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GnRH Administration in the Synchronization of Receptors Cows for Fixed-Time Embryo Transfer

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Abstract

The bovine production chain in Brazil is highlighted worldwide; however, researchers are seeking to improve production even further by evaluating the use of reproductive biotechnologies, such as the transfer of embryos at a fixed time. The aim of this study was to analyze the expression of estrus associated or not with the use of gonadotropin releasing hormone (GnRH), on the diameter of the dominant follicle, diameter of the corpus luteum, utilization rate and conception rate of receptors cows. The experiment was carried out in southern Brazil and 400 receptors cows were used, distributed in a 2x2 factorial scheme, considering estrus expression and GnRH administration. All receptors were synchronized and, on Day 10, their ovaries were evaluated and, based on estrus expression, they were distributed into 2 subgroups: with and without GnRH application. On Day 18, they had their ovaries evaluated and they were inovulated, and on Day 41, the pregnancy diagnosis was made. The expression of estrus increased the diameter of the dominant follicle and corpus luteum and the utilization rate of the recipient cows; however, it did not improve the conception rate. The administration of GnRH, with or without the expression of estrus, did not affect any of the studied variables.

Keywords: cattle breeding, estrus expression, reproductive biotechnology

1. Introduction

1.1 Cattle Farming in Brazil

The Brazilian beef production chain is of great importance in the international market, as the country stands out with the largest commercial cattle herd and the largest exporter in the world, however, in order to improve productive and reproductive efficiency, several points must be improved. Thus, studies on the use of reproductive biotechnologies such as artificial

insemination (AI), *in vitro* embryo production (IVEP) and embryo transfer (ET) are essential (Blondin *et al*., 2015).

ET is an important biotechnology for exploring the reproductive potential of genetically superior bovine females (Varago *et al*., 2008). However, several factors influence their results (Tribulo *et al*., 2016), such as the quality of the corpus luteum (CL) and, consequently, the plasma concentrations of progesterone (P4) of the receptors (Diskin & Morris, 2008). Progesterone, secreted by the CL, modifies the uterine environment, ensuring the growth and development of the conceptus (Mann *et al*., 2003).

So, for the establishment of pregnancy to occur, an interaction between the embryo, the uterine environment and the CL is necessary (Lima & Souza, 2009). In fact, researchers emphasize the importance of the quality of the CL of receptors at the time of inovulated (ET), due to its direct relationship with the plasma concentration of P4 (Luttgenau *et al*., 2011).

Smaller sized CL result in lower conception rates in bovine receptors (Vasconcelos *et al*., 2001). On the contrary, Baruselli *et al.* (2003) reported that larger diameter CL elevate plasma P4 concentrations and improve the conception rate in receptors.

Aiming to increase the conception rate in animals submitted to Fixed Time Artificial Insemination (FTAI), some techniques have been studied, such as the administration of Gonadotropin Releasing Hormone (GnRH). The application of GnRH at the time of the FTAI may be an option to increase the conception rate in beef cows (Hill *et al*., 2016; Oliveira *et al*., 2017), especially in cows that do not show estrus 48 hours after removal of the P4 device (Sa Filho *et al*., 2011). It is known that GnRH induces the pre ovulatory peak of Luteinizing Hormone (LH), synchronizing the time of ovulation and aiding in the formation of a functional CL (Gottschall *et al*., 2008; Perry & Perry, 2009).

Another important point to be considered in the use of reproductive biotechnologies in cattle is the effect of estrus expression and estradiol (E2) concentrations on the conception and gestation rate. The expression of estrus is associated with a uterine environment that favors conceptus establishment and embryonic survival (Bridges *et al.*, 2013) and is related to higher conception rates and reduced embryonic losses in cattle (Pereira *et al.*, 2016; Bó & Cedeño, 2018).

1.2 Research Justification and Objectives

Thus, considering the expression of estrus and the effect of GnRH on the CL, and verifying that studies on the use of GnRH in synchronization protocols of receptors for ET are scarce in the literature, the aim of this work was to evaluate the expression of estrus associated or not with the use of GnRH, on the diameter of the dominant follicle, diameter of the corpus luteum, utilization rate and conception rate of receptors cows of the Nelore breed.

2. Method

2.1 Ethical Animal Experimentation and Local

The procedures used in this research were approved by the Ethics Committee on the Use of Animals at the University Cesumar/ Unicesumar, under protocol No. 014.2/2019. The

experiment was carried out at the Biotechnology Center/ Biotec, at farm school, Cesumar University / Unicesumar, Maringá, Paraná, Brazil (23°25'S, 51°57'W and altitude of 550 meters), in 2019.

