Animal (2019), **13:11,** pp 2569–2575 © The Animal Consortium 2019
doi:10.1017/S1751731119000636

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Estrous expression during a fixed-time artificial insemination protocol enhances development and *interferon-tau* messenger RNA expression in conceptuses from *Bos indicus* beef cows

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(Received 28 September 2018; Accepted 18 January 2019; First published online 10 April 2019)

Expression of estrus (EST) near the time of fixed-time artificial insemination (FTAI) increases pregnancy success in beef females. This outcome has been associated with improved pregnancy establishment and maintenance, although research is still warranted to validate this theory. Hence, this experiment compared ovarian, uterine and conceptus factors associated with pregnancy establishment in Bos indicus *beef cows according to estrous expression during a FTAI protocol. One hundred lactating multiparous Nelore cows received a 2 mg injection of estradiol benzoate and an intravaginal progesterone (P4) releasing device on day −11, a 12.5 mg injection of prostaglandin $F_{2\alpha}$ on day −4, P4 device removal in addition to 0.6 mg injection of estradiol cypionate and 300 IU injection of equine chorionic gonadotropin on day −2, and FTAI on day 0. An estrous detection patch was attached to the tailhead of each cow on day −2, and estrous expression was defined as removal of >50% of the rub-off coating from the patch at FTAI. Overall, 39 cows expressed EST, 55 did not express EST (NOEST), and six cows lost their patch and were discarded from the experiment. Ovarian ultrasonography was performed at FTAI, and on days 7 and 15 of the experiment. Blood samples were also collected on days 7 and 15. Only cows without a corpus luteum (CL) on day 0, and with a CL on days 7 and 15 remained in the experiment (EST,* n *= 36; NOEST,* n *= 48). On day 15, cows were randomly selected within each group (EST,* n *= 29; NOEST,* n *= 30) for conceptus collection via transcervical flushing, followed by endometrial biopsy in the uterine horn ipsilateral to the CL. Within cows not assigned to conceptus collection, blood samples were collected for whole blood RNA extraction (day 20) and pregnancy status was verified by transrectal ultrasonography (day 30). Diameter of dominant follicle on day 0 and plasma P4 concentrations on day 7 were greater (*$P \leqslant 0.02$*) in EST* v. *NOEST cows. Conceptus length and messenger RNA (mRNA) expression of* prostaglandin E synthase *and* interferon-tau *were greater (*$P \leqslant 0.04$*) in EST* v. *NOEST cows. Moreover, EST cows diagnosed as pregnant on day 30 had greater (*$P < 0.01$*) blood mRNA expression of* myxovirus resistance 2 *on day 20 compared with NOEST. In summary, estrous expression near the time of FTAI enhanced pregnancy establishment factors in* B. indicus *cows, including conceptus development and mRNA expression of* interferon-tau.

Keywords: beef cattle, insemination, physiology, pregnancy, reproduction

Implications

This experiment provides novel insights on mechanisms by which estrous expression near the time of artificial insemination improves reproductive function and performance in *Bos indicus* cows. Cows that expressed estrus (EST) had enhanced ovarian function and initial pregnancy development, which promoted embryo-mediated processes responsible for pregnancy signaling to maternal tissues. These responses are known to increase pregnancy success in beef females, across *B. indicus* and *Bos taurus* breeds. Hence, management strategies to promote estrous expression are beneficial to reproductive and overall efficiency of beef cattle operations.

Introduction

Reproductive efficiency is a critical response in cow–calf operations, and contributes to the number of cattle available for beef production. Given the increasing worldwide demand for beef, management to optimize reproductive efficiency in cow–calf systems is warranted to ensure profitability of cattle producers (Lamb *et al.*, 2010) and meet the global

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https://doi.org/10.1017/S1751731119000636 Published online by Cambridge University Press

demand for animal protein (FAO, 2009). Fixed-time artificial insemination (FTAI) is an example of such management, and directly contributed to the recent advances in beef production in the United States (Patterson *et al*., 2016). Yet, understanding the mechanisms that optimize pregnancy success to FTAI is still needed to encourage its adoption by cattle producers (Lamb *et al*., 2010).

