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Efficiency and accuracy of different ovulation inducers after progesterone device removal in crossbred multiparous cows

Eficiência e acurácia de diferentes indutores de ovulação após a remoção do dispositivo de progesterona em vacas mestiças multíparas

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Abstract
The aim of this study was to verify the efficiency and ovulation time after the administration of different inducers for synchronization of ovulation in beef cows. One hundred and eight non-lactating cows were distributed into the control group (CG; untreated; n=28), estradiol benzoate (EB) group (EBG; n=28); 17 beta-estradiol (17ßE) group (17ßEG; n=28), and deslorelin (DES) group (DESG; n=24). On day minus 11 (D-11) of the protocol, the CG underwent application of cloprostenol and ultrasound examination (US); on D0, progesterone (P4) was inserted plus EB; on D7, cloprostenol was applied; on D9, P4 was removed and cloprostenol plus 400 IU of equine chorionic gonadotropin (eCG) was injected. The EBG was subjected to treatment identical to that of the CG, except on D10, when the cows received EB. The 17ßE was subjected to the same protocol used in the CG except for the administration of 17ßE on D10. And, the DESG was subjected to the same treatment as the CG, except on D10, when the group received DES acetate. Twelve hours after the administration of EB, 17ßE and DES, ovarian US were performed every 6 hours. The preovulatory follicle (POF) diameters measured before ovulation were 19.5; 14.7; 18.7 and 19.8 mm respectively for CG, EBG, 17ßEG and DESG; and the time intervals between inducer application and ovulation were 20.2; 18.9; 21.0 and 22.5 hours respectively. In conclusion, all ovulation inducers were efficient in promoting ovulation; the inducers caused ovulation between 18.9 and 22.5 hours; EB promoted ovulation in a shorter time (P<0.05); 17ßE and DES showed greater variation in application/ovulation time between groups.
Keywords: Ovulation inductors; Deslorelin acetate; Cows; Ovulation synchronization; 17 beta-estradiol.

Resumo
O objetivo do estudo foi verificar a eficiência e a ovulação após a administração de diferentes indutores para a sincronização da ovulação em vacas de corte. Cento e oito vacas não-lactantes foram distribuídas em grupo controle (GC; não tratadas; n=28); grupo benzoato de estradiol (BE) (GBE; n=28); grupo 17 beta-estradiol (17ßE) (G17ßE; n=28) e grupo deslorelina (DES) (GDES; n=24). No dia menos 11 (D-11) do protocolo, o GC recebeu cloprostenol e exame ultrassonográfico (US); ao D0, dispositivo de progesterona (P4) foi inserido mais BE; ao D7, cloprostenol foi aplicado; ao D9, a P4 foi removida e cloprostenol mais 400 UI de gonadotrofina coriônica equina (eCG) foi injetada. O GBE foi submetido a tratamento idêntico ao do GC, exceto ao D10, quando as vacas receberam BE. o G17ßE foi submetido ao mesmo protocolo usado no CG exceto pela administração de 17ßE ao D10. E, o GDES foi submetido ao mesmo tratamento que o CG, exceto ao D10, quando o grupo recebeu o acetato de DES. Doze horas após a administração de BE, 17ßE e DES, US ovarianos foram realizados a cada 6 horas. O diâmetro do folículo pré-ovulatório (FPO) medido antes da ovulação foi de 19,5; 14,7; 18,7 e 19,8 mm respectivamente para GC, GBE, G17ßE e GDES; e o intervalo de tempo entre a aplicação do indutor e ovulação foi 20,2; 18,9; 21;0 e 22,5 horas respectivamente. Em conclusão, todos os indutores da ovulação foram eficientes em promover a ovulação; os indutores acarretaram ovulação entre 18,9 e 22,5 horas; o BE promoveu a ovulação em menor espaço de tempo (P<0,05); 17ßE e DES demonstraram maior variação em aplicação/tempo de ovulação entre os grupos.
Palavras-chave: Indutores da ovulação; Acetato de deslorelina; Vacas; Sincronização da ovulação; 17 beta-estradiol.

