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# Gonadorelin at the outset and/or end of an ovulation synchronization progesterone estradiol benzoate-based protocol in Nellore females

# Gonadorelina no início e/ou no final do protocolo de sincronização da ovulação a base de progesterona e benzoato de estradiol em fêmeas Nelore

Carla Cristian Campos[1*]; Mayara Oliveira[2];
Renata de Freitas Ferreira Mohallem[1]; Ricarda Maria dos Santos[3]

## Abstract

This study aimed to evaluate the effects of gonadorelin (GnRH), used both at the outset of a timed artificial insemination (TAI) protocol to synchronize follicular wave recruitment and at the end to synchronize ovulation, on the conception rate (CR) in Nellore cows. The experiment was conducted on two beef cattle farms in the Vale do Araguaia, MT. The body condition score (BCS) evaluation and ultrasound examination to determine ovarian status [follicle diameter < 10 mm, ≥ 10 mm or presence of corpus luteum (CL)] were performed at the beginning (Day 0) of the TAI protocol. Multiparous cows (n = 494) were subjected to the following protocol: Day 0 – insertion of intravaginal progesterone device ($P_4$ DIB®, MSD) previously used for 8 or 16 days and IM administration of 2.0 mg of estradiol benzoate (EB, Gonadiol®, MSD); Day 8 – $P_4$ device withdrawal, IM injection of 300 IU of equine chorionic gonadotropin (eCG, Folligon®, MSD), 1.0 mg of estradiol cypionate (ECP®, Zoetis) and 0.265 mg of Sodium cloprostenol ($PGF_{2\alpha}$, Ciosin®, MSD); Day 10 – TAI, performed by a single inseminator. Animals were randomly assigned to one of four groups: control (n = 126), GnRH D0 (n = 123), GnRH D10 (n = 123) and GnRH D0 + D10 (n = 122). Animals treated with GnRH received 50 mcg of gonadorelin (GnRH, Cystorelin®, Merial). Pregnancy was diagnosed by ultrasound 39 ± 10 days after TAI. Data were analyzed using the GLIMMIX procedure of SAS program ($P < 0.05$). The overall CR was 42.71%. Effect of treatment was not detected ($P = 0.2482$) on CR, which was 36.89% ± 0.19 (47/126) for the control group, 46.08% ± 0.19 (56/123) for GnRH D0, 48.30% ± 0.18 (61/123) for GnRH D10 and 38.48% ± 0.19 (47/122) for GnRH D0 + D10. An effect of farm was detected; the CR was 47.32% ± 0.13 at Farm A and was 38.24% ± 0.13 at Farm B ($P = 0.0249$). No effects of the interaction between farm and treatment were observed ($P = 0.7662$), nor of those among ovarian condition and treatment ($P = 0.1225$) on CR. Cows with follicles < 10 mm had lower CR (16.44% ± 0.08; $P = 0.0001$) than cows with follicles ≥ 10 mm (46.15% ± 0.07) and cows with CL (48.35% ± 0.07). It was concluded that GnRH used to both synchronize the follicular wave recruitment and synchronize ovulation as part of a TAI protocol, did not improve the conception rate in Nellore beef cows.

**Key words:** Gonadotropin-releasing hormone, Nellore, pregnancy, synchronization, timed AI

[1] Discentes de Doutorado em Ciências Veterinárias, Universidade Federal de Uberlândia, UFU, Uberlândia, MG, Brasil. E-mail: carlacristian_vet@yahoo.com.br; renataffm@yahoo.com.br
[2] Discente de Mestrado em Ciências Veterinárias, UFU, Uberlândia, MG, Brasil. E-mail: may.udi@gmail.com
[3] Profª Drª, Faculdade de Medicina Veterinária, UFU, Uberlândia, MG, Brasil. E-mail: ricasantos@famev.ufu.br
[*] Author for correspondence

