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Peri-ovulatory endocrine regulation of the prostanoid pathways in the bovine uterus at early dioestrus

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Abstract. We hypothesised that different endocrine profiles associated with pre-ovulatory follicle (POF) size would impact on uterine prostanoid pathways and thereby modulate the histotroph composition. Beef cows ($n = 15$ per group) were hormonally manipulated to have small (SF-SCL group) or large (LF-LCL group) pre-ovulatory follicles (POF) and corpora lutea (CL). Seven days after induction of ovulation, animals were slaughtered and uterine tissues and flushings were collected for quantification of prostanoids. The POF and CL size and the circulating progesterone concentrations at Day 7 were greater ($P < 0.05$) in the LF-LCL cows than in the SF-SCL group, as expected. The abundance of 5 out of 19 genes involved in prostanoid regulation was different between groups. Transcript abundance of prostaglandin F2α, E2 and I2 synthases was upregulated ($P < 0.05$) and phospholipase A2 was downregulated ($P < 0.05$) in endometrium of the LF-LCL group. No difference ($P > 0.1$) in prostanoid concentrations in the endometrium or in uterine flushings was detected between groups. However, prostaglandin F2α and E2 concentrations in the uterine flushings were positively correlated with the abundance of transcripts for prostaglandin endoperoxide synthase 2 (0.779 and 0.865, respectively; $P < 0.002$). We conclude that endometrial gene expression related to prostanoid synthesis is modulated by the peri-ovulatory endocrine profile associated with POF size, but at early dioestrus differences in transcript abundance were not reflected in changes in prostanoid concentrations in the uterine tissue and fluid.

Additional keywords: endometrium, oestrogen, physiology, prostaglandins.

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Introduction

A significant proportion of bovine females fail to become pregnant after insemination (Diskin *et al.* 2012; Pohler *et al.* 2012) and this has a negative economic impact on beef cattle operations. This high proportion of non-pregnant animals is mainly caused by early embryo loss (Diskin and Sreenan 1980; Diskin and Morris 2008). Therefore, necessary improvements in the reproductive efficiency depend on a greater understanding of the endocrine, cellular and molecular mechanisms involved in reproductive events during early dioestrus. The oviductal and uterine environments play a relevant role during the establishment and maintenance of pregnancy (Bauersachs *et al.* 2003; El-Sayed *et al.* 2006; Ulbrich *et al.* 2013). Indeed, previous studies determined that specific transcriptomic profiles at early dioestrus are necessary for adequate uterine receptivity (Forde *et al.* 2009; Mansouri-Attia *et al.* 2009; Walker *et al.* 2012; Beltman *et al.* 2014; Binelli *et al.* 2015; Mesquita *et al.* 2015). It is known that the timing and magnitude of oestradiol (E2) exposure during pro-oestrus and oestrus, followed by progesterone (P4) at dioestrus, modulate gene expression in the endometrium and histotroph composition and function (Forde *et al.* 2009; Bridges *et al.* 2012; Ramos *et al.* 2015). In this

context, Mesquita *et al.* (2014) also showed that the size of the pre-ovulatory follicle (POF) alters the peri-ovulatory endocrine milieu (i.e. the concentrations of E2 in pro-oestrus and P4 in dioestrus) and acts on the uterus to alter endometrial gene expression.

It is proposed that the uterine environment and receptivity might be modulated in response to peri-ovulatory endocrine changes. Several molecules and associated pathways have been proposed as key factors to determine uterine receptivity and the endocrine regulation of these pathways has been studied in detail by our group (Ramos *et al.* 2014, 2015; Araújo *et al.* 2015; França *et al.* 2015) and others (Bauersachs *et al.* 2006). However, the search continues for regulatory signals that might be involved in the critical processes of maternal receptivity in order to understand and subsequently tackle the possible causes for high rates of early embryonic death in beef cattle. In this context, reports have focussed on unravelling the complex role of the endometrial eicosanoid lipid mediators in the control of a range of reproductive processes (Weems *et al.* 2006). Prostanoids are well-described eicosanoids, which exert pivotal roles in regulation of reproductive processes such as ovulation, implantation, luteolysis and parturition in mammals (Lim *et al.* 1997; Wiltbank and Ottobre 2003). However, prostaglandin (PG) synthesis pathways are also important and active before maternal recognition of pregnancy in ruminants.

Previous studies have shown that bovine embryos at morula and blastocyst stages were susceptible to elevated prostaglandin F2α (PGF2α) concentrations in the uterine lumen, which could negatively influence embryo viability and pregnancy rates (Schrick *et al.* 1993; Buford *et al.* 1996; Seals *et al.* 1998; Hockett *et al.* 2004). Regarding prostaglandin E2 (PGE2), the expression of its main synthase (prostaglandin E synthase (PTGES)) was downregulated in the endometrium of heifers with a retarded embryo at Day 7 after oestrus (Beltman *et al.* 2010), indicating that lack of PGE2 embryotrophic stimulus (Arosh *et al.* 2004; Ulbrich *et al.* 2009) could have contributed to the decreased fertility in these beef heifers. In addition, PGE2 is known to stimulate embryo implantation, luteal function and to modulate the uterine immune response and embryo development mainly by exerting anti-inflammatory effects (Arosh *et al.* 2004; Cong *et al.* 2006; Mosher *et al.* 2012; Vilella *et al.* 2013). Prostaglandin I2 (PGI2) improves the developmental competence of embryos, as the supplementation of *in vitro* culture medium with a PGI2 analogue improved embryonic quality by increasing the proportion of bovine embryos that developed to the expanded blastocyst stage (Song *et al.* 2009). Expression of genes involved in prostaglandin synthesis was reported by Dorniak *et al.* (2011). These authors concluded that PGF2α and PGE2 are important regulators of conceptus elongation and mediators of endometrial responses to P4 in sheep. Therefore, because of critical effects of prostaglandins on embryo development during early dioestrus, deregulation of their biosynthesis may be one of the mechanisms associated with early embryonic loss in cattle.

Herein, we propose that prostanoids are a possible class of endocrine-modulated molecules that are important for embryo receptivity and thus female fertility at early dioestrus. In the present study, we are the first to evaluate the endocrine influences on prostanoid pathways in Day-7 endometrial tissue and uterine flushings; a timing that coincides with the moment of embryo reception by the maternal uterus. Therefore, we used a bovine fertility model as previously described by Mesquita *et al.* (2014, 2015), Ramos *et al.* (2014, 2015), Araújo *et al.* (2015) and França *et al.* (2015) and associated with fertility (Pugliesi *et al.* 2015) in order to evaluate whether peri-ovulatory variations in circulating steroids, positively associated with the ovulatory follicle size, regulate: (1) the expression of endometrial genes involved in the synthesis, transport, signalling and catabolism of eicosanoids and (2) the concentration of eicosanoids in endometrial tissue and uterine secretions.

Materials and methods

Animal procedures

This study was conducted at the Universidade de São Paulo, Pirassununga, São Paulo, Brazil. Animal procedures were approved by the Ethics and Animal Handling Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo with protocol number 2287/2011. Thirty multiparous Nelore cows (*Bos taurus indicus*) without reproductive abnormalities and with body-condition scores between 3 and 4 (0 being emaciate, 5 being obese) were kept on grazing conditions supplemented with sugarcane or corn silage (or both), concentrate and mineral salt. Animals received water *ad libitum*.

