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Use of prostaglandin F2α as ovulatory stimulus for synchronizing dairy cattle

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ABSTRACT

The aim of this study was to evaluate if prostaglandin F2α (PGF) can be used to induce ovulation in a GnRH-progesterone based protocol. In Experiment 1 crossbred dairy cows (n = 32) were synchronized with a progesterone-GnRH based protocol for seven days, where the luteolytic dose of 150 μg PGF was given 24 h prior progesterone device removal (CIDR). On Day 8 cows were separated into two groups to receive: 1) 2 mL of Saline (Control Group, n = 15) or 2) 150 μg of PGF (PGF Group, n = 17). Ovulation rate was higher in the PGF than Control group (100% vs 53.3%, P = 0.001, Odds ratio = 30.88). The percentage of cows that ovulated synchronously tended to be higher in the PGF than Control group (P = 0.1, Odds ratio = 9.6). Experiment 2 was performed in a cross-over (3 × 3) design. Crossbred dairy cows (n = 25) received a CIDR for seven days and GnRH on Day 0. Seven days later 150 μg of PGF was given and the progesterone device was removed, and 24 h later cows were distributed into three groups to receive: 1) 2 mL of Saline (Control Group, n = 25), 2) 150 μg of PGF (PGF Group, n = 25) or 3) 1 mg of ECP (ECP Group, n = 23). Diameter of ovulatory follicle was larger in the PGF and Control than ECP Group (P = 0.002, Effect size > 4.0). Synchronized ovulation rate (between 72 and 96 h after CIDR removal) tended to be higher in PGF group in Control group (P = 0.1, Odds ratio = 0.35). Results suggest that PGF is equally efficient to ECP to induce synchronized ovulation in dairy cows subjected to progesterone-GnRH based protocols.

Estrous cycle synchronization protocols have been widely used in cattle since they allow the widespread use of fixed time artificial insemination (FTAI). Over the last few decades several protocols have been developed (Yilmazbas-Mecitoglu et al., 2014). The substitution of GnRH for estradiol cypionate (ECP) to induce ovulation was an important modification for reducing the costs of the Ovsynch protocol (Stevenson et al., 2004). However, the use of estradiol esters has been restricted in several countries. Therefore, the development of efficient alternative low-cost ovulation inducers is needed, and represents a current challenge.

Prostaglandin F2α (PGF) has been shown to induce ovulation by a mechanism independent of luteolysis (Leonardi et al., 2012). Although this mechanism is not yet fully understood, it was suggested that PGF increases the ability of the pituitary to respond to GnRH in postpartum cows (Randel et al., 1996). Additionally, there is an increase in the expression of PGF and PGE2 receptors in theca and granulosa cells of preovulatory follicles, suggesting a local effect can also take place (Bridges and Fortune, 2007). Furthermore, PGF has a similar effect to ECP and estradiol benzoate (EB) for inducing ovulation in cows subjected to estradiol-progesterone based FTAI protocols in beef (Pfeifer et al., 2014) and dairy cows (Pfeifer et al., 2016). However, the effect of PGF on estradiol-free protocols is not yet well defined. Therefore, the aims of this study were to: 1) evaluate whether a PGF analogue is able to induce ovulation in a synchronized manner; and 2) to compare a PGF analogue with ECP as ovulation inducer in GnRH-progesterone based TAI protocols.

The Committee for Ethics in Animal Experimentation from the Brazilian Agricultural Research Corporation (Embrapa – Rondônia) approved all procedures performed in this experiment (Number F.02/2014).

