

Effect of injectable progesterone on follicular development in lactating beef cows treated with estradiol plus a low-concentration progesterone device

Efeito da progesterona injetável no desenvolvimento folicular em vacas de corte lactantes tratadas com estradiol e dispositivo de progesterona de baixa concentração

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Abstract

The effect of injectable progesterone was evaluated along with estradiol benzoate (EB) on the fate of the dominant follicle (DF) present in the ovary at the beginning of low progesterone-based TAI protocol. All cattle were given 500 µg cloprostenol im (PGF; Schering-Plough Animal Health for Estrumate, Pointe-Claire, QC, Canada) twice, 11 d apart, and allocated into two groups: Estradiol group (E group, n = 11) and Estradiol-Progesterone group (EP group, n = 11). Ten days after the second PGF (Day 0), all cattle were given an intravaginal progesterone device with half progesterone concentration (Cue-Mate with a single pod containing 0.78 g progesterone). Concurrently, all cattle were given 1.5 mg im of estradiol benzoate in 3 mL of canola oil and PGF im on Day 0 of the protocol in a crossover design, in which each cow received both treatments. Cows in the EP group also received 100 mg im progesterone (Sigma) in 2 mL of canola oil. On Day 8, progesterone devices were removed and all cattle were given PGF im. All statistical analyses were performed with SAS 9.0. The DF present on Day 0 ovulated in 76% (16/21) of cows from E group and 28.6% (6/21) of cows from EP group (P = 0.002). After progesterone device removal, the size of ovulatory follicle did not differ between groups (E group, 15.5 ± 0.43 mm vs EP group, 15.8 ± 0.98 mm; P = 0.82). These follicles ovulated in 81.3 ± 3.1 h in E group and 71.0 ± 6.1 h in EP group (P = 0.13). In conclusion, injectable progesterone reduced the proportion of cows that ovulate the dominant follicle present in the ovary at the beginning of estradiol-progesterone-based protocols. However, no difference was detected on time of ovulation after progesterone device removal between groups.

Keywords: Dominant follicle. Beef cattle. Ovulation. Livestock.

Resumo

Foi avaliado o efeito da progesterona injetável e do benzoato de estradiol (BE) no destino do foliculo dominante (FD) presente no ovário no início do protocolo de IATE. Todas as vacas receberam duas injeções de 500 µg de cloprostenol im (PGF; Schering-Plough Animal Health for Estrumate, Pointe-Claire, QC, Canadá) em um intervalo de onze dias e foram alocadas em dois grupos: Estradiol (grupo E, n = 11) e Estradiol-Progesterona (grupo EP, n = 11). Dez dias após a segunda injeção de PGF (Dia 0), elas receberam um implante intravaginal de progesterona com metade da concentração hormonal (Cue-Mate com apenas uma haste contendo 0,78 g de progesterona). Além disso, todas as vacas receberam 1,5 mg im de BE dissolvido em óleo de canola e PGF im no Dia 0 do protocolo, em um delineamento em *crossover* no qual cada vaca recebeu ambos tratamentos. Vacas do grupo EP ainda receberam uma injeção de 100 mg im de progesterona (Sigma) em 2 mL de óleo de canola no Dia 0. No Dia 8, os dispositivos de progesterona foram removidos e todas as vacas receberam PGF im. A análise estatística foi realizada por meio do pacote estatístico SAS 9.0. O FD presente no Dia 0 ovulou em 76% (16/21) das vacas do grupo E e em 28,6% (6/21) das vacas do grupo EP (P = 0,002). Após a remoção do dispositivo de progesterona, o diâmetro do foliculo ovulatório não apresentou qualquer diferença entre os grupos (grupo E, 15,5 ± 0,43 mm; grupo EP, 15,8 ± 0,98 mm; P = 0,82). Esses foliculos ovularam em 81,3 ± 3,1 h no grupo E e em 71,0 ± 6,1 h no grupo EP (P = 0,13). A conclusão obtida foi que o uso de progesterona injetável reduziu a proporção de vacas que ovularam o foliculo dominante presente no ovário no início do protocolo à base de estradiol e progesterona. No entanto, entre os grupos não houve diferença no momento da ovulação após a remoção do dispositivo de progesterona.

Palavras-chave: Foliculo dominante. Gado de corte. Ovulação. Rebanho.

