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Effect of estradiol cypionate and amount of progesterone in the intravaginal device on synchronization of estrus, ovulation and on pregnancy rate in beef cows treated with FTAI based protocols

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ABSTRACT

Three experiments were conducted to evaluate the effect of estradiol cypionate (ECP) and amount of progesterone in the intravaginal device (PID) on synchronization of estrus and ovulation, follicular dynamics, luteal dynamics and function and on pregnancy rate in beef cows treated with fixed-time artificial insemination (FTAI) based protocols. In Experiment 1, we evaluated the synchronization of ovulation using 1 mg of ECP at PID removal (day 8 after PID insertion) or 1 mg of estradiol benzoate (EB) 24 h later, in cows treated with 0.558 or 1 g of progesterone (P4). The final subgroups were: 0.558 g + ECP: $n = 10$; 0.558 g + EB: $n = 11$; 1 g + ECP: $n = 10$; 1 g + EB: $n = 10$. Ovarian ultrasonic examinations were performed to detect the dominant follicle and ovulation. There was no effect of treatments on the diameter of dominant follicle at any time, and on the mean interval to estrus and to ovulation ($P > 0.05$); however, ECP treated cows had scattered distribution of estrus ($P < 0.03$) and ovulation ($P < 0.03$). In Experiment 2, cows received the following treatments: 0.558gP4 + ECP: $n = 52$; 0.558gP4 + EB: $n = 52$; 1gP4 + ECP: $n = 50$; 1gP4 + EB: $n = 52$; and FTAI. Pregnancy rate did not differ ($P > 0.05$) between progesterone content (0.558 g: 52.9%, 55/104; 1 g: 56.9%, 58/102) but differed between estradiol esters ($P < 0.05$; ECP: 48.9%, 49/102; EB: 61.5%, 64/104). In Experiment 3, cows received: 0.558gP4 + ECP: $n = 55$; 0.558gP4 + EB: $n = 53$; 1gP4 + ECP: $n = 54$; 1gP4 + EB: $n = 53$; and FTAI. Pregnancy rate did not differ ($P > 0.05$) between progesterone content (0.558 g: 48.1%, 52/108; 1 g: 53.3%, 57/107) and estradiol esters (ECP: 47.7%, 52/109; EB: 53.8%, 57/106). In conclusion, ECP administration at device removal and progesterone content of PID has no influence on the synchronization of estrus, follicular dynamics, luteal dynamics and function. However, ECP administration affected the synchronization of ovulation and pregnancy rate in non-suckling beef cows, but did not affected pregnancy rate in suckling beef cows. Future studies should evaluate the distribution of ovulations in suckling *Bos taurus* beef cows.

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1. Introduction

It is known that estradiol esters are capable of inducing ovulation in beef cows, most studies that used estradiol as an ovulatory stimulus employed estradiol benzoate (EB) 24 h after progesterone intravaginal device (PID) removal (Macmillan and Burke, 1996; Lane et al., 2001; Diskin et al., 2002). However, their plasma kinetics differ (Vynckier et al., 1990).

When short-acting estradiol formulation (5 mg E-17β) was used to synchronize follicular wave emergence, the timing of estradiol cypionate (ECP) treatment (0 or 24 h following PID removal) did not significantly affect the interval to the LH surge, ovulation time, or pregnancy rate to fixed-time artificial insemination (FTAI) in beef heifers (Colazo et al., 2003).

Additionally, similar pregnancy rates between ECP and EB administration have been obtained in *Bos indicus* cows (Meneghetti et al., 2009; Sales et al., 2012). However, ECP administration at device removal produced precocious ovulations (Colazo et al., 2003) and a wider hourly range of ovulation in *Bos taurus* heifers (Callejas et al., 2011). There is no information on *B. taurus* beef cows. Therefore, ECP is an interesting alternative to replace EB in FTAI protocols in order to reduce animal handling. Considering its convenient time of administration that facilitates animal management, it is relevant to further study the efficiency of ECP in inducing synchronized estrus and ovulation, and adequate pregnancy rates in *B. taurus* beef cows.

On the other hand, it is known that progesterone (P4) levels were correlated with the follicular dynamics (Callejas et al., 2006); accordingly, different amounts of P4 in the PID could generate different ovarian situations at the end of the treatment, which generate different ovulatory responses to the estradiol treatment, and could influence the distribution of ovulation. Additionally, it is important to study the characteristics of the corpus luteum (CL) generated from the induced ovulation to evaluate the effect of treatments on CL characteristics.