Expression of EST near the time of FTAI is known to increase pregnancy success in beef and dairy cattle (Pereira *et al*., 2013; Rodrigues *et al*., 2018). This outcome has been attributed to several factors, including improved ovarian function, embryo development, as well as pregnancy establishment and maintenance (Davoodi *et al*., 2016; Rodrigues *et al*., 2018). More specifically, Larimore *et al*. (2015) reported advanced growth and quality of embryos recovered on day 6 of gestation from cows that expressed EST before FTAI. Early embryonic development is critical for establishment and maintenance of the pregnancy, particularly via the interferon-tau (IFNt) cascade (Ealy and Yang, 2009). Accordingly, Rodrigues *et al*. (2018) reported greater blood messenger RNA (mRNA) expression of interferon-stimulated genes (ISG) on day 20 of gestation in *B. indicus* cows that expressed EST near the time of FTAI. The ISGs have been used to gauge IFNt response and conceptus development (Fricke *et al*., 2016), given that IFNt synthesis upregulates mRNA expression of ISGs in circulating leukocytes (Stevenson *et al*., 2007).

Davoodi *et al*. (2016) reported greater conceptus size, but similar IFNt mRNA expression in conceptuses collected from *B. indicus* cows that expressed EST during the FTAI protocol. However, Davoodi *et al*. (2016) collected conceptuses on day 19 of gestation, whereas the IFNt-based processes responsible for pregnancy signaling to maternal tissues occur around day 15 to 17 of gestation (Ealy and Yang, 2009). Based on this rationale, we hypothesized that estrous expression near the time of FTAI will result in enhanced conceptus development and IFNt-signaling cascade on day 15 of gestation; the beginning of the pregnancy recognition period. Hence, this experiment compared ovarian, uterine and conceptus factors associated with pregnancy establishment in *B. indicus* beef cows according to estrous expression during the FTAI protocol.

Material and methods

Animals and estrous expression

This experiment was conducted in a commercial cow–calf operation located in Aruanã, GO, Brazil. All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee. Moreover, cows evaluated herein were part of a companion project focused on fatty acid supplementation after FTAI, and fully described by Cipriano *et al*. (2016). Pertaining to the current experiment, 100 lactating multiparous Nelore cows (mean ± SE; initial BW = 430 ± 5 kg, initial body condition score (BCS) = 2.87 ± 0.02; age = 8.5 ± 0.2 years; days postpartum = 152 ± 1 days) were enrolled in an estrous synchronization + FTAI protocol (Meneghetti *et al*., 2009) from day −11 to 0. Cows received a 2 mg intramuscular injection of estradiol benzoate (Gonadiol; Zoetis, São Paulo, SP, Brazil) and an intravaginal progesterone (P4) releasing device (CIDR, containing 1.9 g of P4; Zoetis) on day −11, a 12.5 mg intramuscular injection of prostaglandin $F_{2\alpha}$ (Lutalyse; Zoetis) on day −4, P4 device removal in addition to 0.6 mg intramuscular injection of estradiol cypionate (Zoetis) and 300 IU intramuscular injection of equine chorionic gonadotropin (Novormon; Zoetis) on day −2, and FTAI on day 0. Cows were inseminated on day 0 by the same technician, using semen from the same sire and collection. Estrous detection aids (Estrotect; Rockway Inc., Spring Valley, WI, USA) were applied on day −2 to all cows, and occurrence of EST was recorded at FTAI. Estrus was defined as removal of >50% of the rub-off coating on the patch. Overall, 39 cows expressed EST (EST), 55 did not express EST (NOEST) and six cows lost their patch and were removed from the experiment.

