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ANIMAL REPRODUCTION

# Prostaglandin F2α treatment concurrent with artificial insemination does not affect bovine embryo production

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**ABSTRACT**: *Treatment with prostaglandin F2α (PGF) induces ovulation and increases conception rates in cows, while improving embryo production in buffalos. However, its effect on superovulated cows is unknown. This study verified whether single PGF administration concurrent with artificial insemination (AI) improves fertilization and embryo production rates in superovulated cows. In each replicate, embryo donor cows were equally allocated to two groups: the untreated control and PGF groups. The latter of which received 482 µg of cloprostenol concurrent with the first AI. Each cow (n = 35) was subjected to two superovulations (SOV) in a crossover design (total = 70 embryo collections). In the control and PGF groups, respectively, the observed responses were [median (95% CI)]: 12 (10–18) and 15 (12–18) total structures, 9 (7–11) and 7 (6–10) viable embryos, 1 (0–1) and 1 (1–3) degenerated embryos, and 1 (0–3) and 2 (0–5) oocytes (P > 0.05). In conclusion, single PGF treatment concurrent with the first AI did not affect embryo production in superovulated cows.*
**Key words**: *embryo transfer, fecundation, reproduction, cow, superovulation.*

## Administração de prostaglandina F2 alfa no momento da inseminação artificial não afeta a produção de embriões bovinos

**RESUMO**: *A prostaglandina F2α (PGF) pode induzir a ovulação e melhorar tanto a concepção em vacas, como a produção de embriões em búfalas, mas o efeito em vacas superovuladas é desconhecido. Esse estudo teve como objetivo verificar se a administração de uma dose de PGF na inseminação artificial (IA) após a superovulação (SOV) melhora as taxas de fecundação e produção embrionária em vacas. Em cada replicação, vacas doadoras de embriões foram equilibradamente alocadas em dois grupos: controle, não tratado, ou PGF, que recebeu 482 µg de cloprostenol no momento da primeira IA. Cada doadora (n = 35) foi submetida a duas SOV em um delineamento crossover (total = 70 coletas de embriões). Nos grupos controle e PGF, respectivamente, foram observados [medianas (IC 95%)]: 12 (10-18) e 15 (12-18) estruturas totais; 9 (7-11) e 7 (6-10) embriões viáveis; 1 (0-1) e 1 (1-3) embriões degenerados; e 1 (0-3) e 2 (0-5) ovócitos (P > 0,05). Conclui-se que uma única administração de PGF no momento da primeira IA não afeta a produção embrionária de vacas superovuladas.*
**Palavras-chave**: *transferência de embrião, fecundação, reprodução, vaca, superovulação.*

Induction of multiple ovulation and embryo transfer (MOET) contributes to the dissemination of superior genetics. Even though such techniques are efficient, the number of ovulatory follicles is variable after superovulation (SOV), and the recovery of non-fertilized oocytes may correspond to nearly 20% of the total collected structures (LIMA et al., unpublished data). The recovery of such a proportion of non-fertilized oocytes may reflect the ovulation of oocytes with low viability due to the dispersion of the ovulations, and/or alteration in gamete transport (MAPLETOFT et al., 2015), leading to negative effects on the production of transferable embryos; consequently, increasing the costs related to the preparation of unused embryo recipients.

Protocols for fixed-time AI (FTAI) in cows require ovulation induction, which can be accomplished with the use of estradiol (E2) or analogs of gonadotropin-releasing hormone (GnRH). For SOV, GnRH analogs such as gonadorelin, buserelin, and lecirelin are preferred, allowing adequate fertility rates. Additionally, PGF is apparently capable of inducing ovulation in cows (LEONARDI et al., 2012). In superovulated cows, there is a drastic

