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Comunication

[*Comunicação*]

Field comparison of GnRH and PGF2α treatments in cows not expressing estrus at timed-AI

[*Comparação a campo entre GnRH e PGF2α no momento da IATF, em vacas sem expressão de cio*]

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Timed artificial insemination (timed-AI) programs have been used worldwide as reproductive management tools because it provides an organized approach to enhance the use of AI and to improve reproductive efficiency for beef and dairy cattle (Pursley *et al*., 1995; Bó *et al*., 2003; Baruselli *et al*., 2004; Vasconcelos *et al*., 2018). An important factor for high ovulation and pregnancy rates (PR) in timed-AI protocols is the size of dominant follicle (DF) and/or the intensity of estrus behavior (Perry *et al*., 2007; Sa Filho *et al*., 2010b, 2011; Pugliesi *et al*., 2016) regarding the fact that expression of estrus during timed-AI has been positively associated with increased fertility (Pereira *et al*., 2016; Pohler *et al*., 2016; Rodrigues *et al*., 2018).

The GnRH has been successfully used to improve the fertility of cows not expressing estrus (and/or in animals with smaller follicles) at timed-AI by increasing ovulatory rates, ovulatory synchrony and pregnancy rates (Sa Filho *et al*., 2010a; Rodrigues *et al*., 2019). However, it is worth mentioning that the administration of GnRH at the time of AI can induce the ovulation of smaller follicles and, therefore, the formation of smaller corpus luteum (CL), which can reduce the ability of pregnancy maintenance (Vasconcelos *et al*., 2001; Busch *et al*., 2008; Perry & Perry, 2009; Bishop *et al*., 2017).

Recently, our research group demonstrated that PGF2α (12.5mg of Dinoprost) administered at the time of AI tended to increase the pregnancy success of suckled Nelore cows with reduced estrus behavior at timed-AI in an estradiol (E2)/progesterone(P4) based protocol. These results were observed both in cows that received conventional or sexed-sorted semen (Diniz *et al*., 2021). Moreover, other authors have demonstrated several benefits of additional doses of PGF2α in Timed-AI protocols which do not appear to be solely related to the induction of luteolysis (Pereira *et al*., 2015; Noronha *et al*., 2020). It is believed some influence of this treatment also on hypothalamic-pituitary axis, ovulatory process (Sirois 1994, Randel *et al*., 1996; Leonardi *et al*., 2012; Pfeifer *et al*., 2014) and, eventually, on the uterine response (Zhang *et al*., 2017; Gao *et al*., 2018; Fu *et al*., 2020). However, the effectiveness of these two treatments (GnRH or PGF2α at the time of AI) on the fertility of cows not expressing estrus has not yet been compared. Therefore, the present work aimed to analyze the field data of a commercial beef farm in order to compare conception rates (CR) and pregnancy loss (PL) of Nelore cows not expressing estrus that received PGF2a or GnRH at the time AI in the same reproductive program.

The commercial beef farm where this data was obtained is located in Cumaru do Norte

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(equatorial climate), in the state of Pará, Brazil (latitude 07º49'30" S, longitude 50º46'22" W). In the farm, all females are kept on the same pasture of Braquiaria brizantha and receive free access to mineralized salt and water.

The present study was performed using the reproductive data of cows submitted to the same timed-AI protocol, during the reproductive program of the referred commercial farm (February 2021 to March 2022). Hence, data from 1347 suckled, multiparous Nelore (Bos indicus) cows, ranging from 30 to 40 d postpartum (DPP) were analyzed.

In the reproductive program of this farm, cows are allocated into breeding groups of approximately 140 cows per group. The groups of cows are allocated according to calving date following the general management practices of the farm. All cows adhered to the same timed-AI protocol. On day 0 (D0), cows received 2 mg (IM) of estradiol benzoate (Sincrodiol®; Ouro Fino, Cravinhos, SP, Brazil) and an intravaginal P4 releasing device (Sincrogest®; Ouro Fino) was inserted. On day 8 (D8), P4 device was removed and 1.0 mg (IM) of estradiol cypionate (SincroCP®; Ouro Fino), 300 IU (IM) of eCG (SincroeCG®; Ouro Fino), and 526 µg (IM) of cloprostenol (Sincrocio®; Ouro Fino) were administered. Additionally, on D8, all animals were marked with a stick at the base of the tail for the detection of estrus. At 48 h after P4 device removal (D10), estrus behavior was determined and the timed-AI was performed with commercial semen from seven different sires (randomly distributed), by two experienced AI technicians (inseminators alternated every 30 cows inseminated).