Sampling

During the experimental period (day −11 to 30), cows were maintained in *Panicum maximum* pastures and received supplementation as described by Cipriano *et al*. (2016). Cow BW and BCS (Wagner *et al*., 1988) were assessed on day −11 of the experiment. Blood samples were collected immediately on days 7 and 15 of the experiment from either the coccygeal vein or artery into blood collection tubes (Vacutainer, 10 ml; Becton Dickinson, Franklin Lakes, NJ, USA). Samples were placed immediately on ice, centrifuged (2500 × **g** for 30 min; 4°C) for plasma harvest, and stored at −20°C on the same day of collection. Transrectal ultrasonography (7.5-MHz transducer; 500 V, Aloka, Wallingford, CT, USA) was performed concurrently with FTAI (day 0) to verify dominant follicle diameter, and concurrently with each blood collection to estimate corpus luteum (CL) volume (days 7 and 15). Corpus luteum volume was estimated using the formula for volume of a sphere; volume $= 4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter. Cows diagnosed without the presence of a CL on day 0, but with a CL greater than 0.38 cm^3 in volume on days 7 and 15 were classified as responsive to the estrous synchronization protocol (Cooke *et al*., 2014; Cipriano *et al*., 2016). This criterion was based on the smallest diameter of a functional CL detected in *B. indicus*-influenced cows following induced ovulation, as reported by Figueiredo *et al*. (1997). Only cows that responded to the estrous synchronization protocol and that were successfully classified according to estrous expression remained in the experiment (EST, $n = 36$; NOEST, $n = 48$).

Upon ultrasonography on day 15, cows that responded to the estrous synchronization protocol were randomly selected within each estrous expression group (EST, $n = 29$; NOEST, $n = 30$) for conceptus collection and endometrial biopsy. Conceptus collection was performed via transcervical flushing of the uterine horn ipsilateral to the CL as described by Ribeiro *et al*. (2016) and Brandão *et al*. (2018). Up to five flushings of phosphate-buffered saline (20 ml each) were

https://doi.org/10.1017/S1751731119000636 Published online by Cambridge University Press

performed if no conceptus tissue was recovered. The recovered conceptuses were transferred to a sterile 100 × 15 mm Petri-dish (CRAL Artigos para Laboratórios Ltda., São Paulo, Brazil) and measured for length. Following conceptus collection, endometrial biopsy was performed in the uterine horn ipsilateral to the CL according to Cerri *et al.* (2011) and Brandão *et al.* (2018). Conceptus and endometrial samples were stored in 5-ml sterile cryogenic tubes (CRAL Artigos para Laboratórios Ltda.) containing 2 ml of RNA stabilization solution (RNAlater; Ambion Inc., Austin, TX, USA). On day 20, blood samples were collected from the non-flushed cows (EST, $n = 7$; NOEST, $n = 18$) from either the coccygeal vein or artery into PAXgene tubes (BD Diagnostics, Sparks Glencoe, MD, USA) for whole blood RNA extraction. Pregnancy status of the non-flushed cows was evaluated via transrectal ultrasonography (5.0-MHz transducer; 500 V, Aloka) on day 30.

Laboratorial analyses

Plasma samples were analyzed for P4 concentrations using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Samples were analyzed within a single assay, with intra-assay CV of 5.6% and minimum detectable concentration of 0.1 ng/ml.

Total RNA was extracted from tissue samples collected from cows that had a conceptus using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA, USA). Total RNA was extracted from whole blood samples using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA, USA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE, USA) at 260 and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). Extracted RNA (100 ng for conceptus, 200 ng for endometrium, 120 ng for whole blood) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA, USA) as described by Brandão *et al.* (2018). Real-time reverse transcription PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems) and gene-specific primers (20 pM each; Table 1) with the StepOne Real-time PCR system (Applied Biosystems) according to Cooke *et al.* (2008). Responses were quantified based on the threshold cycle (C_T), the number of PCR cycles required for target amplification to reach a predetermined threshold. The C_T responses were normalized to the geometrical mean of C_T values of the reference genes *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19* (conceptus), *suppressor of zeste 12 homolog* and *zinc finger protein 131* (endometrium) and *β2-microglobulin and β-actin* (whole blood). The CV for the geometrical mean of reference genes across all samples was 1.7% in the conceptus, 2.4% in endometrial samples and 2.1% in whole blood samples. Results are expressed as relative fold change ($2^{-\Delta\Delta C_T}$).

