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Prostaglandin F2α and its interference on pregnancy rates in heifers

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Abstract: The present study aimed to evaluate the pregnancy rate of Nellore and Crossbred heifers submitted to the induction of puberty with prostaglandin PGF2α. Control group (n=80), heifers were submitted to a fixed-time artificial insemination protocol (FTAI) without pre-exposure to any hormone, induction group (n=80). Nellore (n=80) and Crossbred (n=80) females were submitted to two applications of prostaglandin PGF2α before the FTAI protocol, 24 days before the beginning of the protocol (D-24), and twelve days before the start of the protocol (D-12). All heifers were aged between 12 and 14 months with an average weight of 311 kg and a body condition score of 3. For the FTAI protocol, both groups were performed on day 0 (D0) with the application of estradiol benzoate and insertion of the intravaginal implant with progesterone. On day eight (D8), the implant was removed and equine chorionic gonadotropin, prostaglandin, and estradiol cypionate were applied. On day 10, artificial insemination was performed. Thirty days after FTAI, pregnancy was diagnosed by transrectal ultrasound. The animals were individually weighed to verify the interaction between weight and pregnancy rates. For statistical analysis, SAS 9.3 software was used. The results were considered different with a significance level of 5%. For animals in the Induction group, pregnancy rates at 30 days after FTAI ($P>0.05$) were 57.5% (46/80) for Nellore heifers and 52.5% (42/80) for Crossbred heifers. Control group, heifers had a pregnancy rate of 60% (48/80) ($P>0.05$). No interaction was observed between heifer weight and conception rate in the FTAI, regardless of the induction treatment ($P=0.1897$). Furthermore, there was no difference in the pregnancy rate between Nellore ($P=0.5389$) and Crossbred females ($P=0.4425$), which were submitted to the application of PGF2α before starting the FTAI protocol. Thus, it can be concluded that there was no difference in the pregnancy rate with the induction of puberty using the PGF2α.
Keywords: Cattle. Cyclicity. Hormone. Induction. Pregnancy.

1. Introduction

Livestock in Brazil plays a very significant role in the gross domestic product (GDP), representing 31% of the total country value. Associated with this, Brazil has approximately 224.6 million cattle distributed in an area of 164.70 million hectares, with a stocking rate of 1.33 head/ha (IBGE, 2022). Thus, the extensive territory allows the country to be in the competitive market for the production and export of meat, considering that the costs of the bovine diet become reduced when it is based on forage, while in other countries the diet has a higher proportion of grains (ANUALPEC, 2022). More than 80% of the Brazilian herd is composed of *Bos taurus indicus*. These animals show good adaptability to higher temperatures and humidity, which are predominant climate characteristics (Valero et al., 2020). Even with such favorable factors for bovine production, Brazil has a low production rate compared to other countries.

Given these data, the need to produce more meat (15 kg) per hectare without the expansion of territories is remarkable. Therefore, the use of tools that improve the efficiency of our herds is indispensable. In this sense, protocols for reducing the calving interval are directly related to the efficiency of herd production (Claro Junior et al., 2010). Knowing that reducing the age at first birth is of paramount importance for increasing the profitability of the farm, the search for efficient protocols in this regard has increased. Thus, for the success of the advancement of the reproductive period, the prior preparation of females, with a diet and offer of foods that favor body development, physiologically contributes to the acceleration of puberty, with fertile estrous cycles. Those heifers with an adequate body condition score have uneventful pregnancy and puerperium and therefore produce better quality calves (Pfeifer et al. 2009; Silva et al., 2022).

Therefore, the cyclicity induction protocols used in heifers expose the animal to exogenous progesterone implants. However, prolonged exposure to a progestogen has not been effective in increasing the pregnancy rate in prepubertal and pubescent heifers on FTAI (Pfeifer et al. 2009). Prostaglandin, on the other hand, has a luteolytic function, that is, it acts in the lysis of the corpus luteum so that a new follicular dominance occurs. Its effect allows for greater capillary permeability, attracting cells such as macrophages, and then carrying out the phagocytosis of cell debris resulting from the inflammatory process (Freitas, 2015; Ferreira, 2018).