The manifestation of estrus was verified by the use of a marker stick at the base of the tail and visualization of the total or partial loss of the marking on the day of the timed-AI. Cows with the stick paint removed were considered as "estrus cows" and did not receive any additional treatment on the day of timed-AI. On the other hand, cows not expressing estrus at the time of AI (cows with the presence of stick paint at the base of the tail) received one of the three different treatments, as described below: Upon entering the chute for AI, the cows not expressing estrus randomly received 12.5 mg (IM) of Dinoprost (PGF2α analogue, dinoprost tromethamine; Lutalyse®; PGF treatment), or 100 mcg (IM) of GnRH (GnRH analogue, buserelin acetate, Sincroforte®; Ouro Fino, Cravinhos, SP, Brazil; GnRH treatment) or no treatment at all (Saline treatment).
As is customary on the farm practice, the Body Condition Score (BCS) of each cow was also recorded at the time of AI, using a 1 to 5 scale [1 = severely emaciated, 5 = obese; Houghton *et al*., 1990).

For the assessment of conception rates (CR; Pregnancy/AI), the pregnancy status was verified on D70 (60 days after timed-AI) by detecting a viable conceptus using transrectal ultrasonography (5.0-MHz transducer; 500 V, Aloka, Wallingford, USA).

For the assessment of pregnancy losses (PL), the pregnancy status was confirmed on D210 by transrectal palpation of the reproductive tract for pregnancy determination.

The data of CR and PL were analyzed as a binary response variable using logistic regression by from Minitab Software (version 21.4.1.0). Variables considered in the model were BCS, bull, AI technician, estrus behavior, treatment for cows not expressing estrus (Saline, GnRH or PGF treatment), and their interactions. Significant difference was considered when $P < 0.05$.

The overall CR in the present reproductive program was 50.3% (678/1347), being 51.6% (545/1057) for cows with estrus behavior and 45.9% (133/290) for animals not expressing estrus at timed-AI. No effect of Bull (P=0.1214) and no effect of AI technician (P=0.2637) were detected, whereas BCS ($P<0.0001$) and estrus behavior ($P = 0.0427$) were factors affecting CR. Regarding to pregnancy losses, overall PL was 6.2% (42/678), being 3.3% (18/545) for animals with estrus behavior and 18.1% (24/133) for animals not expressing estrus ($P < 0.0001$) at timed-AI (Table 1).

Table 1. Field data of a timed-AI program (Estradiol/Progesterone-based timed-AI protocol) of suckled, multiparous Nelore (Bos indicus) cows, ranging from 30 to 40 d postpartum (DPP) in a commercial beef farm

Variables	BCS	CR (60 days after timed-AI)	PL (CR on D210 - D70)
Cows expressing estrus	3.22 ± 0.22 a	51.6% (545/1057) a	3.3% (18/545) a
Cows not expressing estrus	2.98 ± 0.21 b	45.9% (133/290) b	18.1% (24/133) b
P value	< 0.0001	0.0427	< 0.0001

a,b: different letters in the same column indicate P<0.05
BCS: body condition score

The positive effect of estrus expression on CR observed in this study has been previously reported (Perry *et al*., 2005; Thomas *et al*., 2014; Richardson *et al*., 2016; Rodrigues *et al*., 2018). For instance, Sá Filho *et al*. (2010a) reported increased CR in Nelore cows with estrus behavior at timed-AI (58.5% vs. 32.1%), and Cedeño *et al*. (2021) reported an overall increase in pregnancies per AI in bovine females expressing estrus compared to animals not expressing estrus at timed-AI.

