Theriogenology 120 (2018) 56–60

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Different protocols using $PGF_2\alpha$ as ovulation inducer in Nelore cows subjected to estradiol-progesterone timed AI based protocols

Luiz Francisco Machado Pfeifer [a], Walvonvitis Baes Rodrigues [b], Karine Casanova da Silva [c], Natalia Albanese Anache [c], Natália Ávila Castro [d], Eduardo Madeira Castilho [d], Eriklis Nogueira [c, e, f, *]

[a] Embrapa Rondônia, Rodovia BR-364, Km 5,5, Zona Rural Caixa, Postal: 127, CEP: 76815-800, Porto Velho, RO, Brazil
[b] DCR Fundect, Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul, Brazil
[c] Programa de Pós-Graduação em Ciências Veterinárias, UFMS - Universidade Federal de Mato Grosso do Sul, Av. Senador Felinto Muller, 2443, 79070-900 Campo Grande, MS, Brazil
[d] Universidade Federal de Pelotas, Campus Universitário, s/n^0, CEP, 960100-900, Capão do Leão, RS, Brazil
[e] Embrapa Pantanal, Rua 21 de Setembro, 1880, 79320-900, Corumbá, MS, Brazil
[f] Division of Animal Sciences and Department of Obstetrics, Gynecology & Women's Health, University of Missouri, Columbia, MO, 65211, USA

ARTICLE INFO

Article history:
Received 12 February 2018
Received in revised form
25 June 2018
Accepted 29 June 2018
Available online 4 July 2018

Keywords:
Beef
Conception
Gametes
Ovulation

ABSTRACT

The objective of this study was to evaluate the effect of a $PGF_2\alpha$-analogue (PGF) on ovulation and pregnancy rates after timed artificial insemination (TAI) in cattle. In Experiment 1 cows received an intravaginal progesterone-releasing device (CIDR) plus 2 mg im of estradiol benzoate (EB) on Day 0. The CIDR devices were removed on Day 8, and all cows received 150 μg im of d-cloprostenol ($PGF_2\alpha$-analogue), 300 IU of eCG and 1 mg of estradiol cypionate (ECP) im. On Day 9, cows were randomly assigned into two groups: 1) ECP Group (n = 17), that did not receive any further treatment; and 2) ECP-PG Group (n = 14) that were given 150 μg of d-cloprostenol (PGF) as adjuvant stimulus for ovulation. No difference between groups was detected in interval for ovulation (P = 0.5), and in the proportion of cows ovulating (P = 0.09). In Experiment 2, multiparous suckling crossbred Aberdeen Angus cows (n = 260), were treated into two groups, similarly as Experiment 1; ECP group (n = 122), and ECP-PG group (n = 138). All females were TAI on Day 10. The proportion of cows treated with ECP that became pregnant was 54.9% (67/122), and cows treated with ECP plus PGF was 55.1% (76/138; P = 0.9). In Experiment 3, 686 Nelore cows, 40 to 50 days postpartum, were treated as Experiment 1 (ECP group), however, on Day 8 cows were divided into 3 groups: ECP Group (n = 216); PGF-SC Group (n = 228), in which cows did not receive ECP and were given an additional subcutaneous injection of PGF on Day 8; and PGF–IM Group (n = 242), in which cows also did not receive ECP on Day 8 and were given an additional injection of PGF im on Day 9. On Day 10, estrus was evaluated at timed AI (TAI). There was no difference in the diameter of the dominant follicle at CIDR removal and at TAI, and pregnancy per AI among groups (P > 0.05). However, the proportion of cows that displayed estrus between CIDR removal and TAI was higher in ECP group than in PGF-SC and PGF-IM groups (P < 0.001). Cows that displayed estrus has higher P/AI than cows that did not (P = 0.008). In conclusion, these results suggested that intramuscular or subcutaneous injection of $PGF_2\alpha$ could be successfully used to induce ovulation in cattle undergoing TAI, with similar pregnancy rates when compared with ECP. The subcutaneous injection of PGF on the same day of CIDR removal could be an interesting alternative due it reduces cattle management to obtain similar results.