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Introduction

Artificial insemination (AI) has become one of the most important techniques in bovine production, promoting the genetic improvement of the herd and optimizing the reproductive management of animals. Advances in conventional AI have supported the development of timed artificial insemination (TAI), evidenced by a 68.7% increase in beef cattle semen sales volume in the last year [1]. The use of hormonal protocols for the synchronization of ovulation allows the implementation of TAI at a predetermined time, excluding the need for estrus detection[1]. TAI aims to increase herd productivity by manipulating mechanisms involved in the reproductive physiology of female bovines[2]. The use of this tool allows anticipation of conception and, consequently, calving, thus concentrating births in a predetermined period[3]; it can be used in cyclic or anestrus cows, regardless of the estrous cycle phase[4]. To maximize reproductive efficiency during TAI, it is necessary to synchronize ovulation. Ovulation inducers are used to enable the execution of AIs at a time closer to ovulation. Hormonal protocols for TAI also aim to induce the emergence of a new wave of follicular growth, control the duration of follicular growth until the preovulatory follicle (POF) development stage, and synchronize insertion and removal of exogenous and endogenous progesterone (P4) sources [5].

Ovulation inducers promote ovulation when gametes are viable and have a critical effect on the POF. Ovulation of the dominant follicle (DF) depends on P4 exogenous source removal (after five to nine days of exposure), performed simultaneously with the application of an ovulation inducer[6]. Analogs (gonadorelin) or superanalogs (buserelin, lecirelin, and fertirelin) of gonadotropin-releasing hormone (GnRH), estradiol esters[7, 8] (estradiol benzoate, EB; and estradiol cypionate, EC), prohibited in the European Community, despite their efficiency and cost-effectiveness[9], human chorionic gonadotropin (hCG), and luteinizing hormone (LH) are some of the drugs used as inductors of ovulation[10]. Deslorelin (DES), a GnRH superagonist, is used in ovulation induction protocols in mares[11] and cows[12, 13]. Several of these hormones participate in the feedback mechanisms of the hypothalamic–hypophysis–gonadal axis; namely, after GnRH release (hypothalamus), the release of FSH occurs, acting on the development of several ovarian follicles and the selection of a DF. This produces estrogen, which acts at the level of the hypothalamus and hypophysis and requires the release of LH, which promotes the final maturation of the DF, ovulation, and corpus luteum (CL) formation [14].

There are four commercial formulations of estradiol (under different molecular constitutions) that can be used in protocols for synchronizing follicular growth and ovulation in cows: EB, estradiol valerate (EV), EC, and 17 beta-estradiol (17ßE), which are synthetic but identical to natural estradiol[15]. The EB has been used to induce ovulation 24 h after P4 removal[16]. A comparative study between the use of EB and EC demonstrated that EB induced ovulation at a shorter interval after P4 removal[17]. Administration of EB 24 h after exogenous P4 removal results in ovulation between 66 and 78 h [18]. Crepaldi et al.[19] evaluated the ovulation rate and pregnancy in TAI protocols, administering EB on day 8.5, concomitantly with the removal of the P4 device, and concluded that the interval between P4 removal and ovulation was lower in this protocol compared to the others, as well as reduced the number of animals handled, without affecting the reproductive efficiency in beef cows.

Kozicki et al.[12] administered deslorelin acetate to crossbred cows to induce ovulation after previous intravaginal P4 treatment, obtaining an ovulation rate of over 22.3 hours. Bartolome et al. [20] used deslorelin in subcutaneous implant form in postpartum cows and verified ovulation induction owing to the development of physiological CL. Oliveira et al.[13] used DES as an ovulation inducer 6 h before and on the day of artificial insemination. They found an increase in the pregnancy rate in the treated groups (53.3% and 43.8%) compared to the control group (40.6%) that had not been treated.

Ovulation inducers have been systematically used in cattle TAI programs[21]. Estradiol benzoate has been used as an ovulation inducer for several years[22]. However, 17ßE (the same group as EB), which is not yet available for cattle in the Brazilian market, may be a promising inducer of ovulation, as well as DES acetate, which is already used in equine reproduction[11]. DES is still not widely used in bovine reproduction[12] and deserves further study on the two drugs (17ßE and DES). EB should be administered in two stages of TAI, the first on the day of insertion of the intravaginal device with P4 (start of the protocol), aiming at follicular wave atresia and favoring the emergence of a new follicular wave, as well as after P4 removal, as an ovulation inducer[22]. In turn, 17ßE can induce ovulation in a shorter period of time because it is an ester that is quickly metabolized in the body [22]. In contrast, deslorelin is considered a potent GnRH agonist capable of inducing ovulation in a shorter period of time than GnRH[12].

The aim of the present study was to verify the efficiency (ovulation rate) and precision (number of hours required) of synchronization of ovulation caused by EB, 17ßE and DES acetate in crossbred beef cows (*Bos taurus taurus* and *Bos taurus indicus*) in TAI protocols.