Several benefits, including improved ovarian function and early pregnancy development are associated with estrus expression (Cooke *et al*., 2019). The presence of a dominant and mature follicle on the day of AI and its ability to release high levels of estradiol are strong indicators of successful AI for dairy and beef cows (Lopes *et al*., 2007; Bridges *et al*., 2012; Pereira *et al*., 2016). High levels of circulating estrogen (commonly found in cows expressing estrus) induces the pituitary LH surge to promotes ovulation (Taft *et al*., 1996; Kinder *et al*., 1996; Inskeep *et al*., 2002). Additionally, it benefits endometrial glands proliferation (Gray *et al*., 2001; Forde *et al*., 2014), sperm transport (Hawk, 1983) and fertility capacity (Suarez and Pacey, 2006). Moreover, larger dominant follicles are commonly associated with larger CLs presenting increased capacity of P4 production (Rodrigues *et al*., 2018; Cooke *et al*., 2019; Lonergan and Sánchez, 2020), improved composition of the uterine luminal fluid, improved capacity for the development, elongation and interferon production of the conceptus, higher conception rates, and increased pregnancy success and gestational maintenance (Forde *et al*., 2014; Davooid *et al*., 2016; Rodrigues *et al*., 2018). Therefore, the benefits of estrus expression may have contributed to the reduced pregnancy loss (from days 60 to 210) observed in the group of cows expressing estrus behavior (3.3%) compared to cows not expressing estrus (18.1%; Table 1). Although recent studies in beef cows showed no correlations between estrus behavior and embryonic loss from 30 to 60 days of gestation (Franco *et al*., 2018, 2020), Pereira *et al*. (2015) had already demonstrated decreased embryonic mortality in lactating dairy cows expressing estrus at timed-AI and Pohler *et al*. (2016) published interesting results of positive effects from estrus expression on pregnancy maintenance. The authors demonstrated that estrus behavior at Timed-AI is associated with higher concentrations of circulating Pregnancy Associated Proteins (PAGs) and lower probability of pregnancy loss (Pohler *et al*., 2016).

The early embryonic loss takes place during initial embryonic development such as fertilization, maternal-fetal recognition, trophoblast expansion, embryo implantation and initial placentoma formation (Schlafer *et al*., 2000; Reese *et al*., 2020; Filho *et al*., 2020). Franco *et al*. (2020) reported a 5.5% gestational loss during days 24 to 31 of gestation but, according to Reese *et al*. (2019), an average of 32% of gestational loss occurred between fertilization and day 16, while 15% occurred between days 16 and 32. On the other hand, late embryonic and early fetal loss are reported at an average rate of 5.1% (Reese *et al*., 2020) and 6.7% (Franco *et al*., 2020) between 30 and 60 days and 6.2% between days 30 and 100 of gestation (Franco *et al*., 2018). Although it remains unclear, poor embryonic development and inadequate placenta formation may contribute to gestational loss during this period. Additionally, paternal genetics may also play a critical role in placenta formation, particularly in later stages of embryonic development (Pohler *et al*., 2016; Franco *et al*., 2020). Lastly, late gestational loss, evaluated in the present study, may be associated with infectious or non-infectious causes (Mee *et al*., 2023) and, in general, remains below 10% (Smith *et al*., 2022). Unfortunately, for the present data, the inability to diagnose diseases or toxins in the herd prevents us from eliminating this hypothesis to explain the PL here observed. Furthermore, it should be noted that maternal immune system modulation is crucial for pathogen elimination and adequate embryotrophic environment, which are essential for successful pregnancy maintenance (Innes *et al*., 2005; Walker *et al*., 2010; Oliveira *et al*., 2013). High concentrations of E2 and subsequent P4 levels (observed in cows exhibiting estrus behavior) have been also associated to increased endometrial cytokines that affect the endometrial immune system and the differentiation of T cells into Th2 (Bauersachs and Wolf., 2013); which are pivotal factors in maintaining pregnancy and avoiding abortion process (Entrican, 2002; Innes *et al*., 2005). Thus, the proper comprehension of hormonal influence in the maternal immune system may assist the understanding of fetal loss variances observed between animals with and without estrus expression.

Considering the cows not expressing estrus, no effects of BCS (P=0.8282) or AI technician (P=0.7044) were detected. Additionally, no treatment effect was observed for CR (P=0.3004) or PL (P=0.7757), as observed in Table 2.

Table 2. Conception rates (CR; pregnancy/AI) and pregnancy loss (PL) of suckled, multiparous Nelore cows not expressing estrus that received 12.5 mg of Dinoprost (PGF2α treatment), 100 mcg of GnRH (GnRH treatment) or no treatment (Saline) at the time of AI, in an Estradiol/Progesterone-based timed-AI protocol

Treatment	*N*	CR (60 days after timed-AI)	PL (CR on D210 - D70)
Saline	59	37.3% (22/59)	18.2% (4/22)
GnRH	114	46.5% (53/114)	20.1% (11/53)
PGF2α	117	49.6% (58/117)	15.5% (9/58)
P value		0.3004	0.7757

Although no treatment effect was detected on CR among animals with no estrus behavior (Table 2), it was interesting to note that cows not expressing estrus that received GnRH (CR=46.5%) or PGF2α (CR=49.6%) presented similar (P>0.05) CR than cows expressing estrus (CR=51.6%), whereas a tendency was detected for lower (P=0.0629) CR of cows not expressing estrus and receiving no treatment (saline group; CR=37.3%) compared to cows expressing estrus.

