UNIVERSIDADE ESTADUAL PAULISTA JÚLIO DE MESQUITA FILHO

FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

EXPRESSÃO GÊNICA DO CORPO LÚTEO APÓS PULSOS INTRAUTERINOS COM DOSES BAIXAS DE PROSTAGLANDINA E1 E F-2 ALFA EM VACAS.

JULIAN CAMILO OCHOA CUERVO.

Botucatu – SP

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Julian Camilo Ochoa Cuervo

Dissertação apresentada à Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista Júlio de Mesquita Filho, Campus Botucatu, para obtenção do titulo de mestre em Biotecnologia Animal, área de Reprodução Animal.

Orientador: Prof. Dr. João Carlos Pinheiro Ferreira

Co-orientador: Prof. Dr. Milo C. Wiltbank

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Nome do autor: Julian Camilo Ochoa Cuervo.

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COMISSÃO EXAMINADORA

_____

Prof. Dr. João Carlos Pinheiro Ferreira

Presidente e Orientador

Departamento de Reprodução Animal e Radiologia Veterinária

FMVZ – UNESP – Botucatu.

_____

Prof. Dr Cezinande de Meira

Membro

Departamento de Reprodução Animal e Radiologia Veterinária

FMVZ – UNESP – Botucatu

_____

Prof. Dr. Roberto Sartori Filho

Membro

Departamento de Zootecnia. ESALQ.

Universidade de São Paulo - Piracicaba

_____

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Dedicado a: Ulises y Martha Lucia

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LISTA DE ABREVIAÇÕES E SIGLAS

AA	Acido Araquidônico
CL	Corpo Lúteo
CLG	Células Luteais Esteroidogênicas Grandes
CLP	Células Luteais Esteroidogênicas Pequenas
cPGES	Cytosolic Prostaglandina E Sintetase
CypA	Ciclofilina A
CYP11A1	Citocromo P450, família 11, subfamília A, polipéptido 1.
DGLA	Ácido di-homo-ɣ-linolênico
E2	17β-Estradiol
EGR1	Early growth response 1
FAS	Fas, membro da superfamília de receptores TNF.
FASLG	Fas ligando
FGF2	Factor de crescimento fibroblastico 2.
FOS	FBJ Murine Osteosarcoma Viral Oncogene Homolog
FSH	Hormônio Folículo Estimulante
GnRH	Hormônio Liberador de Gonadotrofina
HPGD	Hydroxyprostaglandin Dehydrogenasa 15-[NAD]
IL1B	Interleucina 1, Beta
IL8	Interleucina 8
IU	Intrauterino
JUN	Jun Proto-oncogene

LH	Hormônio Luteinizante
LHCGR	Receptor de hormônio luteinizante/coriogonadotropina
mPGES-1	Prostaglandina E Sintetase-1
NR4A1	Receptor nuclear subfamília 4, grupo A, membro 1
NR5A1	Receptor nuclear subfamília 5, grupo A, membro 1
OXT	Ocitocina
P4	Progesterona
PLA2	Fosfolipase $A_{2\alpha}$
PGD	Prostaglandina D_2
PGH	Prostaglandina H_2
PGDH	15-Hydroxyprostaglandin Dehydrogenase
PGE1	Prostaglandina E_1
PGE2	Prostaglandina E_2
PGEM	13,14-Dihydro-15-Keto-PGE_2 / 13,14-Dihydro-15-Keto-PGE_1
PGFM	13,14-Dihydro-15-Keto-$PGF_{2\alpha}$
PGF	Prostaglandina $F_{2\alpha}$
PTGFS	Prostaglandina-F Sintetase
PTGFR	Receptor de prostaglandina-F
PTGS1	Prostaglandina-endoperoxido Sintetase 1
PTGS2	Prostaglandina-endoperoxido Sintetase 2
StAR	Proteína Reguladora Aguda da Esteroidogênese.
RPS15	Proteína ribosomal S15
VEGFA	Factor de crescimento endothelial vascular

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OCHOA, J.C. Expressão gênica do corpo lúteo após pulsos intrauterinos com doses baixas de prostaglandina E1 e F-2 alfa em vacas. Botucatu 2016, 68p. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP.

RESUMO

Palavras-chave: Corpo luteo, luteólise, prostaglandina E1.

Em ruminantes a luteólise natural é caraterizada pela liberação de vários pulsos de prostaglandina F2alfa (PGF) produzidos pelo útero. A PGF é o hormônio luteolítico, enquanto a prostaglandina E1 (PGE1) é considerada um mediador luteoprotetor. Em estudos anteriores, infusões com doses baixas de PGF no útero com intervalos de 6 horas (h) resultou em regressão do corpo luteo (CL). A proposta deste experimento é desenvolver um modelo para avaliar o efeito de baixas doses de PGE1, também infundidas no lúmen uterino sobre a resposta luteal à PGF intrauterina (IU). Vacas no dia 10 do ciclo estral receberam infusões IU de salina (0,1 ml de salina + 0,1 ml de DMSO), PGE (2 mg de PGE1 em 0,1ml de DMSO) ou PGF (0,25 mg of PGF em 0,1 ml de salina) em intervalos de 6 h em um desenho experimental 2 X 2. Portanto os animais foram agrupados em quatro tratamentos: SALINA (4 infusões de salina; n=5), PGE (4 infusões de PGE1; n=5), PGF (4 infusões de PGF; n=5) e PGE+PGF (4 infusões de PGE1+PGF; n=5). As concentrações plasmáticas de progesterona (P4) foram dosadas por radioimunoensaio e o volume luteal foi determinado por ultrassonografia transretal. As concentrações circulantes de PGFM e PGEM foram dosadas antes e 10 minutos após as primeiras duas infusões. Biopsias luteais foram coletadas de cada vaca 30 minutos após cada infusão para determiner a expressão de genes em resposta a cada tratamento. As concentrações circulantes de PGFM 10 minutos após as infusões foram maiores em vacas que receberam tratamentos com PGF e PGE+PGF em comparação com as vacas tratadas com salina e PGE. Da mesma forma, as concentrações de PGEM 10 minutos após cada infusão foram maiores em vacas tratadas com PGE e PGE+PGF em comparação com vacas dos grupos salina e PGF. As concentrações de P4 diminuiram no grupo PGF em comparação com o grupo

Salina no tempo 12-h (48,9% do controle) após a primeira infusão de PGF, no tempo 24-h (20,2% do controle), e em todos tempos subsequentes ($P < 0,05$). Não foram encontradas diferenças nas concentrações circulantes de P4 entre os grupos Salina, PGE e PGF+PGE. Houve também uma diminuição do volume luteal entre o grupo PGF e os outros três grupos que foi observada nos tempos 24-h (56,4% do controle), 48-h (30,6% do controle), e 72-h (20,4% do controle) após o tratamento com PGF ($P<0.05$). Não houve diferenças no volume luteal entre os tratamentos salina, PGE e PGE+PGF. Por tanto, infusões IU simultâneas de baixas doses de PGE1 bloquearam a ação luteolitica de pulsos IU de PGF em vacas, como observado nas mudanças circulantes de P4 e volume luteal. Análises da expressão génica nas biopsias luteais coletadas após o terceiro pulso de PGF, indicam o padrão tipico de expressão de genes em resposta ao tratamento com PGF (FGF2, EGR1, FOS e FAS aumentaram; PTGFR, VEGFA, NR5A1 e STAR diminuiram) e o tratamento PGE+PGF bloqueou completamente as mudanças na expressão destes genes. Infusões IU de PGF e PGE1 parecem ser um excelente modelo para determiner o padrão de expressão de genes envolvidos no efeito luteoprotetor da PGE1.

OCHOA, J.C. Gene expression in the corpus luteum following intrauterine pulses of low doses of prostaglandins E1 and F-2 alpha in cattle. Botucatu 2016, 68p. Dissertação (Mestrado)– Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP.