In order to form two groups of cows with different POF sizes and subsequent corpus luteum (CL) volume and P4 concentration, a hormonal protocol was used in all cows to manage endocrine patterns of the peri-ovulatory period as described by Mesquita *et al.* (2014). The model was based on the pharmacological control of follicle growth to result in a group exhibiting larger (LF-LCL group) or smaller (SF-SCL group) POF and CL and consequently resulting in different circulating P4 concentrations during early dioestrus. To reach this goal, cows ($n = 15$ per group) received two intramuscular (i.m.) doses of prostaglandin F2α (PGF; 0.5 mg; sodium cloprostenol; Sincrocio; Ourofino, Cravinhos, Brazil) 14 days apart. Following this pre-synchronisation procedure, ovaries were visualised using transrectal ultrasound scanning in order to confirm the presence of a PGF-responsible CL 10 days after the second PGF administration, (Day −10 of the experiment; D–10). On D–10, all cows were treated with 2 mg of oestradiol benzoate (Sincrodiol; Ourofino) and received a P4 intravaginal releasing device (1 g; Sincrogest; Ourofino) to stimulate recruitment of a new follicular wave. Females assigned to the larger POF and subsequent larger CL group (LF-LCL) additionally received a PGF injection on D–10 (0.5 mg; sodium cloprostenol; Sincrocio; Ourofino) to induce CL regression during follicle development, whereas cows assigned to the smaller POF followed by smaller CL group (SF-SCL) did not. Sixty hours before the induction of ovulation, P4 devices were removed and a PGF dose (0.5 mg; Cloprostenol) was administered i.m. to cows in the LF-LCL group, whereas cows in the SF-SCL group received the same treatment 12 h later (D–2.5 and D–2, respectively). Ovulation was induced on Day 0 by an i.m. administration of gonadotrophin-releasing hormone (GnRH; 10 μg buserelin acetate, Sincroforte;

Ourofino). Seven days after induction of ovulation, cows that ovulated in response to GnRH within 48 h ($n = 11$ cows in the SF-SCL group and 12 cows in LF-LCL group) were slaughtered and their reproductive tracts were collected for further analysis. More details about the animal model are available in previous publications by our group (Mesquita *et al.* 2014; França *et al.* 2015).

Ultrasonography

The ovaries were evaluated by transrectal ultrasonography on D–10, D–6 and every 24 h, starting on D–2 until D7, using both an Aloka SSD-500 attached to a 5-MHz linear probe (Hitachi Aloka, Tokyo, Japan) and an Esaote MyLab 30 attached to a multi-frequency probe (Esaote, Genoa, Italy) set between 6 and 7.5 MHz. Ultrasonography was performed to evaluate the presence and size of dominant follicles and CL. The maximum diameter and perpendicular diameter of the largest ovarian follicle were measured using a B-mode still image and an averaged diameter was calculated. Ovulation was defined as the disappearance of the largest ovarian follicle followed by the presence of a new CL at the same location. For evaluation of size of the CL, the maximum CL area was determined using a B-mode still image and the tracing function. For CL with an anechoic fluid-filled cavity, the area of the cavity was subtracted from the total area (Kastelic *et al.* 1990; Pugliesi *et al.* 2014).

Sample collection and processing

At D7 the animals were slaughtered for collection of the reproductive tract. The reproductive tissues were transported on ice to the laboratory within 15 min. The uterine horn ipsilateral to the ovulation was flushed with 20 mL of phosphate-buffered saline (PBS). The uterine flushing was centrifuged at 3000*g* for 30 min at 4°C and the supernatant was stored at −80°C for quantification of eicosanoids. After the flushing, the ipsilateral uterine horn was dissected and fragments of the intercaruncular area were taken. This region was chosen because most endometrial glands responsible for secretion of histotroph are found in this region on the uterine tissue (Dhaliwal *et al.* 2002). The uterine samples were stored at −80°C for quantification of RNA and prostanoid metabolites.

Quantification of progesterone concentrations

Blood samples were taken from the jugular vein on the day of slaughter (D7). Plasma was removed after blood centrifugation at 1500*g* for 30 min at 4°C. P4 plasma concentrations were measured in the samples using a commercial kit (Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), previously validated for bovine plasma samples (Garbarino *et al.* 2004). The intra- and inter-assay CV and sensitivity for P4, were 0.3%, 7.0% and 0.076 ng mL^{-1}, respectively.

Transcript quantification by real-time reverse transcription polymerase chain reaction (PCR)

Approximately 30 mg of endometrial tissue was macerated in liquid nitrogen (SF-SCL, $n = 11$; LF-LCL, $n = 12$) and submitted to total RNA extraction using the RNeasy Mini columns kit (Qiagen Laboratories, Valência, CA, USA) according to the manufacturer's instructions. The RNA concentration was measured spectrophotometrically (NanoDrop; Thermo Scientific, Wilmington, MA, USA). Before the reverse transcription (RT), the RNA samples were treated with DNase I (deoxyribonuclease I, Pure Link Genomic DNA Purification; Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. Briefly, the treatment with DNase was done at room temperature using 1.0 µg of RNA in a 10-µL reaction volume. After 15 min of incubation, 1.0 µL of ethylenediamine tetraacetic acid (EDTA, 25 mM; Invitrogen) was added and warmed to 65°C for 10 min. Synthesis of cDNA was performed using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corporation, Frederick, MD, USA). A master mix (9.0 µL) was added to the 11.0 µL of the treated samples. The samples were incubated at 25°C for 10 min and then at 37°C for 2 h, followed by an enzymatic inactivation period at 85°C for 5 min.

The primers were designed using the Oligo Analyzer 3.1 software (Integrated DNA Technologies, Inc., Coralville, IA, USA, http://www.idtdna.com/calc/analyzer, accessed 15 June 2012) and Software Primer Express 3.0.1 (Life Technologies, Frederick, MA, USA) or were obtained from previous reports. The qPCR reactions were performed using SYBR Green Chemistry for the amplification analysis in a thermocycler (Step One Plus Real Time System; Life Technologies, Frederick, MA, USA). The thermocycler was programmed to start in a holding stage (95°C for 10 min), followed by 40 cycles. Each cycle had a denaturation step (95°C for 15 s) and an annealing phase (60°C for 1 min). A dissociation ('melting') curve was obtained immediately after the amplification and then maintained at 95°C for 15 min, at 60°C for 1 min and then at 95°C for 15 min. The criteria for validation of primers were: amplification efficiency between 85 and 110%, absence of amplification of the negative control, a single peak in the melting curve and the smallest cycle threshold. Standard curves for each primer were validated using five progressive dilutions and using duplicates. This was obtained using a pool of endometrial cDNA samples in dilutions of 1 : 20, 1 : 40, 1 : 80, 1 : 160 and 1 : 320 (cDNA : H_2O). Determination of PCR efficiency and Cq (quantification cycle) values per sample was performed with LinReg PCR software (http://linregpcr.nl/, verified 28 August 2015). Quantification was obtained after normalisation of the target gene expression values (Cq values) by the endogenous control expression in triplicate values of peptidylprolyl isomerase A (cyclophilin A, *PPIA*), using the equation described by Pfaffl (2001) and expressed as a ratio of target gene-to-endogenous control. PCR products of reactions using the primers designed were submitted to electrophoresis and sequencing. Details of primers are provided in Table 1 and the validation data is given in Table S1, available as Supplementary Material to this paper.