To the best of our knowledge, this was the first work comparing the effects of PGF2a and GnRH treatment at AI in cows not expressing estrus behavior.

In cows not expressing estrus, GnRH treatment has been demonstrated to increase pregnancy rates, as evidenced by current and earlier studies, confirming the potential utility of GnRH as a strategy for improving fertility of cows without estrus behavior in timed-AI protocols (Perry and Perry, 2009; Thomas *et al*., 2014; Hill *et al*., 2016; Bishop *et al*., 2017; Alves *et al*., 2021). Since GnRH treatment induces a LH surge, ovulation occurs around 26-30h after administration if a dominant follicle is present (Twagiramungu *et al*., 1992, 1995; Bishop *et al*., 2017). Hence, this strategy has been used to ensure an LH surge in animals not expressing estrus, reducing the percentage of delayed (or absent) ovulation in those animals and improving their fertility outcomes (Madureira *et al*., 2020; Prata *et al*., 2020).

Alike the GnRH treated group, the CR of the PGF2a treated group was similar to cows expressing estrus, which can be attributed to the advantages of this treatment, already demonstrated to go beyond luteolysis (Pereira *et al*., 2015; Noronha *et al*., 2020; Diniz *et al*., 2021). These eicosanoids act on the hipotalamic-pituitary axis stimulating a significant increase in LH secretion (Warberg *et al*., 1976). Randel *et al*. (1996) also observed that the combined treatment GnRH + PGF2a increased LH release in suckled cows compared with cows that received only GnRH treatment, demonstrating that PGF2a favors the GnRH action in pituitary, stimulating LH secretion. The authors also observed an increase in the pituitary response to the GnRH in anestrus postpartum cows previously exposed to PGF2a (Randel *et al*., 1996). Moreover, PGF2a was found to prolong LH surge duration and to increase the proportion of follicles with higher E2 levels in both dairy and anestrous beef cows (Lopes Jr *et al*., 2020; Noronha, *et al*., 2020).

PGF2a may be also associated with ovulation process (Armstrong and Grinwich *et al*, 1972; Yang *et al*., 1974; Tsang *et al*., 1979) since it plays a key role in the proteolytic cascade of ovulation triggered by the LH surge (Robker *et al*., 2000; Li *et al*., 2006); and study demonstrated that prostaglandin inhibitors administered before the LH peak caused an ovulation blockage (Sirois, 1994). In addition to the beneficial effects on the ovulation process, other studies also suggest that PGF2a plays a fundamental role in preparing the uterine environment for a new pregnancy. Treatment with PGF2a increased the expression of uterine cell growth factors (CTGF), angiogenesis (VEGF), cell differentiation (FGF2), epithelial remodeling (Zhang *et al*., 2017; Gao *et al*., 2018), and cell proliferation (Fu *et al*., 2020). In accordance with the present results, Diniz *et al*. (2021) observed that Nelore suckled cows not expressing estrus and receiving PGF2a at the time of AI, tended to present increased CR compared to animals in the same physiological conditions (without estrus behavior at timed-AI) but without the treatment.

Thus, in the present study, since cows not expressing estrus that received no treatment demonstrated reduced CR compared to animals expressing estrus, and because CR of cows not expressing estrus that received GnRH or PGF2a at the time of AI were similar to CR of cows expressing estrus behavior, it can be inferred that both treatments improved the fertility of the animals without estrus behavior at the timed of AI.

Hence, this study demonstrated that the treatment with PGF2α or GnRH at the time of AI had similar effect on the fertility of cows not expressing estrus.