Estradiol and progesterone-based synchronization protocols have been successfully used to control follicular and luteal dynamics and to synchronize ovulation, enabling AI without estrous detection (Bó et al., 2003; Macmillan et al., 2003; Baruselli et al., 2004; Sá Filho et al., 2009). Currently, these protocols are the main commercial treatment in South America to synchronize follicular wave emergence and ovulation for FTAI in beef cows (Bó et al., 2007; Sá Filho et al., 2009). The standard treatment consists of: Day 0, EB + P4 device insertion; Day 8, PGF + P4 device removal; Day 9, EB; and Day 10, FTAI at 52–56 h (Macmillan and Burke, 1996; Bó et al., 2003).

Thus, based on the distinct pharmacokinetic profiles of estradiol esters and aiming to reduce FTAI-related cow handling, three experiments were conducted to evaluate the effect of ECP and amount of P4 in the PID on synchronization of estrus and ovulation, follicular dynamics, luteal dynamics and function (Experiment 1), and on pregnancy rate in non-suckled (Experiments 2) and suckled (Experiments 3) beef cows submitted to FTAI based protocols.

2. Materials and methods

2.1. Location of study

The experiments were carried out on commercial farms located in southeast Argentina (37°S, 60°W). The experimental designs and animal care were performed in compliance with regulations of the Animal Welfare Committee of the Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Buenos Aires, Argentina.

2.2. Experiment 1

2.2.1. Animals and treatments

Forty one multiparous non-suckling cycling *B. taurus* beef cows, grazing on a ryegrass pasture (*Lolium perenne*), weighing 487.4 ± 43.0 kg (mean ± SD); with a body condition score (BCS) of 3.4 ± 0.5 (mean ± SD) as assessed on a scale from 1 to 5 (Houghton et al., 1990) and more than 60 days of post-partum at the start of the experiment were randomly allocated to each of four treatments. The day 0 of the study was designated as the day when PID (Cronipres®, Biogénesis-Bagó, Argentina) was inserted and 2 mg of EB (Bioestrogen, Biogénesis-Bagó, Argentina) administered intramuscularly (i.m.). At the moment of PID removal (Day 8), cows received 0.150 mg of D-Cloprostenol (Enzaprost D-C, Biogénesis-Bagó, Argentina), i.m.

The experimental design was a 2 × 2 factorial arrangement with the amount of progesterone in the intravaginal device and estradiol salt as the main effects. The amount of progesterone was 0.558 (n = 21) or 1 g (n = 20). Half of the animals on each group were treated with 1 mg of ECP, i.m. (Estradiol Cypionate, König, Argentina; n = 20) at the time of PID removal, whereas the other half were treated with 1 mg EB 24 h later (n = 21; utilized as a positive control). The final subgroups after randomized were: 0.558 g + ECP: n = 10; 0.558 g + EB: n = 11; 1 g + ECP: n = 10; 1 g + EB: n = 10. Animals were not inseminated, because they were only to evaluate the effect of the amount of P4 and estradiol esters on ovarian response.

Estrus detection was performed twice daily from PID removal to ovulation. Onset of estrus was determined as the first of three mounts within a 4-h period that lasted 2 s or longer in duration (Fields et al., 2012). To help heat detection, tail paint was applied at the time of device removal, to estimate the amount of tail paint rubbed off by mounts. Cows with >30% rubbed off were considered to be in estrus (Stahringer et al., 2011).

2.2.2. Blood collection and radioimmunoassay (RIA)

To characterize plasma progesterone concentrations, blood samples were collected via jugular venipuncture from day 7 to 12, every 24 h, and on day 22. Blood samples were collected into heparinized tubes and centrifuged (2000 × g for 15 min) within 1 h after collection. Plasma was removed and stored at −20 °C until assayed.

Plasma samples were assayed at the Facultad de Ciencias Veterinarias, U.N.C.P.B.A., Tandil, Argentina. Samples were analyzed using a progesterone RIA kit (COAT A COUNT, Siemens Healthcare Diagnostic Inc., CA, USA) previously validated for use with bovine plasma (Toribio et al.,

1994). The intra-assay coefficient of variation was less than 7% for concentrations between 0.1 and 40 ng/mL, the inter-assay coefficient of variation was less than 3.5% and assay sensitivity was 0.01 ng/mL.