ABSTRACT

Keywords: Corpus luteum, luteolysis, prostaglandin E1.

In ruminants, natural luteolysis is characterized by the release of several pulses of prostaglandin F2alpha (PGF) produced by the uterus. Prostaglandin F2alpha is the luteolytic hormone, whereas prostaglandin E1 (PGE1) is considered to be a luteoprotective mediator. In previous studies, low doses of PGF infused into the uterus at 6 hour (h) intervals resulted in regression of the corpus luteum (CL). This study was designed to develop a model to study the effect of low doses of PGE1, also infused into the uterine lumen, on the luteal responses to intrauterine (IU) PGF. Cows on day 10 of the estrous cycle received IU infusions of saline (0,1 ml of saline + 0,1 ml of DMSO), PGE (2 mg of PGE1 in 0,1ml of DMSO) or PGF (0,25 mg of PGF in 0,1 ml of saline) at 6-h intervals in a 2 X 2 experimental design. Thus, there were four treatment groups: SALINE (4 saline infusions; n=5), PGE (4 PGE1 infusions; n=5), PGF (4 PGF infusions; n=5), and PGE+PGF (4 PGE1+PGF infusions; n=5). Radioimmunoassay was used to measure circulating progesterone (P4) concentrations and luteal volume was determined by transrectal ultrasonography. Circulating concentrations of PGFM and PGEM were measured before and 10 minutes after the first two infusions. A luteal biopsy was collected from each cow at 30 minutes after each infusion for later determination of gene expression in response to each treatment. Circulating concentrations of PGFM 10 minutes after infusions were greater in cows receiving treatments with PGF and PGE+PGF than in Saline or PGE-treated cows. In the same way, concentrations of PGEM 10 minutes after infusions, were greater in cows that were treated with PGE and PGE+PGF than in saline and PGF-treated cows. Concentrations of P4 in the PGF group decreased compared to those in the saline group by 12-h (48.9% of control) after first infusion of PGF, at 24-h (20,2% of control), and all subsequent time points ($P < 0,05$). No

differences in circulating P4 concentrations were found between Saline, PGE, and PGF+PGE. There was also a decrease of luteal volume between the PGF group and the other three groups that was detectable at 24 (56,4% of control), 48 (30,6% of control), and 72 (20,4% of control) h after PGF treatment ($P<0.05$). There were no differences in luteal volume between Saline, PGE, or PGE+PGF. Thus, simultaneous IU infusion of a low dose of PGE1 blocked the luteolytic actions of IU PGF pulses in cattle, as measured by changes in circulating P4 and luteal volume. Analyses of gene expression in the luteal biopsy taken after the third PGF pulse indicate a typical pattern of gene expression in response to the PGF treatments (FGF2, EGR1, FOS and FAS increased; PTGFR, VEGFA, NR5A1 and STAR decreased) and that simultaneous PGE1 treatment completely blocked these gene expression changes. Thus, IU infusion of PGF and PGE1 seems to provide an excellent model for determining the patterns of gene expression involved in the luteoprotective effect of PGE1.

CAPÍTULO 1

1. Introdução.

O corpo lúteo (CL) bovino é uma glândula endócrina temporária, que se desenvolve a partir das células da teca e células da granulosa remanescentes do folículo ovulado (1) , e que desempenha papel essencial no estabelecimento e manutenção da gestação em ruminantes, devido à produção de progesterona (P4). O CL da vaca, é composto principalmente por células esteroidogênicas grandes e pequenas, assim como células endoteliais vasculares que interagem para regular de maneira eficiente a produção e secrecão de P4 pelo CL (2-5). A cada ciclo estral, as células luteínicas esteroidogênicas sintetizam e liberam P4 na circulação sistêmica, que promove quiescência do miométrio, desenvolvimento glandular do endométrio e o estabelecimento de um ambiente uterino adequado para o desenvolvimento do concepto. Na ausência de um sinal embrionário, ou em animais não gestantes, a vida funcional do CL é terminada pela ação da prostaglandina $F_{2\alpha}$ (PGF) liberada pelo útero, em padrões pulsáteis no final do ciclo estral (6-8)

Durante o início da gestação na vaca, é discutível se o embrião resgata o CL, produzindo uma substância luteotrópica ou inibindo uma luteolítica. Neste aspecto, o interferon tau (IFNT) é tipicamente reconhecido por exercer um efeito antiluteolitico no endométrio, tendo assim papel fundamental para o reconhecimento da gestação em ruminantes, agindo através da inibição dos pulsos de PGF (9). No entanto, cada vez mais provas suportadas por interações moleculares e celulares desenvolvidas em vacas e ovelhas, sugerem que o mecanismo envolvido no resgate do CL, poderia depender não só da inibição dos pulsos uterinos de PGF mas também da produção de prostaglandina E pelo embrião e útero, que agirá como um mediador com capacidade luteoprotetora (10-13).

A proposta deste este estudo, é utilizar um modelo in vivo de infusões intrauterinas (IU) que simula a liberação natural de PGF e PGE, a fim de entender as respostas luteais às prostaglandinas secretadas pelo embrião e útero durante

a gestação inicial. O estudo da expressão gênica induzida após as infusões, permitirá avançar no entendimento do efeito luteoprotector que a PGE confere ao CL durante o reconhecimento materno da gestação na vaca.

2. Revisão de literatura.

2.1. Ciclo estral na vaca.

A melhor compreensão da fisiologia do ciclo estral em vacas, especialmente a relacionada ao funcionamento das estruturas ovarianas (14-16) melhorou consideravelmente nos últimos 20 anos devido à implementação da ultrassonografia transretal em tempo real. Por tanto, o uso desta ferramenta diagnóstica tem aumentado de modo acelerado o conhecimento do ciclo estral na vaca.

O ciclo estral representa o padrão cíclico das atividades ovarianas que permitem a fêmeas ir de um período de não receptividade reprodutiva a um período receptivo, possibilitando o acasalamento e subsequente estabelecimento da gestação. O estabelecimento dos ciclos estrais começa a partir da puberdade, quando o animal entra em atividade reprodutiva. Essa ciclicidade vai continuar ao longo da maior parte da sua vida (17, 18).

Em novilhas Pardo-Suíço e Brahman, o inicio da pubertade foi reportado em média a os 9,4 e 12,3 meses de idade respetivamente, quando os animais independentemente da raça atingiram um peso de 233 kg (19). Porém, a idade estimada de puberdade em novilhas *Bos taurus* está entre os 16 a 18 meses de idade, enquanto que para os animais Zebu em regiões tropicais e subtropicais está entre os 16 e 40 meses, com media de 25 meses, ou seja de 6 a 12 meses mais tarde do que o *Bos taurus* (20, 21). A duração normal do ciclo estral é de 18-24 dias. O ciclo estral esta constituído por duas fases: a fase luteal (14-18 dias) e a fase folicular (4-6 dias). A fase luteal ocorre após a ovulação quando o CL é formado, enquanto que a fase folicular é o período posterior à regressão do CL (luteólise) até a ovulação. A maturação final e a ovulação do folículo ovulatório, ocorrem durante a fase folicular. O oócito é liberado no oviduto permitindo a fertilização (17). Em vacas, os ciclos são caraterizados pelo

crescimento e regressão dos folículos e do CL, apresentando um comportamento de estro que se repete em média a cada 21 dias (17, 22).