Keywords: bovine, estrus behavior, fertility, Timed-AI

RESUMO

Objetivou-se comparar as taxas de prenhez (TP) e a perda gestacional (PG) de vacas sem expressão de estro (NEE), as quais receberam PGF2α ou GnRH no momento da IATF. Vacas multíparas da raça Nelore (n=1347) receberam, no D0, 2mg (IM) de benzoato de estradiol e um dispositivo intravaginal de progesterona (P4). No D8, para detectar o cio, as vacas foram marcadas com um bastão e o dispositivo P4 foi removido; além disso, elas receberam (IM) 526µg de cloprostenol sódico, 1,0mg de cipionato de estradiol e 300IU de eCG. No D10, foi realizada IATF, e as vacas NEE receberam (IM) 12,5mg de dinoprost, 100mcg de GnRH ou nenhum tratamento. A TP geral foi de 50,3% (678/1347), 51,6% (545/1057), para vacas que expressaram estro (EE), e de 45,9% (133/290), para animais NEE na IATF (P=0,0427). A PG geral foi de 6,2% (42/678), 3,3% (18/545), para animais EE, e de 18,1% (24/133), para animais NEE (P<0,0001). Considerando-se as vacas NEE, não foi detectado efeito de tratamento para TP (P=0,3004) ou PL (P=0,7757); no entanto, as vacas que receberam GnRH (CR=46,5%) ou PGF2α (CR=49,6%) apresentaram CR semelhante (P>0,05) ao das vacas EE (51,6%), enquanto uma tendência foi detectada para TP menor (P=0,0629) das vacas NEE e para aquelas que não receberam tratamento (37,3%), em comparação com as vacas EE. O tratamento com PGF2α ou GnRH no momento da IATF teve efeito semelhante sobre a fertilidade das vacas NEE.

Palavras-chave: bovino, comportamento de estro, fertilidade, IATF

REFERENCES

ALVES, R.L.O.R.; SILVA, M.A.; CONSENTINI, C.E.C. *et al.* Hormonal combinations aiming to improve reproductive outcomes of Bos indicus cows submitted to estradiol/progesterone-based timed AI protocols. *Theriogenology*, v.169, p.89-99, 2021.

ARMSTRONG, D.T.; GRINWICH, D.L. Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin biosynthesis. *Prostaglandins*, v.1, p.21-28, 1972.

BARUSELLI, P.S.; REIS, E.L.; MARQUES, M.O. *et al*. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim. Reprod. Sci.*, v.82-83, p.479-486, 2004.

BAUERSACHS, S.; WOLF, E. Immune aspects of embryo-maternal cross-talk in the bovine uterus. *J. Reprod. Immunol.*, v.97, p.20-26, 2013.

BISHOP, B.E.; THOMAS, J.M.; ABEL, J.M. *et al.* Split-time artificial insemination in beef cattle: III. Comparing fixed-time artificial insemination to split-time artificial insemination with delayed administration of GnRH in postpartum cows. *Theriogenology,* v.99, p.48-52, 2017.

BÓ, G.A.; BARUSELLI, P.S.; MARTÍNEZ, M.F. Pattern and manipulation of follicular development in Bos indicus cattle. *Anim. Reprod. Sci.*, v.78, p.307-326, 2003.

BRIDGES, G.A.; MUSSARD, M.L.; PATE, J.L. Impact of preovulatory estradiol concentrations on conceptus development and uterine gene expression. *Anim. Reprod. Sci*., v.133, p.16-26, 2012

BUSCH, D.C.; ATKINS, J.A.; BADER, J.F. *et al.* Effect of ovulatory follicle size and expression of estrus on progesterone secretion in beef cows. *J. Anim. Sci*., v.86, p.553-563, 2008.

CEDEÑO, A.V.; CUERVO, R.; TRÍBULO, A. *et al*. Effect of expression of estrus and treatment with GnRH on pregnancies per AI in beef cattle synchronized with an estradiol/progesterone-based protocol. *Theriogenology,* v.161, p.294-300, 2021.

COOKE, R.F.; POHLER, K.G.; VASCONCELOS, J.L.M.; CERRI, R.L.A. Estrous expression during a fixed-time artificial insemination protocol enhances development and interferon-tau messenger RNA expression in conceptuses from Bos indicus beef cows. *Animal*, v.13, p.2569-2575, 2019.

DAVOODI, S.; COOKE, R.F.; FERNANDES, A.C.C. *et al.* Expression of estrus modifies the gene expression profile in reproductive tissues on Day 19 of gestation in beef cows. *Theriogenology,* v.85, p.645-655, 2016.

DINIZ, J.H.W.; PERES, R.F.G.; TEIXEIRA, A.C.B. *et al.* Administration of PGF2a at the moment of timed-AI using sex-sorted or conventional semen in suckled nelore cows with different intensity of estrus behavior. *Theriogenology*, v.174, p.169-175, 2021.

ENTRICAN, E. Immune regulation during pregnancy and host–pathogen interactions in infectious abortion. *J. Comp. Pathol*., v.126, p.79-94, 2002

FORDE, N.; MCGETTIGAN, P.A.; MEHTA, J.P. *et al*. Proteomic analysis of uterine fluid during the pre-implantation period of pregnancy in cattle. *Reproduction,* v.147, p.575-587, 2014.