The last sample (day 22) was collected to assess the functionality of the corpus luteum (CL) (Carvalho et al., 2008). A cow was considered to have a functional CL if its plasma P4 concentration was >1 ng/ml (Chebel et al., 2006; Bicalho et al., 2008).

2.2.3. Ultrasonography examinations

Ovaries of all cows were examined once daily on day 0, 7 and 8 to determine the ovarian status (OS), using an ultrasound scanner fitted with a 5 MHz transducer (Chison D600VET, 2007, China). OS on day 0 were: presence of corpus luteum (CL), Follicle ≥ 10 mm (without CL) or Follicle < 10 mm (without CL, without $F \geq 10$ mm). After PID removal, ultrasonographic examinations were carried out every 12 h until ovulation or 108 h. Follicular diameters of 5 mm or greater were recorded. During each examination, a sketch of the ovaries was made, recording the location and the diameter of each follicle.

The dominant follicle (DF) of a wave was defined as that one that reached the largest diameter (Ginther et al., 1989). In the present study, the dominant follicle detected on day 7 belonged to the new follicular wave induced by the progesterone plus estradiol treatment. The time of ovulation was defined as the average time between the last observation of the dominant follicle and the time at which the dominant follicle disappeared (ovulation), which also determined the ovulatory follicle diameter (Manes et al., 2012).

Follicular growth rate (mm/day) was calculated from the maximum diameter reached by the follicle minus the diameter of the dominant follicle at device removal, divided by the interval of days (adapted from Henao et al., 2000; Henao Restrepo and González Cadavid, 2008). On day 22 (approximately 12 days after ovulation), another ultrasound was performed to confirm ovulation (Carvalho et al., 2008).

2.3. Experiment 2

2.3.1. Animals and treatments

The experiment was performed using 207 multiparous, non-suckling cyclic *B. taurus* beef cows with a body condition score of 3.2 ± 0.4 (mean ± SD), and more than 60 days of post-partum, grazing on a ryegrass pasture (*L. perenne*). Hormonal treatments were the same as those described in Experiment 1.

The experimental design was a 2 × 2 factorial arrangement with the amount of progesterone in the intravaginal device and estradiol salt as the main effects. The amount of progesterone was 0.558 ($n = 104$) or 1 g ($n = 103$). Half of the animals on each group were treated with 1 mg of ECP, i.m. (Estradiol Cypionate, König, Argentina; $n = 103$) at the time of PID removal, whereas the other half were treated with 1 mg EB 24 h later ($n = 104$). The final subgroups after randomization were: 0.558 g + ECP: $n = 52$; 0.558 g + EB: $n = 52$; 1 g + ECP: $n = 51$; 1 g + EB: $n = 52$. FTAI was performed at 52–54 h after PID removal using frozen-thawed semen from one bull of proven fertility.

2.3.2. Ultrasonographic examinations

Ovarian structure was determined on day 0 as previously described in Experiment 1. Another two ultrasounds were performed on days 30 and 60 post FTAI to determine pregnancy rate and pregnancy loss rate.

2.4. Experiment 3

2.4.1. Animals and treatments

The experiment was performed using 215 multiparous, suckling *B. taurus* beef cows with a body condition score of 3.4 ± 0.5 (mean ± SD), and more than 45 days of post-partum, grazing on a ryegrass pasture (*L. perenne*). Thirty one percent of cows had a CL, indicating that approximately half of the animals were cycling. Hormonal treatments were the same as those described in Experiment 1 (0.558gP4: $n = 108$; 1gP4: $n = 107$; ECP: $n = 109$; EB: $n = 106$). The final subgroups after randomization were: 0.558 g + ECP: $n = 55$; 0.558 g + EB: $n = 53$; 1 g + ECP: $n = 54$; 1 g + EB: $n = 53$. FTAI was performed at 52–54 h after PID removal using frozen-thawed semen from one bull of proven fertility.

2.4.2. Ultrasonographic examinations

Ovarian structure was determined on day 0 as previously described in Experiment 1. Another two ultrasounds were performed on days 30 and 60 post FTAI to determine pregnancy rate and pregnancy loss rate.