O folículo ovariano é uma das estruturas mais estudadas, devido seu papel como parte integral do processo reprodutivo na vaca e sua importância no controle do ciclo estral assim como na determinação do comportamento de estro. As caraterísticas do folículo irão exercer papel fundamental na função do CL após a ovulação e na síntese de P4 (18). Na fêmea bovina, o crescimento dos folículos antrais ocorre em padrões de crescimento semelhantes a ondas, durante o ciclo ovariano (22). A duração do ciclo estral tem sido relacionada com o número de ondas foliculares. Em animais *Bos indicus* foram descritas duas e três ondas de crescimento folicular, resultando em ciclos com durações médias de 20.7 e 22 dias respetivamente (23). Em novilhas e vacas *Bos taurus* também existe incidência variável no número de ondas foliculares, com animais exibindo 2, 3 e 4 ondas por ciclo, sendo maior a incidência de duas ondas. Cada onda de crescimento envolve emergência, seleção e dominância seguido por atresia ou ovulação do folículo dominante (17, 24, 25). A duração do ciclo estral para vacas em lactação tem sido maior (23 d) em comparação com novilhas (20.8 d) (24).

2.1.1. Regulação endócrina do ciclo estral.

O ciclo estral é regulado pelos hormônios do hipotálamo: hormônio liberador de gonadotrofina (GnRH), da pituitária anterior: hormônio folículo estimulante (FSH) e hormônio luteinizante (LH), dos ovários: P4, 17β-estradiol (E2), ocitocina (OXT), inibina e o útero: PGF. A capacidade regulatória do ciclo estral destes hormônios está baseada num sistema de *feed back* positivo e negativo (26). O controle da GnRH é mediado através das suas ações na pituitária anterior que regulam a secreção das gonadotropinas FSH e LH (27). O GnRH liga-se a receptores específicos e desencadeia a liberação de Ca^{2+} intracelular causando liberação transitória de FSH e LH. A frequência na liberação de LH é determinada pelo *feedback* da P4 ou E2 enquanto que a liberação de FSH não é pulsátil e geralmente não é coincidente com a liberação de LH (26).

Durante a fase folicular do ciclo estral as concentrações de P4 são baixas devido à regressão do CL. A alta concentração de E2 derivada a partir da rápida proliferação celular no folículo dominante pré-ovulatório, concomitante com a

diminuição das concentrações de P4 circulantes, induz um pico de GnRH e permite a exibição do comportamento de estro durante o qual as fêmeas são sexualmente receptivas ao macho (28). O pico pré-ovulatorio de GnRH induz um pico de FSH e LH (29). A ocorrência do pico de LH é seguida pela ovulação do folículo 24-32 horas após (22) e induz a diferenciação de células foliculares residuais (células da granulosa e células da teca) que formarão o CL com a consequente produção de P4 (30). As concentrações de P4 permanecem elevadas durante toda a vida do CL, e são essenciais para o desenvolvimento, manutenção e estabelecimento da gestação (22).

Durante o diestro ou fase luteal, as concentrações de P4 permanecem elevadas enquanto que ondas recorrentes de crescimento folicular continuam a ser iniciadas pela liberação de FSH pela pituitária anterior. Apesar de haver a formação de folículos dominantes resultantes das ondas de crescimento durante a fase luteal, estes folículos geralmente não ovulam. A P4, hormônio dominante durante a fase luteal, promove um *feed back* negativo, que permite a secreção de pulsos de LH de menor frequência que são inadequados para induzir o crescimento do folículo dominante (31). Durante a fase luteal, as elevadas concentrações circulatórias de P4 regulam negativamente os receptores de P4 (32) no epitélio luminal do endométrio permitindo assim um aumento nos receptores de E2 (ESR1) no útero (33). A subsequente ativação dos receptores de E2 pelo E2 circulante, estimula a síntese de receptores endometriais de OXT que se ligam à OXT circulante, induzindo a secreção uterina de PGF (34, 35). Em bovinos, o aumento inicial nos receptores de OXT, que antecedem a luteólise ocorre 15-16 dias após o estro (36, 37). Em consequencia as concentraços circulantes de OXT induzem a liberação pulsatil de PGF uterina entre o dia 18 e 19 (8, 38).

2.2. Corpo lúteo

O CL é considerado um órgão endócrino temporário que se origina a partir das células foliculares que permanecem depois da ovulação (39). Devido a seu papel critico no estabelecimento e manutenção da gestação, existem muitos estudos na literatura que descrevem em detalhe a formação e os mecanismos de funcionamento do CL (2, 40). Os ruminantes domésticos são uma excelente

opção para o estudo do CL, porque o ciclo estral pode ser facilmente manipulado pelo uso de hormônios exógenos e o grande tamanho em ruminantes como a vaca, permite a extração de quantidades significativas de tecido para análise. Por outro lado o uso da ecografia transretal facilita consideravelmente a avaliação do CL rapidamente em tempo real. Por estas razões, uma proporção significativa de conhecimento disponível sobre o CL está baseado em experimentos realizados em bovinos e ovinos (40).

O CL é uma glândula altamente vascularizada e um local de intensa angiogênese. Ali ocorre o desenvolvimento de uma elaborada rede de vasos sanguíneos que dota o CL com uma das taxas mais altas de fluxo de sangue por unidade de massa de tecido do corpo (41). Devido a essa conformação anatômica no CL maduro cerca de 60% das células esteroidogênicas luteais estão diretamente adjacentes a um capilar (42-44).

Sob a influência do pico pré-ovulatório de LH da pituitária anterior, o folículo maduro sofre ruptura e expele o oócito. A parede do folículo colapsa e o espaço é ocupado por sangue, fibroblastos, células musculares lisas e células do sistema imune (45-47), assim como células da teca interna e células da granulosa (48). Esse conjunto de células promove inicialmente a formação do corpo hemorrágico (39). Existem três tipos principais de células que habitam o CL: células luteais esteroidogênicas grandes (CLG), células luteais esteroidogênicas pequenas (CLP) e células endoteliais vasculares. As CLP (15-22 μm) são derivadas das células da teca teca interna, enquanto que as CLG (>22.5 μm) são derivadas das células da granulosa que revestem a parede do folículo (49, 50). Adicionalmente existem outros tipos celulares importantes no CL incluindo fibroblastos, células vasculares e células do sistema imune (5). A taxa de crescimento do CL na vaca é extremamente acelerada. Em vacas, o peso do CL 3 dias após a ovulação é aproximadamente 640 mg, enquanto que no dia 14, o peso médio é de 5,1 g (51). A maior parte deste rápido incremento é devido a hipertrofia das células da granulosa e da teca.

A pesar das CLG ocuparem um grande volume da massa luteal relativa; 40 a 60% delas representam apenas 3.5% do numero total de células no CL da vaca (50, 52). O processo de diferenciação que resulta na formação da CLG, envolve

um fenômeno de hipertrofia das celulas da granulosa que aumentam de volume cerca de 50 vezes (52, 53). A produção de esteroides nas células da granulosa também aumenta dramaticamente após o pico de LH e lutetinização. Em vacas e novilhas, as concentrações de P4 aumentam até o dia 14 do ciclo estral, embora o volume do CL não aumente significativamente após o dia 7. Neste aspecto ainda que o volume luteal em vacas leiteiras lactantes seja maior que em novilhas devido ao maior tamanho do folículo pré-ovulatório em vacas (24, 54) as concentrações circulantes de P4 são mais baixas nas vacas lactantes que em novilhas. Esta diminuição nas concentrações em sangue representa uma taxa de depuração metabólica de P4 muito maior nas vacas em lactação (55, 56).