Quantification of prostanoid abundance

Liquid chromatography – mass spectrometry (LC–MS/MS)

The prostanoid concentrations were measured in the endometrial tissue and in the uterine flushings from a subgroup of cows selected randomly from each experimental group ($n = 4$–6 samples per group). The oxylipin analysis was basically performed as described by Lundström *et al.* (2013) using the

Table 1. Primer name, forward (F) and reverse (R) sequence, representative identification number and amplicon size of genes from the prostanoid synthesis and signalling pathway

Gene symbol	Gene name	Sequence 5′–3′	ID	Software/reference[A]	Size (bp)
AKR1B1	Aldo–keto reductase family1, member B1	F-ATACAAGCCGGCGGTTAAC R-TGTCTGCAATCGCTTTGATC	NM_001012519	Ulbrich *et al.* 2009	188
AKR1C3	Aldo–keto reductase family1, member C3	F- GACTCAGTTCTTTGTGCCATTGC R-TCAGTTCAAAGTCAAACACCTGTATG	NM_001038584.1	Primer Express	154
AKR1C4	Aldo–keto reductase family1, member C4	F- TCCTGTCCTGGGATTTGGAACCTT R-ATCGGCAATCTTGCTTCGAATGGC	NM_181027.2	PrimerQuest	166
CBR1	Carbonyl reductase 1	F- TTGCCTTCAAGACTGCTGACA R-CACTGACAAAGCTGGATACATTCAC	NM_001034513.1	Primer Express	153
HPGD	Hydroxyprostaglandin dehydrogenase	F- TGATCAGTGGAACCTACCTGG R-TGAGATTAGCAGCCATCGC	NM_001034419	Ulbrich *et al.* 2009	183
PPIA	Peptidylprolyl isomerase A (cyclophilin A)	F- GCCATGGAGCGCTTTGG R-CCACAGTCAGCAATGGTGATCT	NM_178320.2	Bettegowda *et al.* 2006	69
PLA2G10	Phospholipase A2, group X, transcript variant	F- TGTGCCCGAAGGTAGGGCTGTT R-GGCGAGGGCCAACACAGTCAAT	XM_864950.4	Ulbrich *et al.* 2009	138
PTGDR	Prostaglandin D2 receptor	F- TTCAGCACAGCAACAAGCTCACAG R-ATCTTACCATCTCCACCAAGGGCA	NM_001098034.1	PrimerQuest	115
PTGDS	Prostaglandin D2 synthase	F-AGGTCAAGGAACACTTCACCACCT R-TTGTCAGTCTTCGGCAGGAACACA	NM_174791.4	PrimerQuest	82
PTGER2	Prostaglandin E receptor 2	F- CTACTTGCCTTTTCCATGACC R-GATGAAGCACCACGTCCC	NM_174588	Ulbrich *et al.* 2009	210
PTGER4	Prostaglandin E receptor 4	F- CGATGAGTATTGAGCGCTACC R-AGCCCGCATACATGTAGGAG	NM_174589	Ulbrich *et al.* 2009	237
PTGES	Prostaglandin E synthase	F- GCTGCGGAAGAAGGCTTTTGCC R-GGGCTCTGAGGCAGCGTTCC	NM_174443.2	PrimerQuest	101
PTGES2	Prostaglandin E synthase 2	F- GTGGGCGGACGACTGGTTGG R-CGGAGGTGGTGCCTGCGTTT	NM_001166554.1	PrimerQuest	192
PTGES3	Prostaglandin E synthase 3	F- CAGTCATGGCCAAGGTTAACAAA R-ATCACCACCCATGTTGTTCATC	NM_001007806.2	Primer Express	150
PTGS1	Prostaglandin endoperoxide synthase 1	F- CACCCGCTCATGCCCGACTC R-TTCCTACCCCCACCGATCCGG	NM_001105323.1	PrimerQuest	155
PTGS2	Prostaglandin endoperoxide synthase 2	F- CCAGAGCTCTTCCTCCTGTG R-GGCAAAGAATGCAAACATCA	NM_174445.2	PrimerQuest	161
PTGIS	Prostaglandin I2 synthase	F-AAGATGGGAAGCGACTGAAG R-ATCAGCTCCAGGTCAAACTG	NM_174444.1	PrimerQuest	136
SLCO2A1	Solute carrier organic anion transporter family, member 2A1	F- TGTGGAGACGATGGGATTGA R-GGGACACGGGCCTGTCTT	NM_174829.3	Primer Express	150
TBXA2R	Thromboxane A2 receptor	F- TGTCCTTCCTGCTCAACACCATCA R-AAATGCTGGCCACCACCATAATGC	NM_001167919.1	PrimerQuest	141
TBXAS1	Thromboxane A synthase 1	F- TCACCAACACTCTCTCTTTCGCCA R-TCCTTGCTGAAACAGTCCACCTCT	NM_001046027.1	PrimerQuest	94

[A]Software used for alignment of primer sequences or literature reference.

LC–MS/MS approach and is only briefly described here. The analytical standards and deuterated surrogates were obtained from Cayman Chemical (Ann Arbor, MI, USA), Larodan Fine Chemicals AB (Malmö, Sweden) or Biomol International (Plymouth Meeting, PA, USA). The oxylipins were extracted from 2 mL of uterine flushing using Waters Oasis-HBL cartridges (Waters, Milford, MA, USA) preconditioned with wash solution (H_2O : MeOH; 95 : 5, in 0.1% acetic acid). The uterine flushing aliquots, 200 μL of wash solution, 10 μL of surrogate standards (400 nM per standard in MeOH), 10 μL anti-oxidant and enzyme inhibitor solution (0.2 mg mL^{-1} of butylated hydroxytoluene (BHT), EDTA, thiamine pyrophosphate and indomethacin) were applied to the cartridge, rinsed with wash solution, eluted with 500 μL of methanol and then with 1.5 mL of ethyl acetate and collected into polypropylene tubes containing 6 μL of 30% glycerol in methanol. The solvent was stripped and the sample was suspended in 50 μL of methanol containing the technical standard 1-cyclohexyl-dodecanoic acid urea (CUDA; 800 nM). The samples were then centrifuged at 10 000*g* for 30 min at 4°C and the supernatants were stored at −20°C until analysis. Oxylipin profiling was performed using 10-μL sample injections on a Waters ACQUITY UPLC system via a 2.1 × 150 mm, 1.7-μm Waters Acquity BEH column maintained at 60°C coupled to an XEVO TQ triple quadrupole mass spectrometer (Waters). The samples were maintained at 4°C before injection. Solvents A (0.1% acetic acid in water) and B (acetonitrile : methanol : acetic acid, 88 : 12 : 0.1) were used in the following gradient: 15% B for 0.74 min, 30% B at 1.5 min, 47% B at 3.5 min, 54% B at 6 min, 60% B at 10.5 min, 70% B at 15 min, 80% B at 16 min, 100% B from 17 to 19 min, 30% B from 19.3 to 21 min. The oxylipins detected above the limit of quantitation (LOQ) were quantified, recalculated based on the original uterine flushing concentrations and normalised to the uterine flushing recovery (V[recovered volume]/V[instilled volume]). The normalisation to uterine flushing recovery did not affect the overall trends in the samples. For the endometrial tissue, the samples were previously extracted with organic solvents before solid-phase extraction. For this step, 100 mg of cryo-pulverised endometrial tissue was added to amber vials (2 mL, polytetrafluoroethylene (PTFE) caps; National Scientific Co., Rockwood, TN, USA) prepared with 5 μL of BHT–EDTA (0.2 mg mL^{-1} in 1 : 1 MeOH : H_2O) and 20 μL of the surrogate standards (1000 nM per standard in MeOH). Then, 500 μL of MeOH was added and the vials were capped and then briefly vortexed. The samples were then centrifuged at 3.000*g* and 0°C for 5 min. The supernatant was collected and saved. Then, 350 μL of isopropyl alcohol (IPA) was added to the remaining tissue. The samples were treated in an identical manner using methanol and the IPA extract was added to the MeOH fraction. The remaining tissue was mixed with 350 μL of cyclohexane. The cyclohexane extract was treated as described above and the supernatant was pooled with IPA and MeOH. The combined fraction was dried at reduced pressure (Genevac Inc., Stone Ridge, NY, USA) for ~1 h. The dried samples were reconstituted in 200 μL of MeOH : toluene (1 : 1) and 100 μL of a subaliquot of the extract was loaded into the solid phase extraction (SPE) cartridges and extracted as described above for the uterine flushing.