2.5. Statistical analysis

In Experiment 1 the following variables were subjected to Kolmogorov–Smirnov test for normality of the residues and analyzed by analysis of variance (ANOVA) in SAS (PROC GLM; SAS, 1998) and subjected to Bartlett's test (transformed when necessary) to assess homogeneity of variances: diameter of the dominant follicle at the time of device removal and 24 h later, diameter of the ovulatory follicle, follicular growth rate, interval between device removal and estrus, interval between device removal and ovulation. The model included the main effects of device amount of progesterone, estradiol ester and their interaction. The diameter of the dominant follicle at the time of PID removal was analyzed with a model that included only the device amount of progesterone. Distribution of estrus and ovulations was analyzed by Proc FREQ of SAS (SAS, 1998). Plasma concentrations of progesterone from day 8 to 12, and on day 22 (Experiment 1), were analyzed by ANOVA for repeated measures with SAS (PROC MIXED; Littell et al., 1998). The statistical model included the effects of the treatment, day and their interaction. All values are expressed as mean ± SEM.

One cow was excluded of the statistical analysis because suffered a rectal injury (of the 0.558 g + ECP subgroup). Therefore, the final subgroups were: 0.558 g + ECP: $n = 9$; 0.558 g + EB: $n = 11$; 1 g + ECP: $n = 10$; 1 g + EB: $n = 10$.

In Experiment 2 and 3, pregnancy rate and pregnancy loss rates between days 30 and 60 were analyzed by Proc

Table 1
Diameter (mean ± SEM) of the dominant follicle (DF) at device removal and 24 h later and diameter of the ovulatory follicle and follicular growth rate in non-suckling *B. taurus* beef cows treated with progesterone intravaginal devices and estradiol esters for synchronization of ovulation in Experiment 1.

	Principal effects			
	Progesterone amount		Estradiol esters	
Diameters	0.5 g	1 g	EB	ECP
DF at device removal (mm)	13.15 ± 0.42 (10–17)	12.45 ± 0.54 (8–17)	13.19 ± 0.40 (11–17)	12.37 ± 0.56 (8–17)
DF at EB administration (mm)	14.30 ± 0.42 (10–17)	13.65 ± 0.59 (9–19)	14.48 ± 0.38 (12–19)	13.42 ± 0.61 (9–18)
Ovulatory follicle (mm)	14.40 ± 0.52 (11–20)	13.85 ± 0.46 (11–18)	14.52 ± 0.51 (11–20)	13.68 ± 0.46 (11–18)
Growth rate (mm/day)	1.30 ± 0.24	1.50 ± 0.21	1.58 ± 0.23	1.20 ± 0.21

CATMOD of SAS (SAS, 1998) to determine the effect of device amount of progesterone, estradiol salt and their interaction. Results are expressed as means along with the standard error of the mean. The level of significance was set at 0.05. In Experiment 2, one cow (treated with 1 g of P4 and ECP) was not inseminated because suffered a rectal injury. Therefore, the final subgroups were: 0.558 g + EB: $n = 52$; 0.558 g + ECP: $n = 52$; 1 g + EB: $n = 52$; 1 g + ECP: $n = 50$.

3. Results

3.1. Experiment 1

There was no effect of device amount of progesterone ($P > 0.05$) and device amount of progesterone by day interaction ($P > 0.05$) on plasma progesterone concentrations, but there was an effect of day ($P < 0.01$). Progesterone concentrations on days 7 and 8 were higher than the following days.

The diameter of the DF at device removal and 24 h later, the diameter of the ovulatory follicle and the growth rate of the DF did not differ between treatments ($P > 0.05$; Table 1).

Overall 77.5% (31/40) of the treated cows showed estrus within 24–72 h after PID removal, irrespective of the treatments. Out of those cows showed estrus, 84% (26/31) synchronized into estrus between 36 and 48 h after device removal. The relative distribution of ECP and EB treated cows showing estrus at different interval after PID removal is presented in Fig. 1. Expression of estrus for each subgroup were: 0.558gP4 + ECP: (6/9) 66.7%; 1gP4 + ECP: (7/10) 70.0%; 0.558gP4 + EB: (9/11) 81.8%; 1gP4 + EB: (9/10) 90.0%.

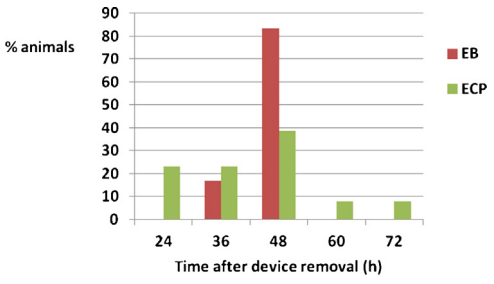

Fig. 1. Distribution pattern of estrus occurrence in non-suckling beef cows treated with progesterone intravaginal devices and estradiol esters.