As CLP, que se originaram a partir das células da teca do folículo ovulado, tem cerca de um décimo do volume das CLG (3,000 vs 30,000 μm^3). Essa proporção expressada em volume, significa que as CLP ocupam 27,7% do volume do CL entre o dia 8 e 12 do ciclo estral (52). Existem caraterísticas morfológicas que diferenciam as CLG das CLP tais como núcleo esférico em CLG e irregular na CLP assim como a presença de reticulo endoplasmático rugoso e grânulos secretórios na CLG mas não na CLP (43, 53, 57, 58). Outra diferença reside nos receptores de LH que geralmente estão em maior quantidade nas CLP e os receptores para E2 e PGF estão em maior número nas CLG (53, 59-61)

Para a produção de P4, a célula luteal utiliza colesterol. As principais fontes de colesterol para as células luteais são as lipoproteínas circulantes (61, 62) particularmente lipoproteinas de alta e de baixa densidade. Devido o colesterol ser considerado uma molécula hidrofóbica, a movimentação dentro do sistema circulatório (lipoproteínas) ou dentro da célula é dependente de proteínas de transporte. No interior da celula luteal, várias proteínas têm sido postuladas como transportadoras de colesterol, contudo StAR (proteína reguladora aguda da esteroidogênese) parece ser a proteína encarregada de regular o movimento de colesterol desde o exterior para o interior da membrana mitocondrial na célula luteal (63). Previamente foi demostrada uma diminuição notável na expressão gênica de StAR, quando vacas em lactação foram submetidas a infusões IU de $PGF_{2\alpha}$ que provocaram luteólise funcional da glândula (59). Outra enzima

envolvida na produção de P4 é a P450scc. Esta enzima está localizada na membrana mitocondrial interna e catalisa a conversão de colesterol para pregnenolona (30). A pregnenolona se difunde da mitocôndria para o reticulo endoplasmático liso, onde é convertida a P4 pela enzima 3βHSD. Em seguida a P4 se difunde para fora da célula luteal para ser transportada aos tecidos alvos pela corrente sanguínea (64).

2.3. Prostaglandinas

As prostaglandinas regulam várias funções em fisiologia reprodutiva, entre elas: ovulação, regressão luteal, implantação, manutenção da gestação, parto e têm sido utilizadas em técnicas de reprodução assistida como sincronização de cio em protocolos de inseminação artificial e transferência de embriões (7, 65-67).

2.3.1. Estrutura

Os prostanóides (prostaglandinas, leucotrienos e tromboxanos), são moléculas de 20 carbonos. A maioria das prostaglandinas como PGF e prostaglandina E_2 (PGE2), têm ligações duplas nos carbonos 5-6 e 13-14 e são derivados do ácido araquidônico (AA), enquanto a prostaglandina E_1 (PGE1) só tem uma ligação dupla nos carbonos 13-14 e é derivada do ácido graxo poli-insaturado de 20 carbonos: ácido di-homo-ɣ-linolênico (DGLA). As ligações duplas nos carbonos 13-14 e o grupo OH no C-15 são essenciais para a atividade biológica (68, 69).

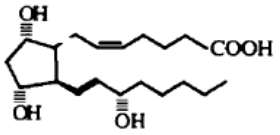
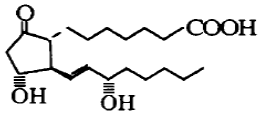
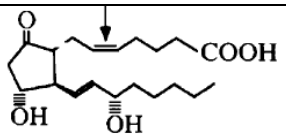
Prostaglandina	Estrutura química
PGF	
PGE1	
PGE2	

Tabela 1. Estrutura química das prostaglandinas $F_{2\alpha}$, E_1 e E_2. Anderson et al. (69)

2.3.2. Biossíntese

A biossíntese das prostaglandinas começa pela liberação do AA da membrana fosfolipídica, após a ativação da Fosfolipase $A_{2\alpha}$ (PLA2) (70). A seguinte etapa (Fig 1) é considerada um limitante na velocidade de produção e é catalisada por prostaglandina-endoperoxido sintases que convertem o AA no prostanóide intermédio prostaglandina H_2 (PGH). Tem sido identificadas duas isoformas das enzimas: PTGS1 que é uma enzima constitutiva e PTGS2 que é induzida por vários estímulos incluindo citocinas, fatores de crescimento, e lipopolisacarídeos. A segunda etapa limitante é a conversão terminal de PGH por diferentes sintetases terminais nos principais prostanóides produzidos in vivo: prostaglandina D_2 (PGD), PGE2, PGF, prostaciclina (PGI_2) e tromboxanos (TXA_2) (71, 72).

PGES.

Dois genes caraterizados, codificam as enzimas PGES identificadas como cytosolic PGES (cPGES) (73) e PGES ligada a membranas (mPGES), ou também chamada mPGES-1. A cPGES é uma proteína de 23kDa, idêntica à Hsp90 ou heat shock protein 90 (73, 74). A cPGES, está funcionalmente ligada a PTGS1 e suas funções estão direcionadas principalmente para

produzir prostaglandina E com o objetivo de manter a homeostase celular (75). Em contraste mPGES-1 converte preferencialmente a PGH derivada de PTGS2 em PGE2. Tanto PTGS2 como mPGES-1 estão acopladas funcionalmente e são enzimas induzíveis que essencialmente contribuem na formação fisiopatológica massiva de PGE2 sob condições inflamatórias (76). Resultados obtidos de CL, indicaram que a expressão proteica para mPGES-1 se incrementa consideravelmente em ovelhas gestantes simultaneamente com um incremento nas concentrações de PGE2 no CL (12). Achados baseados na expressão gênica do útero de vacas não gestantes, determinaram que a via PTGS2 e mPGES-1 é o mecanismo primário envolvido na produção endometrial de PGE2 (77).

Síntese de PGF

A PGF é sintetizada por duas vias diferentes a partir de PGE2 e PGH, pelas vias PGE 9-ketoreductase, e PGH 9-, 11-endoperoxido redutase, respetivamente (78). PGF_2 tem dois estereoisomeros que são produzidos in vivo: $PGF_{2\alpha}$ e 9α-, 11β-PGF_2. As duas $PGF_{2\alpha}$ e 9α-, 11β-PGF_2, causam contração bronquial, vascular e arterial no músculo liso, mas só 9α-, 11β-PGF_2 causa inibição da agregação plaquetária (79, 80).

Síntese de PGF a partir de PGE.

PGE 9-ketoreductase catalisa a conversão de PGE2 para PGF (78). Adicionalmente a enzima é classificada como um membro da superfamília aldo-keto reductase (AKR) e é idêntica a 20α-hidroxiesteroid dehidrogenase (HSD) que pertence ao grupo AKR1C e é mesma enzima que metaboliza P4 no metabolito inativo 20α-OHP (81). PGE 9-ketoreductase e PGF synthase demostraram ser enzimas com um papel fundamental na regulação de prostaglandinas específicas no endométrio durante o período peri implantação (82, 83).

Sintese de PGF a partir de PGH.

PGH9-,11-endoperoxido redutase catalisa a conversão de PGH para $PGF_{2\alpha}$. Esta enzima também é chamada PG endoperoxido redutase ou PGF sintetase (PTGFS) e é membro da familia AKR (78, 84).

2.3.3. Metabolismo

Ensaios em porcos da india, indicaram que a PGE2 tem uma meia vida de aproximadamente 30 segundos em circulação periferica antes de ser metabolizada na circulação pulmonar (85). Por outro lado, experimentos em ovelhas reportaram que mais de 90% da PGF é metabolizada em uma única passagem através dos pulmões (86). O metabolismo das prostaglandinas está regulado enzimaticamente pela ação da 15-Hydroxyprostaglandin dehydrogenase (PGDH) que converte PGF e PGE2 nos respetivos metabolitos inativos PGFM e PGEM (87). PGE1 tambem é considerada um excelente substrato para a enzima PGDH que resuta na produção do metabólito 13,14-dihydro-15 keto PGE_1 em tecidos animais e humanos (87-89).

A atividade enzimática de PGDH foi medida no CL em ovelhas, pela taxa de conversão de PGF para PGFM, mostrando uma atividade maior no CL do dia 4 do ciclo estral e no dia 13 em animais gestantes, quando comparado com animais não gestantes no dia 13 do ciclo estral (90).