Enzyme-linked immunosorbent assay (ELISA)

Because a reduced number of samples was used for mass spectrometry and considering that PGE2 and PGF2α are the most important prostanoids in the uterus, enzyme-linked immunosorbent assays (ELISAs) to measure the concentrations of PGE2 and PGF2α in uterine flushing samples were validated using commercial kits for PGE2 and PGF2α (both from Cayman Chemical Co.). Initially, a uterine flushing pool was treated with activated charcoal to remove prostaglandins (Turzillo and Fortune 1990). Briefly, 500 mg of activated charcoal was added for each mL of uterine flushing and this mixture was incubated for 45 min and then centrifuged at 12 000*g* and 4°C for 1 h. The supernatant was filtered and stored at −80°C. This prostaglandin-free matrix was used only to prepare PGE2 and PGF2α standards from 15.6 to 1000 pg mL^{-1} and from 7.8 to 500 pg mL^{-1}, respectively. The matrix volume added to each standard was equal at each standard-curve point (five points were used in each curve). The standard curves were compared with the curves produced using only the manufacturer's enzyme immunoassay (EIA) buffer and validated by parallelism. All of the assay-specific reagents were prepared as described and suggested by the manufacturers. After the plate setup and incubations, the absorptions were read spectrophotometrically at a wavelength of 414 nm (Labsystems Multiskan – MS; Thermo Fisher Scientific, Waltham, MA, USA). After validation, the concentrations of PGE2 and PGF2α in the uterine flushings collected from a subset of cows in the LF-CL group ($n = 10$) and the SF-SCL group ($n = 10$) were assayed in duplicate. Concentrations were calculated in reference to a regression equation generated from a standard curve prepared with increasing concentrations of PGE2 or PGF2α, diluted in prostaglandin-free uterine flushing.

Statistical analyses

Outlying observations greater than two standard deviation ranges from the mean were not used in the statistical analyses. The data were tested for normality of the residues using the Shapiro–Wilk test and for homogeneity of variance using the F-max test and natural log-transformed if needed. The ovarian and endocrine variables were analysed by one-way ANOVA to test the effect of the treatment using the PROC GLM procedure of the SAS software (Version 9.2; SAS Institute, Cary, NC, USA). The eicosanoid concentrations and relative gene expression levels were analysed through non-paired Students' *t*-test. Pearson's correlation coefficients were calculated between P4 concentrations, POF and CL size or P4 concentrations and abundance of transcripts and concentrations of prostanoids in the uterus, and between abundance of transcripts and concentrations of prostanoids in the endometrial tissues or uterine flushings. A probability of $P \leq 0.05$ indicated that an effect was significant and a probability of $P > 0.05$ to $P \leq 0.1$ indicated that significance was approached.

Results

Ovarian responses and circulating P4 concentrations: animal model

The hormonal treatments successfully resulted in two groups of cows with distinct ovarian characteristics, as previously

described by Mesquita *et al.* (2014). More specifically, cows assigned to the LF-LCL group had larger POF diameters when compared with animals from the SF-SCL group (12.7 ± 0.3 mm and 11.2 ± 0.4 mm, respectively; $P < 0.05$). Furthermore, the larger POFs resulted in larger (2.5 ± 0.31 vs 1.6 ± 0.1 cm^3; $P < 0.05$) and heavier (2.9 ± 0.4 vs 2.0 ± 0.1 g; $P < 0.05$) corpora lutea on Day 7 after induction of ovulation. Mean P4 concentrations were higher in cows from the LF-LCL group when compared with cows from the SF-SCL group (4.4 ± 0.4 vs 3.5 ± 0.3 ng mL^{-1}; $P < 0.05$; Fig. 1).

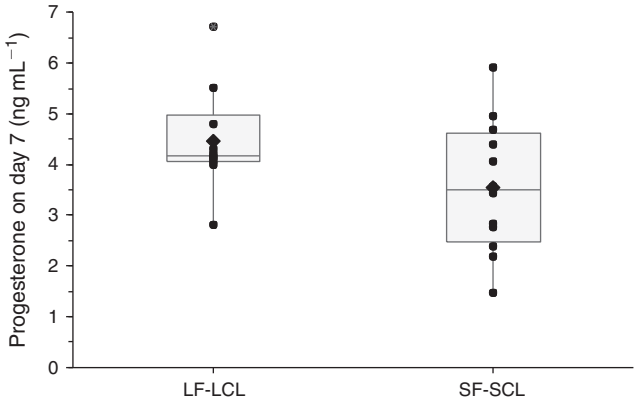

Fig. 1. Box plot showing the mean (diamond), median (continuous horizontal line) and individual values (dots) for progesterone concentrations (ng mL^{-1}) on Day 7 after induction of ovulation in cows treated to achieve a large pre-ovulatory follicle and corpus luteum (LF-LCL group; $n = 12$) and cows treated to achieve a small pre-ovulatory follicle and corpus luteum (SF-SCL group; $n = 11$).