Material and methods

The present study was conducted in accordance with international standards on animal welfare and breeders' consent (Directive 2010/63/EU of the European Parliament, CEUA-PUCPR number 01742). One hundred and eight non-lactating crossbred cows (*Bos taurus* [Red and Aberdeen angus] × *Bos indicus* [Nellore]), age (26 to 45 months), weighing 360 to 450 kg, were used. The study was located in a beef cattle farm at coordinates 25º25'40"S and 49º16'23"W. The animals stayed in paddocks under an extensive grazing system having *Cynodon dactylon* (crude protein 14.56; FDN 71.58; FDA 31.76; lignin 31.54[23]; Ca 0.48; P 0.53; K 1.73; Mg 0.24(dag/kg); Fe 431.6; Mn 90.04;

Cd 2.76; Pb 20.9 (mg/kg)[24] with a supply of mineral salt for beef cows (FOSBOVI® 40, Zoetis, São Paulo-Brazil); each kg of product contains: calcium 223 g/kg; calcium (max) 260 g/kg; phosphorus (min) 174 g/kg; sulfur (min) 24 g/kg; cobalt (min) 100 mg/kg; copper (min) 1.250 mg/kg; iron (min) 1.795 mg/kg; iodine (min) 90 mg/kg; manganese (min) 2.000 mg/kg; selenium (min) 15 mg/kg; zinc (min) 5270 mg/kg and fluorine (max) 1.740 mg/kg). Water was provided *ad libitum*. At the beginning of the study (breeding season October to December), the cows had a mean body condition score (BCS) of 2.8 (2.5, and 3.5; scale from 1 to 5, where 1 = very thin and 5 = obese [25].

Cows were distributed into the control group (CG; n=28), estradiol benzoate group (EBG; n=28), 17 beta-estradiol group (17ßEG; n=28), and deslorelin group (DESG; n=24). The CG was submitted on day minus 11 (D-11) to the application of prostaglandin (PG) (500µg, cloprostenol; im, Croniben,Biogenesis Bago, Curitiba - Paraná–Brazil) plus ultrasound examination (US) (SonosCape® A6v, L531v 3.5 to 7.5 MHz straight transducer, China) of the ovaries, aiming to verify the ovarian cyclicity (presence of CL in the ovary[26]); on D0, an intravaginal device (1 g of P4, Cronipress,Biogenesis Bago) was inserted plus the application of 2 mg (im) of EB (Bioestrogen, Biogenesis Bago) + US performed; on D7, PG was applied; on D9, the device was removed and PG was again applied, plus 400 IU of equine chorionic gonadotropin (eCG; im, EcEgon, Biogenesis Bago) and ovarian US; 36 h after removal of the intravaginal device, US examinations of the ovaries every 6 h were performed, to quantify the time (h) until the detection of ovulation of the POF.

The EBG was subjected to the same treatment as the CG, except that on D10, the cows received 1 mg (im) of EB; The 17ßEG group was subjected to the same protocol used in the CG except for the administration of 2 mg of 17ßE (im, 17 Beta, Botupharma) on D10; and the DESG group was subjected to the same treatment as the CG except that on D10, it received 1 mg of DES acetate (im, Deslorelina, Botupharma).

Twelve hours after the administration of EB, 17ßE, and DES, US of the ovaries was performed every 6 h to monitor the preovulatory follicle. The diameter of the POF was measured by dividing the largest diameter plus the smallest diameter by 2[27]. The presence of ovulation was considered when the preovulatory follicle on the previous day was not present in the ovary by US visualization of the ovulation site. Figure 1 shows the hormonal protocols used in the respective groups of cows.