There is still a gap in the use of prostaglandin in inducing cyclicity in heifers. However, studies show that it conditions a better corpus luteum and a larger ovulatory follicle diameter. Therefore, it is a hormone that can contribute to a better pregnancy rate. There is still doubt about when to use it in reproduction protocols. Prostaglandin, when used as an inducer of cyclicity, causes a decrease in the serum level of progesterone and does not show improvement in the conception rate when used on the same day of insemination (Marques, 2018). This study aimed to evaluate the pregnancy rate of Nellore and Crossbred heifers, whether or not subjected to induction using the prostaglandin hormone being applied 24 and 12 days before the start of the protocol of FTAI.

2. Material and Methods

The experiment was approved by the Ethics Committee on Research Involving Animal Experimentation of the Universidade Paranaense (UNIPAR), with protocol number 37709/2020.

2.1. Animals and experimental groups

The management activities, synchronization, gynecological examinations, and procedures were carried out on a private farm located in the municipality of Diamante Do Oeste, located at latitude 25º01'04.2" S and longitude 54º05'50.9" W, in the western region from the state of Paraná, Brazil; and at the Animal Reproduction Department of UNIPAR, located in the municipality of Umuarama, located at the latitude of 23º45'59" S and a longitude 53º19'30" W, in the northwest region of the state of Paraná, Brazil.

Therefore, the study was divided into two groups. In the control group (C), 80 Nellore heifers were used with an average age between 12 and 14 months, submitted to weighing and body condition scores between 3 and 3.5 (scale of 1-5). These animals were not subjected to the application of prostaglandin.

In the induction group (I), 80 heifers were divided into two groups: Nellore group (N): 80 Nellore animals (*Bos indicus*) and Crossbred group (C): 80 crossbred animals (*B. taurus* x *B. indicus*). All animals had a mean age between 14 and 16 months and a body condition score between 3 and 3.5 (scale of 1-5). All animals in this group received 150 µg of prostaglandin (PGF2α; Croniben, IM, Biogenesis Bagó, Curitiba, Brazil) 24 days (D-24) before starting the fixed-time artificial insemination protocol. (FTAI), followed by a new administration of 150 µg prostaglandin (PGF2α), 12 days (D-12) after the first application.

For the FTAI protocol, animals in the Control and Induction groups were submitted on day 0 (D0) to the application of 2 mg of estradiol benzoate (Bioestrogen; IM; Biogenesis Bagó, Curitiba, Brazil) and impregnated intravaginal implants of progesterone were inserted (Cronipres Mono dose; 1 g; Biogenesis Bagó, Curitiba, Brazil). On day eight (D8), the implants were removed and 400 I.U. of equine chorionic gonadotropin (eCG; Ecegon; IM; Biogenesis Bagó), 150µg of prostaglandin (PGF2α, IM) and 1 mg of estradiol cypionate (Croni-Cip; IM) were applied. On day 10 (D10), 48 h after D8, artificial insemination was performed. Thirty days after FTAI, the pregnancy was diagnosed by transrectal ultrasound of the female genital tract using the SonoScape A5v device, probe 5-16 MHz (city, country). All females were kept on pasture feeding, and receiving mineral supplementation according to the management of the property and *ad libitum* water intake.

2.2. Statistical analysis

For statistical analysis, the Statistical Analysis System software (SAS Institute Inc., 2001, (city, country)) was used. Initially, the Shapiro-Wilk test (Proc Univariate) was used for the normality test of the residuals and the Chi-square test for homogeneity. The averages generated were adjusted using the LSMEANS command before statistical analyses. To prepare the graphics, the computer software GraphPad Prism version 5.0 (city, country) was used. All results are presented as mean ± standard error of the mean. Differences were considered when $P < 0.05$.

3. Results

No differences were found in animal weight at baseline ($P > 0.05$). Control group heifers had an initial mean weight (standard deviation) of 305.65 ± 13.7 kg, that is, 24 days before the beginning of the FTAI protocol. The females in the Induction group had an initial weight of 311.2 ± 23.2 kg for the Nellore breed and 305.65 ± 13.7 kg for the Crossbreds. Furthermore, according to the results obtained, there was no interaction between heifer weight and conception rate in the FTAI, regardless of treatment with prostaglandin or not ($P = 0.1897$).

Likewise, there was no difference in the ovarian area (cm^2), as shown in Table 1, nor the diameter of the largest follicle (cm) between the experimental groups, as shown in Table 2. Additionally, no structures similar to the corpus luteum were identified in the analyses performed.