Transcript abundance in endometrial tissue

Gene expression analyses showed that 5 out of 19 analysed genes displayed a significantly modulated expression in response to the differential peri-ovulatory endocrine profiles (Table 2; Fig. 2). More specifically, the expression of phospholipase A2 (*PLA2G10*), encoding an enzyme that releases arachidonic acid for eicosanoid synthesis, was decreased (fold change (fc) 0.58; $P < 0.05$) in the endometrium of cows belonging to the LF-LCL group when compared with the SF-SCL group. The abundance of prostaglandin E synthase (*PTGES*) transcripts in the LF-LCL group was higher than in the SF-SCL group (fc 1.32; $P < 0.05$). The latter prostaglandin synthase E2 enzyme converts prostaglandin H2 (PGH2) into PGE2. Furthermore, the gene expression levels of aldo–keto reductase family 1, member C4 (*AKR1C4*) and aldo–keto reductase family 1, member C3 (*AKR1C3*), involved in PGF2α synthesis, were upregulated (fc 1.65; $P < 0.05$ and fc 1.84; $P < 0.05$, respectively) in the LF-LCL group compared with the SF-SCL counterparts. Also the expression of prostaglandin

Table 2. Mean ± s.e.m. of relative transcript abundance of target genes involved in prostanoid biosynthesis, signalling and catabolism in endometrial tissue at Day 7 after ovulation induction in cows with large pre-ovulatory follicle (POF) and large corpus luteum (LF-LCL, $n = 11$) and cows with small POF and small corpus luteum (SF-SCL, $n = 10$)

NS, no significant difference

Gene symbol	Gene name	LF-LCL	SF-SCL	Fold change LF-LCL/SF-SCL	*P* value
Prostanoid synthesis					
PLA2G10	Phospholipase A2, group X, transcript variant	0.07 ± 0.08	0.12 ± 0.01	0.58	0.04
PTGS1	Prostaglandin endoperoxide synthase 1	0.002 ± 0.0003	0.001 ± 0.0001	1.32	NS
PTGS2	Prostaglandin endoperoxide synthase 2	0.001 ± 0.0002	0.001 ± 0.0003	1.00	NS
PTGES	Prostaglandin E synthase	0.004 ± 0.0004	0.003 ± 0.0002	1.32	0.05
PTGES2	Prostaglandin E synthase 2	0.01 ± 0.0006	0.01 ± 0.0005	1.06	NS
PTGES3	Prostaglandin E synthase 3	0.22 ± 0.02	0.2 ± 0.03	1.20	NS
AKR1B1	Aldo–keto reductase family1, member B1	0.34 ± 0.02	0.3 ± 0.03	1.09	NS
AKR1C4	Aldo–keto reductase family1, member C4	0.007 ± 0.001	0.004 ± 0.0005	1.65	0.04
AKR1C3	Aldo–keto reductase family1, member C3	0.004 ± 0.0008	0.002 ± 0.0003	1.84	0.02
CBR1	Carbonyl reductase 1	0.018 ± 0.002	0.01 ± 0.002	1.25	0.07
PTGDS	Prostaglandin D2 synthase	0.06 ± 0.009	0.06 ± 0.006	1.06	NS
PTGIS	Prostaglandin I2 synthase	0.036 ± 0.008	0.029 ± 0.007	1.22	0.04
TBXAS1	Thromboxane A synthase 1	0.001 ± 0.0002	0.001 ± 0.0002	1.08	NS
Prostanoid transporter					
SLCO2A1	Solute carrier organic anion transporter family, member 2A1	0.003 ± 0.0007	0.002 ± 0.0005	1.22	NS
Prostanoid receptors					
PTGER2	Prostaglandin E receptor 2	0.001 ± 0.0001	0.001 ± 0.0001	0.92	NS
PTGER4	Prostaglandin E receptor 4	0.006 ± 0.001	0.006 ± 0.0009	1.09	NS
PTGDR	Prostaglandin D2 receptor	0.0007 ± 0.0001	0.0006 ± 0.0001	1.03	NS
TBXA2R	Thromboxane A2 receptor	0.001 ± 0.0001	0.0004 ± 0.0001	3.39	NS
Prostanoid Catabolism					
HPGD	Hydroxyprostaglandin dehydrogenase	0.17 ± 0.017	0.1441 ± 0.0139	1.20	NS

I synthase (*PTGIS*), an enzyme that converts PGH2 into PGI2, was greater (fc 1.22; $P < 0.05$) in the endometrium from the LF-LCL group than in the SF-SCL group. The transcript abundance of carbonyl reductase 1 (*CBR1*), which uses PGE2 as a substrate for the synthesis of PGF2α, tended to be upregulated in the LF-LCL compared with the SF-SCL tissue (fc 1.25; $P = 0.07$). No difference ($P > 0.1$) between groups was detected for the transcripts of the main gene related to PGF2α synthesis (aldo–keto reductase family 1, member B1 (*AKR1B1*); Fig. 2).

Eicosanoid abundance in endometrial tissue and in uterine flushings

LC–MS/MS data did not ($P > 0.1$) reveal significant changes in the concentrations of prostanoids between the LF-LCL and SF-SCL endometrial tissue or uterine fluid (Tables 3 and 4). Moreover, concentrations of PGE2 and PGF2α were also measured in a large number of samples ($n = 10$ cows per group) using ELISA techniques in the uterine flushing samples. Consistently, no differences ($P > 0.1$) in either PGE2 or PGF2α concentrations were observed in the Day-7 uterine flushings when comparing LF-LCL versus SF-SCL treatments (Fig. 3).

Correlations between P4 concentrations, POF and CL size

Among the ovarian variables analysed, a significant positive correlation with the P4 concentrations was observed for CL diameter (0.599; $P = 0.004$), CL area (0.579; $P = 0.006$), CL volume (0.554; $P = 0.009$) and CL weight (0.554; $P = 0.01$). A tendency for positive correlation was also observed between

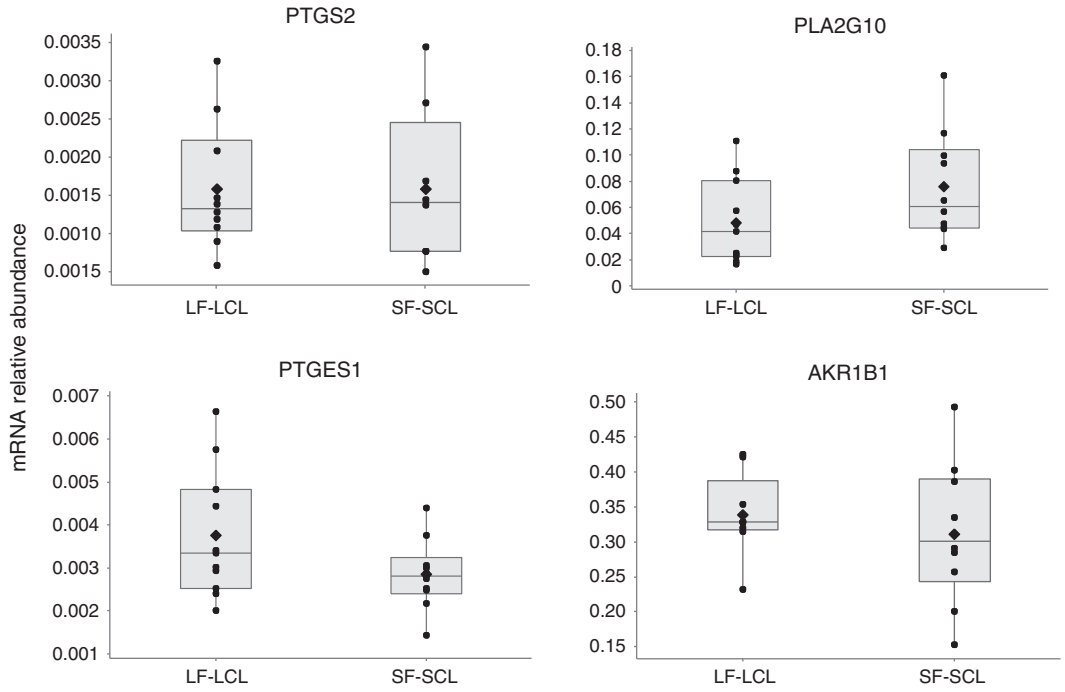

Fig. 2. Box plot showing the mean (diamond), median (continuous horizontal line) and individual values (dots) for relative abundance of mRNA for PTGS2, PLA2G10, PTGES1 and AKR1B1 on Day 7 after induction of ovulation in cows treated to achieve a large pre-ovulatory follicle and corpus luteum (LF-LCL group; $n = 12$) and cows treated to achieve a small pre-ovulatory follicle and corpus luteum (SF-SCL group; $n = 11$).