In the Experiment 1, 32 crossbred dairy cows (Gyr × Holstein) were used. On Day 0, all females were given 100 μg of lecirelin (GnRH-analogue, Gestran plus®, Tecnopec, São Paulo, Brazil) i.m., plus an intravaginal progesterone-releasing device (CIDR®, 1.9 g progesterone, Pfizer Animal Health, São Paulo, Brazil). On Day 6, 150 μg of D-Cloprostenol (PGF-analogue, Croniben®, Biogénesis-Bagó, Curitiba, Brazil) i.m. was given and 24 h later the CIDRs were removed. On Day 8, cows were randomly assigned into one of the two treatments: 1) 2 mL

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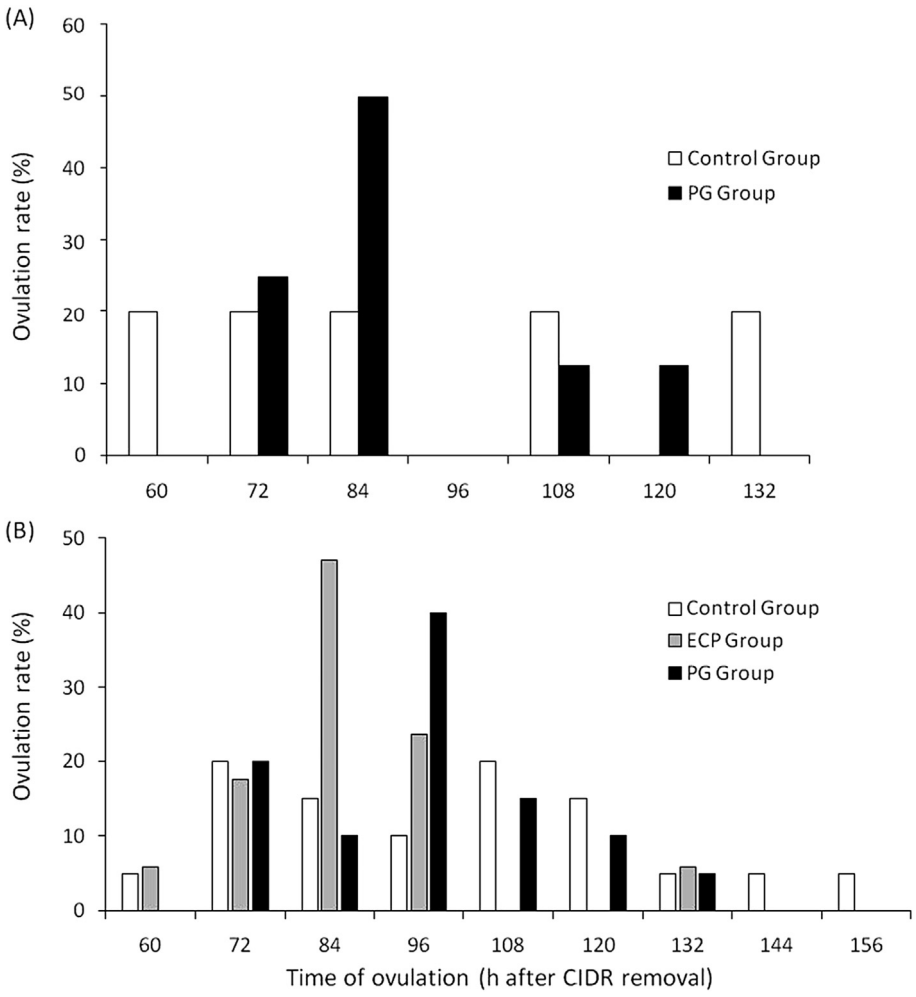

Fig. 1. Distribution and percentage of cows ovulating after CIDR removal in the (A) Control and PGF groups of Experiment 1; and (B) Control, PGF and ECP groups of Experiment 2; PGF: prostaglandin F2α; ECP: estradiol cypionate.

Table 1
Ovarian responses in cows synchronized with a progesterone estradiol based protocol and treated with Saline (Control Group), D-Cloprostenol (PGF Group) or estradiol cypionate (ECP Group) as ovulatory inducer in Experiment 2.