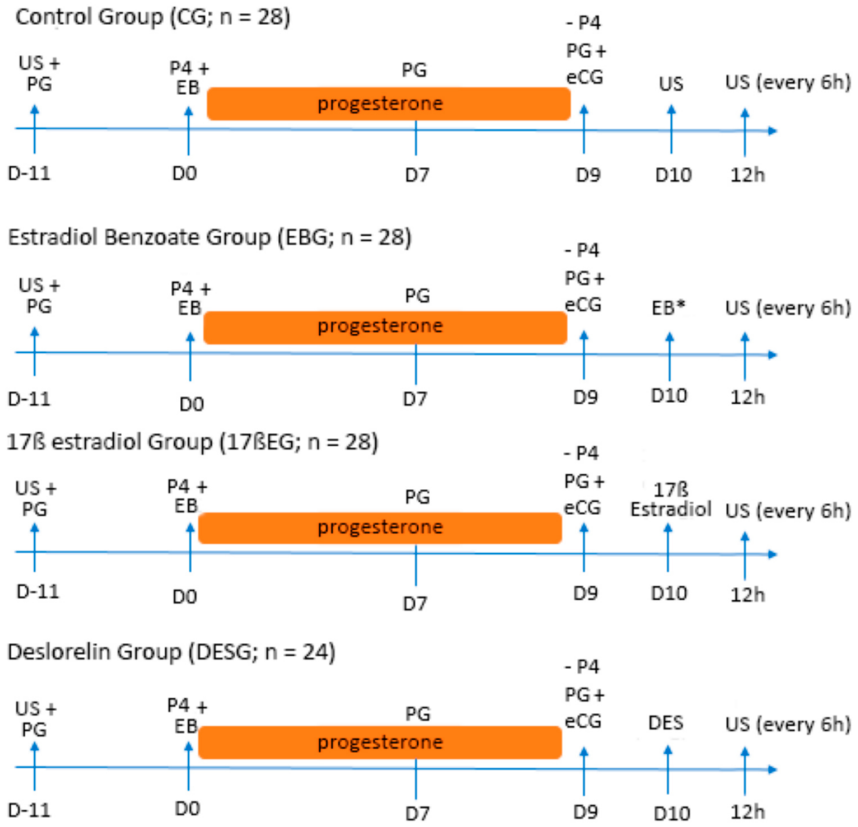

Figure 1. Diagram of the protocols applied to crossbred cows, for synchronization of ovulation. US (ultrasonography); PG (cloprostenol; 500 µg, im, Biogenesis Bago, Curitiba, Paraná, Brazil); P4 (intravaginal progesterone device; 1 g; Biogenesis Bago); EB (estradiol benzoate; 2 mg; EB*=1 mg, im; Biogenesis Bago); eCG (equine chorionic gonadotropin; 400 UI; im; Biogenesis Bago); 17ß estradiol (2 mg; im, Botupharma; Botucatú, São Paulo); DES (deslorelin acetate; 1 mg; im; Botupharma).

Statistical analysis

The differences in the mean dimensions of the POF diameter between the groups, as well as the time interval between the application of the inducer and ovulation, were compared using the Bonferroni test, and the homogeneity of variance was compared using the Levene test. To verify the intragroup and intergroup correlations between the diameter of the POF and the number of hours required for ovulation, the Pearson correlation test was applied. The level of significance was set at $p<0.05$.

Results and discussion

Synchronization of ovulation for TAI has emerged as one of the most advanced reproductive biotechnologies in recent decades[28]. Since Pursley et al.[29] developed a protocol for FTAI in dairy cattle, significant developments have been observed regarding AI, with emphasis on the increase in the volume of commercialized doses of semen in Brazil[1]. This fact was fundamental to the increase in herd fertility rates because new knowledge available in this area improves the possibility of increasing fertility rates. This project aims to provide knowledge to help professionals and breeders who work with TAI in cattle herds. Ovulation inducers, such as those used in this study, can result in increased reproductive performance, therefore, they can be routinely used in FTAI protocols by better predicting ovulation time [8].

To synchronize ovulation, EB, 17ßE and DES acetate were used in the present study. The mean diameter of the POF was significantly different between the 17ßEG, DESG, and CG compared to the EBG ($p=0.0001$), clarifying the variability of the responses of the preovulatory follicles, even in the face of identical treatment applied to the groups up to D9 of the protocol (Table 1). Physiologically, the growth and diameter of POF are related to estradiol concentrations, due to the greater number of granulosa cells, enabling an increase in the conception rate[30], due to the action of the preovulatory LH peak, optimizing the ovulation rate[31].

The study data showed that estradiol benzoate significantly induced ovulation in a shorter period of time than the other groups ($p=0.000$; Table 1). In fact, the POF of animals in the estradiol benzoate group had a lower mean diameter. Hypothetically, these follicles carry fewer granulosa cells because they are smaller in size. Even so, the EBG responded to ovulation induction, 18.0 hours after its administration, even before than that of the groups treated with 17ßE, a synthetic ester with identical characteristics to natural [15], or DES, a GnRH superagonist used in mares[11] or in cows[12, 13]. Intragroup and between-group tests were performed to verify the correlation between POF diameter and the number of hours required for ovulation. Intragroup correlation was observed for the DESG ($p<0.009$) but not for the other groups; GDES presented the highest average POF diameter among the groups (19.8 mm) and required more hours for ovulation (22.5h). EB, even with an average diameter of 14.7 mm, induced ovulation 18.0 hours after application. In this sense, the number of hours between the application of the inducers and the absence of the POF (disregarding the formation of the CL) must be considered, as well as POF monitoring was done every 6 h, providing better accuracy of ovulation time, which resulted in a significant correlation ($p<000.1$) (Table 2).