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increase in the concentration of PGF and prostaglandin E2 (PGE) in the follicular fluid prior to ovulation. This suggests that both hormones may act on follicle rupture (BERISHA et al., 2019). In addition, prostaglandins are involved in capturing and transporting gametes in many species and in oviduct contraction in cows (WIJAYAGUNAWARDANE et al., 2001). Although such mechanisms are unknown, PGF has been used as a replacement for E2 in FTAI protocols for cows, with satisfactory results (CASTRO et al., 2018). Recently, the use of PGF during the periovulatory period has been associated with increased fecundation rates and embryo development in superovulated buffalos (CARVALHO et al., 2020). Despite its well-known luteolytic effect in cows (PHILLIPPO & ROWSON, 1975), the use of PGF during the peri-ovulatory period under basal progesterone concentrations has not yet been elucidated. The present study evaluated the effect of administering PGF after SOV in cows concurrent with the first AI on fecundation rates and embryo production. Our hypothesis was that treatment with PGF during the peri-ovulatory period increases the number of transferable embryos in superovulated cows.

*Bos taurus* and *Bos taurus x Bos indicus* (n=35) embryo donor cows allocated to the Biotec Embryo Collection and Processing Center (Protásio Alves, RS) were subjected to SOV after estrus synchronization (Figure 1). Synchronization occurred eleven days prior to estrus (D -11) through the insertion of an intravaginal device (IVD) containing 1 g progesterone (P4) plus the administration of 2 mg estradiol benzoate (EB). At D -4, the cows received 482 µg of cloprostenol (PGF, Estron, Agener União). The IVDs were removed on D -2, to allow the cows to show estrus. Between 9 and 11 days after estrus, cows presenting a corpus luteum (CL) with a diameter greater than 1 cm were selected. The ablation of follicles greater than 8 mm was conducted on D0 (LIMA et al., 2007) and the SOV started one day after follicular ablation. For each cow, the FSH dose (160–260 mg; Folltropin, Vetoquinol) was determined individually, according to their breed and performance in previous programs. The FSH treatment started on the first day of SOV, at 12 hour-intervals (M/A), in eight decreasing doses (20% M/A, 15% M/A, 10% M/A, and 5% M/A). Simultaneously with the sixth and seventh doses (at D3 and D4, respectively), 482 µg of cloprostenol (PGF) was administered to induce luteolysis and decrease circulating P4 levels. On the morning after the last FSH treatment (D5), all cows detected in estrus were treated with 50 µg gonadorelin acetate (GnRH; Gestran Plus, Agener União) to synchronize ovulation, whereas cows that did not show estrus were treated with GnRH in the afternoon. Cows in the PGF group received 482 µg sodium cloprostenol (Estron; Tecnopec) immediately after the first AI (12 h after GnRH), whereas the control cows remained untreated. The two groups were compared simultaneously in each replicate. As each cow was subjected to two SOVs, all cows were included in both groups, totaling 70 embryo collections. After collection (at D12; 7 days after estrus), the number, stage of development, and quality of recovered structures were recorded. As the data did not present normality, between-group comparisons were performed through the Wilcoxon signed rank test for paired data, using Prism 8 software, and $P < 0.05$ was considered significant.

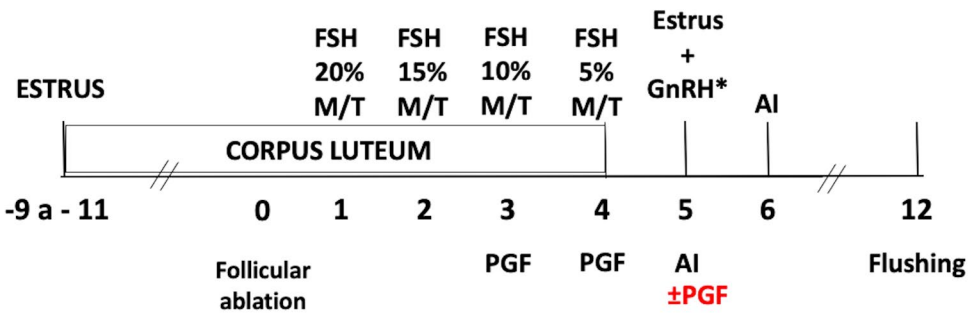


Figure 1 – Protocol for estrus synchronization and superovulation. Concurrently with the first AI (12 h after GnRH treatment), cows were treated with 482 µg sodium cloprostenol (PGF) or untreated (control). *On the morning of D5, cows detected in estrus were immediately treated with GnRH and cows not detected in estrus were treated with GnRH in the afternoon. The AIs were conducted 12 and 24 h after the GnRH treatment.