FRANCO, G.; REESE, S.; POOLE, R. *et al*. Sire contribution to pregnancy loss in different periods of embryonic and fetal development of beef cows. *Theriogenology,* v.15, p.84-91, 2020.

FRANCO, G.A.; PERES, R.F.G.; MARTINS, C.F.G. *et al*. Sire contribution to pregnancy loss and pregnancy-associated glycoprotein production in Nelore cows. *J. Anim. Sci*., v.6, p.632-640, 2018.

FU, C.; MAO, W.; GAO, R. *et al*. Prostaglandin F2a-PTGFR signaling promotes proliferation of endometrial epithelial cells of cattle through cell cycle regulation. *Anim. Reprod. Sci.,* v.213, p.106276, 2020.

GAO, L.; GAO, R.; MAO, W. *et al*. PTGFR activation promotes the expression of PTGS-2 and growth factors via activation of the PKC signaling pathway in bovine endometrial epithelial cells*. Anim. Reprod. Sci.,* v.199, p.30-39, 2018.

GRAY, C.A.; TAYLOR, K.M.; RAMSEY, W.S. *et al*. Endometrial glands are required for preimplantation conceptus elongation and survival. *Biol. Reprod*., v.64, p.1608-1613, 2001.

HAWK, H.W. Sperm survival and transport in the femalere productive tract. *J. Dairy Sci.*, v.66, p.2645-2660, 1983.

HILL, S.L.; GRIEGER, D.M.; OLSON, K.C. *et al*. Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program. *J. Anim. Sci*., v.94, p.3722-3728, 2016.

HOUGHTON, P.L.; LEMENAGER, R.P.; MOSS, G.E. *et al*. Prediction of postpartum beef cow body composition using weight to height ratio and visual body condition score. *J. Anim. Sci.*, v.68, p.1428-1437, 1990.

INNES, E.A.; WRIGHT, S.; BARTLEY, P. *et al*. The host–parasite relationship in bovine neosporosis. *Vet. Immunol. Immunopathol*., v.108, p.29-36, 2005.

INSKEEP, E.K. Factors that affect embryonic survival in the cow: Application of technology to improve calf crop. In: FIELDS, M.J.; SAND, R.S.; YELICH, J.V. *Factors affecting calf crop*: biotechnology of reproduction. Boca Raton: CRC Press, 2002. p. 255-279.

KINDER, J.E.; KOJIMA, F.N.; BERGFELD, E.G.M.; WEHRMAN, M.E.; FIKE, K.E. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J. Anim. Sci.*, v.74, p.1424-1440, 1996

LEONARDI, C.E.P.; PFEIFER, L.F.M.; RUBIN, M.I.B. *et al*. Prostaglandin F2alpha promotes ovulation in prepubertal heifers. *Theriogenology*, v.78, p.1578-1582, 2012.

LI, Q.; JIMENEZ-KRASSEL, F.; KOBAYASHI, Y. *et al*. Effect of intrafollicular indomethacin injection on gonadotropin surge-induced expression of select extracellular matrix degrading enzymes and their inhibitors in bovine preovulatory follicle. *Reproduction*, v.131, p.533-543, 2006.

LONERGAN, P.; SÁNCHEZ, J.M. Review: progesterone effects on early embryo development in cattle. *J. Dairy Sci.*, v.103, p.8698-8707, 2020.

LOPES JR, F.R.; SILVA, L.M.; ZIMPEL, R. *et al*. Prostaglandin F2a influences pre-ovulatory follicle characteristics and pregnancy per AI in anovular dairy cows. *Theriogenology*, v.153, p.122-132, 2020.

LOPES, A.S.; BUTLER, S.T.; GILBERT, R.O.; BUTLER, W.R. Relationship of preovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. *Anim. Reprod. Sci.*, v.99, p.34-43,2007.

MADUREIRA, G.; MOTTA, J.C.L.; DRUM, J.N. *et al*. Progesterone-based timed AI protocols for Bos indicus cattle I: evaluation of ovarian function. *Theriogenology*, v.145, p.126-137, 2020.

MEE, J.F.; HAYES, C.; STEFANIAK, T.; JAWOR, P. Review: bovine foetal mortality - risk factors, causes, immune responses and immuno-prophylaxis. *Animal*, Suppl.1, p.100774, 2023.