Statistical analysis

All data were analyzed using cow as experimental unit, with the MIXED or GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA) for quantitative and binary data, respectively. Satterthwaite approximation was used to determine the denominator degrees of freedom for the tests of fixed effects, and cow(estrous expression) as random effect. The model statement used for cow BW, BCS, days-postpartum, dominant follicle diameter, presence of conceptus, conceptus length, and all endometrial and conceptus gene expression results contained the effect of estrous expression. The model statement used for blood gene expression results contained the effects of estrous expression, pregnancy status on day 30, and the resultant interaction. The model statement used for CL volume and plasma P4 concentrations contained the effects of estrous expression, day, and the resultant interaction. The specified term for the repeated measure analyses was day, cow(estrous expression) was the subject, and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means, and separated using least square differences. Significance was set at $P \leqslant 0.05$ and tendencies were determined if $P > 0.05$ and $\leqslant 0.10$

Results

The interaction of estrous expression (EST or NOEST) with the treatments imposed by Cipriano *et al.* (2016) was tested across all variables investigated, and no significant interactions were observed ($P \geqslant 0.51$). Hence, results reported herein were independent of the nutritional treatments from Cipriano *et al.* (2016). Cow BW, BCS and days postpartum on day −11 were similar ($P \geqslant 0.51$) between EST and NOEST cows (Table 2). Diameter of the dominant follicle on day 0 was greater ($P = 0.01$) in EST *v.* NOEST cows (Table 2). An estrous response × day interaction was detected ($P = 0.03$) for plasma P4 concentrations, which were greater ($P = 0.02$) on day 7 for EST *v.* NOEST cows, but similar ($P = 0.48$) between groups on day 15 (Table 2). Conversely, no estrous response effects were noted ($P \geqslant 0.62$) for CL volume (Table 2).

A similar proportion ($P \geqslant 0.65$) of EST and NOEST cows had a conceptus on days 15 and 30 of the experiment (Table 3). Conceptuses collected from EST cows had increased ($P = 0.03$) length compared with conceptuses collected from NOEST cows (Table 3). Expression of *prostaglandin E synthase* and IFNt mRNA were greater ($P \leqslant 0.04$) in conceptuses collected from EST *v.* NOEST cows (Table 4). No estrous expression effects were detected ($P \geqslant 0.62$) for mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* in endometrial samples (Table 4).

An estrous response × pregnancy status interaction was detected ($P < 0.01$) for blood mRNA expression of the ISG *myxovirus resistance 2* on day 20 of the experiment (Table 4). Expression of this ISG was similar ($P = 0.81$) within cows diagnosed as non-pregnant on day 30, but greater ($P < 0.01$) for EST *v.* NOEST cows diagnosed as pregnant. Estrous response, however, did not impact ($P \geqslant 0.69$) mRNA expression of the ISGs *interferon-stimulated gene 15* and

https://doi.org/10.1017/S1751731119000636 Published online by Cambridge University Press

Table 1 *Primer sequences and accession number for all gene transcripts analyzed by real-time reverse transcription PCR*

Target gene	Primer sequence	Accession n°
Genes of interest		
20,50-Oligoadenylate synthetase		
Forward	ACCCTCTCCAGGAATCCAGT	NM 001040606
Reverse	GATTCTGGTCCCAGGTCTGA	
Cyclooxygenase-2		
Forward	TCCTGAAACCCACTCCCAACA	NM_174445
Reverse	TGGGCAGTCATCAGGCACAG	
Interferon-stimulated gene 15		
Forward	GGTATGAGCTGAAGCAGTT	NM_174366
Reverse	ACCTCCCTGCTGTCAAGGT	
Interferon-tau		
Forward	GCCCTGGTGCTGGTCAGCTA	AF238612
Reverse	CATCTTAGTCAGCGAGAGTC	
Myxovirus resistance 2		
Forward	CTTCAGAGACGCCTCAGTCG	NM 173941
Reverse	TGAAGCAGCCAGGAATAGTG	
Prostaglandin E synthase		
Forward	CGCTGCTGGTCATCAAAAT	NM_174443.2
Reverse	GGAAGGGGTAGATGGTCTCC	
Reference genes		
β-Actin		
Forward	CTGGACTTCGAGCAGGAGAT	AY141970
Reverse	GGATGTCGACGTCACACTTC	
β2-Microglobulin		
Forward	GGGCTGCTGTCGCTGTCT	NM_173893
Reverse	TCTTCTGGTGGGTGTCTTGAGT	
Glyceraldehyde-3-phosphate dehydrogenase		
Forward	ACCCAGAAGACTGTGGATGG	NM_001034034
Reverse	CAACAGACACGTTGGGAGTG	
Ribosomal protein L19		
Forward	ATTGACCGCCACATGTATCA	NM_001040516
Reverse	GCGTGCTTCCTTGGTCTTAG	
Suppressor of zeste 12 homolog		
Forward	GAACACCTATCACACACATTCTTGT	XM_582605
Reverse	TAGAGGCGGTTGTGTCCACT	
Zinc finger protein 131		
Forward	AGAAAGAAGCTTTATGAATGTCAGG	NM_001101218
Reverse	GTTTATCTCCAGTGTGTATCACCAG	

Sources are described in Cipriano *et al.* (2016).