1. Introduction

Prostaglandins affect several pathways of reproduction, such as ovulation, luteal regression, the implantation and maintenance of pregnancy, parturition, and postpartum physiology [1]. The prostanoid prostaglandin F2α (PGF), derived from arachidonic acid, is a

* Corresponding author. Embrapa Pantanal, Rua 21 de Setembro, 1880, 79320-900, Corumbá, MS, Brazil.
E-mail address: luiz.pfeifer@embrapa.br (E. Nogueira).

https://doi.org/10.1016/j.theriogenology.2018.06.030
0093-691X/

biologically potent substance with several applications in the control of reproduction. In cattle, the most common uses are estrus synchronization, regression of persistent CL, and induction of abortion or parturition [2] due to its luteolytic properties. PGF is widely used alone to induce luteolysis and estrus, however, PGF is also used associated to intravaginal progesterone device and estradiol esters in beef cows subjected to timed artificial insemination (TAI) protocols. Previously, intramuscular (im) injection of PGF has also been reported to act as an ovulatory stimulus in prepubertal heifers [3,4]. These studies support the hypothesis that the PGF can induce ovulation of dominant follicle (DF) by an independent mechanism of luteolysis. More recently, im injection of PGF has been successfully used as an ovulation inducer in TAI protocols for beef [5] and dairy cows [6].

In TAI programs, the effect of an extra im dose of PGF given 24 h after the progesterone device removal on hastening luteolysis has been studied [7–10]. Moreover, an extra im PGF injection was also given concurrently with AI [11,12]. Authors suggest that this treatment may affect pregnancy per AI (P/AI) by increasing uterine contractility, thereby enhancing sperm transport. Although the effects of im PGF given at removal, 24 h later or at AI have been studied, to our knowledge, no study was performed to investigate the effect of im PGF as adjuvant for ovulation, or of subcutaneous (sc), as ovulatory stimulus in timed AI beef cows.

Although label indications for use in cattle recommended im injection of PGF, alternative routes have resulted in longer intervals from treatment to estrus [13]. The recommended dose (500 μg of cloprostenol) was equally efficacious when given either im or sc in inducing luteolysis, however sc via presented longer interval from injection to estrus [13,14].

The above mentioned considerations led us to believe that a sc via of PGF injection has similar effect on the P/AI in TAI programs as im via. We tested the hypotheses that an extra dose of PGF will induce more synchronized ovulations, and that the injection of sc PGF, given on CIDR removal, would provide similar P/AI as cows treated with im PGF, given 24 h after CIDR removal. The objectives of this study were to: 1) evaluate the ovulatory response and pregnancy of beef cows treated with an extra dose of PGF in conventional estradiol-progesterone based protocols, and 2) compare via of injection (sc vs. im) of PGF in beef cows subjected to TAI protocols.

2. Material and methods

The Committee for Ethics in Animal Experimentation from the Embrapa approved all of the procedures performed in the experiments described in this manuscript (Protocol F02.2014).

2.1. Experiment 1

This study was performed at the experimental research farm of Embrapa Rondônia (Brazilian Agricultural Research Corporation, Rondônia, Brazil; $08^0 48' 12'' S$, $63^0 50' 56'' W$).

Thirty one non lactating dual purpose crossbred cows (Gyr × Holstein), 3–7 years old, 450–650 kg of body weight, and with a 3–4 of body condition score (BCS; range 1–5 where 1 = emaciated and 5 = obese; [15]) were used. Cows were kept in an outdoor grazing system (*Brachiaria brizantha* pasture) with *ad libitum* access to mineral salt and water.