NORONHA, I.M.; COOKE, R.F.; MARTINS, C.F.G. *et al*. Administering an additional prostaglandin F2a injection to Bos indicus beef cows during a treatment regimen for fixed-time artificial insemination. *Anim. Reprod. Sci.*, v.219, p.106535, 2020.

OLIVEIRA FILHO, R.V.O.; FRANCO, G.A.; REESE, S.T. *et al*. Using pregnancy associated glycoproteins (PAG) for pregnancy detection at day 24 of gestation in beef cattle. *Theriogenology*, v. 141, p.128-133, 2020

OLIVEIRA, L.J.; MANSOURI-ATTIA, N.; FAHEY, A.G. *et al*. Characterization of the Th profile of the bovine endometrium during the oestrous cycle and early pregnancy. *PLoS One*, v.8, p.e75571, 2013.

PEREIRA, M.H.C.; WILTBANK, M.C.; BARBOSA, L.F.S.P. *et al*. Effect of adding a gonadotropin-releasing-hormone treatment at the beginning and a second prostaglandin F2a treatment at the end of an estradiol-based protocol for timed artificial insemination in lactating dairy cows during cool or hot seasons of the year. *J. Dairy Sci.*, v.98, p.947-959, 2015.

PEREIRA, M.H.C.; WILTBANK, M.C.; VASCONCELOS, J.L.M. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. *J. Dairy Sci*., v.99, p.2237-2247, 2016.

PERRY, G.A.; PERRY, B.L. GnRH treatment at artificial insemination in beef cattle fails to increase plasma progesterone concentrations or pregnancy rates. *Theriogenology*, v.71, p.775-779, 2009.

PERRY, G.A.; SMITH, M.F.; LUCY, M. *et al*. Relationship between follicle size at insemination and pregnancy success. *Proc. Natl. Acad. Sci*., v.102, p.5268-5273, 2005.

PERRY, G.A.; SMITH, M.F.; ROBERTS, A.J. *et al*. Relationship between size of ovulatory follicle and pregnancy success in beef heifers. *J. Anim. Sci*., v.85, p.684-689, 2007.

PFEIFER, L.F.M.; LEONARDI, C.E.P.; CASTRO, N.A. *et al*. The use of PGF2a as ovulatory stimulus for timed artificial insemination in cattle. *Theriogenology*, v.81, p.689-695, 2014.

POHLER, K.; PERES, R.; GREEN, J. *et al*. Use of bovine pregnancy associated glycoproteins to predict late embryonic mortality in postpartum Nelore beef cows. *Theriogenology*, v.85, p.1652-1659, 2016.

PRATA, A.B.; MADUREIRA, G.; ROBL, A.J. *et al*. Progesterone-based timed AI protocols for Bos indicus cattle III: Comparison of protocol lengths. *Theriogenology*, v.152, p.29-35, 2020.

PUGLIESI, G.; SANTOS, F.B.; LOPES, E. *et al*. Improved fertility in suckled beef cows ovulating large follicles or supplemented with long-acting progesterone after timed-AI. *Theriogenology*, v.85, p.1239-1248, 2016.

PURSLEY, J.R.; MEE, M.O.; WILTBANK, M.C. Synchronization of ovulation in dairy cows using PGF2 and GnRH. *Theriogenology*, v.44, p.915-923, 1995.

RANDEL, R.D.; LAMMOGLIA, M.A.; LEWIS, A.W. *et al*. Exogenous PGF2α enhanced GnRH-induced LH release in postpartum cows. *Theriogenology*, v.45, p.643-654, 1996.

REESE, S.T.; FRANCO, G.A.; POOLE, R.K. *et al.* Pregnancy loss in beef cattle: a meta-analysis. *Anim. Reprod. Sci*, v.212, p.106251, 2020.

REESE, S.T.; GEARY, T.W.; FRANCO, G.A. *et al*. Pregnancy associated glycoproteins (PAGs) and pregnancy loss in high vs sub fertility heifers. *Theriogenology*, v.135, p.7-12, 2019.

RICHARDSON, B.N.; HILL, S.L.; STEVENSON, J.S. *et al*. 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*., v.166, p.133-140, 2016.

ROBKER, R.L.; RUSSELL, D.L.; YOSHIOKA, S. *et al*. Ovulation: a multi-gene, multistep process. *Steroids*, v.65, p.559-570, 2000.

RODRIGUES, A.D.; COOKE, R.F.; CIPRIANO, R.S. *et al*. Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in Bos indicus-influenced beef cows. *J. Anim. Sci.*, v.96, p.236-249, 2018.