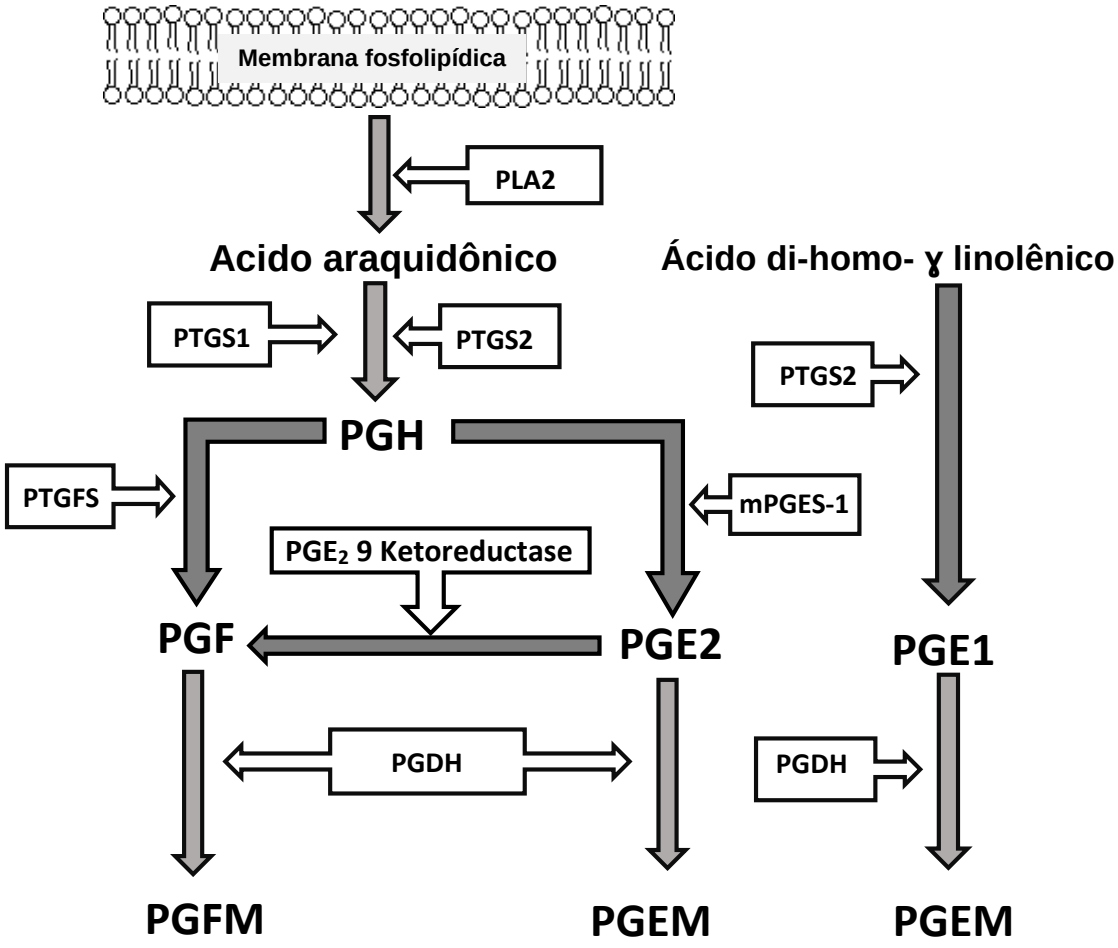


Figura 1. Esquema representativo da biossíntese e metabolismo de PGF, PGE2 e PGE1. Esquema baseado em (75, 78, 87, 89). As abreviaturas estão descritas no sumario.

2.3.4. Especificidade de ligação das prostaglandinas aos receptores no CL.

Ainda que PGE1 e PGE2 sejam semelhantes na estrutura química, vários resultados sugerem que estas prostaglandinas induzem efeitos fisiológicos diferentes na duração do CL (91, 92). As prostaglandinas do tipo E (PGE2, PGE1) e PGF atuam principalmente através da sua ligação com receptores identificados como EP e FP respetivamente. Existem vários subtipos de receptores que se ligam às prostaglandinas do tipo E (EP_1, EP_2, EP_3, EP_4) (93). Uma avaliação farmacológica, determinou a especificidade e a afinidade na ligação de diferentes prostaglandinas aos receptores EP e FP no tecido luteal bovino (69). Tanto PGF quanto PGE1, se ligaram com alta

afinidade e especificidade aos receptores FP e EP respectivamente. Semelhante a PGE1 a PGE2 teve uma alta afinidade pelos receptores EP, no entanto, a PGE2 mostrou reação cruzada com os receptores FP, ainda que a afinidade foi quase 10 vezes menor que a demonstrada pela PGF para os receptores FP. Portanto, experimentos nos quais o objeto de avaliação sejam os efeitos fisiológicos mediados por receptores EP, deve-se considerar o uso de PGE1 ou outro agonista específico para estes tipos de receptores, a fim de evitar uma reação cruzada com receptores FP (69).

Diferentes estudos demonstraram que PGE1 e PGE2 podem ter diferentes efeitos fisiológicos no CL. Por exemplo em ovelhas nas quais o pedículo ovariano foi tratado com PGF, houve uma diminuição no peso luteal e nas concentrações séricas de P4. Porém as concentrações de P4 foram restauradas aos valores observados em ovelhas tratadas com veículo, quando foram tratadas simultaneamente com PGE1 mas não com PGE2 (92). Em outro estudo a administração de doses altas de PGE1 em ratas pseudogestantes atrasou a luteólise, enquanto a administração de altas doses de PGE2 acelerou o início da luteólise (91).

2.4. Luteólise.

2.4.1. PGF como hormônio luteolitico em ruminantes

Nos ruminantes, a regressão do CL no final do ciclo estral é causada pela liberação uterina de PGF (7). A primeira evidência de que a PGF está principalmente envolvida no controle da vida meia do CL foi reportada em 1969 através de ensaios feitos em ratas que receberam infusões de PGF durante 2 dias, induzindo uma diminuição nos níveis ovarianos de P4 (94). Além disso, o fato de que a PGF é o hormônio responsável pela luteólise em ruminantes, é suportado por diversas linhas de evidência revisadas por Knickerbocker et al (95). Em síntese, o período de luteólise coincide com concentracões elevadas de PGF no tecido uterino, drenagem venosa e lavados uterinos. Por outro lado a inibição farmacologica na producão uterina de PGF, bloqueia a regressão espontânea do CL. Adicionalmente, a luteólise pode ser impedida após imunização passiva contra PGF. Finalizando, de acordo com o conceito do mecanismo local de transporte veno-arterial entre

o utero e o ovario, tem sido demostrado que a PGF tem a capacidade de ser transportada desde a drenagem venosa uterina para a corrente arterial ovariana em ruminantes (95, 96).

2.4.2. Liberação pulsátil de PGF durante a luteólise.

Na vaca não gestante, a regressão do CL é caraterizada pela liberação uterina de PGF em pulsos que têm sido associados com a diminuição nas concentrações de P4 (97, 98). Um estudo feito em duas novilhas não gestantes demonstrou um patrão pulsátil muito semelhante em ambos animais que se manteve por 2-3 dias. Ainda que ambos os animais demonstraram uma liberação pulsátil regular definida por 4 pulsos com duração similar de 4 horas cada um, o dia de início dos pulsos foi diferente entre animais (8). Um padrão semelhante foi descrito em outro estudo utilizando 6 vacas, sugerindo que a luteólise resulta de 5 episódios de liberação de PGF, cada um deles com 4 horas de duração aproximadamente (35). Adicionalmente, a necessidade dos pulsos sequenciais foi demostrada através de infusões IU de PGF a cada 12 horas, que resultaram em uma diminuição gradual da concentracão plasmatica da P4 a valores <1ng/ml em aproximadamente 36 horas (98).