A hundred percent (40/40) of treated cows ovulated in response to the hormonal treatment. The mean interval from device removal to ovulation did not differ between treatments ($P > 0.05$; Table 2). However, 90.5% (19/21) of EB treated cows ovulated between 54 and 66 h after device removal (12 h interval), while 89.5% (17/19) of ECP treated cows ovulated between 42 and 78 h after device removal (36 h interval) ($P < 0.03$; Fig. 2). There was no effect of treatments on corpus luteum area (4.14 ± 0.28 mm^2; range: 1.19–11.76 mm^2) and plasma progesterone concentration (5.99 ± 0.32 ng/mL; range: 2.79–10.00 ng/mL) on day 22 ($P > 0.05$).

3.2. Experiment 2

There was no effect ($P > 0.05$) of device amount of P4 on pregnancy rate after FTAI (0.558 g: 52.9%, 55/104; 1 g: 56.9%, 58/102), but there was an effect ($P < 0.05$) of estradiol esters (ECP: 48.9%, 49/102; EB: 61.5%, 64/104). Pregnancy rates for each subgroup were: 0.558gP4 + ECP: (23/52) 44.2%; 1gP4 + ECP: (26/50) 52.0%; 0.558gP4 + EB: (32/52) 61.5%; 1gP4 + EB: (32/52) 61.5%. All treated cows showed no incidence of pregnancy loss between days 30 and 60 after insemination.

3.3. Experiment 3

Pregnancy rates after FTAI did not differ ($P > 0.05$) between progesterone (0.558 g: 48.1%, 52/108; 1 g: 53.3%, 57/107) and estradiol esters (ECP: 47.7%, 52/109; EB: 53.8%, 57/106) groups in this study. Pregnancy rates for each subgroup were: 0.558gP4 + ECP: (24/55) 43.6%; 1gP4 + ECP: (28/54) 51.9%; 0.558gP4 + EB: (28/53) 52.8%; 1gP4 + EB:

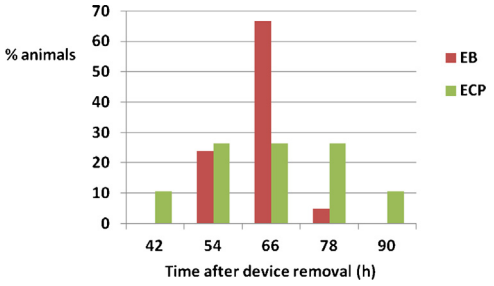

Fig. 2. Distribution pattern of ovulation occurrence in non-suckling beef cows treated with progesterone intravaginal devices and estradiol esters.

Table 2
Intervals (mean ± SEM) from device removal to estrus and to ovulation in non-suckling *B. taurus* beef cows treated with progesterone intravaginal devices and estradiol esters for synchronization of ovulation in Experiment 1.

Intervals (h)	Estradiol esters		*P* value
	EB	ECP	Mean
Removal-estrus	46.00 ± 1.08 (n = 18)	42.46 ± 3.99 (n = 13)	>0.05
Estrus range	36–48	24–72	
Removal-ovulation	65.43 ± 2.26 (n = 21)	66.0 ± 3.31 (n = 19)	>0.05
Ovulation range	54–78	42–90	

(29/53) 54.7%. There was an effect of the ovarian status at the beginning of the treatment on pregnancy rate; hence, cows that had a follicle ≥10 mm and received a PID with 1 g of P4 showed a higher pregnancy rate (68.0%; 34/50; $P < 0.05$) than cows that had a corpus luteum and received 1 g of P4 (36.4%; 12/33). All treated cows showed no incidence of pregnancy loss between days 30 and 60 after insemination.

4. Discussion

The present study has special value in terms of application of FTAI protocols in *B. taurus* beef cows, particularly for pregnancy rate. Despite differences between the ovulation ranges of the two estradiol esters, diameter of the dominant follicle at the time of PID removal and 24 h later, diameter of the ovulatory follicle and follicular growth rate were similar in Experiment 1. The pregnancy rate differed between estradiol esters in non-suckling beef cows (Experiment 2), but it was similar in suckling beef cows (Experiment 3) submitted to a FTAI protocol.