Ovarian responses	Treatment			P value			Odds ratio/effect size[a] (CI)		
	Control	PGF	ECP	Treat	Category[b]	Categ * Treat	PGF vs Control	ECP vs Control	PGF vs ECP
Ovulation rate	80% (20/25)	80% (20/25)	74% (17/23)	0.23	0.90	0.21	1.0 (0.5–2.0)	0.71 (0.44–1.60)	0.71 (0.44–1.60)
Synchronized ovulation rate[c]	45% (9/20)^{A}	70% (14/20)^{AB}	88% (15/17)^{B}	0.02	0.93	0.72	0.35 (0.32–1.12)	0.10 (0.25–0.78)	0.31 (0.37–1.11)
Time (h) from CIDR removal to ovulation (range)[d]	102 (60–156 h)	96 (72–132 h)	84 (60–132 h)	0.10	0.54	0.89	1.07 (−10.71–20.31)	3.07 (−1.49–30.86)	2.06 (−6.29–26.06)
Pre-ovulatory follicle diameter (mm)[e]	14.3 ± 0.4^{A}	13.9 ± 0.4^{A}	12.3 ± 0.4^{B}	0.002	0.67	0.51	1.0 (−0.98–1.54)	5.0 (0.56–3.19)	4.0 (0.29–2.92)

^{A,B}Values without a common superscript differed between groups.
[a] Odds ratio and Effect size were calculated for categorical and continuous data, respectively.
[b] Pubertal heifer or lactating cow.
[c] Synchronized ovulation rate was considered females that ovulated in a 24 h window, between 72 and 96 h post CIDR removal.
[d] Data are presented as Median.
[e] Data are presented as Mean ± Standard Error.

of Saline (Control Group, 0.9% NaCl, n = 15) or 2) 150 μg D-Cloprostenol (PGF Group, n = 17). Cows were examined by transrectal ultrasonography every 24 h from day 0 to day 7 of the protocol to monitor follicular wave development, and every 12 h from Day 7 to ovulation or up to five days after the removal of the CIDR, in the absence of ovulation.

In the Experiment 2, 25 crossbred dairy cattle (Gyr × Holstein) were used (12 pubertal heifers and 13 lactating cows). A cross-over (3 × 3) design was used, in which all cows received all three treatments, with a month between the end of a protocol and the beginning of the next one. On Day 0, all females were given 100 μg of lecirelin i.m., plus a CIDR. On Day 7, CIDRs were removed and 150 μg of D-

Cloprostenol i.m. was given. On Day 8, females were randomly assigned to receive: 1) 2 mL of Saline (0.9% NaCl; Control Group, n = 25) i.m., 2) 150 μg D-Cloprostenol i.m. (PGF Group, n = 25) or 3) 600 μg (heifers) or 1 mg (cows) of ECP (E.C.P.®, Pfizer Animal Health, São Paulo, Brazil) i.m. (ECP Group, n = 23). The cows were examined by transrectal ultrasonography on the day of insertion (Day 0) and removal (Day 7) of the CIDR. After removal of the CIDR, examinations were performed every 12 h until ovulation or, in the absence of ovulation, up to five days after CIDR removal. In both experiments, ovulation was defined as the disappearance, from one examination to the next, of a previously identified follicle ≥ 8 mm in diameter (Martinez et al., 2005).

All statistical analysis was performed on SAS 9.0 software (SAS Institute Inc., Cary, NC, USA, 1998). Single-point outcome variables were analyzed by one-way ANOVA and Tukey's post-hoc test. Two-way ANOVA was used to evaluate the effect of animal category (heifer or lactating cow), treatment and its interaction. Ovulation rate and synchronized ovulation rate was analyzed using Fisher's exact test and the confidence interval (CI) was calculated at a 95% level for all variables analyzed. For the categorical variables (ovulation and synchronized rates) and CI was also calculated. The effect size was estimated by Cohen's d for continuous variables and is represented by the odds ratio for categorical variables. The synchronized ovulation rate was calculated by the proportion of females ovulating between 72 and 96 h after CIDR removal (24 h window). Differences among groups were considered statistically significant when P value ≤ 0.05. Tendency was considered when P value was > 0.05 and ≤ 0.1.