20,50-oligoadenylate synthetase (Table 4). Moreover, mRNA expression of all ISGs were greater ($P < 0.01$) in pregnant *v.* non-pregnant cows across both estrous expression groups (Table 4).

Discussion

Reproductive function of beef females is largely modulated by nutritional status and interval from calving (Vasconcelos *et al.*, 2014). Given the similar BW, BCS and days postpartum between EST and NOEST cows on day −11, the estrous expression effects on reproductive responses reported herein should not be associated with these variables. The greater dominant follicle diameter noted in EST cows is coherent with the existing literature (Carvalho *et al.*, 2016; Rodrigues *et al.*, 2018), and typically result in increased preovulatory estradiol concentrations in cows that express EST. Although estradiol was not evaluated in this experiment, estrous expression is primarily determined by circulating concentrations of this steroid, which triggers the hypothalamus to initiate estrous behavior (Allrich, 1994). Accordingly, estrous expression has been considered a biomarker for estradiol concentrations in beef females (Perry *et al.*, 2005; Larimore *et al.*, 2015), whereas dominant follicle diameter and circulating estradiol have been positively associated with pregnancy success in beef cattle (Perry *et al.*, 2005; Pohler *et al.*, 2012).

Ovulation of a larger follicle often yields a larger CL and greater P4 synthesis (Vasconcelos *et al.*, 2001). Rodrigues *et al.* (2018) reported greater CL size on day 7 after FTAI, which resulted in greater serum P4 concentration in

https://doi.org/10.1017/S1751731119000636 Published online by Cambridge University Press

Table 2 *Cow and ovarian parameters according to estrous expression (EST = estrous behavior,* n = *36; NOEST = no estrous behavior,* n = *48) in* Bos indicus *beef cows during a fixed-time artificial insemination (FTAI) protocol*[1]

Item	EST	NOEST	SEM	*P*
Cow parameters				
BW (day −11), kg	421	425	8	0.73
Body condition score (day −11)[2]	4.70	4.71	0.05	0.89
Days post-partum (day −11)	153	152	1	0.51
Ovarian parameters				
Dominant follicle diameter (day 0), mm	13.8	12.7	0.30	0.01
Corpus luteum volume, cm^3				
Day 7	2.27	2.16	0.16	0.62
Day 15	3.78	3.81	0.32	0.95
Plasma progesterone, ng/ml				
Day 7	3.37	2.70	0.22	0.02
Day 15	5.81	5.41	0.38	0.48

[1]Cows were assigned to a estrous synchronization + FTAI protocol (Meneghetti *et al*., 2009) from day −11 to 0 of the experiment. An estrous detection patch was attached to the tailhead of each cow on day −2, and estrous expression was defined as removal of >50% of the rub-off coating from the patch on day 0 (FTAI).
[2]According to Wagner *et al.* (1988).

B. indicus cows that expressed estrous behavior during the FTAI protocol. Similar outcomes were detected herein for plasma P4 concentration on day 7, although differences noted for dominant follicle diameter at FTAI were not translated into greater CL volume in EST cows. Yet, preovulatory estradiol promotes proliferation of follicular granulosa cells, which differentiate into luteal cells after ovulation and are responsible for 80% of progesterone secretion by the CL (Murdoch and Van Kirk, 1998). Hence, the CL of EST cows were likely capable of synthesizing more P4 on day 7 after FTAI compared with CL from NOEST cows, while this outcome disappeared by day 15 after FTAI. Nevertheless, circulating P4 concentrations on day 7 after breeding has been shown to modulate pregnancy success in beef and dairy females (Demetrio *et al*., 2007; Rodrigues *et al*., 2018).