RODRIGUES, W.B.; SILVA, A.S.; BORGES SILVA, J.C. *et al*. Timed artificial insemination plus heat II: gonadorelin injection in cows with low estrus expression scores increased pregnancy in progesterone/estradiol-based protocol. *Animal*, v.10, p.2313-2318, 2019.

SA FILHO, M.F.; AYRES, H.; FERREIRA, R.M. *et al*. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (Bos indicus) cows. *Theriogenology*, v.73, p.651-658, 2010a.

SA FILHO, M.F.; CRESPILHO, A.M.; SANTOS, J.E.P. *et al*. Ovarian follicle diameter at timed insemination and estrus response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. *Anim. Reprod. Sci.*, v.120, p.23-30, 2010b.

SA FILHO, M.F.; SANTOS, J.E.P.; FERREIRA, R.M. *et al*. Importance of estrus on pregnancy per insemination in suckled Bos indicus cows submitted to estradiol/progesterone-based timed insemination. *Theriogenology*, v.76, p.455-463, 2011.

SCHLAFER, D.; FISHER, P.; DAVIES, C. The bovine placenta before and after birth: placental development and function in health and disease. *Anim. Reprod. Sci.*, v.60, p.145-160, 2000.

SIROIS, J. Induction of prostaglandin endoperoxide synthase-2 by human chorionic gonadotropin in bovine preovulatory follicles in vivo. *Endocrinology*, v.135, p.841-848, 1994.

SMITH, B.D.; POLIAKIWSKI, B.; POLANCO, O. *et al*. Decisive points for pregnancy losses in beef cattle. *Reprod. Fertil. Dev*., v.35, p.70-83, 2022.

SUAREZ, S.S.; PACEY, A.A. Sperm transport in the female reproductive tract. *Hum. Reprod. Update*, v.12, p.23-37, 2006.

TAFT, R.; AHMAD, N.; INSKEEP, E.K. Exogenous pulses of luteinizing hormone cause persistence of the largest bovine ovarian follicle. *J. Anim. Sci.*, v.74, p.2985-2991, 1996.

THOMAS, J.M.; LOCK, S.L.; POOCK, S.E. *et al*. Delayed insemination of nonestrous cows improves pregnancy rates when using sex-sorted semen in timed artificial insemination of suckled beef cows. *J. Anim. Sci.*, v.92, p.1747-1752, 2014.

TSANG, B.K.; AINSWORTH, L.; DOWNEY, B.R.; ARMSTRONG, D.T. Preovulatory changes in cyclic AMP and prostaglandin concentrations infollicular fluid of gilts. *Prostaglandins*, v.17, p.141-148, 1979.

TWAGIRAMUNGU, H.; GUILBAULT, L.A.; PROULX, J.; DUFOUR, J.J. Buserelin alters the development of the corpora lutea in cyclic and early postpartum cows. *J. Anim. Sci.,* v.73, p.805-811, 1995.

TWAGIRAMUNGU, H.; GUILBAULT, L.A.; PROULX, J.; DUFOUR, J.J. Synchronization of estrus and fertility in beef cattle with two injections of buserelin and prostaglandin. *Theriogenology*, v.38, p.1131-1144, 1992.

VASCONCELOS, J.L.; SARTORI, R.; OLIVEIRA, H.N. *et al*. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology*, v.56, p.307-314, 2001.

VASCONCELOS, J.L.M.; PEREIRA, M.H.C.; WILTBANK, M.C. *et al*. Fertility of programs using E2/P4 for FTAI in dairy cattle. *Anim. Reprod.*, v.15, p.940-951, 2018.

WALKER, C.G.; MEIER, S.; LITTEJOHN, M.D. *et al*. Modulation of the maternal immune system by the pre-implatation embryo. *BMC Genom*., v.11, p.474, 2010.

WARBERG, J.; ESKAY, R.L.; PORTER, J.C. Prostaglandin-induced release of anterior pituitary hormones: structure-activity relationships. *Endocrinology*, v.98, p.1135-1141, 1976.

YANG, N.S.T.; MARSH, J.M.; LEMAIRE, W.J. Post-ovulatory changes in the concentrations of prostaglandins in rabbit graafian follicles. *Prostaglandins*, v.6, p.37-44, 1974.

ZHANG, S.; LIU, B.; GAO, L. *et al*. Prostaglandin F2a-PTGFR signaling activation, growth factor expression and cell proliferation in bovine endometrial explants. *Reprod. Fertil. Dev.*, v.29, p.2195-2205, 2017.