Em vacas, ovelhas e cabras, as interações entre o E2 ovariano, P4 e OXT com o endométrio uterino, regulam o tempo e magnitude na produção de PGF durante o ciclo estral (38, 95). A OXT tem sido postulada como o principal hormônio regulador do padrão de liberação pulsátil de PGF. Este conceito foi apoiado pela descoberta de que os pulsos de OXT que são liberados desde a neuro-hipófise, ocorrem em sincronia com picos plasmáticos de PGFM durante a luteólise na ovelha (99). Diversos estudos reportaram pulsos de OXT durante o periodo de luteólise em cabras (100) e vacas (101). Estes estudos sugerem que as liberações episódicas de OXT em ruminantes que interagem com os receptores uterinos de OXT que se encontram concentrações crescentes, são as causas das liberações episódicas de PGF que irão consequentemente causar a luteólise nestas espécies. No entanto, posteriormente foi descoberto que o CL da ovelha contém grandes quantidades de OXT (102), e que a liberação dessa OXT

armazenada no CL, era induzida pela administração sistémica de um análogo de PGF (103). Assim sendo, durante o período luteolítico na ovelha, não é claro qual é a proporção de OXT liberada pela pituitária posterior e qual pelo CL.

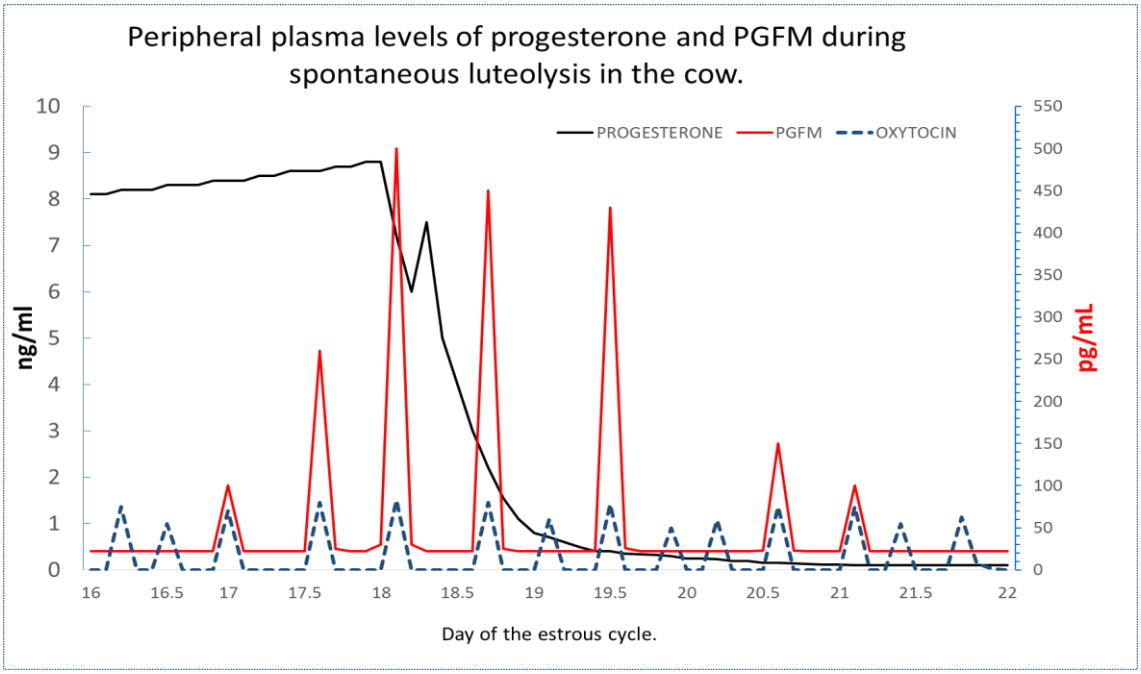


Figura 2. Esquema representativo das concentrações de PGFM, ocitocina e P4 na vaca não gestante, durante a luteólise natural. Baseado em dados de Ginther et al. (97), Mann and Lamming (35), Kotwica et al. (104).

As prostaglandinas são metabolizadas rapidamente principalmente nos pulmões em ruminantes (105). Uma vez na circulação sistêmica, a PGF é metabolizada rapidamente e transformada em PGFM durante a primeira passagem através dos pulmões. Ensaios desenvolvidos em vacas Jersey sugerem que 65% da PGF é metabolizada em uma única passagem pelos pulmões (86). A infusão IU de 0,25 mg de PGF em novilhas (98), incrementou as concentrações de PGFM 2 minutos após a infusão e atingiu a concentração máxima depois de 10 minutos. Neste aspecto, a dose IU luteolítica mínima efetiva (1-2 mg) liberada no corno uterino ipsilateral ao CL na vaca é equivalente a um décimo da dose sistêmica (15 mg) (106). A eficiência na dose IU é atribuída

ao mecanismo de contracorrente útero-ovariano no qual a relação anatômica entre a veia útero-ovariana e a artéria ovariana, permite a transferência de PGF da veia para a artéria (96, 107).

2.5. Expressão gênica do CL após infusões intrauterinas de PGF

O delineamento do presente experimento, foi baseada num estudo recente (59), que utilizou quatro infusões IU com doses baixas (0,5 mg) de PGF em vacas leiteiras, numa tentativa de imitar os pulsos fisiológicos deste hormônio e caracterizar o processo luteolítico. A seguir serão descritos os achados mais importantes deste experimento que aportaram consideravelmente ao entendimento do processo luteolítico através do estimulo com infusões IU sequenciais de PGF.

Neste estudo o intervalo entre as infusões IU foi de 6 horas. Trinta minutos após cada infusão, foram realizadas biopsias luteais a fim de avaliar a expressão gênica do tecido após cada pulso de PGF. Os resultados deste experimento permitiram determinar o padrão temporal de expressão gênica, associado com cada pulso de PGF e assim diferenciar mudanças associadas com a regressão completa do CL. O primeiro pulso de PGF, induziu um aumento na expressão dos “genes precoces imediatos” tais como JUN e FOS, os quais têm sido propostos como mediadores das cascatas de sinalização de apoptoses (108). Adicionalmente FOS e JUN parecem estar envolvidos na programação da expressão gênica durante a luteólise induzida e a sua expressão se incrementa depois da administração de uma dose supra fisiológica de PGF (109, 110). Do mesmo jeito, durante a segunda, terceira e quarta infusão, unicamente as vacas tratadas com PGF tiveram um incremento na expressão destes genes.

O gene StAR, codifica a proteína essencial no transporte de colesterol intracelular e consequente produção de P4 pela célula luteal (63). Ainda de acordo com o mesmo estudo, a expressão deste gene, foi incrementada no momento da primeira biopsia. Posteriormente os níveis de expressão caem gradualmente durante a segunda, terceira e quarta biopsias (59).

O gene HPGD codifica para a produção da enzima PGDH, que executa o passo enzimático limitante na taxa de inativação de prostaglandinas da série E e F. Esta enzima metaboliza PGF no metabolito inativo PGFM em diferentes

tecidos incluindo o CL. Portanto, esta enzima poderia estar envolvida na resistência do CL à atividade luteolítica da PGF devido a sua capacidade de inativar a PGF produzida no tecido luteal (88, 90). A expressão do gene HPGD se incrementa no primeiro pulso, para depois diminuir dramaticamente durante o segundo, terceiro e quarto pulso de PGF (59).

Por outro lado o mRNA para PTGS2 e PTGFS, foram induzidos 0,5 e 6,5 horas respetivamente depois do primeiro pulso de PGF. Houve um incremento dramático nas concentrações de mRNA de PTGS2 e PTGFS durante as quatro biopsias nos grupos que regressaram o CL, de acordo com o mecanismo de auto amplificação da PGF.