The proportion of cows that had a conceptus on days 15 and 30 did not differ between EST and NOEST, although this experiment was not designed nor had the statistical power to compare pregnancy rates between groups (Pereira *et al*., 2013; Rodrigues *et al*., 2018). Yet, estrous expression near the time of FTAI enhances uterine environment for embryo development (Davoodi *et al*., 2016), whereas estradiol promotes secretion of oviductal glycoproteins (Buhi, 2002) and endometrial nutrients that contribute to early conceptus growth (Gray *et al*., 2001). Embryo development during the initial 11 days of gestation is also dependent on the histotroph as a source of nutrients, enzymes and growth factors (Forde *et al*., 2014), whose secretion and composition are directly regulated by circulating P4 (Brooks *et al*., 2014). Indeed, Forde *et al*. (2009) reported that circulating P4 concentrations during the initial 7 day of gestation were positively associated with conceptus size on day 14 of gestation. Collectively, these research findings corroborate the greater conceptus length observed in EST cows on day 15 of gestation herein, likely as a result of increased preovulatory estradiol and post-FTAI progesterone concentrations in cows that expressed EST (Perry *et al*., 2005; Rodrigues *et al*., 2018).

Size and stage of development of the conceptus during the pregnancy recognition period are critical for establishment and maintenance of the pregnancy (Ealy and Yang, 2009). The conceptus initiates the IFNt-based processes responsible for pregnancy signaling to maternal tissues, including production of prostaglandins from the two-series, such as prostaglandin E_2, which modulate synthesis and endometrial activity of IFNt. Accordingly, mRNA expression of *prostaglandin E synthase* and IFNt were greater in conceptuses from EST cows, supporting our hypothesis that estrous expression during the FTAI protocol promotes the IFNt-signaling cascade on day 15 of gestation. Conversely, mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* in the endometrium were not impacted by estrous expression. Davoodi *et al*. (2016) reported that *cyclooxygenase-2* and *oxytocin* mRNA expression, genes that regulate prostaglandin F2α biosynthesis, were downregulated in the endometrium of cows that expressed EST during the FTAI protocol. Yet, Davoodi *et al*. (2016) collected samples on day 19 after FTAI, while results from this experiment suggest that estrous expression does not modulate prostaglandin-related genes in endometrial tissues on day 15 of gestation. Supporting this rationale, dramatic

Table 3 *Pregnancy responses according to estrous expression (EST = estrous behavior,* n = *36; NOEST = no estrous behavior,* n = *48) in* Bos indicus *beef cows during a fixed-time artificial insemination (FTAI) protocol*[1,2]

Item	EST	NOEST	SEM	*P*
Proportion of cows with conceptus, %				
Day 15	44.9 (13/29)	33.0 (10/30)	9.23	0.37
Day 30	42.5 (3/7)	46.6 (9/18)	17.7	0.86
Cumulative (days 15 and 30)	44.4 (16/36)	39.7 (19/48)	7.80	0.65
Conceptus length, cm	2.74	0.98	0.59	0.02

[1]Cows were assigned to a estrous synchronization + FTAI protocol (Meneghetti *et al*., 2009) from day −11 to 0 of the experiment. An estrous detection patch was attached to the tailhead of each cow on day −2, and estrous expression was defined as removal of >50% of the rub-off coating from the patch on day 0 (FTAI).
[2]On day 15, cows (EST, *n* = 29; NOEST, *n* = 30) were assigned to conceptus collection via transcervical flushing, followed by endometrial biopsy in the uterine horn ipsilateral to the corpus luteum (Cerri *et al*., 2011; Ribeiro *et al*., 2016). On day 30, pregnancy status was verified by transrectal ultrasonography in cows not assigned to conceptus collection (EST, *n* = 7; NOEST, *n* = 18).

https://doi.org/10.1017/S1751731119000636 Published online by Cambridge University Press

Table 4 *Expression of genes associated with pregnancy establishment in the endometrium, conceptus and blood according to estrous expression (EST = estrous behavior,* n = *36; NOEST = no estrous behavior,* n = *48) in* Bos indicus *beef cows during a fixed-time artificial insemination (FTAI) protocol*[1,2]

