paradoxical effect of the PG treatment at device insertion that occurred in Experiment 1, could have an unknown, if not negative, outcome on fertility.

In the present study, even with the differences in the circulating progesterone concentrations and ovulatory follicle diameter among PG treatment groups, there were similar results in pregnancy rates after FTAI. In the present study, the AI was performed at 48 or 54 h, and the ovulation occurred approximately 64 to 78 h after progesterone device removal (*i.e.*, on average, 10 to 30 h after insemination). In estrous cycling 2-year-old heifers that were treated with the two-PG dose regimen and when FTAI was performed at 54 h after device removal, the pregnancy rate was less than in heifers that were treated with PG only at the time of device removal and FTAI occurred at 48 h. It is hypothesized that the lesser pregnancy rate in heifers where there was FTAI at 54 h was related to a shorter interval from insemination to ovulation. In the two-PG treatment group, however, P/AI percentage was similar to when FTAI was performed at 48 h, and thus this result does not fully support this hypothesis. Based on this finding, it does not appear to be beneficial to treat with the half PG dose at the time of device insertion in *Bos taurus* heifers. Results from previous studies in *Bos indicus* and cross-bred heifers indicated females that had a CL at the beginning of treatment and in which there was relatively greater circulating progesterone concentrations induced by intravaginal device insertion as compared with females not treated with the device could have a lesser growth of dominant follicles, which would negatively affect ovulation and pregnancy rate (Cutaia et al., 2003; Sá Filho et al., 2015; Sales et al., 2015; Vasconcelos et al., 2013). Results of the present study in *Bos taurus* heifers are not consistent with these lines of thought in *Bos indicus* heifers and indicate there was a differentiated effect among these females. Significant differences have been reported in progesterone pharmacokinetics among *Bos indicus* and *Bos taurus* heifers (Carvalho et al., 2008, 2017). Additionally, when the same progesterone treatment are administered in both genotypes, circulating progesterone concentrations, which suppress follicular growth and lead to lesser pregnancy rates after AI, are greater in *Bos indicus* heifers (Carvalho et al., 2017; Sartori et al., 2016a, 2016b). The differences between the benefit of PG treatment in *Bos indicus* heifers reported previously and the neutral effect in *Bos taurus* heifers that was ascertained in the present study seems to be supported by the differences in progesterone pharmacokinetics reported previously.

The control of circulating progesterone concentrations by the administration of PG at device insertion may also be affected by the amount of progesterone contained in the intravaginal device. Findings in the present study are valid only for treatment with devices containing 0.5 g of progesterone, and in the present study, associated with an eCG treatment at the time of progesterone-device removal. This protocol for FTAI, consisting of an intravaginal device with 0.5 g of progesterone, EB, ECP and eCG, is the most popular treatment in South American countries (Bó et al., 2018), where more than 16 million cows and heifers are inseminated annually using FTAI protocols (Mapletoft et al., 2018). Using this protocol, the administration of a half dose of PG at the time of progesterone device insertion does not appear to be beneficial for enhancing P/AI percentage in *Bos taurus* heifers, and the complete dose is recommended in a single administration at the time of device removal. From a practical perspective, in addition to the easy implementation of the single PG treatment, the longer period (*i.e.*, from 48 to 56 h) for conducting inseminations may be maintained with use of the single PG treatment at the time of progesterone device removal. This is relevant for large-scale FTAI programs with 400 to 500 females per day (insemination am and pm), and in small herds, allows the time of insemination to be more convenient.

## 5. Conclusions

The administration of PG at the time of insertion of an intravaginal progesterone device (0.5 g) with FTAI results in relatively lesser progesterone concentrations throughout the treatment period as compared with when there is treatment with a single dose of PG at the time of device removal. Consequently, there is a larger size of the ovulatory follicle and shorter interval to ovulation in *Bos taurus* beef heifers treated using the split PG treatment regimen at the time of insertion and removal of the intravaginal progesterone device. Pregnancy rate is not different with use of a split dose of PG at the time of progesterone device insertion and removal as compared with when PG is only administered at the time of device removal, therefore, PG may be administered in a single dose at the time of device removal without detrimental effects on P/AI percentage.

## Declaration of Competing Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartially of the research reported.

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