2.2 Management, Estrus Synchronization Protocol and Experimental Groups

Were used 400 cows receptors of the Nelore breed, kept in paddocks of brachiaria (*Brachiaria brizantha* cv MG-5), with mineral supplementation and water *ad libitum*, and submitted to hygienic and sanitary management adopted on the property.

Initially, all receptors were submitted, on Day 0 (D0), a random day of the estrous cycle, to the estrus synchronization protocol, receiving 2.0 mg of Estradiol Benzoate (EB) (Estrogin®, Zoetis, São Paulo, SP, Brazil) intramuscular, and the insertion of a multipurpose P4 intravaginal device (CIDR® Zoetis, São Paulo, SP, Brazil). On Day 8 (D8), 10 mg of Dinoprost Trometamina ($PGF_2\alpha$) (Lutalyse® , Zoetis, São Paulo, SP, Brazil) was applied, the P4 implant was removed and 0.8 mg of Estradiol Cypionate was applied (ECP®, Zoetis, São Paulo, SP, Brazil) and 300UI Equine Chorionic Gonadotropin (ECG) (Novormon®, Zoetis, São Paulo, SP, Brazil). At that time, the animals were also marked with a marker stick (LA-CO Industries) in the dorsal sacrum (base of the tail).

On Day 10 (D10), estimated day of estrus, all receptors had their ovaries evaluated by ultrasonography (US) (Aloka SSD-500™) and, at this time, the diameter of the ovulatory follicle (dominant) was identified and measured.

On that same day, the receptors were also evaluated for estrus expression, which was verified according to the status of the stick mark at the base of the tail (Loiola *et al*., 2018). Based on this criterion, the cows were distributed into two groups:

- Group 1: animals without the stick mark (absent mark) - estrus group;

- Group 2: animals with the stick mark (intact mark) - non-estrus group;

At this time, within each group, the animals were distributed into 2 subgroups, according to the application or not of 0.02 mg of GnRH (Sincroforte®, Buserelin Acetate, Ouro Fino, Cravinhos, SP, Brazil).

On Day 18 (D18), all receptors again had their ovaries evaluated by US, being observed the presence and diameter of CL and, at this moment, regardless of CL diameter, all receptors who presented CL had an inovulated embryo. On Day 41 (D41) the diagnosis of pregnancy was performed, that is, 23 days after inovulation.

Thus, the animals were distributed in a 2x2 factorial scheme, considering the expression of estrus by the stick mark (absent: estrus; intact: no estrus) and the administration of GnRH (with or without application of GnRH) (Plate 1).

To obtain the embryos, 25 Wagyu bovine donors were aspirated, and their oocytes were quantified, classified, matured *in vitro* (MIV), fertilized *in vitro* (FIV) with sexed semen from a Wagyu bull, and after fertilization, the embryos were cultured *in vitro* (CIV) and, finally, inovulated in the receptors on D18 of the protocol.

2.3 Statistics and Data Analysis

The data collected were presence and diameter of the ovulatory follicle, presence and diameter of the corpus luteum, utilization rate, which is the proportion of receptors submitted to the estrus synchronization protocol who presented CL at the time of inovulation and conception rate, which is the percentage of cows that became pregnant, in relation to the total number of cows that were inovulated.

The experimental design was completely randomized and the variables were analyzed by the PROC GENMOD procedure of the statistical program SAS (2000), version 8.01, using binomial distribution and identity linkage function.