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Received: 15/8/2017
 Approved: 13/1/2018

Introduction

Artificial insemination (AI) is one of the main techniques used worldwide to disseminate desirable genetics characteristics among beef and dairy herds (BO et al., 2016) and the development of timed AI (TAI) protocols contributed to the widespread use of the AI technique. The typical TAI protocol involves the use of an intravaginal progesterone insert to mimic the luteal phase, estradiol benzoate (EB) to synchronize follicular wave emergence, prostaglandin F2a analogues (PGF) to induce luteolysis, and an ovulation inducer that could be either an ester of estradiol (benzoate or cypionate) or a GnRH analogue, such as gonadorelin and buserelin acetate (KIM et al., 2007; SÁ FILHO et al., 2009).

Although the current progesterone–estradiol-based TAI protocol are well established and have achieved acceptable fertility, changes in progesterone concentrations of intravaginal devices may affect fertility of TAI protocols (COLAZO et al., 2004; EL-TARABANY, 2016). Low progesterone-based protocols used to synchronize estrus and ovulation, in which plasma concentrations of progesterone was 1-2 ng/mL in cows have been associated with high LH pulse-frequency (BERGFELD et al., 1996; PFEIFER et al., 2009), the development of persistent follicles (SHAHAM-ALBALANCY et al., 2000) and decreased fertility attributed to premature oocyte maturation (REVAH; BUTLER, 1996; MIHM et al., 1999). In contrast, Pfeifer et al. (2009) and Cerri et al. (2011) reported that low progesterone protocols promote overgrowth of the dominant follicle, which improved corpus luteum (CL) function without negative effects on fertility when timed artificial insemination was used.

Cattle treated with estradiol and high concentrations of progesterone (a progesterone-releasing device in presence of a natural CL) had a new follicular wave emerge 4 days later (BO et al., 1995). However, failure to induce a new follicular wave after the estradiol-progesterone treatment late in the cycle has been reported (KASTELIC et al., 2004). Instead of regression,

the injection of estradiol-17 β induced ovulation of 50% of dominant follicle present at the time of treatment (KASTELIC et al., 2004). Although progesterone-releasing devices have been devised to inhibit estrus, LH surges, and subsequent ovulation (MACMILLAN et al., 1991), elevated plasma progesterone concentrations did not prevent an estradiol-induced LH surge in ovariectomized cows (MARTÍNEZ et al., 2007). These data may suggest that inserts with low progesterone concentrations, which achieve 1-2 ng/mL of plasma concentrations, may have not been enough to block estradiol-induced LH release and, consequently, to inhibit ovulation during hormonal treatment period. Although the effects of progesterone and estradiol on follicular wave dynamics have been intensively studied (BO et al., 1994, 1995, 2000, 2016), the effects of estradiol esters, given at the beginning of protocol, in a low progesterone environment have not been critically examined.

The present investigation evaluated the effect of injectable progesterone along with EB on the fate of the dominant follicle present in the ovary at the beginning of estradiol-low progesterone-based protocol. This study tested the hypothesis that the progesterone injection, given at the beginning of the low-progesterone TAI protocol, will inhibit ovulation of the extant dominant follicle and improve ovarian response in lactating beef cows.

Materials and Methods

The experimental protocols were approved by the University Committee on Animal Care and Supply and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Animals and treatments

This experiment was conducted with 22 suckled beef cows (Hereford and Hereford x Charolais crosses) that were 7 to 14 years of age, 60 to 150 d postpartum, 500 to 650 kg body weight, and maintained at the University of Saskatchewan Goodale Research Farm, near Saskatoon, SK, Canada. The experiment was conducted in two replicates using a crossover design, i.e., each cow received both treatments.

The experimental design and treatments are shown in Figure 1. All cattle were given 500 μ g cloprostenol im (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, QC, Canada) twice, 11 d apart, and allocated into two groups: Estradiol group (E group, n = 11) and Estradiol-Progesterone group (EP group, n = 11). Ten

days after the second PGF (Day 0), corresponding to approximately 5 to 8 d after ovulation, all cattle were given a Cue-Mate vaginal device equipped with one progesterone-releasing pod (containing 0.78 g progesterone) and a second blank pod. Concurrently, all cattle were given 1.5 mg im of estradiol benzoate dissolved in 3 mL of canola oil and PGF im. Cows in the EP group were also administered 100 mg im progesterone (Sigma) dissolved in 2 mL of canola oil.