No evaluated parameters differed (P > 0.05) between the treatments (Figure 2). The median (95% CI) for the control and PGF groups, respectively, were 12 (10–18) and 15 (12–18) for total structures, and 9 (7–11) and 7 (6–10) for viable embryos. The rates of embryo production also did not differ (P > 0.05): 78% (61–85%) for the control and 61% (43–83%) for PGF, which are similar to those reported in previous studies (LIMA et al., 2007; GUERRA et al., 2012; WILEY et al., 2019).The number of freezable

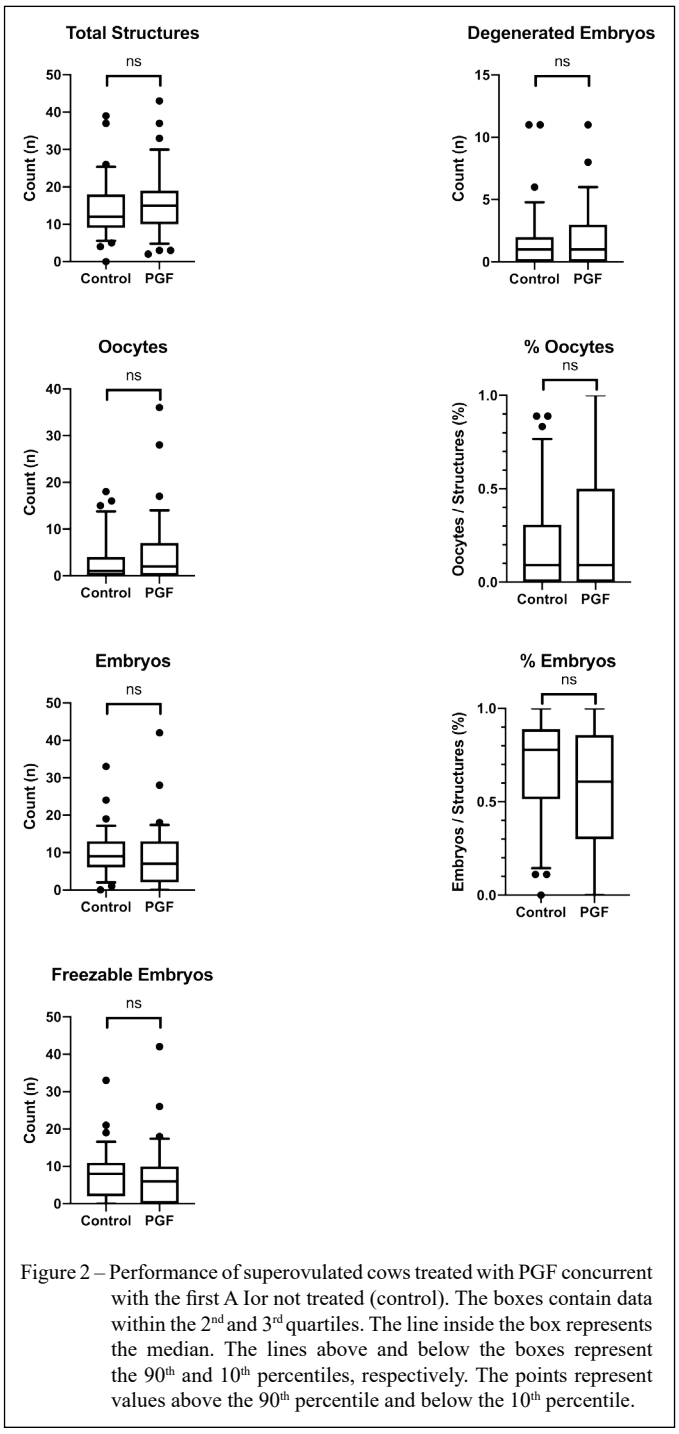


Figure 2 – Performance of superovulated cows treated with PGF concurrent with the first A Ior not treated (control). The boxes contain data within the 2nd and 3rd quartiles. The line inside the box represents the median. The lines above and below the boxes represent the 90th and 10th percentiles, respectively. The points represent values above the 90th percentile and below the 10th percentile.

embryos for the control (8; 5–9) and PGF (6; 2–10) cows did not differ (P > 0.05) and were similar to those reported by Wiley et al. (2019). The number of degenerated embryos for the control (1, 0–1) and PGF (1, 1–3) groups were also similar (P > 0.05). In previous studies, the number of oocytes varied from 5.6 to 5.8 (GUERRA et al., 2012; WILEY et al., 2019).However, in our study, those numbers were inferior: 1 (0–3) for control and 2 (0–5) for PGF. This may reflect differences in methodology, especially those related to the protocols used for SOV and ovulation induction. There was no difference in the percentage of oocytes: 9% (0–23%) for the control and 9% (0–43%) for PGF (P > 0.05). Although there are no data on the effect of PGF treatment concurrent with AI in superovulated cows, in buffalos, such treatment resulted in an increase in fecundation rates and in the number of viable embryos (CARVALHO et al., 2020). However, as the rate of embryo recovery in buffalos is lower than that in cattle, the lack of effect observed in the present study may be because most structures were fertilized, even in the control group. Furthermore, in a study by CARVALHO et al. (2020), buffalos received four doses of PGF every 12 h from the moment of ovulation induction, which likely prolonged the PGF action. In the present study, PGF was administered during the first AI session. Thus, it is possible that a greater number of administrations could be effective.