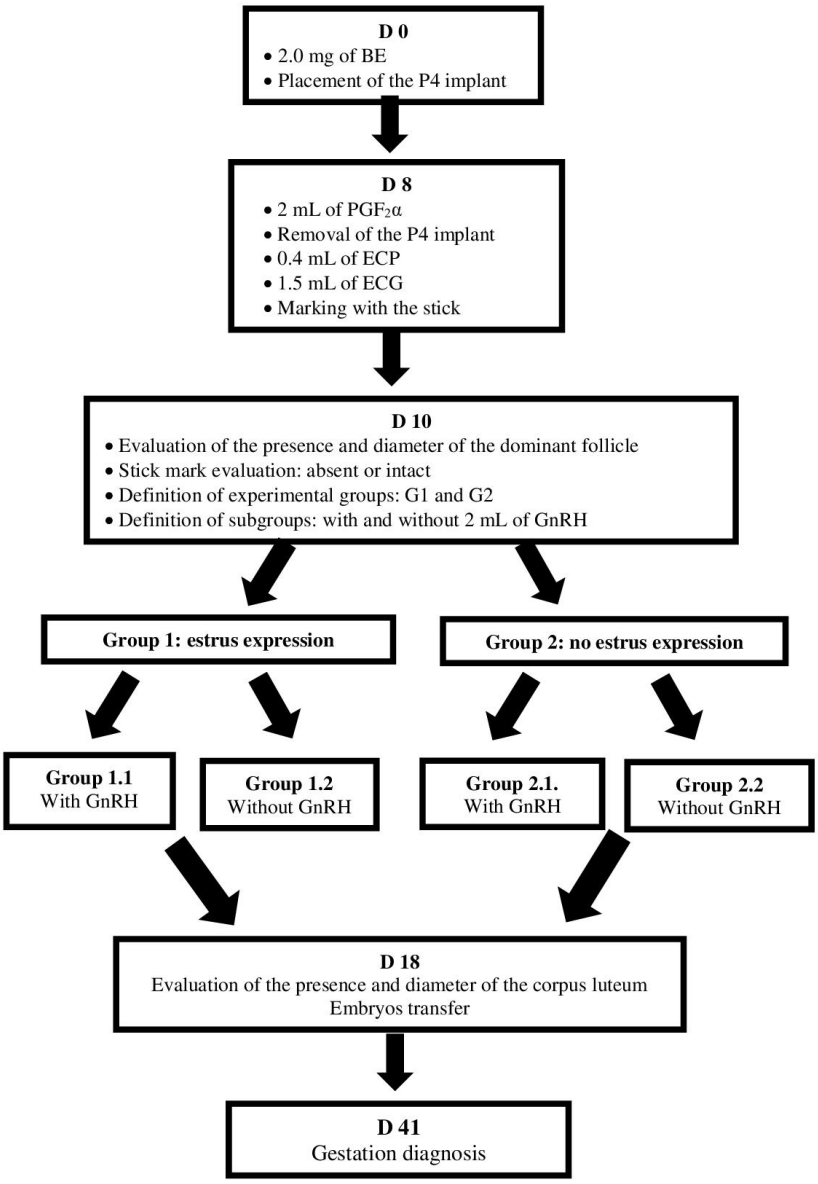

Plate 1. Experimental protocol

3. Results

The result of the evaluation of the ovulatory follicle diameter performed on D10 of the synchronization protocol of receptors cows of the Nelore breed showed a greater diameter (P<0.05) of the ovulatory follicle (10.02 ±0.16 mm) for cows in estrus (Table 1).

The animals that presented a larger diameter of the ovulatory follicle in the D10 of the synchronization protocol also presented a larger diameter of the CL (P<0.05), however, there was no effect (P>0.05) of the application of GnRH and neither interaction between the expression of estrus and GnRH (P>0.05) on the CL diameter at the time of embryo transfer, eight days after ovulation (Table 2).

Table 1. Mean ovulatory follicle diameter (mm) as a function of estrus expression in Nelore receptors cows

	Estrus expression		p value
	Yes	No	
Number of animals (n)	283	117	
Ovulatory follicle diameter (mm) (M±SE)	10.02 ± 0.16	7.20 ± 0.19	0.001

M±SE: mean plus or minus standard error of the mean.

Table 2. Mean corpus luteum diameter (mm) as a function of estrus expression and GnRH administration in Nelore recipient cows

Variable	Main effects		p value
Corpus luteum diameter (mm) (M±EP)	Estrus expression		
	Sim	Não	
	18.46 ± 0.14	16.89 ± 0.36	0.0004
	GnRH		
	Yes	No	
	17.69 ± 0.30	17.66 ± 0.31	0.9442

M±SE: mean plus or minus standard error of the mean.

The utilization rate, which is the proportion of receptors who presented CL at the time of ET, was higher (P<0.05) in animals that expressed estrus, however, the application of GnRH did not affect (P>0.05) this rate (Table 3).

Table 3. Average utilization rate (%) as a function of estrus expression and application of GnRH in Nelore receptors cows

Variable	Main effects		p value
Utilization rate (%) (M±SE)	Estrus expression		
	Yes	No	
	76.86 ± 2.12	61.22 ± 2.46	0.001
	GnRH		
	Yes	No	
	72.83 ± 3.00	66.25 ± 2.73	0.1044

M±SE: mean plus or minus standard error of the mean

There was no effect (P>0.05) of the expression of estrus and the application of GnRH on the conception rate of the evaluated animals (Table 4).