On Day 8, progesterone devices were removed and all cattle were given PGF im.

Two cows (one from each group) did not ovulate at the end of first replicate and were removed from the remaining experiment; the second replicate was performed with 20 cows.

Treatments in the second replicate (PGF, EB and Cue-Mate insertion) were initiated 6 d after the ovulation that occurred at the end of Replicate 1.

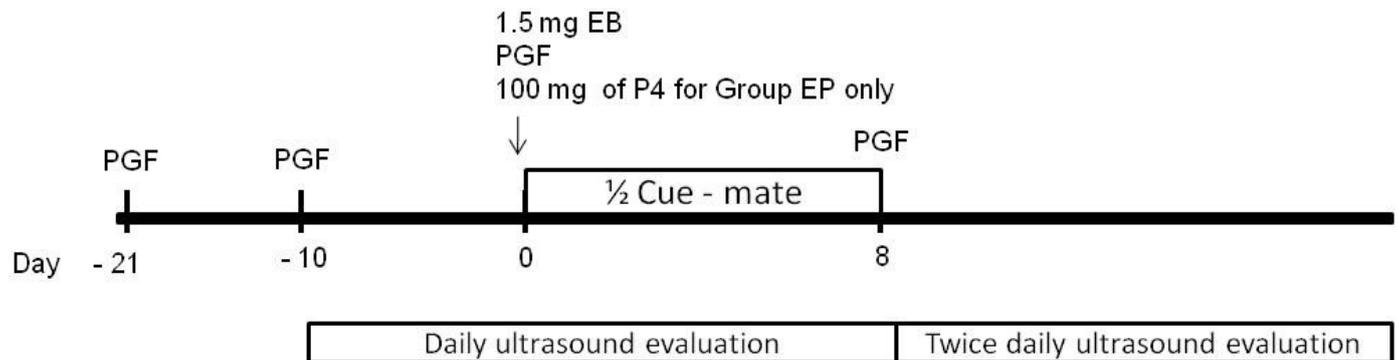


Figure 1 – Experimental design used to induce cows to low-progesterone treatment. To investigate the effect of injectable progesterone, cows in the EP group were given 100 mg of injectable progesterone

Ultrasonographic evaluations and definitions

Before the beginning of the experiment, ovarian function was assessed twice, 11 d apart, by transrectal ultrasonography, and all cows presented a CL in at least one exam. Further ultrasound examinations were performed in all cows once daily to monitor ovarian follicular development from the time of the second PGF injection (Day 10) to progesterone device removal, and then every 12 h until ovulation (both replicates) or until 10 days after progesterone device removal in the absence of ovulation (only two cows in first replicate). The presence of CL on Day 0 and its regression during treatment was monitored to assure that, during progesterone treatments, cows were under low-progesterone environment. At each examination, a sketch of each ovary was made, and the diameter and location of ≥ 3 mm follicles and CL were recorded (GINTHER et al., 1989). Ovulation during the progesterone treatment and after the progesterone device removal was defined as the disappearance of a previously identified follicle ≥ 8 mm in diameter from one ultrasound examination to the next (MARTÍNEZ et al., 2005). The day of follicle wave emergence was defined retrospectively as the day when the dominant follicle was first detected at a diameter of 4 to 5 mm (GINTHER et al., 1989) after Cue-mate insertion.

Persistent follicles were defined as those dominant follicles that were present in the ovary at the time of progesterone device insertion, grew during the treatment period and were present until ovulation or 10 days after progesterone device

removal (in the absence of ovulation). Healthy ovulatory follicles were those that originated from a follicular wave that emerged after progesterone device insertion. Induced follicular wave emergence was expected to occur 4 d after treatment with estradiol (BO et al., 1995) and was defined as the wave that occurred with no evidence of ovulation within first six days of progesterone device treatment. Thus, failure in induction of new follicular wave emergence was defined when the ovulation of the dominant follicle present in the ovary at the beginning of the treatment was detected or when persistent follicle was formed.

The fate of the dominant follicle present in the ovary at the beginning of low-progesterone-based protocol was defined as: 1) regression; 2) ovulation; and 3) become a persistent follicle.

Statistical analyses

All statistical analyses were performed with SAS 9.0 (SAS Institute Inc., Cary, NC, USA). The initial statistical model included the effects of treatment (E vs EP), BCS and replicate. Replicate and BCS had no effect and were excluded from the final model. Single-point measurements (e.g., ovulatory and dominant follicle diameters, and time of ovulation) were analyzed by one-way Anova. Chi-square test was used to examine the effects of treatment on the percentage of cows that developed persistent follicles or had new follicular wave and ovulation rates.