Cows at random stages of the estrous cycle, received an intravaginal progesterone-releasing device (1.9 g progesterone, CIDR®, Pfizer Animal Health, São Paulo, Brazil) plus 2 mg im of estradiol benzoate (EB; Bioestrogen®, Biogénesis-Bagó, Curitiba, Brazil) on Day 0. The CIDR devices were removed on Day 8, and all cows received 150 μg im of d-cloprostenol ($PGF_2\alpha$-analogue; Croniben®, Biogénesis-Bagó, Curitiba, Brazil), 300 IU of eCG (Novormon®, Syntex, Buenos Aires, Argentina) im, and 1 mg of estradiol cypionate (ECP®, Zoetis, São Paulo, SP) im. On Day 9, cows were randomly assigned into two treatments groups: 1) ECP Group (n = 17), that did not receive any further treatment; and 2) ECP-PG Group (n = 14) that were given 150 μg of d-cloprostenol as adjuvant stimulus for ovulation.

In this experiment no AI procedures were performed and the status of the ovulatory follicle was followed by ultrasonography (SIUI CTS-900, equipped with a 5 MHz linear-array transducer (Guangdong, China)), at 12 h intervals from CIDR removal to ovulation. Ovulation was defined as the disappearance (from one scanning session to the next) of a previously identified follicle greater than or equal to 8 mm in diameter [16].

2.2. Experiment 2

This trial was carried at a commercial farm located in the southern region of Brazil ($32^0 20' 46'' S$ and $52^0 32' 39'' W$).

Multiparous suckling crossbred Aberdeen Angus cows (n = 260), 40 to 50 days postpartum with a body condition score (BCS) ranging from 2.5 to 3.5 (from 1 - emaciated to 5 - obese) were enrolled in this experiment. Cows were treated and separated into 2 groups, similarly as Experiment 1; ECP group (n = 122), in which cows did not receive any further treatment after CIDR removal; and ECP-PG group (n = 138), in which cows were given 150 μg of PGF on Day 9. All females were timed inseminated on Day 10.

Ultrasonic examinations were performed 30 days post-TAI to assess pregnancy status. Visualization of the embryonic vesicle and detection of the embryo were the positive criteria for determining pregnancy.

2.3. Experiment 3

The trial was carried in two commercial farms in center west region of Brazil ($19^0 59' 59.57'' S$ and $55^0 3' 6.05'' W$). The animals were kept in a grazing system (*Brachiaria brizantha*) with *ad libitum* access to mineral salt and water. For this experiment, 686 Nelore cows, 40 to 50 days postpartum with a body condition score (BCS) ranging from 2.5 to 3.5 were enrolled. Animals were treated as Experiment 1, however, on Day 8 cows were divided into 3 groups: ECP Group (n = 216); PGF-SC Group (n = 228), in which cows received an additional subcutaneous (in the neck region) injection of PGF on Day 8; and PGF–IM Group (n = 242), in which cows received an additional injection of PGF im on Day 9. The PGF-SC and PGF-IM groups did not receive ECP at CIDR removal. On Day 8, cows were painted with chalk marker in the sacrocaudal region to identify cows that displayed estrus. On Day 10, estrus was evaluated at TAI, and deemed to have occurred in cows without a tail-head chalk mark.

Semen from two Aberdeen Angus and two Nellore bulls were used for TAI and was equally distributed among treatments.

Ultrasonic examinations were performed at CIDR removal and at TAI in a subset (n = 150), to measure the diameter of the largest DF, and 30 days post-TAI to assess pregnancy status in all cows.

2.4. Statistical analyses

All statistical analyses were performed using the SAS. The statistical model included the effect of treatment, bull, inseminator, BCS, lactation number, and days postpartum. The variables bull, inseminator, lactation number, and days postpartum had no significant effect on the model and were therefore excluded from it. Single-point outcome variables (e.g., time of ovulation, diameter of DF) were analyzed using analysis of variance (PROC GLIMMIX; SAS

Inst. Inc., Cary, NC) and the means were compared among groups using Tukey's post hoc test.

Proportions with dichotomous outcomes (yes or no), such as ovulation, estrus detection and pregnancy rates (P/AI) were analyzed using the Chi-square test. The effect of BCS on the probabilities of P/AI were determined by Logistic regression using PROC LOGISTIC, and curves were created using the coefficients provided by the interactive data analysis from SAS and the formula $y = \exp(x + b)/[1 + \exp(x + b)]$.