Item	EST	NOEST	SEM	*P*-Value
Endometrium				
Cyclooxygenase-2	5.22	5.86	1.67	0.78
Prostaglandin E synthase	4.63	5.19	0.79	0.62
Conceptus				
Interferon-tau	22.24	3.98	5.62	0.04
Prostaglandin E synthase	8.17	2.78	1.72	0.03
Blood cells				
Interferon-stimulated gene 15				
Pregnant	29.66	26.01	3.57	0.49
Non-pregnant	4.09	4.92	2.55	0.82
Myxovirus resistance 2				
Pregnant	57.50	27.08	5.05	<0.01
Non-pregnant	7.65	6.43	3.60	0.81
20,50-Oligoadenylate synthetase				
Pregnant	42.31	39.27	5.20	0.69
Non-pregnant	8.89	6.54	3.70	0.66

[1]Cows were assigned to a estrous synchronization + FTAI protocol (Meneghetti *et al*., 2009) from day −11 to 0 of the experiment. An estrous detection patch was attached to the tailhead of each cow on day −2, and estrous expression was defined as removal of >50% of the rub-off coating from the patch on day 0 (FTAI).

[2]On day 15, cows (EST, *n* = 29; NOEST, *n* = 30) were assigned to conceptus collection via transcervical flushing, followed by endometrial biopsy in the uterine horn ipsilateral to the corpus luteum (Cerri *et al*., 2011; Ribeiro *et al*., 2016). Additional blood samples were collected for whole blood RNA extraction (day 20) and pregnancy status was verified by transrectal ultrasonography (day 30) in cows not assigned to conceptus collection (EST, *n* = 7; NOEST, *n* = 18).

increases in endometrial mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* are observed near day 15 of the bovine estrous cycle, which stabilize after day 16 (Arosh *et al*., 2002). Such robust response, which is inherent to the bovine estrous cycle, may have prevented the detection of estrous expression effects on these genes on day 15 of gestation herein, but not on day 19 in Davoodi *et al.* (2016).

Expression of ISGs in whole blood can be used to assess conceptus development and IFNt production from day 15 to 22 of gestation, as well as pregnancy diagnosis 18 days after breeding (Fricke *et al*. 2016). More specifically, IFNt production by the conceptus upregulates mRNA expression of ISGs, particularly *myxovirus resistance 2*, in body tissues such as whole blood cells (Stevenson *et al*., 2007). Accordingly, mRNA expression of all ISGs reported herein were upregulated in pregnant cows across EST and NOEST groups. Yet, only *myxovirus resistance 2* was upregulated in EST *v*. NOEST cows, despite differences detected for conceptus length and IFNt mRNA expression on day 15 of gestation. Rodrigues *et al*. (2018) also noted that estrous expression during near the time of FTAI upregulated mRNA expression of *myxovirus resistance 2* in pregnant cows on day 20 of gestation, without similar outcomes for *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase.* The reasons for such outcomes herein and in Rodrigues *et al*. (2018) are unclear, despite the beneficial impacts of estrous expression on conceptus development in beef females.

Conclusions

Results from this experiment provide novel information about the mechanisms by which estrous expression near the time of FTAI improves reproductive function and performance in *B. indicus* cows. Cows that expressed EST had greater dominant follicle diameter at FTAI, greater plasma P4 concentration 7 days later, increased length and mRNA expression of IFNt-related genes in 15-day old conceptuses, resulting in increased mRNA expression of the ISG *myxovirus resistance 2* on 20 of gestation. All these responses are known to increase pregnancy success to FTAI in beef females, across *B. indicus* and *B. taurus* breeds (Cipriano *et al*., 2016; Brandão *et al*., 2018; Rodrigues *et al*., 2018). Collectively, this experiment demonstrates that estrous expression increases pregnancy rates to FTAI by enhancing pregnancy establishment events, including IFNt-based processes responsible for pregnancy signaling to maternal tissues. Hence, management strategies and synchronization protocols that promote expression of EST near the time of FTAI are warranted to optimize reproductive efficiency of beef females.

Acknowledgments

Dr Reinaldo F. Cooke is also affiliated as permanent professor to the Programa de Pós-Graduação em Zootecnia/São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu 18168-000, Brazil. Results reported herein were preliminarily published as a conference abstract by Schubach *et al*. (2018).

Declaration of interest

No conflict of interest to report.

Ethics statement

All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee.

Software and data repository resources

No software, data or models were deposited in official repositories.

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