O mRNA relacionado com a resposta imune (IL1B e IL8) incrementou sua expressão 6,5 horas após o primeiro pulso de PGF, demonstrando um papel claro dos mediadores e células do sistema imune na diminuição da esteroidogênese e morte do tecido luteal (5). Em geral a maioria dos mecanismos intracelulares tende a impedir a regressão do CL após um único pulso de PGF. Porém o primeiro pulso parece amplificar os efeitos luteolíticos dos pulsos subsequentes de PGF no CL em regressão (5).

2.6 Mecanismos envolvidos no resgate do CL

A maioria dos dados é consistente em que existe um “período crítico” para o reconhecimento materno da gestação e consequente resgate do CL, sendo por volta do dia 16 após o estro na vaca. Northey e colaboradores (111), demonstraram que quando o embrião é removido do útero antes do dia 16, o ciclo estral da vaca apresentou uma duração normal, enquanto que as as vacas nas quais o embrião era removido no dia 16 ou depois, havia uma fase luteal prolongada. Apoiando este resultado, foi descrito que os embriões transferidos no dia 16 do ciclo mantiveram a gestação, porém quando os embriões foram transferidos no dia 17 ou depois as gestações não foram mantidas (112).

Efeitos semelhantes foram achados em novilhas Holandesas, através da infusão IU de embriões homogenizados com 17 a 18 dias de desenvolvimento. As infusões entre os dias 14 e 18 após ovulação, prolongaram a vida do CL quando comparados com animais do grupo controle. Os resultados destes experimentos, indicam que entre o dia 15 e 17 de gestação, o embrião bovino

produz uma substância que prolonga a vida do CL (111). Os possíveis sinais envolvidos durante o periodo critico que regulam o resgate do CL tem sido divididós em 3 abordagens teóricas.

2.6.1 Efeito antiluteolítico do IFNT

A maioria dos modelos atuais que explica como o embrião promove o resgate do CL durante o perido luteolítico, envolve o IFNT como parte fundamental no mecanismo da sinalização do embrião (113-115). O IFNT foi descoberto por meio da cultura de conceptos ovinos na presença de aminoácidos radiomarcados. Através deste meio foi detectada uma proteína de baixo peso molecular abundante, inicialmente chamada de proteína X. Mais tarde a mesma proteína foi chamada proteína trofoblástica ovina 1 ou oTP1 (116, 117). Quando o gene para oTP1 foi clonado e sequenciado, verificou-se que a proteina corresponde a um interferon tipo 1, que foi logo designado como IFNT (118, 119).

O reconhecimento materno da gestação em ruminantes (ovelha, vaca, cabra) requer que o concepto mude de uma forma esférica para tubular e posteriormente para uma forma filamentosa para produzir IFNT, impedindo o desenvolvimento do mecanismo luteolítico endometrial (120-122). As celulas mononucleares do trofoectodermo sintetizam e secretam o IFNT entre os dias 10 e 25, atingindo uma produção máxima entre os dias 14 a 16 (122, 123). Um estudo recente desenvolvido utilizando embriões fertilizados *in vivo,* demonstrou que o mRNA para IFNT, se incrementa gradualmente desde o dia 12 até o dia 18 e diminui depois do dia 20. Consequentemente, as quantidades de IFNT no útero incrementam gradualmente desde o dia 12 até o dia 20 e diminuem após o dia 22 (124).

O modelo de ação local, argumenta que o IFNT produzido pelo embrião age sobre o epitélio glandular superficial e luminal do utero para silenciar a expressão dos receptores de E2 (ESR1) e consequntemente os receptores de OXT, anulando assim a liberação dos pulsos luteolíticos de PGF dependentes de OXT pelo epitélio uterino. Por conseguinte, o CL continua produzindo a P4 necessária para a manter a gestação (115).

2.6.2 Produção de PGE.

O segundo modelo está baseado na produção incrementada de PGE2 e PGE1 por estimulo da gestação sobre as celulas endometrias. O equilíbio no transporte de prostaglandinas para o ovário muda em favor da PGE2 durante o estabelecimento da gestação incrementando também a biossíntese de PGE2 e do seu receptor na celula luteal resultando num efeito luteoprotetor (10, 12, 125). Ver seção 2.7.

2.6.3 Genes estimulados por interferon (ISG).

O terceiro modelo promove o papel sistêmico do IFNT que escapa do lúmen uterino agindo sobre o CL. Recentemente foi reportado que a infusão direta de IFNT de origem recombinante na veia uterina induziu a expressão de ISG no tecido luteal e prolongou a vida do CL em ovelhas (126, 127).

2.7 Prostaglandinas E (PGE1 e PGE2) como fatores antiluteoliticos.

Ainda que o mecanismo de ação do IFNT provavelmente altere a produção de PGF no utero, experimentos desenvolvidos utilizando embriões ovinos produzidos *in vivo* com 8-17 dias de desenvolvimento, demonstraram que a expressão de PTGS2 nas celulas do trofoblasto, atinge níveis máximos entre os dias 14 e 16 (128). A produção de PTGS2 é modulada positivamente por IFNT no endometrio, miometrio e CL de ovelhas (11). Portanto, resultados em ruminantes sugerem que as prostaglandinas produzidas pelo concepto e pelo útero poderiam desenvolver um papel importante no mecanismo de resgate do CL.

Em ruminantes, a PGF e PGE2, são as principais prostaglandinas produzidas no endométrio, mas seus padrões de secreção são diferentes. A expressão de mRNA para mPGES no útero, sugere que a produção de PGE2 é moderada durante os dias 1-3 do ciclo estral, baixa durante os dias 4-12, e alta entre os dias 13-21 na vaca (77). O embrião bovino, também foi identificado como produtor de PGE2 através de avaliações feitas em embriões coletados entre os dias 6 e 17 de desenvolvimento. Um estudo que utilizou embriões coletados de vacas submetidas a superovulação, observou que os embriões coletados entre o dia 6 e 10 após fertilização, metabolizaram AA primariamente

em PGE2, enquanto que os embriões no dia 13 encaminham o metabolismo para a produção de PGE2 e de PGF. Depois do dia 15 metabolizam o AA para PGE2, PGF e PGI_2 (129).

As prostaglandinas E1 e/ou E2 são postuladas como agentes antiluteolíticos/luteotrópicos (130). Por serem moléculas de tamanhos semelhantes à PGF (68), e podem ser transportadas através de um mecanismo local similar. Em ovelhas que receberam infusões IU de PGE2 a cada 4 horas nos cornos ipsilateral e contralateral ao CL desde o dia 10 até o dia 17, a função luteal se manteve somente quando as infusões foram feitas no corno ipsilateral ao CL, apoiando a teoria do transporte local de prostaglandinas (125). A infusão IU de PGE1 ipsilateral ao CL, também estendeu o intervalo interestro até 38,2 dias em outra avaliação (10).

Um experimento *in vivo* que utilizou implantes intraluteais de PGE durante os dias 13-19 do ciclo estral em vacas Angus, demonstrou que nas vacas tratadas com implantes de PGE2 e PGE1, o peso do CL bem como as concetrações de P4 não diminuiram, quando comparadas com o grupo controle no final do ciclo estral (13). Ainda que os mecanismos que governam este efeito não sejam discutidos amplamente, este experimento sugere claramente que as prostaglandinas E1 e E2 podem exercer um papel antiluteolítico/luteotrópico.

Um recente estudo (12) desenvolvido em ovelhas gestantes, indicou que a proteína do IFNT não é transportada do útero até o ovário, através do plexo útero-ovariano. Os resultados sugerem que a biossíntese luteal de prostaglandinas está seletivamente encaminhada para a produção de PGF no momento da luteólise, porém a proporção muda em favor de PGE2 durante o estabelecimento da gestação. A dosagem nas concentrações de prostaglandinas no útero, veia uterina e artéria ovariana, evidenciou que uma grande proporção de PGE2 endometrial é transportada do útero para o ovário através do plexo útero-ovariano e que esse transporte foi favorecido na ovelha gestante entre os dias 12 e 16 de gestação (12).