Based on our hypothesis, it was expected that the cows in the PGF group would present greater ovulation synchrony and/or a positive effect on the transport of gametes, resulting in a lower proportion of non-fertilized oocytes and more viable embryos. However, Morrison et al. (1988) also did not report positive effects of PGF treatment concurrent with AI, on the number of spermatozoa present in the oviduct and on pregnancy rates in cows. Taken together with our results, it contradicted the initial hypothesis. A recent study conducted with superovulated cows reported that, although PGF is related to ovulation in cows, the level of PGE in the follicular fluid immediately before ovulation (484.21 ng/mL) was greater than the concentration of PGF (101.01 ng/mL). This suggested that PGE may be the main paracrine mediator of the LH peak in cows, playing a relevant role in ovulation and in the formation of CL (BERISHA et al., 2019).

Ambrose et al. (2015) reported an increase in the conception of cows treated with PGF concurrent with FTAI at the beginning of lactation (possibly facing a negative energy balance); however, no subsequent effect was observed after mid-lactation. Additionally, the rates of pregnancy and gestation maintenance did not differ in dairy cows treated with PGF, GnRH, or PGF plus GnRH concurrent with FTAI (MOHAMMADI et al., 2019). Therefore, PGF treatment concurrent with AI may be efficient for cows subjected to stressful conditions that may impair ovulation (such as negative energy balance and heat stress) but would not benefit cows with adequate reproductive performance (LOPEZ-GATIUS et al., 2004), as observed in the present study. In conclusion, a single PGF administration concurrent with the first AI had no effect on embryo production in superovulated cows.

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## ETICS AND BIOSECURITY COMMITTEE

CEEA Universidade Federal de Pelotas (# 57360).

## DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## AUTHORS CONTRIBUTION

All authors contributed equally for the article.

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