The results are expressed as means ± SEM or as percentages. In all analyses, differences were considered significant when $P \leq 0.05$, whereas $0.05 < P \leq 0.10$ was considered tendencies.

3. Results

3.1. Experiment 1

The ovarian responses of the Experiment 1 are summarized in Table 1. No difference between groups was detected in diameter of the ovulatory follicle ($P = 0.9$), in interval for ovulation ($P = 0.5$), in the proportion of cows ovulating ($P = 0.09$). All cows from ECP-PG group ovulated in a 12 h interval (10/10), while in the ECP group 83.3% (10/12) occurred in the same interval ($P = 0.48$).

The distribution and percentage of heifers ovulating after CIDR removal is shown in Fig. 1.

3.2. Experiment 2

No difference in the pregnancy per AI (P/AI) was detected between groups ($P = 0.9$). Pregnancy per AI of cows treated with ECP was 54.9% (67/122), and cows treated with ECP plus PGF was 55.1% (76/138).

3.3. Experiment 3

Ovarian and estrus responses and pregnancy per AI of the Experiment 3 are summarized in Table 2. There was no difference in the diameter of the DF at CIDR removal and at TAI. Similarly, the pregnancy per AI did not differ among groups ($P > 0.05$). However, the proportion of cows that displayed estrus between CIDR removal and TAI was higher in ECP group than in PGF-SC and PGF-IM groups ($P < 0.001$).

The overall occurrence of estrus was 41% (280/686). Cows that displayed estrus had a similar DF at CIDR removal (9.28 vs 8.85 mm, $P = 0.3$) and larger DF at TAI (13.8 vs 12.7 mm, respectively; $P = 0.05$) than cows that did not displayed estrus. Disregarding group, the P/AI of cows that displayed estrus was higher than cows that did not present (Table 3; $P = 0.05$).

The BCS did not differ among groups ($P > 0.05$). However, the probability of pregnancy increased with the increasing BCS ($P = 0.018$; Fig. 2).

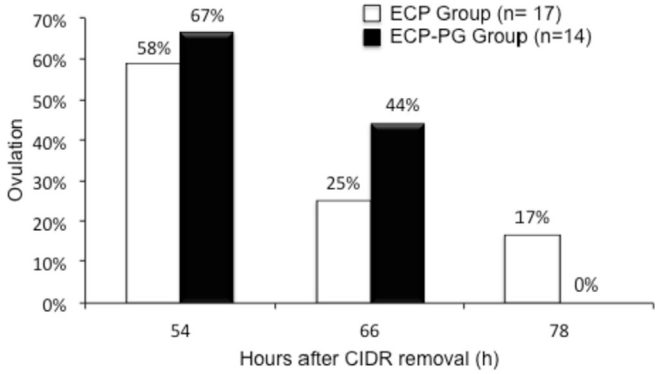

Fig. 1. The percentage of cows ovulating after CIDR removal, and the distribution of ovulation by time in the ECP and ECP-PG groups.

4. Discussion

The results of the current study did not support the hypotheses tested. Although, the interval to ovulation and ovulation rate were not different between treatments in Experiment 1, all cows treated with an extra injection of PGF ovulated in a 12 h interval. This result led us to believe that PGF as adjuvant of ovulation could increase P/AI in TAI programs. However, such synchrony in ovulations did not increase P/AI. Cows treated with sc PGF have similar P/AI of cows treated with im PGF or ECP. However, only the ECP group achieved P/AI (~50%) consistent with what others observed when using a conventional TAI procedure in early postpartum Nelore cows [17–19].