Table 3. Mean ± s.e.m. of eicosanoid metabolite concentrations (pg mg^{-1}) in the endometrial tissue at Day 7 after ovulation induction in cows with large pre-ovulatory follicle (POF) and large corpus luteum (LF-LCL, $n = 4$) and cows with small POF and small corpus luteum (SF-SCL, $n = 5$)
NS, no significant difference

Eicosanoid metabolite (pg mg^{-1})	LF-FCL	SF-SCL	Fold change LF-LCL/SF-SCL	*P* value
Prostaglandin E2	9.72 ± 2.51	9.50 ± 1.41	1.02	NS
8-Iso-prostaglandin E2	1.37 ± 0.34	1.15 ± 0.58	1.19	NS
Prostaglandin F2α	14.7 ± 1.6	17.6 ± 2.4	0.83	NS
6-Keto-prostaglandin 1α	53.0 ± 1.1	76.0 ± 23.0	0.70	NS
11β-Prostaglandin F2α	1.14 ± 0.44	0.94 ± 0.25	1.21	NS
Prostaglandin D2	90.6 ± 16.1	86.9 ± 18.3	1.04	NS
Δ-12 Prostanglandin J2/prostaglandin J2	0.08 ± 0.01	0.12 ± 0.02	0.72	NS
Thromboxane B2	0.56 ± 0.12	0.48 ± 0.08	1.16	NS

Table 4. Mean ± s.e.m. of concentrations (pg mL^{-1}) of prostanoid metabolites in uterine flushings at Day 7 after ovulation induction in cows with large pre-ovulatory follicle (POF) and large corpus luteum (LF-LCL, $n = 6$) and cows with small POF and small corpus luteum (SF-SCL, $n = 5$)
NS, no significant difference

Prostanoid metabolite	LF-LCL	SF-SCL	Fold changeLF-LCL/SF-SCL	*P* value
Prostaglandin E2	115.2 ± 28.0	75.4 ± 36.6	1.53	NS
8-Iso-prostaglandin E2	7.1 ± 3.1	3.8 ± 0.3	1.85	NS
Prostaglandin F2α	675.3 ± 154.8	664.4 ± 249.4	1.02	NS
Prostaglandin D2	53.1 ± 14.9	40.7 ± 21.4	1.31	NS
Prostaglandin J2/Δ Prostaglandin J2	1.9 ± 0.2	1.9 ± 0.2	1.03	NS
15-Deoxy-Δ-12.14 prostaglandin J2	7.6 ± 0.7	8.4 ± 0.8	0.91	NS
6-Keto-prostaglandin F1α	508.8 ± 118.7	507.4 ± 164.5	1.00	NS
Thromboxane B2	9.75 ± 5.1	18.1 ± 8.8	0.54	NS

P4 concentrations on D7 and POF size (0.414; $P = 0.062$). Also, a positive correlation was detected between POF size and CL diameter (0.613; $P = 0.002$), area (0.614; $P = 0.002$), volume (0.612; $P = 0.002$) and weight (0.697; $P = 0.001$).

Correlation between POF size and P4 concentrations with the abundance of transcripts or concentrations of prostanoids in the uterus

There was no significant correlation between POF size and P4 concentrations and the abundance of transcripts for the enzymes involved in prostanoid synthesis, nor between POF size and P4 concentrations and concentrations of uterine metabolites analysed.

Correlation between abundance of transcripts and concentrations of prostanoids in the endometrial tissues and uterine flushings

There were significant correlations between abundance of transcripts and concentrations of prostanoids in the endometrial tissues and uterine flushings. The transcript for prostaglandin endoperoxide synthase 2 (*PTGS2*) was positively correlated with the concentrations of PGE2 (0.865; $P = 0.0001$) and PGF2α (0.779; $P = 0.002$) measured by ELISA. In contrast, the abundance of *PTGS2* was negatively correlated with the PGF2α concentrations in the endometrial tissue (−0.936; $P = 0.002$) measured by MS/MS. In addition, no significant correlation was observed between prostaglandin synthase and its metabolites. The abundance of *CBR1* transcript, the gene encoding the enzyme responsible for PGE2 conversion to PGF2α, was positively correlated with PGF2α concentrations in the uterine fluid (0.668; $P = 0.005$).

No difference ($P > 0.1$) in the ratio between PGF2α and PGE2 (PGF2α : PGE2) was detected between the LF-LCL (3.68) and SF-CL (4.97) groups.

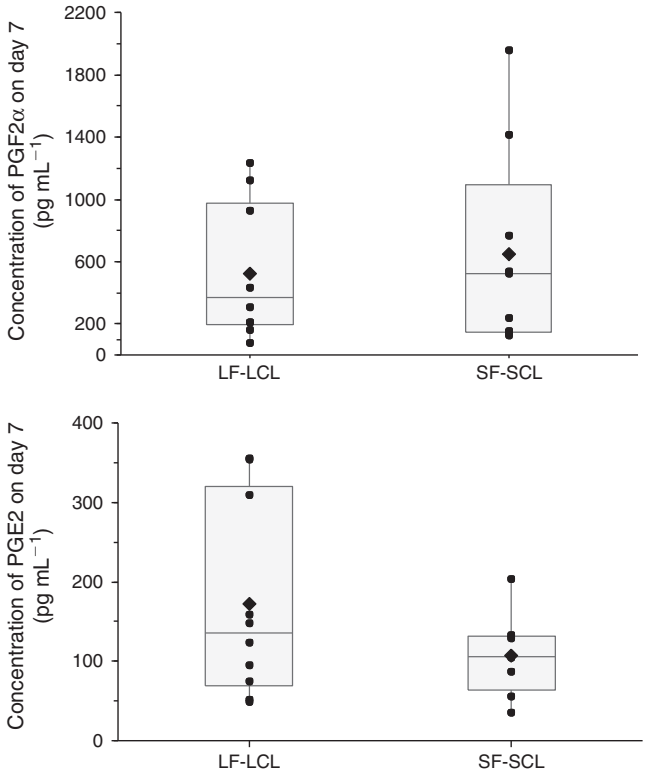

Fig. 3. Box plot showing the mean (diamond), median (continuous horizontal line) and individual values (dots) for PGF2α and PGE2 concentrations (pg mL^{-1}) in uterine flushings at Day 7 after induction of ovulation in cows treated to achieve a large pre-ovulatory follicle and corpus luteum (LF-LCL group; $n = 10$) and cows treated to achieve a small pre-ovulatory follicle and corpus luteum (SF-SCL group; $n = 10$).