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## Resumo

Objetivou-se avaliar os efeitos da gonadorelina (GnRH) utilizada para sincronização da emergência da onda de crescimento folicular e para indução da ovulação sincronizada ou em ambos sobre a taxa de concepção (TC) de vacas da raça Nelore. O experimento foi realizado em duas fazendas no Vale do Araguaia, MT. A avaliação do escore de condição corporal (ECC) e a ultrassonografia para determinação da condição dos ovários [folículo com diâmetro < 10 mm, ≥ 10 mm ou presença de corpo lúteo (CL)] foram realizadas no início (Dia 0) do protocolo de inseminação artificial em tempo fixo (IATF). Vacas multíparas (n = 494) foram submetidas ao protocolo: Dia 0 – inserção do dispositivo intravaginal de progesterona ($P_4$, DIB®, MSD) utilizado previamente por oito ou 16 dias e aplicação IM de 2,0 mg de Benzoato de Estradiol (BE, Gonadiol®, MSD); Dia 8 – remoção do dispositivo, aplicação IM de 300 UI de Gonadotrofina Coriônica Equina (eCG, Folligon®, MSD), de 1,0 mg de Cipionato de Estradiol (ECP®, Zoetis) e de 0,265 mg de Cloprostenol Sódico ($PGF_{2\alpha}$, Ciosin®, MSD); Dia 10 – IATF, realizada por um único inseminador. Os animais foram distribuídos aleatoriamente em quatro grupos: controle (n = 126), GnRH D0 (n = 123), GnRH D10 (n = 123) e GnRH D0 + D10 (n = 122), sendo que os animais tratados com GnRH receberam 50 mcg de gonadorelina (GnRH, Cystorelin®, Merial). O diagnóstico de gestação foi realizado por ultrassonografia 39 ± 10 dias após a IATF. As variáveis foram analisadas pelo procedimento GLIMMIX do programa SAS (P < 0,05). Verificou-se uma TC geral de 42,71%. Não foi detectado efeito dos tratamentos sobre a TC (P = 0,2482), que foi de 36,89% ± 0,19 (47/126) para o grupo controle, 46,08% ± 0,19 (56/123) para GnRH D0, 48,30% ± 0,18 (61/123) para GnRH D10 e de 38,48% ± 0,19 (47/122) para GnRH D0 + D10. Observou-se efeito da variável fazenda, sendo que na Fazenda A a TC foi de 47,32% ± 0,13 e na Fazenda B de 38,24% ± 0,13 (P = 0,0249). Não foi verificado efeito das interações entre fazenda e tratamento (P = 0,7662), nem entre condição ovariana e tratamento (P = 0,1225) sobre a TC. Vacas com folículos < 10 mm apresentaram menor TC (16,44% ± 0,08; P = 0,0001), quando comparadas às vacas com folículos ≥ 10 mm (46,15% ± 0,07) e vacas com CL (48,35% ± 0,07). Conclui-se que a utilização de gonadorelina para sincronização da emergência da onda de crescimento folicular e/ou como indutor da ovulação nos protocolos de IATF não promove incremento na taxa de concepção de vacas da raça Nelore.

**Palavras-chave:** Gonadorelina, IATF, Nelore, prenhez, sincronização

## Introduction

Gonadotropin-releasing hormone (GnRH) plays an important role in bovine reproductive function. This hormone is a decapeptide produced by the hypothalamus, responsible for the release of gonadotropin hormones, such as follicle stimulating (FSH) and luteinizing (LH) hormones, which are directly related to ovarian activity, from the pituitary (HAFEZ; HAFEZ, 2004). There are many synthetic agonists of GnRH, such as lecirelin, fertirelin, buserelin and gonadorelin, available on the market for veterinary therapeutic applications (FERREIRA, 2010).

GnRH administration at the beginning of a timed artificial insemination (TAI) protocol is aimed at promoting the ovulation of a possible dominant follicle present in the ovary, followed by the reduction of estradiol and an increase of FSH concentrations, both responsible for the synchronized recruitment of a new follicular wave (MARTÍNEZ et al., 2000; DISKIN et al., 2002). When GnRH is administrated at the end of a TAI protocol, it can induce a LH pre-ovulatory peak, which leads to ovulation of the dominant follicle developed during hormonal treatment (MARTÍNEZ et al., 2002).