Although the effect of injectable PGF on ovulation in beef cows has already been studied previously [5], the mechanism of action remains unclear. Several studies have attempted to understand how PGF acts on inducing ovulation [20–22]. Previous reports have demonstrated an increase in LH pulse frequency occurring 6 h after treatment with a PGF analogue in anestrous cows [20]. In addition, it has been suggested that PGF2α exerts a direct effect on the anterior pituitary [1], which increases pituitary responsiveness to GnRH and thereby enhances the release of LH [20]. PGF also appears to play a local role in the ovary. Prostaglandins (PGE2 and PGF2α) secreted by the granulosa cells [23,24] was closely linked to the ovulatory process [25]. Our recent data, in which spayed cows were treated with 300 μg of PGF analogue (D-cloprostenol), 10.5 μg of GnRH analogue (Buserelin Acetate) or saline (0.9% of NaCl), demonstrated that only cows treated with GnRH had increasing of plasma LH concentration after treatment (not published data). Moreover, when PGF were used as ovulatory stimulus in dairy cows, it was observed an association between follicle vascularization and the time to ovulation [6]. This data led us to further hypothesize that PGF, acting mainly by a local mechanism, requires a higher developmental status of pre-ovulatory follicles compared to the systemic-mediated action of ECP. Even with this evidence,

Table 1
Ovarian responses of cows treated with ECP or ECP plus Prostaglandin F2α (ECP-PG) to induce ovulation in Experiment 1.

Variable	Group		
	ECP	ECP-PG	P-Value
n	17	14	
Diameter of the ovulatory follicle (mm)	13.6 ± 0.5	13.5 ± 0.7	0.9
Interval to ovulation (h)[a]	61 ± 2.7	59 ± 2.0	0.5
Ovulation rate	70.5% (12/17)	71.5% (10/14)	0.9
Synchronized ovulation rate (54–66 h)[a]	83.3% (10/12)	100% (10/10)	0.48

[a] Time of ovulation after CIDR removal.

Table 2
Diameter of largest follicle, proportion of cows in estrus and pregnancy of cows induced to ovulate with ECP or subcutaneous (PGF-SC) or intramuscular (PGF_IM) injection of prostaglandin in Experiment 3.

Variable	Group				
	ECP	PGF-SC	PGF-IM	SE	P- Value
Diameter of the LF at CIDR removal, mm	8.85	8.89	9.14	0.51	0.58
Diameter of the LF at TAI, mm	13.70	12.82	13.01	0.67	0.39
Proportion of cows that displayed estrus, % (n/total)	58.79[a](*127/216*)	30.26[b](*70/228*)	34.29[b](83/242)	–	<0.001
Pregnancy per AI, % (n/total)	49.07(*106/216*)	41.66(*95/228*)	41.32(*100/242*)	–	0.15

Whithin line, different letters indicate difference among groups ($P < 0.05$).
LF, largest dominant follicle.

whether there is a direct action of exogenous PGF in the ovary remains unclear.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is usually given by intramuscular (im) injection to induce luteolysis in cattle. Once $PGF_{2\alpha}$ is absorbed into the blood, it is almost completely inactivated (by oxidation) after passage through the lungs [26]. Numerous attempts have been made by others researchers to delay the absorption or reduce the metabolism of $PGF_{2\alpha}$ to prolong its action. When sc and im via of PGF injection were compared, im via resulted in shorter interval from treatment to estrus, and both via were equally efficient in the induction of luteolysis [13]. In our experiment, PGF sc at CIDR withdraw, reduces cattle management compared with im injection 24 h later, with similar results in P/AI. These findings are consistent with previous reports, in which PGF administered by iv [27], ischiorectal fossa [14], or behind the shoulder-sc injection [13] did not differ from im injection in luteolytic eficacy. Therefore, sc injection appears to be a viable route for the administration of d-cloprostenol in cows submitted to TAI protocols with less cattle handling and, consequently, less stress.