Discussion

The quality of the preimplantation uterine environment encompasses a variety of aspects that potentially affect early embryo survival. Hormonal variations during each bovine oestrous cycle induce uterine changes that are crucial for its receptivity to the embryo, as indicated by the increased pregnancy rates in cows with higher circulating P4 concentrations at Day 7 after insemination (McNeill *et al.* 2006; Peres *et al.* 2009). In the present study, we were the first to evaluate the endocrine influences on prostanoid pathways during early dioestrus, which coincides with the moment of embryo reception by the maternal uterus and may consequently interfere with embryo survival.

Considering that the POF size is positively associated with its capacity to secrete E2 and subsequent CL size and P4 secretion (Vasconcelos *et al.* 2001; Carter *et al.* 2008; Peres *et al.* 2009), we used an experimental model based on the modulation of

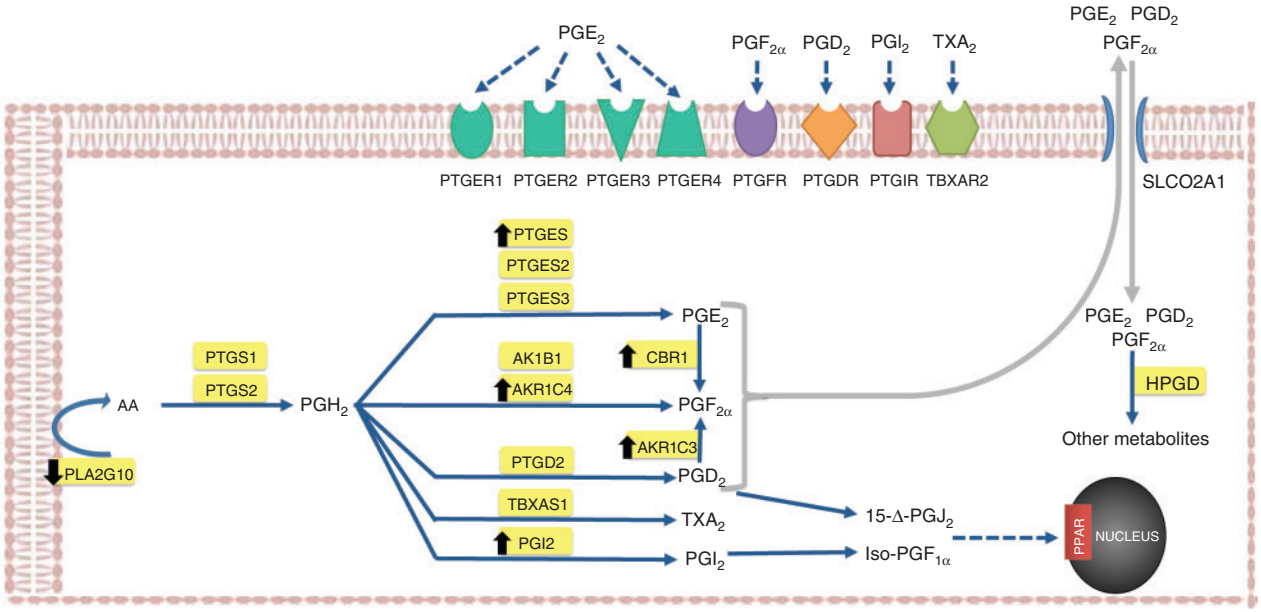

Fig. 4. Biosynthesis, metabolism and regulation pathways of prostanoids in the endometrium. The enzyme phospholipase A2 (PLA2) releases arachidonic acid (AA) from membrane phospholipids. The AA is metabolised by cyclo-oxygenases 1 and 2 (PTGS2 and PTGS1) to PGH2, which is the precursor of all prostanoids. Specific synthases convert PGH2 into PGE2 (PTGES1, PTGES2 and PTGES3), PGF2α (AKR1B1, AKR1C3, AKR1C4 and CBR1), PGD2 (prostaglandin D2 synthase (PTGDS)), PGI2 (PTGIS) and TXA2 (thromboxane A synthase 1 (TBXAS1)). Once synthesised, the transport of prostaglandins through the plasma membrane is done bi-directionally, passively or facilitated by membrane carrier protein (solute carrier organic anion transporter family, member 2A1 (SLCO2A1)). Once outside the cell, prostaglandins can specifically bind to their membrane receptors PTGER1–4, PTGFR, PTGIR, prostaglandin D2 receptor (PTGDR) and thromboxane A2 receptor (TBXAR2). PGI2 and PGD2 promote their biological effects by signalling to nuclear receptors of the peroxisome proliferator-activated receptor (PPAR) family. Prostaglandins can be inactivated by the enzyme hydroxyprostaglandin dehydrogenase (HPDG), which converts prostaglandins to other metabolites. Up and down black arrows in the enzyme symbols indicate the up and downregulated genes, respectively, in the Day-7 endometrial tissue in cows that ovulated larger follicles and had larger corpus luteum (LF-LCL) compared with cows with smaller follicles and smaller corpus luteum. (Adapted from Fortier *et al.* 2008).

follicle growth and CL size, as has been previously described by our group (Mesquita *et al.* 2014, 2015; França *et al.* 2015; Ramos *et al.* 2014). In the present study, positive correlations between P4 concentrations on D7 and POF and CL size were observed. This confirmed that our experimental model not only modulated POF growth but efficiently altered CL growth and function, based on the P4 concentrations during early dioestrus. However, there was no significant correlation between POF size and P4 concentrations with the transcripts involved in the synthesis of PGE2 and PGF2α.

Based on the present results, at Day 7 after induction of ovulation the expression of several enzymes responsible for prostaglandin synthesis was upregulated in the endometrial tissue of cows that ovulated larger follicles compared with the tissue from cows ovulating small follicles and consequently small CLs. Using LC–MS/MS and ELISA techniques, the relevance of the differently expressed genes have been studied in detail, by eicosanoid identification and quantification in the Day-7 endometrial tissue and associated uterine flushings from cows ovulating large or small POF. Interestingly, no differences in concentrations of prostanoids could be observed either in endometrial tissue or in associated uterine flushings when comparing the experimental groups. In Fig. 4, an overview of the prostanoid metabolic pathway and the modulation of gene expression in the LF-LCL group is provided.

When focusing on the genes involved in each eicosanoid synthesis pathway, the endometrium of the cows in the LF-LCL group apparently supported synthesis of PGF2α. The expression of *AKR1C3*, *AKR1C4*, *CBR1*, *PTGES* and *PTGIS* was upregulated in cows with larger POF and CL. Indeed, expression of two enzymes belonging to the aldo–keto reductase family (AKR), which convert PGH2 into PGF2α (Dozier *et al.* 2008; Bresson *et al.* 2011; Phillips *et al.* 2011), was stimulated in the LF-LCL endometrial tissue compared with the SF-SCL counterparts. This effect on expression of prostaglandin synthases may be caused by the combined effect of pre-ovulatory E2 and post-ovulatory P4 modulated by differential POF growth, as correlations between P4 concentrations alone and AKR enzymes and *PTGES* were not detected.