Results

In the first replicate ($n = 11$ per group), one cow from each group did not ovulate after progesterone device removal; thus, these cows were removed from replicate two. Initial analyses indicated that there was no replicate effect; therefore, replicate was removed from further analyses and data were analyzed with 21 cows per group. There was no difference between groups in ovulation rate after progesterone device removal; 95.2% (20/21) for both groups. Ovulatory follicle size did not differ between groups (E group, 15.5 ± 0.43 mm vs EP group, 15.8 ± 0.98 mm; $P = 0.82$). After progesterone device removal, ovulation occurred in 81.3 ± 3.1 h in E group and 71.0 ± 6.1 h ($P = 0.13$) in EP group.

On the basis of the obtained results, for both groups a model is proposed for the three different possible fates of the dominant follicle present in the ovary at the beginning of low-progesterone-based protocol (Figure 2). No difference in the interval from progesterone device removal to ovulation was detected between cows that ovulated following estradiol treatment (Fate 2, 82.0 ± 3.6 h) and cows that had regression of dominant follicle (Fate 1, 77.5 ± 5.1 h).

The dominant follicle present on Day 0 ovulated in 76% (16/21) of cows from E group and 28.6% (6/21) of cows from EP group ($P = 0.002$; Table 1). In contrast, 52.4% and

14.3% of the cows from EP and E Groups had regression of the dominant follicles present in the ovary on Day 0, respectively ($P = 0.02$; Table 1). Disregarding treatment group, one cow ovulated one day, seven ovulated two days and fourteen ovulated three days after progesterone device insertion and estradiol benzoate treatment.

In the present investigation, there was no difference between groups in the proportion of cows that developed persistent follicles (Fate 3), in which the extant dominant follicle continued to grow after E treatment without emergence of a new follicular wave (2 and 4 for E and EP groups, respectively; $P = 0.6$). Thus, disregarding treatment group, the incidence of persistent follicle development was 14% (6/42). However, when cows that ovulated the dominant follicle present on Day 0 were removed from the analyses, 40% (2/5) of cows in E group and 27% (4/15) of cows in EP group developed persistent follicles ($P = 0.28$). All persistent dominant follicles ($n = 6$) ovulated and there was no difference between groups in the time of ovulation of the persistent follicles, 60 ± 6 and 51 ± 5.74 h after progesterone device removal, for E and EP groups, respectively ($P = 0.39$). Persistent follicles were larger than healthy follicles (19.2 ± 1.4 and 14.8 ± 0.5 mm, respectively; $P = 0.003$) and ovulated earlier after progesterone device removal (54.0 ± 4.4 h vs. 80.6 ± 5.2 h; $P = 0.02$).

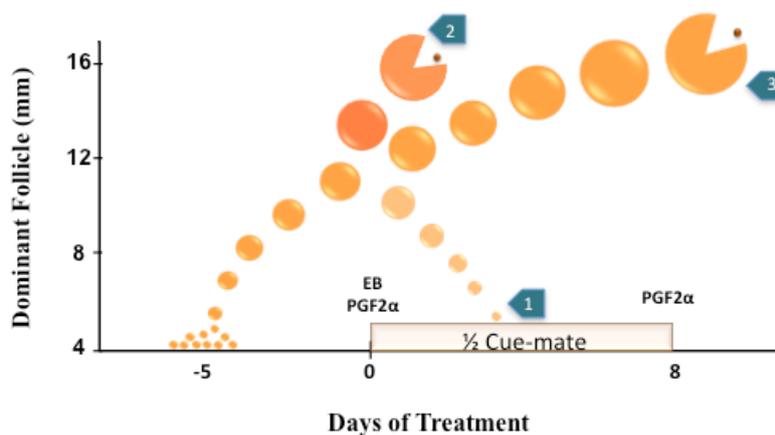


Figure 2 – Fates of dominant follicle present in the ovary at the beginning of low-progesterone treatment in lactating beef cows. Fate 1, Regression; Fate 2, Ovulation; and Fate 3, Become a persistent follicle and ovulation. EB, estradiol benzoate