It is well known that occurrence of estrus between the progesterone source removal and AI positively affect P/AI in TAI protocols. Disregarding treatment, in Experiment 3, cows that displayed estrus were more likely to become pregnant. Cows presenting estrus have larger follicular diameter at intravaginal device removal, larger follicular diameter at TAI, greater ovulation rate, increased subsequent luteal function, and greater P/AI than those that did not display estrus [28]. Moreover, BCS and estrus-cycling status have the greatest influence on the expression of estrus and pregnancy [29,30], as observed in the present experiment, in which cows with higher BCS presents higher pregnancy probability. Similar to the current results, it was reported that increasing BCS during the synchronization protocol enhanced the proportion of Nelore postpartum cows that displayed estrus after the estrus synchronization protocol [28].

The effect of ECP in promoting estrus behaviour was reported previously [20]. When postpartum Nelore cows were induced to ovulate with ECP or GnRH in TAI programs, it was observed that ECP injection increased the proportion of cows displaying estrus after progesterone device removal. Expression of estrus is important,

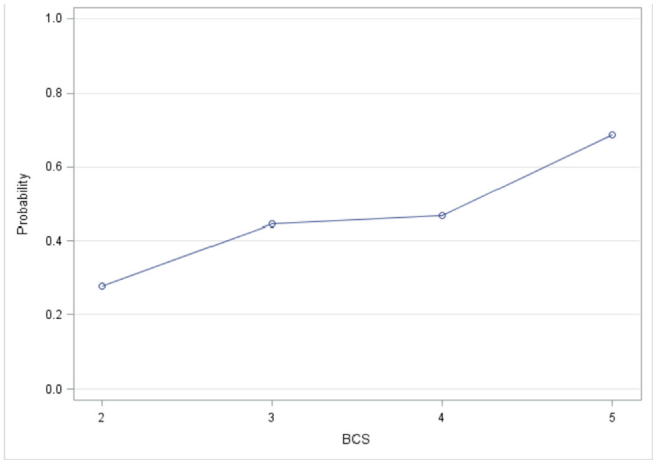

Fig. 2. Pregnancy probability (PAI) according BCS in exp 3 (BCS:1–5; P = 0.018).

and stimulated by increasing concentrations of estradiol at a time when progesterone is low. The preovulatory secretion of estradiol by a DF coordinates a number of physiological processes required for the establishment of pregnancy. Some of these effects occur during the preovulatory period (e.g. estrus expression, induction of the gonadotropin surge that induces ovulation, and sperm transport). In our experiment, groups receiving PGF had less estrous expression compared with ECP; however, albeit not statistically significant, fewer pregnancies (~8%) were detected in PGF treated cows than ECP-treated cows.

One may question the use of dual-purpose (*Bos indicus* x *Bos taurus*) cows in Experiment 1, *Bos taurus* cows in Experiment 2 and *Bos indicus* in Experiment 3. However, as reported by Santos et al. (2001) [31], several studies have observed that the diameter of POF and the time of ovulation of dual-purpose Girolando cows (*Bos indicus* x *Bos Taurus*) are similar to those observed for *Bos taurus* [32–34] and *Bos indicus* [17,35]. Thus, it is assumed that the model used in Experiment 1 to determine the time of ovulation and ovulation rate was proper.

Table 3
Pregnancy per AI (%) according to estrus observed in cows induced to ovulate with ECP, subcutaneous (PGF-SC) or intramuscular (PGF-IM) injection of prostaglandin in Experiment 3.

Group	Estrus		P- value
	No -% (pregnant/non-estrus)	Yes-% (pregnant/estrus)	
ECP	41.5 (*37/89*)	54.3 (*69/127*)	0.09
PG-SC	38.3 (*61/159*)	49.3 (*34/70*)	0.1
PG-IM	38.3 (*61/159*)	47 (*39/83*)	0.19
Total	39 (*159/407*)	50.9 (*142/279*)	0.05

Estrus-evaluate at TAI with aid of tail chalk.

5. Conclusion

These results suggested that an extra injection of PGF did not increase pregnancy per AI in timed AI postpartum beef cows. However, intramuscular or subcutaneous injection of $PGF_{2\alpha}$ could be successfully used to induce ovulation in cattle undergoing TAI, with similar pregnancy rates when compared with ECP. Less corral management when subcutaneous injection of PGF at CIDR removal is used could be an effective alternative to TAI programs.