	Ovarian Area (cm^2)						
	Control		Nellore Induction		Crossbred Induction		P
	RO	LO	RO	LO	RO	LO	
D-24	3.2±0,81	3.1±0,64	3.3±0,67	3.2±0,98	3.1±0,12	3.3±0,12	0.432
D-12	3.5±0,31	3.4±0,47	3.3±0,43	3.4±0,67	3.3±0,78	3.3±0,56	0.654
D0	3.6±0,23	3.5±0,76	3.4±0,21	3.4±0,11	3.6±0,90	3.4±0,93	0.543
D10	3.8±0,45	3.9±0,56	3.5±0,55	3.5±0,12	3.7±0,87	3.6±0,91	0.124

Table 1 – Means (standard error) of the area of the right ovary (RO) and left ovary (LO) for the Control, Nellore Induction and Crossbred Induction groups.

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	Dominant Follicle (DF; mm)						
	Control		Nellore Induction		Crossbred Induction		P
	RO	LO	RO	LO	RO	LO	
D-24	8,9±0,12	8,4±0,42	8,6±0,71	9,2±0,81	9,1±0,21	9,3±0,34	0,234
D-12	8,8±0,15	8,4±0,31	8,7±0,32	9,1±0,71	9,3±0,17	9,3±0,12	0,541
D0	8,5±0,32	9,5±0,37	8,4±0,12	9,4±0,12	9,5±0,91	9,4±0,22	0,323
D10	9,8±0,17	9,9±0,4	9,5±0,12	9,5±0,17	9,7±0,67	9,6±0,11	0,224

Table 2 – Means (standard error) of the diameter of the dominant follicle in the right ovary (DFRO) and the diameter of the dominant follicle in the left ovary (DFLO), between the experimental groups.

Table 3 shows the pregnancy rate of the Control group, Nellore, and Crossbred heifers submitted to the pre-FTAI protocol with prostaglandin PFG2α.

	Pregnancy rate (%)	P
Control	60 (48/80)	
Nellore	57,5 (46/80)	0,5389
Crossbred	52,5 (42/80)	0,4425

Table 3 – Pregnancy rate (%) of the Control group, Nellore and Crossbred heifers submitted a pre-FTAI protocol with two applications of PGF2α (D-24 and D-12), before starting the Fixed-Time Artificial Insemination protocol.

4. Discussion

The exogenous hormones and their analogs are used to manipulate the estrous cycle of female bovine animals and have the advantage of reducing the amount of work and time spent on estrus detection. Despite being compared to hormones, prostaglandins differ from these in that they are formed in almost all tissues and not in specialized glands. Furthermore, they usually act locally, rather than being transported by the blood to the target cell. Arachidonic acid, an essential fatty acid, is the precursor of prostaglandins associated with reproductive processes, such as prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2). Prostaglandins are extremely potent compounds that trigger a wide range of physiological responses, being present in the form of at least six compounds related to numerous metabolites. In reproduction, prostaglandins are involved in gonadotropin release, ovulation, corpus luteum regression, uterine motility, childbirth, and sperm transport (Nishimura and Okuda, 2020). In this present study, two applications of PGF2α were performed, in a presynch protocol. Additionally, PGF2α is commonly administered to cows and heifers as part of estrous synchronization protocols. Administration of PGF2α results in regression of a functional corpus luteum between days 5 and 16 of the estrous cycle (Rowson et al., 1972), and estrus occurs in approximately three days (Tervit et al., 1973). Some studies have evaluated the efficacy of various PGF2α and found no differences in the decrease in serum progesterone concentrations or the estrus-inducing response (Oosthuizen et al., 2018; López-Gatius, 2022), in addition to not showing differences in pregnancy rates (Salverson et al., 2002; Stevenson; Phatak, 2010; Gabriel et al., 2011). The route of administration of PGF2α also did not affect the decline in progesterone concentration, nor the proportion of cows undergoing luteal regression (Chebel et al., 2007).