The response to GnRH administration is variable, since the capacity of the follicle to ovulate depends on the stage of estrous cycle that the female is in at the moment of treatment (GEARY et al., 2000; TWAGIRAMUNGU et al., 1995). For this reason, in case of non-existence of a responsive dominant follicle to GnRH in the ovary, ovulation will not occur. According to Geary et al. (2000), around 66% of crossbred (Hereford x Red Angus) lactating beef cows ovulated in response to GnRH and formed new corpus luteum (CL).

The combination of progesterone and estradiol benzoate (EB) it is one of the most common TAI protocols, the action of which is to promote dominant follicle atresia, and consequently, to induce the recruitment of a new follicular wave around 4 days after hormone administration (BÓ et al., 1995; CACCIA; BÓ, 1998). The use of EB and progesterone has been shown to be efficient for females in post-partum anestrous, since these animals do not respond satisfactorily to GnRH when used to provide ovulation synchronization (BARROS; ERENO, 2004). Meneghetti et al. (2005) reported an 89% of synchronization rate in lactating beef cows subjected to a progesterone EB-based TAI protocol.

New follicular wave recruitment is dependent upon ovarian status at the time of treatment. Some cows may not have follicle regression following EB and progesterone administration, and thus the emergence of a new wave does not occur (FERNANDES, 2005), altering the diameter of the dominant follicle, which becomes persistent. Dominant follicle persistence results in a significant reduction of around 35% in the conception rate of beef cows (SANCHEZ et al., 1993). A possible explanation for this low fertility is the fact that a longer follicular dominance period affects oocyte quality, due to early *in vivo* maturation (REVAH; BUTLER, 1996).

Despite the possible failures related to TAI protocols, considering progesterone combined with EB as well as the use of GnRH, the joint administration of these three hormones could be an alternative to resolve these failures and possibly improve ovulation synchronization and, consequently, the conception rate (CR) in Nellore beef cows.

The aim of this study was to evaluate the effects of gonadorelin used both at the outset to synchronize follicular wave recruitment and at the end to synchronize ovulation during a progesterone estradiol benzoate-based TAI protocol on the CR conception rate (CR) in Nellore beef cows.

## Materials and Methods

This study was conducted on two commercial beef farms located in the Vale do Araguaia region, Mato Grosso state, Brazil. Data were collected from February to May of 2014 during the breeding season 2013/2014. A total of 302 lactating cows at 80 ± 40 days post-partum (DPP) and 192 non-lactating multiparous Nellore beef cows were included in the study. The animals were raised in pasture, receiving mineral supplementation and *ad libitum* access to water.

At the TAI protocol outset, an ultrasonography exam (DP-3300Vet®, Mindray) was performed to determine ovarian status, and the females were subsequently categorized as follows: cows with follicle diameters of less than 10 mm (n = 73), cows with follicle diameters of greater then or equal to 10 mm (n = 208), and cows with CL present (n = 213) in at least one of the ovaries. Thus, only cows diagnosed with CL received an intramuscular (IM) injection of 1.0 ml (0.265 mg) of Cloprostenol Sodium ($PGF_{2\alpha}$,Ciosin®, MSD) on Day zero (D0) of the protocol, with the aim of reducing serum progesterone concentration.

The body condition score (BCS) of the cows was also evaluated at the TAI protocol outset, on a scale of 1 to 5 (1 = very skinny and 5 = obese) as proposed by Houghton et al. (1990). Cows with BCS greater than or equal to 2.5 were included in the trial.

Animals were subjected to the following TAI protocol: Day zero (D0) – insertion of a progesterone slow-release intravaginal device ($P_4$, DIB®, MSD) and an IM injection of 2.0 mg (2.0 ml) of Estradiol Benzoate (EB, Gonadiol®, MSD); Day 8 (D8) – intravaginal device withdrawal, an IM injection of 1.5 mL (300 UI) equine chorionic gonadotropin (eCG, Folligon®, MSD), 0.5 ml (1.0 mg) of Estradiol Cipionate (E.C.P. ®, Zoetis) and 1.0 ml (0.265 mg) of $PGF_{2\alpha}$; Day 10 (D10) – TAI. All inseminations were performed by a single technician on both farms. In each group, 30 intravaginal progesterone devices that had previously been used for 8 days

were used (second use), and the remaining devices had previously been used for 16 days (third use).