Table 1 – Proportion of fate of the dominant follicle present in the ovary at the beginning of low-progesterone treatment in lactating beef cows – Saskatoon, SK, Canada – 2009

Fate of the dominant follicle on Day 0*	Group		P-value
	E Group	EP Group	
Regression	14.3 % (3/21)	52.4 % (11/21)	0.02
Ovulation	76.2 % (16/21)	28.6 % (6/21)	0.002
Became persistent	9.5 % (2/21)	19 % (4/21)	0.6

* Dominant follicle present in the ovary at the beginning of the EB-progesterone-based protocol

A dominant follicle was detected in all cows on Day 0. There was no difference in diameter of the dominant follicle at the beginning of progesterone treatment in cows that ovulated (13.4 ± 0.5 mm) or did not ovulate (13.1 ± 0.55 mm; $P = 0.68$). Also, there was no relationship between the diameter of the dominant follicle at this time point and the formation of persistent follicles. The follicles that became persistent were 14.2 ± 0.6 mm at the time of estradiol treatment, while those that regressed or ovulated were 13.1 ± 0.41 mm ($P = 0.31$).

Discussion

The obtained results partially supported the hypothesis that injectable progesterone, given at the beginning of the low-progesterone-based TAI protocol, will inhibit ovulation of the extant dominant follicle. The progesterone injection reduced dramatically the proportion of cows that ovulated during TAI protocol; however, 28% of the cows still ovulated after estradiol benzoate injection. Although progesterone injection reduced the ovulation during TAI protocol, no difference was detected in time of follicular wave emergence and in interval to ovulation after progesterone device removal, regardless of whether cows ovulated after estradiol benzoate injection given on Day 0.

One could speculate that differently from Replicate 1, cows were not treated with two injections of PGF, 11 d apart, in Replicate 2. However, the goal was to induce cows to an early luteal phase with the presence of a healthy dominant follicle at the beginning of a low-progesterone treatment. Thus, in Replicate 1, the hormonal treatment started five to eight days after ovulations and in Replicate 2, same protocol started six days after the ovulations occurred at the end of Replicate 1. Thus, ovarian environment was similar between replicates 1 and 2. The success of the treatments in inducing such hormonal environment was observed in both replicates, since all cows had CL and dominant follicle on Day 0. Moreover, there was no observed difference in any of the variables analyzed between replicates.

Although no blood samples were collected, and no serum LH and progesterone analyses were performed, the hormonal treatment used in the current study provided low plasma progesterone milieu during the growing and preovulatory phases of the dominant follicle as shown elsewhere (PFEIFER et al., 2009). Peripheral concentrations of progesterone (0.1 – 1.0 ng/mL) have been associated with development of persistent follicles (HATLER et al., 2003; ROBINSON et al., 2006). Although

only six cows developed persistent follicles (14%), when cows that ovulated the dominant follicle present on Day 0 were not considered, a high proportion of cows in E group developed persistent follicles (40%). This result provides evidence that injectable progesterone induces regression of dominant follicle. However, further studies on the effect of injectable progesterone on persistent follicles are still necessary to properly answer this research question.

Surprisingly, the high incidence of ovulation following estradiol treatment at the time of progesterone device insertion, in low-progesterone-based protocols, has apparently not been reported previously (CARVALHO et al., 2008; PFEIFER et al., 2009). On the other hand, Kastelic et al. (2004) reported that treatment with E-17 β and progesterone late in the oestrous cycle resulted in ovulation (50%), atresia (33%) or persistence (17%) of the dominant follicle present at the time of treatment. Although a lower proportion of cows ovulated in the EP group, the dominant follicle ovulated up to three days after estradiol treatment in ~30% of the cows. By using this model to study follicular growth in a low-progesterone environment, a proestrous environment was mimicked, wherein progesterone decreases rapidly after the PGF treatment, allowing the final growth and ovulation of the dominant follicle in response to estradiol-induced LH release (MARTÍNEZ et al., 2007). Similar effect was observed in the present study, in which PGF-luteolysis induction on Day 0 associated with the estradiol-induced LH release induced ovulations, on average, 2.6 d after EB treatment. Although no blood samples were collected in this study, it is known that the injection of EB increases LH plasma concentrations by 12-36 h (MARTÍNEZ et al., 2005).