In the Experiment 1, the median of time from CIDR removal to ovulation (78 h vs 84 h, P = 0.90, Effect Size = 0.18, CI = −16.94–19.11) and ovulatory follicle diameter (14.2 ± 0.6 vs 14.3 ± 0.7, P = 0.90, Effect Size = 0.15, CI = −1.61–0.80) did not differ between the PGF and Control groups, respectively. However, the ovulation rate (100%, 17/17 vs 53.3%, 8/15, P = 0.001, Odds ratio = 30.88, CI = 1.57–607.0) and synchronized ovulation rate (70.6%, 12/17 vs 20%, 3/15, P = 0.006, Odds ratio = 9.6, CI = 1.25–5.92) were higher in the PGF than in the Control group. The distribution of ovulations after CIDR removal is shown in Fig. 1A.

In the last replica of the Experiment 2, two females (one cow and one heifer) from the ECP Group were excluded from the remaining experiment due to the health issues (vulvar lesion). The results of the ovarian responses according to the treatments of the Experiment 2 are described in Table 1. Diameter of the ovulatory follicle was larger in the PGF and Control than ECP Group (P = 0.002). No difference in the synchronized ovulation rate was detected between PGF and ECP Groups (P = 0.2). However, ECP Group had more synchronized ovulations (higher ovulation rate between 72 and 96 h) than the Control group (P = 0.02). Similarly, cows treated with PGF tended to ovulate more synchronously than cows from the Control group (P = 0.1). The distribution of ovulations after CIDR removal is shown in Fig. 1B.

The hypothesis that PGF is able to induce synchronized ovulation in cows and heifers submitted to estradiol-free synchronization protocols was confirmed in the current study. The ovulation rate in PGF treated cows was above 80% in both studies, and 70% of cows treated with PGF ovulated in a 24-hour window (72–96 h after CIDR removal). This is very important, since the occurrence of the highest number of ovulations in the shortest interval is a key factor for the success of FTAI programs (Saacke, 2008). In the first experiment, the luteolytic dose of PGF was injected one day prior to CIDR removal, so that it was possible to differentiate the luteolytic action from the ovulation effect. In this study, only three cows from the Control group ovulated within the same 24-hour interval, demonstrating the need of an ovulation inducer after CIDR removal, and the efficacy of the PGF for this purpose. The low ovulation rate achieved when no ovulation inducer was used may be due to the GnRH used at Day 0 results in high variation in ovulation rate after treatment (Barros et al., 2000; Geary et al., 2000; Stevenson et al., 2000). Studies using similar GnRH based protocols always include some ovulation inducer soon after CIDR removal like GnRH (Stevenson et al., 2004), therefore it is difficult to compare our results with previous reports. However, it is clear the need to induce ovulation at the end of the protocol for desirable results to be achieved.

Previous studies have shown that PGF induced ovarian responses similar to estradiol, and also resulted in adequate pregnancy rates around 50% in beef heifers and cows (Pfeifer et al., 2014) and 40% in dairy cows (Pfeifer et al., 2016). These previous studies were performed with estradiol-progesterone based protocol, which is the most used in South America for synchronizing ovulation in cattle (Bó et al., 2013). However, in the current study, GnRH was injected to synchronize follicular wave emergence at the beginning of the protocol, which is the most used protocol in North America and Europe, where estradiol is prohibited (Bó and Baruselli, 2014). In our current protocol, PGF was equally efficient to induce ovulation; however, more studies are necessary in order to evaluate the pregnancy rates in such protocols.

In the second experiment cows that received PGF to induce ovulation and those that did not receive any ovulatory stimulus had a larger ovulatory follicle diameter than females receiving ECP. In a previous study, Pfeifer et al. (2014) also reported a smaller diameter of the ovulatory follicle in cows receiving BE or ECP compared to PGF treated heifers, when cows received BE at CIDR insertion (Pfeifer et al., 2014). Although the mechanism by which PGF induces ovulation is still unclear, PGF probably allows follicle growth over a longer period than that of estradiol esters before ovulation occurs (Pfeifer et al., 2014), and that could result in the larger ovulatory follicle diameters observed.

In conclusion, the hypothesis that PGF can be used to induce ovulation in Ovsynch + CIDR based protocols has been confirmed. Therefore, PGF induces synchronized ovulations similarly as ECP, and allows the preovulatory follicle to reach a larger diameter.

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