Cows were randomly assigned to one of four groups: Cows in the control group (n =126, 68 lactating and 58 non-lactating) were submitted to the TAI protocol previously described without GnRH addition; GnRH D0 group cows (n =123, 86 lactating and 37 non-lactating) each received an IM injection of 1.0 ml of GnRH (50 mcg of Gonadorelin; Cystorelin® Merial) on D0 of the TAI; The GnRH D10 group (n =123, 63 lactating and 60 non-lactating) cows each received a GnRH injection on D10 of the TAI simultaneously with insemination; Finally, cows in the GnRH D0 + D10 group (n =122, 85 lactating and 37 non-lactating) were treated with GnRH injection on both days D0 and D10.

Inseminations were performed using semen from five different Abeerden Angus sires, from reputable artificial insemination (AI) centres. Pregnancy diagnosis was performed by ultrasonography 39 ± 10 days post-AI.

Data were analyzed using GLIMMIX procedure of SAS program (SAS, 2003), including in the model the effects of farm, treatment, ovarian status, cow category (lactating or non-lactating), sire, number of previous uses of progesterone devices, and BCS at the beginning of the TAI protocol. The interactions between farm and treatments, as well as between ovarian status and treatments were also evaluated. Statistical differences with levels of $P \leq 0.05$ were considered significant.

## Results and Discussion

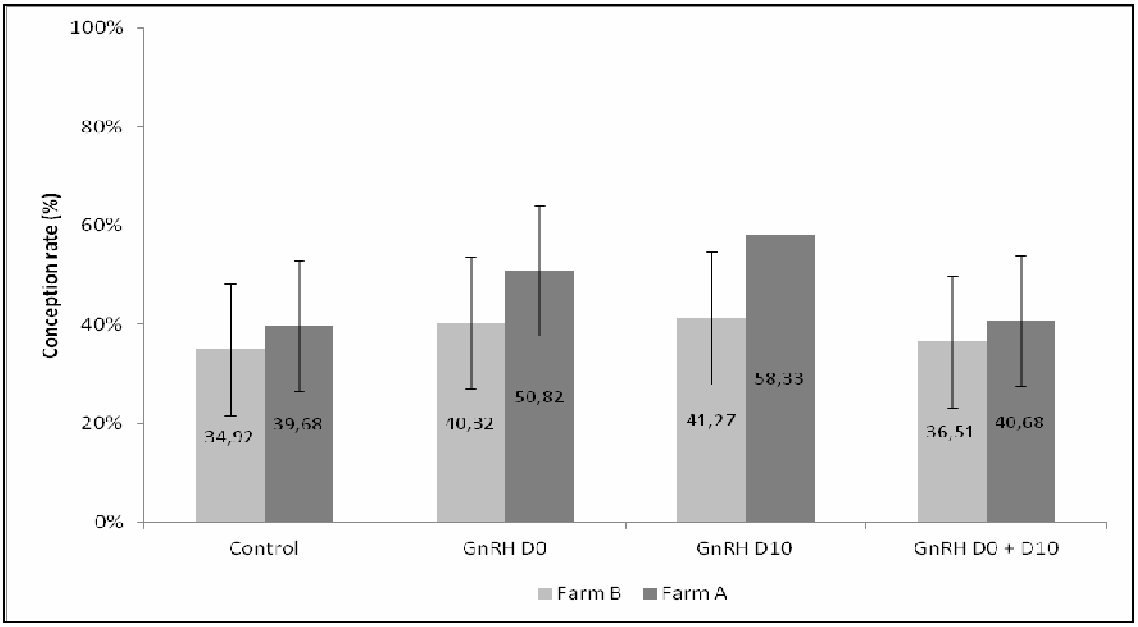
Out of 494 inseminated cows, 211 became pregnant, resulting in an overall CR conception rate (CR) of 42.71%. No effect of treatment on CR was detected ($P = 0.2482$); the CR was 36.89% ± 0.19 (47/126) for the control group, 46.08% ± 0.19 (56/123) for the GnRH D0 group, 48.30% ± 0.18 (61/123) for the GnRH D10 group and 38.48% ± 0.19 (47/122) for the GnRH D0 + D10 group, considering both farms.