Acknowledgment

The authors thank to FUNDECT and CNPq for the financial support (23/200.166/2014). This research was also supported by grants from EMBRAPA (Project MP2 n 02.12.01.021.00.00). The authors thank to Cristopher T Cardoso for help in editing the document, and the farms Alvorada, Primavera and 4 Irmãos for providing animals that were enrolled in these experiments.

References

[1] Weems CW, Weems YS, Randel RD. Prostaglandins and reproduction in female farm animals. Vet J 2006;171:206–28.
[2] Gabriel HG, Wallenhorst S, Dietrich E, Holtz W. The effect of prostaglandin F(2alpha) administration at the time of insemination on the pregnancy rate of dairy cows. Anim Reprod Sci 2011;123:1–4.
[3] Pfeifer LF, Siqueira LG, Mapletoft RJ, Kastelic JP, Adams GP, Colazo MG, et al. Effects of exogenous progesterone and cloprostenol on ovarian follicular development and first ovulation in prepubertal heifers. Theriogenology 2009;72:1054–64.
[4] Leonardi CE, Pfeifer LF, Rubin MI, Singh J, Mapletoft RJ, Pessoa GA, et al. Prostaglandin F2alpha promotes ovulation in prepubertal heifers. Theriogenology 2012;78:1578–82.
[5] Pfeifer LF, Leonardi CE, Castro NA, Viana JH, Siqueira LG, Castilho EM, et al. The use of PGF2alpha as ovulatory stimulus for timed artificial insemination in cattle. Theriogenology 2014;81:689–95.
[6] Pfeifer LFM, Siqueira LGB, Arashiro EKN, Castro NÁd, Viana JHM. Prostaglandin F2α or estradiol benzoate to induce ovulation in timed artificially inseminated dairy cows. Pesqui Agropecuária Bras 2016;51:738–44.
[7] Bisinotto RS, Ribeiro ES, Martins LT, Marsola RS, Greco LF, Favoreto MG, et al. Effect of interval between induction of ovulation and artificial insemination (AI) and supplemental progesterone for resynchronization on fertility of dairy cows subjected to a 5-d timed AI program. J Dairy Sci 2010;93:5798–808.
[8] Santos JE, Narciso CD, Rivera F, Thatcher WW, Chebel RC. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. J Dairy Sci 2010;93:2976–88.
[9] Lima FS, Ribeiro ES, Bisinotto RS, Greco LF, Martinez N, Amstalden M, et al. Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers. J Dairy Sci 2013;96:7054–65.
[10] Garcia-Ispierto I, Lopez-Gatius F. Effects of different five-day progesterone-based fixed-time AI protocols on follicular/luteal dynamics and fertility in dairy cows. J Reprod Dev 2014;60:426–32.
[11] Neglia G, Natale A, Esposito G, Salzillo F, Adinolfi L, Campanile G, et al. Effect of prostaglandin F2alpha at the time of AI on progesterone levels and pregnancy rate in synchronized Italian Mediterranean buffaloes. Theriogenology 2008;69:953–60.
[12] Ambrose DJ, Gobikrushanth M, Zuidhof S, Kastelic JP. Low-dose natural prostaglandin F2alpha (dinoprost) at timed insemination improves conception rate in dairy cattle. Theriogenology 2015;83:529–34.
[13] Colazo MG, Martinez MF, Kastelic JP, Mapletoft RJ. Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. Anim Reprod Sci 2002;72:47–62.
[14] Colazo MG, Martinez MF, Kastelic JP, Mapletoft RJ, Carruthers TD. The ischiorectal fossa: an alternative route for the administration of prostaglandin in cattle. Can Vet J 2002;43:535–41.
[15] Lowman B, Scott N, Somerville S. Condition scoring of cattle, revised edition. 1976. p. 6. East of Scotland College of Agriculture. Bulletin no.
[16] Martinez MF, Kastelic JP, Bo GA, Caccia M, Mapletoft RJ. Effects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. Anim Reprod Sci 2005;86:37–52.