It is not clear what determines whether the follicle ovulates, regresses or persists after estradiol treatment in a low-progesterone environment. Bo et al. (1994) reported that treatment of norgestomet-implanted heifers with 5 mg E-17 β resulted in decreased plasma FSH concentrations by 6 h, with a resurgence of plasma FSH concentrations from 30 to 72 h. The initial decrease in FSH concentrations is probably responsible for regression of FSH-dependent follicles, while it would appear that progesterone might be important in inducing LH-dependent follicles to regress. Apparently, follicle regression does not always occur in a low-progesterone environment (KASTELIC et al., 2004). The high LH pulse frequency may have caused ovulation or continued growth of the dominant follicle preventing its regression. Consequently, in a low-progesterone environment, a dominant follicle present in the ovary at

the time of estradiol treatment is more likely to ovulate or to become persistent rather than regress. In contrast, when the progesterone injection was given, fewer cows ovulated following estradiol treatment, supporting the hypothesis that low progesterone is responsible for the dominant follicle not regressing. The biphasic effect of estradiol on the hypothalamic-pituitary axis (SHORT et al., 1973; SCHOENEMANN et al., 1985; MARTÍNEZ et al., 2005) is likely involved in the mechanism responsible for ovulation in the low-progesterone environment. In ovariectomized cattle, E-17 β initially caused a decline in plasma LH concentrations, followed by an LH surge, approximately 12-24 h later (HAUSLER; MALVIN, 1976; SHORT et al., 1976; SCHOENEMANN et al., 1985; MARTÍNEZ et al., 2005). Cows given E-17 β had a significant increase in LH concentrations, despite concurrent treatment with 100 mg progesterone, whereas cows treated with estradiol benzoate or valerate did not (MARTÍNEZ et al., 2005). The suppression of LH release may depend on the circulating life-span of the estradiol preparation used (MARTÍNEZ et al., 2005). Bo et al. (2000) reported that plasma estradiol concentrations reached 3500 pg/mL by 2 h after an injection of 5 mg E-17 β , while Martínez et al. (2005) reported peak concentrations of 97.7 pg/mL and 64.1 pg/mL estradiol-17 β after injection of 5 mg estradiol benzoate or valerate, respectively, which may explain the failure of a concurrent injection of progesterone to block LH release. Short et al. (1973) also demonstrated the inability of progesterone to inhibit estradiol-17 β -induced LH release; varying doses (ranging from 0.25 to 10 mg) consistently resulted in LH surges in

progesterone-implanted, ovariectomized cows. However, there are no data available regarding the effect of 1.5 mg of estradiol benzoate in cows under a low-progesterone environment. Progesterone in association with estradiol drastically reduced both LH pulse frequency and amplitude (PRICE; WEBB, 1988); however, this effect was apparently not detected in the low-progesterone environment in this study. This result provides evidences that, in addition to estradiol to suppress FSH, the regression of a dominant follicle requires elevated concentrations of progesterone to suppress the LH release.

In conclusion, injectable progesterone reduced the proportion of cows that ovulate the dominant follicle present in the ovary at the beginning of estradiol-progesterone-based protocols. However, no difference was detected on time of ovulation after progesterone device removal between groups.

Acknowledgements

The study was supported by research grants from the Saskatchewan Agriculture Development Fund, Natural Science and Engineering Council of Canada and Westgen and by Embrapa (Project MP2 no. 02.12.01.021.00.00) research grant. The authors thank Bioniche Animal Health for Cue-Mate and Lutropin, and Schering-Plough Animal Health for Estrumate. The authors also thank Drs. Fernanda Caminha, Hayder Al-Shanoon, Irfan Khan, Paula Tribulo and Adriane Zart for help with data collection, and personnel from the Agriculture and Agri-Food Canada, Research Centre, Brandon, MB, and the Goodale Research Farm for providing cattle and technical assistance.