Silveira et al. (2011) compared the use of EB or GnRH both associated with a progesterone intravaginal device on D0 of a TAI protocol, suggesting that there was no significant difference between treatments on the pregnancy rate of beef cows at 34 and 90 days post-partum (55% vs. 41%, respectively); results of the present study corroborate these data.

Studies using similar TAI protocols to the one employed in the present study with the aim of evaluating the effects of GnRH at the time of AI indicated that this hormone did not increase pregnancy rates in lactating and non-lactating *Bos indicus* and crossbred beef cows (AYRES et al., 2006; MARTINS et al., 2009; MOESSA et al., 2014; PAVARINA, 2007). However, Silva et al. (2012) obtained an increase in the pregnancy rate of Nellore non-lactating cows using 0.004 mg of Buserelin Acetate at the time of AI. In the present study, the effect of GnRH administration on the CR of lactating and non-lactating cows was not verified, likely because both categories of cow experienced good synchronization rates with the progesterone EB-based TAI protocol, without improvement of the CR by the addition of GnRH.

There was a significant effect of farm on conception, since the CR obtained was 47.32% ± 0.13 (115/243) in Farm A and 38.24% ± 0.13 (96/251; $P = 0.0249$) in Farm B. There was no significant farm x treatment interaction ($P = 0.7662$), indicating that all treatments initiated the same response, regardless of the location in which they were performed (Figure 1). This effect may be explained by the failure of nutritional management practices adopted on one of the evaluated farms. During the rainy season (spring-summer), in the majority area designated for pasture, flooding occurs on the grass, which reduces dry matter availability for the animals, and thus could compromise the reproductive performance of the cows. However, in the present study, it was not possible to verify the effect of the BCS on the CR, since the average BCS at the onset of TAI was 2.84.

**Figure 1.** Conception rate in Nellore beef cows subjected to the TAI protocol, with gonadorelin addition on Day zero (D0) and/or at the time of TAI (D10) on different farms.



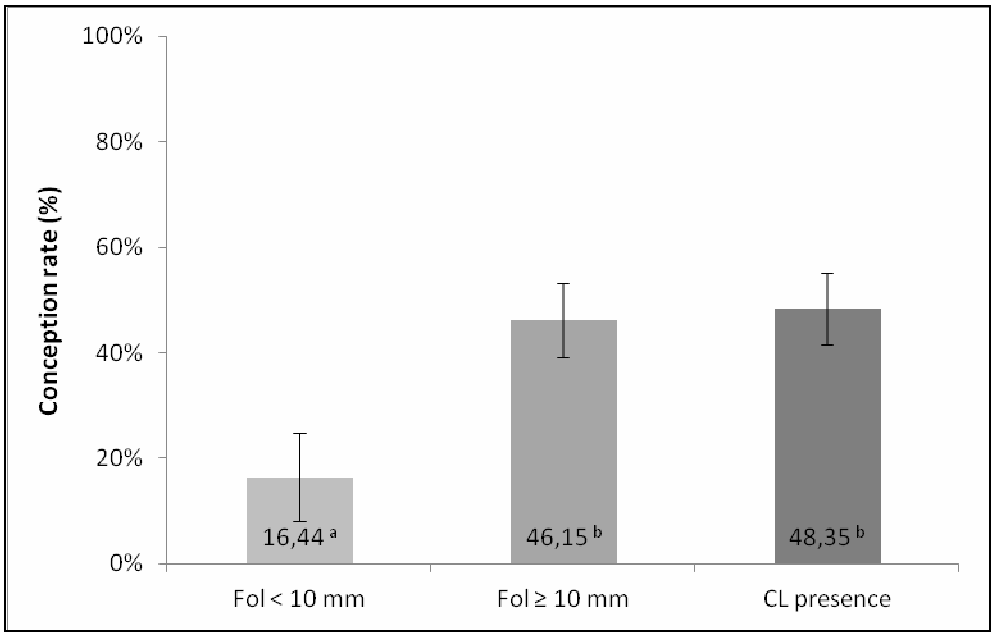

Ereno et al. (2007) reported that BCS in TAI protocols did not interfere with the pregnancy rate in Nellore lactating cows. Nevertheless, Pfeifer et al. (2007) and Toledo et al. (2012) have shown that cows with low BCS also experience low CR, contradicting the results of the present study. The BCS may not have affected the CR, likely because cows with BCS < 2.5 were not included in the trial.