In Experiment 1, plasma progesterone concentration was higher on days 7 and 8 than in the following days, similar to that observed by Carvalho et al. (2008). Thus, animals that received different amounts of progesterone showed similar plasma P4 concentration at PID removal; therefore, there was no effect of P4 amount on the diameter of the DF at PID removal and at ovulation, similarly to that observed by Meneghetti et al. (2009) in *B. indicus* cows. Also, there was no effect of P4 amount or estradiol esters on growth rate of the DF, which was consistent with literature values (Bó et al., 2003; Martinez et al., 2005). These results show that PID with 0.558 or 1 g of P4 did not alter follicular development in the present study.

In general, cows that display estrus have higher chances to ovulate, and cows that do not display estrus have lesser chances (Perry et al., 2007; Hillegas et al., 2008). In Experiment 1, the percentage of cows observed in estrus was lower compared to those by Martinez et al. (2000) and Garcia and De Jarnette (2003). However, we obtained a 100% ovulation rate, in agreement with Martinez et al. (2005) and Sales et al. (2012); therefore, both estradiol esters had similar efficacy in inducing ovulation in beef cows, regardless of the estrus expression.

In disagreement with other reports in *B. indicus* cattle (Peralta-Torres et al., 2010; Sales et al., 2012), in the present study, we observed statistical differences in the distribution of estrus and ovulation between estradiol esters in Experiment 1. It could be explained because of the different pharmacokinetic profiles of the estradiol esters (Vynckier et al., 1990) or by the presence of precocious ovulations in ECP treated cows (Colazo et al., 2003). These results are in agreement with the observations of Callejas et al. (2011) in dairy heifers and have special interest in FTAI programs where the objective was to synchronize ovulations in order to inseminate all cows in an established time.

To the further of our knowledge, this is the first report that shows lower pregnancy rate in non-suckling ECP treated cows in comparison to EB treated cows submitted to FTAI. It could be explained because of the different distribution of ovulation caused by the ECP administration as it has been indicated previously. Based on data from Experiment 1, EB treated cows ovulated between 54 and 78 h after device removal and had higher chances of being fertilized considering that FTAI was performed 52–54 h after PID removal. But ECP treated cows ovulated between 42 and 90 h after device removal and cows that ovulated at 42 h (11%; 2/19) probably had an aged oocyte at the time of semen arrival (Dalton and Saacke, 2007, cited by Ayres et al., 2008) and, according to Roelofs et al. (2006), fertilization rate drastically decreases when AI occurs after ovulation. It has also been estimated that the maximal viability of sperm in the female reproductive tract is 24–30 h (Hiers et al., 2003). Hence, it is probable that an asynchrony between ECP treated cows that ovulated at 90 h (11%; 2/19) and arrival of viable sperm capable of fertilization resulted in decreased pregnancy rates in ECP treated cows in Experiment 2.

On the other hand, pregnancy rate was not influenced by estradiol esters in suckling beef cows (Experiment 3). Similar results were observed by Meneghetti et al. (2009) and Sales et al. (2012), who reported no pregnancy rate differences between cows treated with ECP at P4 device removal or EB 24 h after. Perhaps, there was no difference in the distribution of ovulation between estradiol esters in suckling cows as indicated by Sales et al. (2012) and it could be an explanation for that.

It appears that the synchronization of ovulation and differences in fertility between different AI sires are just some of the factors contributing to unacceptable FTAI pregnancy rates (Hiers et al., 2003). Additional research must be conducted to further study the effect of sires on pregnancy rates and develop a test that can identify AI sires that could be used specifically in FTAI protocols and yield acceptable and consistent pregnancy rates.

In suckling beef cows (Experiment 3), there was an effect of ovarian status at the beginning of the treatment on pregnancy rate in cows treated with the higher amount of progesterone (1 g of P4); hence, cows that presented follicles ≥10 mm in diameter showed higher pregnancy rates than cows that had a CL, which may have been due to lower frequency of LH pulses in cycling cows, because there are two sources of progesterone (IVP device and CL) and, consequently, lower rates of dominant follicle growth and ovulation (Dias et al., 2009; Meneghetti et al., 2009). It is possible that an optimal range of circulating progesterone concentration during the protocol exists in cows without CL that have less circulating concentrations of progesterone and it could reduce these negative impacts on reproduction (Dias et al., 2009).

In conclusion, ECP administration at device removal and progesterone content of PID has no influence on the synchronization of estrus, follicular dynamics, luteal dynamics and function. However, ECP administration affected the synchronization of ovulation and pregnancy rate in non-suckling beef cows, but did not affect pregnancy rate in suckling beef cows. Future studies should evaluate the distribution of ovulations in suckling *B. taurus* beef cows.

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