Despite the fact that abundance of mRNAs for *AKR1C3* and *AKR1C4* was upregulated in cows with larger POF and CL, the concentrations of PGF2α were not increased in the endometrium and uterine flushings. Additional support for this mismatch was that a positive correlation between PGF2α synthases and PGF2α concentrations in uterine tissue and flushings were not detected. Several possible explanations should be taken into consideration. The first intuitive reason for this inconsistency is that the synthesis of PGF2α is not dependent only on the conversion of PGH2 by the PGF synthases but also on the expression of other mediators, such as the cyclo-oxygenases (PTGS1 and PTGS2)

to convert the arachidonic acid (AA) into PGH2. In this regard, the production of PGH2 by the cyclo-oxygenase PTGS1 (constitutive) and PTGS2 (regulatory) is considered the rate-limiting step of prostaglandin biosynthesis in the endometrium (Smith *et al.* 2000; Parent *et al.* 2003). This was supported in the present study by the strong positive correlations between *PTGS2* and the concentrations of PGF2α and PGE2 in the uterine flushings. Secretion of PGF2α by endometrial explants is also correlated with their PTGS2 content, suggesting that the increase in the ability of the uterus to produce prostaglandin during the luteal phase of the oestrous cycle is due to the increase in PTGS2 levels (Charpigny *et al.* 1997). In addition, the expression of PTGS2 increases 70–100 times before PGF2α elevation at parturition, whereas PGF2α synthase (*AKR1B1*) increases only 2.6 times (Schuler *et al.* 2006). Steroid hormones may modulate the expression of PTGS2 in endometrial cells (Madore *et al.* 2003), but the significant increase in P4 concentrations in cows with large POF and CL did not result in altered expression of this gene in the present study.

A second consideration is that the abundance of *AKR1B1* was also similar between cows with large and small POF and CL. AKR1B1 is considered to be the main synthase enzyme in the ARK family responsible for PGF2α biosynthesis in the human and bovine endometrium (Madore *et al.* 2003; Bresson *et al.* 2011) and its expression is positively associated with PTGS2 abundance (Charpigny *et al.* 1997; Xiao *et al.* 1998; Schuler *et al.* 2006). Consequently, the similar abundance of transcripts for *PTGS2* and *AKR1B1* in cows with large or small POF and CL and the absence of a significant correlation between P4 concentrations and the abundance of these transcripts may be the main explanations for the lack of difference in PGF2α concentrations in the endometrium and uterine flushings between groups. Therefore, the upregulation of *AKR1C3* and *AKR1C4* in cows with large POF and CL was possibly a response to the greater P4 concentrations on D7, as enzymes in the AKR family have a double function of prostaglandin synthesis and P4 catabolism (Pelletier *et al.* 1999; Madore *et al.* 2003; Ito *et al.* 2006). This result is also a novel finding, as a previous study reported that AKR1C family members were not expressed in the bovine endometrium during dioestrus (Madore *et al.* 2003).

Similarly, the concentrations of PGE2 were not increased in consequence of the greater abundance of *PTGES1* transcript in cows with larger POF and CL. This mismatch between a synthase and its prostanoid may also be caused by the absence of the modulation of *PTGS2* by the different peri-ovulatory endocrine profiles. In line with this, Arosh *et al.* (2002) suggested that the increased PGE2 production in endometrial cells is mainly caused by the associative upregulation of PTGES1 with PTGS2. In addition, lower levels of PGE synthase and PTGS2 in the bovine endometrium were detected between Days 1 and 12 of the oestrous cycle (Arosh *et al.* 2002), indicating a limited capacity of the uterus to secrete PGE2 during early and mid dioestrus. This also suggested that the cyclo-oxygenases might be the key component monitoring final prostaglandin concentrations in the bovine endometrium at early dioestrus. Furthermore, there was an increased abundance of *CBR1* transcripts in the LF-LCL group. This enzyme uses PGE2 as a substrate for the synthesis of PGF2α (Kankofer and Wierciński 1999; Asselin and Fortier 2000; Kankofer *et al.* 2002). Therefore, part of the PGE2 converted by PTGES1 could be instantly transformed into PGF2α by CBR1 activity. The concentrations of PGF2α were greater than the concentrations of PGE2 in the uterine flushings and in the endometrial tissue on D7 of the oestrous cycle. Thus, at least part of this abundance of PGF2α may be caused by the conversion of PGE2 into PGF2α through *CBR1*, as indicated by the positive correlation between PGF2α and CBR1 in the uterine flushings.

A third consideration is related to the gene expression results of the PLA2G10 enzyme. This phospholipase comes into view as a potential regulator of eicosanoid homeostasis, as its downregulated expression in the LF-LCL compared with SF-SCL tissue might result in a limited substrate provision towards effective production of prostaglandins. In this regard, the PLA2 acts on the release of AA, the primary precursor of prostanoids (Godkin *et al.* 2008).

Another consideration regarding the mismatch between gene expression and prostaglandin concentrations is that our results are primarily based on the transcript abundance data. It is not clear whether all transcripts will be translated or even post-translationally modified (Robert 2010). A previous report (Ulbrich *et al.* 2009) documented a similar mismatch when comparing eicosanoid transcripts and metabolite concentrations in the uterus, although in a reverse way and during a later time window during dioestrus. Finally, post-transcriptional effects regulating activities should be considered as well. The importance of the latter assumption has been recently emphasised by Walker *et al.* (2013); DNA methylation is involved in early pregnancy events, which might point towards potential post-transcriptional alterations.

The complete role of prostaglandins in the fertility of cows still needs to be elucidated, but during early embryo development the evidence is that specific prostanoids are needed for adequate embryonic viability during early dioestrus. Previous research has revealed that development of bovine embryos is impaired by increased PGF2α levels (Scenna *et al.* 2004, 2005) and is stimulated by PGE2 (Arosh *et al.* 2004; Ulbrich *et al.* 2009). Prostaglandins are also essential for elongation of the conceptus, as intrauterine infusions of a selective PTGS2 inhibitor prevented conceptus elongation in early-pregnant sheep (Simmons *et al.* 2010; Dorniak *et al.* 2011). In the present study, a bovine model was used in order to screen for endocrine preparation of maternal receptivity without the presence of the embryo. Considering the previous studies and our working model where cows with large POF and CL had an 80% increase in pregnancy rates (Pugliesi *et al.* 2015), the expectation was that cows in the LF-LCL group could stimulate PGE2 synthesis and inhibit PGF2α in the endometrium. However, as the concentrations of PGE2 and PGF2α were correlated only with abundance of transcripts for *PTGS2*, the study of other important metabolic pathways in uterine tissue at early dioestrus are indicated to understand the positive effects of greater steroid concentrations during the peri-ovulatory period on bovine fertility.

In conclusion, the peri-ovulatory endocrine changes associated with the size of the POF regulate transcript abundance of genes belonging to prostanoid synthesis pathways in the bovine endometrium at early dioestrus (at Day 7 after induction

of ovulation). Specifically, cows that ovulated larger follicles have increased abundance of *AKR1C4*, *AKR1C3*, *PTGIS*, *PTGES* and *CBR1* transcripts in the endometrium, whereas the expression of *PLA2G10* was reduced. These changes in transcription do not result in modifications in the prostanoid concentrations in the endometrium nor in the uterine flushings, which probably result from the lack of modulation of *PTGS2*, the regulatory rate-limiting enzyme in prostaglandin biosynthesis. Indeed, the abundance of transcripts for *PTGS2* is highly and positively correlated with PGF2α and PGE2 concentrations in the uterine flushings. Although the concentrations of prostanoids are not affected by the peri-ovulatory endocrine profiles at this time point, these novel results characterising the prostanoid concentrations at early dioestrus point towards maintenance of homeostasis at the time of early embryo development.

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