References

- BERGFELD, E. G. M.; KOJIMA, F. N.; CUPP, A. S.; WEHRMAN, M. E.; PETERS, K. E.; MARISCAL, V.; SANCHEZ, T.; KINDER, J. E. Changing dose of progesterone results in sudden changes in frequency of luteinizing hormone pulses and secretion of 17 beta-estradiol in bovine females. **Biology of Reproduction**, v. 54, n. 3, p. 546-553, 1996. doi: 10.1095/biolreprod54.3.546.
- BO, G. A.; ADAMS, G. P.; PIERSON, R. A.; MAPLETOFT, R. J. Exogenous control of follicular wave emergence in cattle. **Theriogenology**, v. 43, n. 1, p. 31-40, 1995. doi: 10.1016/0093-691X(94)00010-R.
- BO, G. A.; ADAMS, G. P.; PIERSON, R. A.; TRÍBULO, H. E.; CACCIA, M.; MAPLETOFT, R. J. Follicular wave dynamics after estradiol-17 β treatment of heifers with or without a progestogen implant. **Theriogenology**, v. 41, n. 8, p. 1555-1569, 1994. doi: 10.1016/0093-691X(94)90821-Y.
- BO, G. A.; BERGFELT, D. R.; BROGLIATTI, G. M.; PIERSON, R. A.; ADAMS, G. P.; MAPLETOFT, R. J. Local versus systemic effects of exogenous estradiol-17 beta on ovarian follicular dynamics in heifers with progestogen implants. **Animal Reproduction Science**, v. 59, n. 3-4, p. 141-157, 2000. doi: 10.1016/S0378-4320(00)00140-8.

- BO, G. A.; MATA, J. J.; BARUSELLI, P. S.; MENCHACA, A. Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. **Theriogenology**, v. 86, n. 1, p. 388-396, 2016. doi: 10.1016/j.theriogenology.2016.04.053.
- CARVALHO, J. B. P.; CARVALHO, N. A. T.; REIS, E. L.; NICHI, M.; SOUZA, A. H.; BARUSELLI, P. S. Effect of early luteolysis in progesterone-based timed AI protocols in *Bos indicus*, *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. **Theriogenology**, v. 69, n. 2, p. 167-175, 2008. doi: 10.1016/j.theriogenology.2007.08.035.
- CERRI, R. L. A.; CHEBEL, R. C.; RIVERA, F.; NARCISO, C. D.; OLIVEIRA, R. A.; THATCHER, W. W.; SANTOS, J. E. P. Concentration of progesterone during the development of the ovulatory follicle: I. Ovarian and embryonic responses. **Journal of Dairy Science**, v. 94, n. 7, p. 3342-3351, 2011. doi: 10.3168/jds.2010-3734.
- COLAZO, M. G.; KASTELIC, J. P.; WHITTAKER, P. R.; GAVAGA, Q. A.; WILDE, R.; MAPLETOFT, R. J. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. **Animal Reproduction Science**, v. 81, n. 1-2, p. 25-34, 2004. doi: 10.1016/j.anireprosci.2003.09.003.
- EL-TARABANY, M. S. The efficiency of new CIDR and once-used CIDR to synchronize ovulation in primiparous and multiparous Holstein cows. **Animal Reproduction Science**, v. 173, p. 29-34, 2016. doi: 10.1016/j.anireprosci.2016.08.006.
- GINTHER, O. J.; KNOPE, L.; KASTELIC, J. P. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. **Journal of Reproduction and Fertility**, v. 87, n. 1, p. 223-230, 1989. doi: 10.1530/jrf.0.0870223.
- HATLER, T. B.; HAYES, S. H.; LARANJA DA FONSECA, L. F.; SILVIA, W. J. Relationship between endogenous progesterone and follicular dynamics in lactating dairy cows with ovarian follicular cysts. **Biology of Reproduction**, v. 69, n. 1, p. 218-223, 2003. doi: 10.1095/biolreprod.102.012179.
- HAUSLER, C. L.; MALVIN, P. V. Interaction of progesterone, GnRH and estradiol in the control of LH release in castrate heifers. **Journal of Animal Science**, v. 42, n. 5, p. 1239-1243, 1976. doi: 10.2527/jas1976.4251239x.
- KASTELIC, J. P.; COLAZO, M. G.; SMALL, J. A.; WARD, D. R.; MAPLETOFT, R. J. 14 ovarian follicular dynamics in cows treated with a CIDR, estradiol and progesterone late in the estrous cycle. **Reproduction Fertility and Development**, v. 16, n. 2, p. 129-130, 2004. doi: 10.1071/RDv16n1Ab14.
- KIM, U. H.; SUH, G. H.; HUR, T. Y.; KANG, S. J.; KANG, H. G.; PARK, S. B.; KIM, H. S.; KIM, I. H. Comparison of two types of CIDR-based timed artificial insemination protocols for repeat breeder dairy cows. **Journal of Reproduction and Development**, v. 53, n. 3, p. 639-645, 2007. doi: 10.1262/jrd.18147.
- MACMILLAN, K. L.; TAUFA, V. K.; BARNES, D. R.; DAY, A. M. Plasma progesterone concentrations in heifers with a new intravaginal device. **Animal Reproduction Science**, v. 26, n. 1-2, p. 25-40, 1991. doi: 10.1016/0378-4320(91)90063-6.
- MARTÍNEZ, M. F.; KASTELIC, J. P.; BO, G. A.; CACCIA, M.; MAPLETOFT, R. J. Effects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. **Animal Reproduction Science**, v. 86, n. 1-2, p. 37-52, 2005. doi: 10.1016/j.anireprosci.2004.06.005.
- MARTÍNEZ, M. F.; KASTELIC, J. P.; COLAZO, M. G.; MAPLETOFT, R. J. Effects of estradiol on gonadotrophin release, estrus and ovulation in CIDR-treated beef cattle. **Domestic Animal Endocrinology**, v. 33, n. 1, p. 77-90, 2007. doi: 10.1016/j.domaniend.2006.04.009.
- MIHM, M.; CURRAN, N.; HYTTEL, P.; KNIGHT, P. G.; BOLAND, M. P.; ROCHE, J. F. Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in heifers. **Journal of Reproduction and Fertility**, v. 116, n. 2, p. 293-304, 1999. doi: 10.1530/jrf.0.1160293.
- PFEIFER, L. F. M.; MAPLETOFT, R. J.; KASTELIC, J. P.; SMALL, J. A.; ADAMS, G. P.; DIONELLO, N. J.; SINGH, J. Effects of low versus physiologic plasma progesterone concentrations on ovarian follicular development