With the use of hormonal protocols and the performance of the artificial insemination technique, pregnancy rates in heifers increase, mainly in replacement, which favors genetic advancement within a herd. According to Monn et al. (2019), those heifers that demonstrate at least three estrous cycles, before the beginning of the reproductive activity, have higher pregnancy rates when compared to acyclic females. Therefore, these same authors performed a study with a pre-synchronization treatment, with two injections of prostaglandin F2α (PGF2α), on days 21 (D-21) and 7 (D-7), before starting the CO-Synch + protocol 5 d CIDR, to anticipate puberty, presentation of more estrous cycles before AI and better pregnancy outcomes. However, they found that the Presynch treatment reduced pregnancy rates, with 52.2% for the Control group and 38.1 ± 6.3% for the Presynch group ($P = 0.06$), in addition to not resulting in an advance of the onset of puberty in beef heifers ($P > 0.05$). In this sense, the Presynch treatment did not impact pregnancy rates in heifers with reproductive tract scoring (RTS) of 3 or 4 ($P > 0.05$). For the assessment of reproductive status, these authors used a scale from 1 to 5, where 1 and 2 are classified as reproductively immature (prepubertal), 3 with some reproductive capacity and relatively close to reaching puberty (peripubertal), and 4 and 5 are reproductively mature (pubertal), as described by Cooke et al., 2020. When compared to our study, we found that the administration of PGF2α before the FTAI protocol in heifers did not improve pregnancy rates.

As shown in our study, the PGF2α pre-synchronization protocol with two injections is already widely used in postpartum dairy cows (Bishop et al., 2022). Cows that ovulate earlier postpartum have been shown to have an increase in the number of estrous cycles before the first insemination and therefore to increase fertility (Thatcher and Wilcox, 1973; Darwash et al., 1997). However, there are two limitations to this presynchronization protocol: the first is that PGF2α alone does not benefit acyclic cows (Moreira et al., 2000) and the second is that follicular growth is not precisely synchronized (Souza et al., 2008). In the present study, when used in heifers, pre-synchronization did not bring benefits in Nellore and Cross-bred animals. In our work, we used a prostaglandin D-cloprostenol and, in this context, the use of different PGF2α analogs is discussed by several authors. According to Macedo et al.

(2015), the analogs D-cloprostenol sodium and Dinoprost Tromethamine have the same pharmacokinetics. Therefore, there should be no differences in fertility, but there are many debates in defense of one analog or another, with controversial results. Some authors have shown similar pregnancy rate results using the two PGF analogs (Stevenson and Phatak, 2010; Pérez-Marin et al., 2015). On the other hand, Pursley et al. (2012) found that cloprostenol provided higher heat detection rates in primiparous cows (42.4%), general conception (38.3%), and pregnancy (14.4%) than the dinoprost group (34.0, 34, 4 and 12.2%; P < 0.05).

Prostaglandin has been used in different ways in reproduction, either as a gestational increment or as a means of providing a new current follicular wave. With the use of prostaglandin, the serum P4 level decreases causing the conception rate to decrease, thus enabling an association with the application of progesterone, to regulate its level at this stage, generating a greater possibility of conception (Nishmura et al., 2020). However, studies show that there is an increase in the pregnancy rate with the application of PGF2α at the time of FTAI in dairy females (Mohammadi, 2019). Unlike the present study, in which pre-FTAI prostaglandin in Nellore and Crossbred heifers did not change pregnancy rates. (Marques 2018) verified that the use of 10 mg of Dinoprost Tromethamine - PGF2α (Lutalyse, Zoetis) on the day of the FTAI did not influence the conception rates of the treated groups (cattle). In this study, the author demonstrated that the functions of PGF2α at the beginning of FTAI, which are the favoring of sperm transport and the induction of LH release by a mechanism independent of luteolysis, as described by Ambrose et al. (2015), were not efficient enough to increase the conception rate. In contrast, these same authors reported an increase in the pregnancy rate with the application of PGF2α at the time of FTAI in dairy cattle.