None of the other variables analysed, namely, cow category (lactating or non-lactating), sire, number of previous uses of progesterone devices (previously used for 8 or 16 days) influenced the CR of Nellore cows ($P > 0.05$). Results from other studies showed that cow category and sires had no effect on pregnancy rates in Nellore cows subjected to TAI (ERENO et al., 2007; TOLEDO et al., 2012). Studies on beef cows and heifers treated with progesterone devices (DIB®) previously used for 8 or 16 days found no effects of the number of uses of the devices on pregnancy rate (CUTAIA et al., 2004; CHESTA, 2011).

However, ovarian status at the beginning of the TAI protocol affected the reproductive performance of Nellore cows ($P = 0.0001$). Cows with follicle diameters < 10 mm had lower CR (16.44% ± 0.08) than cows with follicles ≥ 10 mm (46.15% ± 0.07) or those with CL (48.35% ± 0.07) (Figure 2). No interaction between ovarian status and treatment ($P = 0.1225$) was detected. Fernandes et al. (2001) also demonstrated that lactating Nellore cows at 60-90 DPP in the anestrous condition had lower pregnancy rate than cows with CL at the beginning of the TAI protocol (14.9% vs. 46.3%).

One of the factors that can influence the success of TAI is the ciclycity at the outset of the protocol, since cows in estrous have greater responses to hormonal treatment than cows in anestrous (MACHADO et al., 2007). Thus, cows diagnosed with the presence of CL at the beginning of the protocol were able to resume reproductive activity naturally during the post-partum period without the necessity of induction.

**Figure 2.** Conception rate in Nellore beef cows subjected to the TAI protocol according to ovarian status at the beginning of the TAI protocol.




GnRH plays a role in TAI protocols to promote the release of a LH surge and subsequently, ovulation. According to Gimenes et al. (2008) in *Bos indicus* heifers, ovulatory response to hormonal treatment is reached when their follicles are up to 7 mm in diameter. In this way, heifers treated with 25 mg of LH showed varying ovulatory capacities of follicles of varying diameter; follicles > 10 mm displayed higher ovulation rates (90%) than those with diameters of 8.5-10 mm (80%) and 7.0-8.4 mm (33%).

Amaral (2009) reported that Gyr cows in anestrous post-partum with dominant follicles ≥ 10 mm in diameter at progesterone EB-based TAI protocol outset had greater pregnancy rates (59.1%) than cows with follicles < 10 mm in diameter (33.3%).Thus, dominant follicle diameter is positively associated with treatment response.

The combination of progesterone and EB at the beginning of TAI (D0) worked efficiently to synchronize the recruitment of a new follicular wave, since the CR of the control group did not differ from that of the other studied groups. According to these results, GnRH addition to a progesterone EB-based TAI protocol for Nellore beef cows can be dispensed with reducing protocol costs.

Hormonal treatments based on progesterone and estradiol may be used effectively to synchronize the recruitment of a new follicular wave in beef cows (MARTÍNEZ et al., 2000). This is due to the fact that estradiol is able to induce both new follicular wave emergence and the LH pre-ovulatory surge in a low progesterone environment, when used for both proposes in place of GnRH.

In conclusion, the addition of gonadorelin to synchronize follicular recruitment (D0) and/or to synchronize ovulation (D10) in a progesterone estradiol benzoate-based TAI protocol does not improve the conception rate in lactating and non-lactating Nellore beef cows. Additionally, cows with follicles above 10 mm in diameter or with a CL in one of the ovaries at the TAI protocol outset have greater conception rates than cows with follicles below 10 mm in diameter.

## Ethical Committee Approval

This research was conducted according to Ethical Principles in Animal Experimentation, approved by the Committee Ethics in the Use of Animals (CEUA) of the Federal University of Uberlândia (UFU), protocol number 041/14.

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