Table 4. Mean conception rate (%) as a function of estrus expression and application of GnRH in Nelore recipient cows

Variable	Main effects		p value
Conception rate (%) (M±SE)	Estrus expression		
	Yes	No	
	36.69 ± 4.89	34.31 ± 4.78	0.6810
	GnRH		
	Yes	No	
	36.66 ± 2.30	34.34 ± 3.01	0.6871

M±SE: mean plus or minus standard error of the mean

4. Discussion

Regarding the larger diameter of the ovulatory follicle observed in recipient cows submitted to the estrus synchronization protocol that expressed estrus signals, this result is attributed to a possible increase in the blood concentration of estradiol. Sá Filho *et al.* (2009) reported that larger ovulatory follicles on day 10 of the FTAI protocol are associated with high concentrations of estradiol and, consequently, with a high incidence of estrus expression, a fact also observed by Atkins *et al.* ,(2010) and Roelofs *et al.*,(2010).

Indeed, there is a positive correlation between pre ovulatory follicle diameter and serum estradiol levels and estrus expression (Perry *et al.*, 2014), because the larger the follicle diameter, the higher the estradiol concentrations and the higher the expression of estrus and the best uterine environment for the establishment of the conceptus (Bridges *et al.*, 2013). Similarly, Ferraz *et al.*, (2017) reported that in Nelore cows the expression of estrus is directly related to the diameter of the ovulatory follicle.

However, it was expected that the application of GnRH could have an effect on follicular dynamics, especially in cows that did not express estrus, as some researchers have pointed out that administering GnRH at the time of FTAI can improve reproductive rates in beef cows that do not present estrus 48 hours after removal of the P4 device (Sa Filho *et al*., 2011; Hill *et al*., 2016; Oliveira *et al*., 2017), a fact not observed in this study.

Regarding the animals that presented the largest diameter of the ovulatory follicle and the largest diameter of the CL, we inferred that the development of a physiological corpus luteum depends on the number of granulosa cells in the pre-ovulatory follicle and the number of LH receptors in the theca and cells of the granulosa, capable of synthesizing sufficient amounts of progesterone after luteinization. Thus, a larger diameter of the ovulatory follicle promotes the formation of a larger diameter corpus luteum (Lonergan *et al.*, 2013), justifying the findings of this study, especially related to the expression of estrus.

However, it was believed that the administration of GnRH could induce a pre ovulatory LH surge, synchronizing the time of ovulation and aiding in the formation of a functional CL, as observed by several researchers (Gottschall *et al*., 2008; Perry & Perry, 2009), but this fact was not found in this study.

GnRH has been widely used in FTAI programs and has shown positive results mainly in animals that do not show signs of estrus at the end of the FTAI protocol (Hill *et al*., 2016; Oliveira *et al*., 2017), so the results that showed the lack of effect of the application of GnRH and the interaction between the expression of estrus and GnRH on the corpus luteum diameter at the time of ET may be associated with the ovulation time and not with the diameter of the CL formed, as observed in this study.

On the result of the higher utilization rate observed in the animals that expressed estrus and on the lack of effect of the estrus expression and the application of GnRH on the conception rate of the evaluated animals, we reported that, although some authors claim that administering GnRH at the time of FTAI can be an option to increase the conception rate in beef cows (Hill *et a*l., 2016; Oliveira *et al*., 2017), especially in cows that do not present estrus 48 hours after the removal of the P4 device (Sá Filho *et al*., 2011), there was no such effect on ET.

As for the results of the highest utilization rate observed in animals that expressed estrus, however, without the effect of the application of GnRH, it appears that researchers claim that the larger the diameter of the follicle, the greater the concentrations of estradiol, the greater the expression of estrus and the better uterine environment for the establishment of the conceptus (Bridges *et al*., 2013). Thus, cows that did not express estrus have higher rates of embryonic loss (Cedeño *et al*., 2018). However, no differences were observed on the conception rate between cows that expressed or not estrus, in addition, we point out that the value found in this study, around 30%, is consistent with the mean values reported in the literature for beef cows submitted to ET.

Some authors (Vasconcelos *et al.*, 2001; Baruselli *et al.*, 2003) have stated that smaller corpus luteum reduces the conception rate in bovine receptors. However, Cavalieri *et al.* (2019)

worked with postpartum Nelore receptors cows and observed, after classifying the CL size into 4 levels, that the conception rate was not influenced by the CL size. Thus, based on the lack of effect of the studied variables on the conception rate in this study, it can be stated that the corpus luteum formed from small follicles produce sufficient levels of progesterone to support pregnancy after ET.

5. Conclusion

Based on the results obtained, it is concluded that the expression of estrus increased the diameter of the ovulatory follicle, the diameter of the corpus luteum and the utilization rate in receptors Nelore cows submitted to ET, however, there was no effect of the expression of estrus on the conception rate.

The administration of GnRH in receptors Nelore cows submitted to ET, with or without the expression of estrus, did not affect the diameter of the ovulatory follicle, the diameter of the corpus luteum and the utilization and conception rates, not being an option to increase the reproductive indices of these animals.

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