As an estrus inducer, the use of prostaglandin PGF2α conditions a better corpus luteum due to the larger diameter of the ovulatory follicle formed. Therefore, it is a hormone that can contribute to an improvement in pregnancy rates. There is still doubt about the ideal time to use this hormone, since in the protocols when used as a cyclicity inducer, it caused a decrease in serum levels of progesterone and did not lead to an improvement in the conception rate when used on the same day of insemination (Marques, 2018). To determine the effects of administering 25 mg of PGF2α seven days before the start of the FTAI protocol in beef heifers (*B. taurus*), Oosthuizen et al., 2018 verified that there was no difference in the pregnancy rate between treatments (Control 45 .4 ± 2.5 vs. pre-FTAI 43.2 ± 2.5%), according to the data obtained here in both Nellore and Crossbred heifers. However, in beef heifers, the administration of PGF2α three days before the FTAI protocol increased the percentage of heifers initiating a new wave of follicular growth, as well as estrus synchronization after removal of a progesterone-impregnated vaginal implant (CIDR, Pfizer) (Grant et al., 2011). (Perry et al., 2012; Moorey et al., 2022), estrus synchronization (Grant et al., 2011), and rates of pregnancy in beef cattle (Perry et al., 2012).

In addition to the use of exogenous hormones to increase pregnancy rates in cattle, it is known that the animal's body condition score (BCS) is directly related to the success of the FTAI. This condition is a determining factor for the reestablishment of ovarian activity in bovine females (Richards et al.1986). In a study carried out with Holstein cows, conception rates were found to be higher in cows that received 10 mg of PGF2α than in cows in the control group (45.8% vs. 36.0%; P < 0.05), in cows with high ECC compared to cows with low ECC (52.1% vs. 30.4%; P < 0.01), and in primiparous cows compared to multiparous cows (47.6 vs. 34.4%, P < 0.01), but their interaction with PGF2α treatment did not affect conception rates. In conclusion, 5 mg of PGF2α administered intramuscularly simultaneously with FTAI did not increase the conception rate in lactating dairy cows, whereas 10 mg of PGF2α significantly increased the pregnancy rate (Ambrose et al., 2015). In this context, it was found that, in the present study, heifers with adequate BCS and weight above 311 kg presented similar pregnancy rate data (pre-synchronized or not), showing that well-nourished animals have no difficulty in having good results in their reproductive activity (Brandt et al., 2023).

Maltthews et al., (2017) showed that dietary energy restriction can directly influence the hypothalamus, by potentiating negative estrogen feedback, reducing the frequency of LH release. This effect of increased sensitivity to estrogen can promote a delay in the emergence of the follicular wave after treatment with progesterone and estrogen in FTAI programs, by directly influencing the hypothalamic-pituitary axis, promoting smaller diameter follicles at the end of the FTAI protocol resulting in lesser synchronization of these animals (Nafziger et al., 2021). It also affects LH levels, due to lower concentrations of progesterone after prolonged energy restriction. Authors suggest that restricting food intake reduces the responsiveness of the *corpus luteum* (CL) to LH stimulation, resulting in CL synthesizing and releasing less progesterone (Bishop et al, 2022). Thus, it is essential to evaluate the body condition, both of cows and heifers, before the beginning of their reproductive activity. This assessment allows for avoiding unnecessary expenses with hormonal protocols since it is known that females with hormonal restrictions do not perform their reproductive activity properly (Nafziger et al., 2021).

In this context, Rutter and Randel (1984) demonstrated that females in postpartum loss of body condition had a longer interval between parturition and estrus than those that kept their weight. Female nutrition may not only affect the synchronization of the estrous cycle but also the quality of the ovulated oocyte and uterine health. Lean cows are often on negative energy balance (NEB) and therefore have higher non-esterified fatty acid (NEFA) concentrations, which is associated with reduced fertility. This may be related to the active absorption of these NEFA by the oocyte during the early postpartum period, which leads to damage in embryonic development, accumulation of mRNA, proteins, and other factors in oocyte maturation, and even disturbances in the uterine environment such as increased uterine expression of inflammation markers and tissue remodeling (Ambrose et al., 2015). Associated with body condition assessment factors, the reproductive assessment of heifers before the beginning of their reproductive life is undeniable. In this sense, ultrasound diagnosis is considered a great technological advance in the research and reproductive clinic of large animals, as described by (Fathoni et al., 2022). With the evaluation of the female genital system by ultrasound, it is possible to evaluate the ovarian dynamics, uterine function, and fetal viability. Thus, it was possible to observe in this study that the prior evaluation of heifers before carrying out the pre-FTAI protocols saves the producer, avoiding unnecessary expenses with hormones.

5. Conclusion

The administration of prostaglandin PGF2α before the fixed-time artificial insemination protocol of Nellore and Crossbred heifers did not interfere with the pregnancy rates of the animals under the conditions of this experiment.

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