Table 1. Effects of different ovulation inducers administered to crossbred beef cows, related to preovulatory follicle (POF) dimensions, time required between inducer administration until ovulation and ovulation ovarian side, to ovulation synchronization

Groups of cows (n)	Size of the POF (mm) (x±s)	Time of inducer application to ovulation (hours) (x±s)	Ovulation rate (%)
Control (28)	19.4 ± 1.0^{ab}	20.2 ± 3.0^{b}	100.0
Estradiol benzoate (28)	14.7 ± 2.0^{c}	$18.0 \pm 0,0^{c}$	100.0
17 beta-estradiol (28)	18.7 ± 1.7^{b}	21.0 ± 3.8^{ab}	100.0
Deslorelin (24)	19.8 ± 1.0^{a}	22.5 ± 2.6^{a}	100.0
P value	0.0001	0.0000	

Values with different letters in the same column are considered statistically different according to the P-values above.

Table 2. Correlation values between the diameter of the preovulatory follicle (POF) and the number of hours between the administration of the inducer until the occurrence of ovulation in crossbred cows

Groups	Pearson's correlation	P value
Intragroups		
Control	0.2512	1.973
Estradiol benzoate (not possible, all values are similar)	-	-
17 beta-estradiol	0.1307	0.5075
Deslorelin	0.05213	0.0090
Intergroups	0.4024	0.0001

In contrast to the data from the present study, Cavalieri et al.[32] used zebu–taurine crossbred beef heifers. They observed an interval of 50 h between EB application and ovulation, a similar time to that obtained by Martinez et al.[33], who reported an interval of 53.30 h for ovulation in taurine cows after the use of EB or even 17ßE. Studies by Sales et al.[34], report that ovariectomized Nellore heifers showed the LH peak induced within 19.6 h after EB application. In another

study, Sellars et al.[35] administered estrogen using an FTAI protocol to monitor ovulation in cows. They observed ovulation between 16 and 32 h after administration, and 100% ovulation. These data reinforce those verified in the present study because the ovulations occurred within 24 h in all groups, including animals in the control group. One of the factors that may have contributed to this short time interval for ovulation can be attributed to the fact that the cows in the present study were not suckling, favoring the LH preovulatory surge.

Another factor to be considered is that Sales et al.[34] and Sellars et al.[35] did not use eCG, contrary to the present study, in which 400 IU of eCG was administered. It is possible that in the present study, the eCG administered on day 9 of the protocol influenced the development of preovulatory follicles by binding to FSH and LH receptors, resulting in an increase in the follicle growth rate and a larger POF, increasing the possibility of ovulation[36]. The inclusion of eCG in ovulation synchronization protocols is related to increased follicular growth, which makes them more responsive to ovulation-inducing hormones [16]. With reference to the action and dosage of eCG on the development of the DF, Prata et al.[37] reported the administration of 300 IU of eCG (im) during the final phase of follicle growth in Nelore cows. No differences were observed in the diameter of the largest follicle on days 8 and 10 between the groups (control, eCG, and three hCG groups), but the DF growth rate was higher in the eCG and hCG groups.

Conclusions

It was concluded that all ovulation inducers were efficient to promote the ovulation in cows; the inducers caused ovulation between 18.9 and 22.5 h; EB carried the ovulation in a shorter time after the administration than the others inducers ($P<0.05$); 17ßE and DES showed greater variation in application/ovulation time between groups.

Conflict of interest

The research team (the authors) declare that there are no conflicts of interest related to this study or its publication.

Author Contributions

Investigation: I. S. Ramos, M. Schaus and L. H. Bacher. *Project management*: L.E. Kozicki. *Visualization*: I. da S. Padilha. *Data curation*: M. S. Segui. *Writing (original draft)*: L. E. Kozicki. *Writing (review and editing)*: G. Gassenferth. *Validation*: R. R. Weiss and J. A. Dell'Aqua Junior.

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