- and fertility in beef cattle. **Theriogenology**, v. 72, n. 9, p. 1237-1250, 2009. doi: 10.1016/j.theriogenology.2009.07.019.
- PRICE, C. A.; WEBB, R. Steroid control of gonadotropin secretion and ovarian function in heifers. **Endocrinology**, v. 122, n. 5, p. 2222-2231, 1988. doi: 10.1210/endo-122-5-2222.
- REVAH, I.; BUTLER, W. R. Prolonged dominance of follicles and reduced viability of bovine oocytes. **Journal of Reproduction and Fertility**, v. 106, n. 1, p. 39-47, 1996. doi: 10.1530/jrf.0.1060039.
- ROBINSON, R. S.; HUNTER, M. G.; MANN, G. E. Supra-basal progesterone concentrations during the follicular phase are associated with development of cystic follicles in dairy cows. **The Veterinary Journal**, v. 172, n. 2, p. 340-346, 2006. doi: 10.1016/j.tvjl.2005.04.004.
- SÁ FILHO, O. G.; MENEGHETTI, M.; PERES, R. F. G.; LAMB, G. C.; VASCONCELOS, J. L. M. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows II: strategies and factors affecting fertility. **Theriogenology**, v. 72, n. 2, p. 210-218, 2009. doi: 10.1016/j.theriogenology.2009.02.008.
- SCHOENEMANN, H. M.; HUMPHREY, W. D.; CROWDER, M. E.; NETT, T. M.; REEVES, J. J. Pituitary luteinizing hormone-releasing hormone receptors in ovariectomized cows after challenge with ovarian steroids. **Biology of Reproduction**, v. 32, n. 3, p. 574-583, 1985. doi: 10.1095/biolreprod32.3.574.
- SHAHAM-ALBALANCY, A.; ROSENBERG, M.; FOLMAN, Y.; GRABER, Y.; MEIDAN, R.; WOLFENSON, D. Two methods of inducing low plasma progesterone concentrations have different effects on dominant follicles in cows. **Journal of Dairy Science**, v. 83, n. 12, p. 2771-2778, 2000. doi: 10.3168/jds.S0022-0302(00)75173-3.
- SHORT, R. E.; BELLOWS, R. A.; CARR, J. B.; STAIGMILLER, R. B.; RANDEL, R. D. Induced or synchronized puberty in heifers. **Journal of Animal Science**, v. 43, n. 6, p. 1254-1258, 1976. doi: 10.2527/jas1976.4361254x.
- SHORT, R. E.; HOWLAND, B. E.; RANDEL, R. D.; CHRISTENSEN, D. S.; BELLOWS, R. A. Induced LH release in spayed cows. **Journal of Animal Science**, v. 37, n. 2, p. 551-557, 1973. doi: 10.2527/jas1973.372551x.