[17] Baruselli P, Reis E, Marques M, Nasser L, Bó G. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim Reprod Sci 2004;82:479–86.
[18] Meneghetti M, Sá Filho O, Peres R, Lamb G, Vasconcelos JLM. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: basis for development of protocols. Theriogenology 2009;72:179–89.
[19] Sá Filho MFd, Crespilho A, Santos J, Perry G, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Anim Reprod Sci 2010;120:23–30.
[20] Randel R, Lammoglia M, Lewis A, Neuendorff D, Guthrie M. Exogenous PGF2α enhanced GnRH-induced LH release in postpartum cows. Theriogenology 1996;45:643–54.
[21] Naor Z, Jabbour HN, Naidich M, Pawson AJ, Morgan K, Battersby S, et al. Reciprocal cross talk between gonadotropin-releasing hormone (GnRH) and prostaglandin receptors regulates GnRH receptor expression and differential gonadotropin secretion. Mol Endocrinol 2007;21:524–37.
[22] Fortune J, Willis E, Bridges P, Yang C. The periovulatory period in cattle: progesterone, prostaglandins, oxytocin and ADAMTS proteases. Anim Reprod 2009;6:60. Colegio Brasileiro de Reproducao Animal.
[23] Evans G, Dobias M, King G, Armstrong D. Production of prostaglandins by porcine preovulatory follicular tissues and their roles in intrafollicular function. Biol Reprod 1983;28:322–8.
[24] Bridges P, Fortune J. Progesterone mediates gonadotrophin induced secretion of prostaglandins E and F2a (PGE, PGF) by follicular cells from bovine preovulatory follicles. Biol Reprod 2003;68:47.
[25] Murdoch W, Peterson T, Van Kirk E, Vincent D, Inskeep E. Interactive roles of progesterone, prostaglandins, and collagenase in the ovulatory mechanism of the Ewe. Biol Reprod 1986;35:1187–94.
[26] Samuelsson B, Granstrom E, Green K, Hamberg M. Metabolism of prostaglandins. Ann NY Acad Sci 1971;180:8–163.
[27] Stevens R, Seguin B, Momont H. Evaluation of the effects of route of administration of cloprostenol on synchronization of estrus in diestrous dairy cattle. J Am Vet Med Assoc 1995;207:214–6.
[28] Sá Filho M, Santos J, Ferreira R, Sales J, Baruselli P. Importance of estrus on pregnancy per insemination in suckled Bos indicus cows submitted to estradiol/progesterone-based timed insemination protocols. Theriogenology 2011;76:455–63.
[29] Richardson BN, Hill SL, Stevenson JS, Djira GD, Perry GA. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim Reprod Sci 2016;166:133–40.
[30] Silva K, Rodrigues W, Silva A, Borges J, Abreu U, DdN Batista, et al. Heat score accessed with aid of tail-chalk influences pregnancy rate of suckled Bos indicus beef cows subjected to P4/estradiol TAI-based protocols. Anim Reprod 2016;13:451.
[31] Santos AS, Oliveira MAL, Caldas JGL, Lima PF, Donato IV. Ovarian follicular dynamics of five-eighths Girolando cows. Reprod Domest Anim 2001;36:207–10.
[32] Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod 1988;39:308–17.
[33] Adams GP, Kot K, Ginther OJ. Selection of a dominant follicle and suppression of follicular growth in heifers. Anim Reprod Sci 1993;30:259–71.
[34] Rivera GM, Goni CG, Chaves MA, Ferrero SB, Bo GA. Ovarian follicular wave synchronization and induction of ovulation in postpartum beef cows. Theriogenology 1998;49:1365–75.
[35] Souza AH, Viechnieski S, Lima FA, Silva FF, Araujo R, Bo GA, Wiltbank MC, Baruselli PS. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. Theriogenology 2009;72:10–21.