A produção endometrial de PGE2 estimulada pela gestação, parece incrementar a biossíntese de PGE2 luteal, ativando mecanismos intracelulares

mediados pelos receptores PTGER2 e PTGER4 resgatando assim o CL durante o estabelecimento da gestação na ovelha (12).

Desta maneira, é importante que sejam realizados estudos que proporcionem informação sobre os mecanismos enzimáticos e hormonais que regulam a produção de prostaglandinas no CL.

3. Justificativa.

O embrião bovino sinaliza sua presença intrauterina por meio da secreção de subtâncias que possuem ações parácrinas e endócrinas. Durante o período de reconhecimento materno da gestação, a comunicação inadequada entre o embrião, útero e ovários resulta na falha da manutenção do CL, dando inicio à liberação pulsátil de PGF que provoca uma queda nas concentrações circulantes de P4 com a consequente perda embrionária.

Estudos prévios em ruminantes, têm permitido progressos consideráveis no entendimento dos mecanismos fisiológicos envolvidos na luteólise. No entanto, é importante observar as diferenças que existem entre as respostas do CL após a injeção de uma dose única supra-fisiológica de PGF comparadas com infusões de doses baixas no corno uterino a fim de imitar o processo luteolitico natural. O segundo método permitiu a descrição clara de várias vias moleculares que são reguladas dinamicamente a cada pulso de PGF.

A prostaglandina E tem sido postulada como um hormônio luteoprotetor que incrementa sua produção por estimulo da gestação, protegendo o CL durante o periodo crítico através do bloqueio da ação luteolítica da PGF no CL em ruminantes. Porém em nosso conhecimento, não existem relatos científicos em vacas, que avaliem os padrões de expressao gênica que induzem o efeito protetor característico da prostaglandina E no CL. A preferência no uso de PGE1 em vez de PGE2, é devido a que PGE1 se liga com alta especificidade aos receptores EP. A PGE2 não foi utilizada devido à possibilidade de provocar uma reação cruzada com os receptores FP.

O objetivo deste estudo, é estudar o mecanismo protetor induzido pela PGE no tecido luteal, através de um modelo *in vivo* que imita a liberação uterina pulsátil de PGF na vaca. O tratamento intrauterino com doses baixas de

prostaglandinas que têm efeitos opostos, deveriam estimular vias metabolicas diferenciais evidentes através da expressão gênica do tecido.

Os resultados deste experimento podem fornecer informação importante sobre os mecanismos enzimáticos e proteicos que regulam a produçao intraluteal de prostaglandinas e que são a causa aparente do efeito luteoprotetor da prostaglandina E.

4. OBJETIVO E HIPÓTESES

O objetivo geral deste estudo é determinar a resposta do CL em termos de padrões de expressão gênica e funcionalidade (produção de P4), em resposta ao estímulo com duas prostaglandinas diferentes envolvidas no processo de luteólise (PGF) e resgate do CL (PGE1).

Hipótese 1. Os pulsos IU de PGF provocam luteólise. Os animais tratados com PGF expressam um padrão de expressão gênica semelhante ao reportado anteriormente em vacas que tiveram regressão completa do CL.

Hipótese 2. Os pulsos IU de PGE1 não alteram o tamanho do CL ou a produção de P4, e induzem um padrão de expressão gênica distinto do grupo PGF.

Hipótese 3. Os pulsos IU de PGE1+PGF mantém o CL estrutural e funcional e induzem um padrão de expressão gênica após cada pulso de PGF que significa a falta na regressão do CL através da inibição das vias especificas que normalmente são ativadas por pulsos fisiologicos de PGF.

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Capítulo 2

Expressão gênica do corpo lúteo após pulsos intrauterinos com doses baixas de prostaglandina E1 e F-2 alfa em vacas

Gene expression in the corpus luteum following intrauterine pulses of low doses of prostaglandins E1 and F-2 alpha in cattle

INTRODUCTION

The corpus luteum (CL) is a transitory endocrine gland, which is essential for establishment of pregnancy in cattle because of the production of progesterone (P4). In nonpregnant animals, or in the absence of the appropriate embryonic signal, function of the ruminant CL is terminated by the action of prostaglandin $F_{2\alpha}$ (PGF) released by the uterus (6, 7, 111). During early pregnancy in the cow, it is still being debated whether the embryo rescues the CL by producing a luteotropic substance or by inhibiting a luteolytic one. Interferon tau (IFNT) has been shown to be the primary embryonic signal from the ruminant embryo that results in maintenance of the CL during pregnancy (115, 121). Most of the early researchers advocated a primary effect of IFNT on inhibition of luteolytic pulses of PGF (9). However, there is evidence from both early research and from recent research that IFNT also alters prostaglandin E2 secretion by the endometrium (11, 12, 131, 132) and this may also be a key factor in maintenance of the CL during early pregnancy, by acting as a luteoprotective agent (10-13).

Prostaglandins are modified fatty acids with 20-carbon molecules that regulate many aspects of reproductive physiology (7, 65, 133). Both PGF and prostaglandin E2 (PGE2) are derived from the essential fatty acid arachidonic acid (AA). Release of AA from membrane phospholipids is through the action of phospholipases, such as phospholipase A2 (134, 135). The resulting AA acid is converted to the unstable peroxide PGH_2 by the action of constitutive PTGS1 and inducible PTGS2 (72, 75). The resulting PGH2 can be converted into various PGs including PGE2 through the action PGE synthase (mPGES-1) or PGF through the action of PGF synthase (PTGFS) (11, 70, 71).

The PGs act through G-protein coupled receptors that have 7 transmembrane domains. For PGF, there is a single receptor, termed FP receptor (136). Activation of the FP receptors leads to inositol phosphate accumulation, protein kinase C activation, and increased free intracellular calcium concentrations (137-142), consistent with coupling of the FP receptor to the Gαq family of G-proteins (142, 143). In contrast, PGE molecules bind to a family of EP receptors that activate a multitude of G-proteins with some activating similar pathways as PGF but others activating opposing intracellular effector systems such as the cAMP/protein kinase A pathways (144, 145). Extensive binding studies with agonists of PG receptors in bovine luteal membranes, have demonstrated the specificity and affinity of different PG agonists for the different luteal EP and FP receptors (69). PGF and PGE1 were found to bind with high affinity and specificity to either the FP or EP receptors, respectively. Similar to PGE1, PGE2 had high affinity for the EP receptors, however, it also had crossreactivity with the FP receptor although the affinity was about 10-fold lower than PGF for the FP receptor. Similarly, some studies have found that high concentrations of PGE2 can produce effects that resemble PGF actions in luteal cells (91). This is important for the present studies because we chose to use PGE1 as the PGE receptor agonist, in order to avoid any potential cross reactivity with the FP receptor. Inactivation of all of these PGs occur through similar pathways by the action of 15-hydroxyprostaglandin dehydrogenase (PGDH) converting PGF, PGE2, or PGE1 into the inactivated metabolites PGFM and PGEM (87).

Regression of the CL in the cow, as well as in many other species, is mediated by production of PGF by the uterus at the end of the luteal phase (7, 38, 95). Functional and structural regression of the CL is characterized by the release of PGF in pulses. In this regard, PGFM has been defined as the main plasma metabolite of PGF and evaluation of circulating PGFM has been used to monitor PGF release during luteolysis (146).

Measurements of PGFM at the end of the estrous cycle have demonstrated that luteolysis, as measured by decreases in circulating P4, is associated with 3-5 pulses of PGFM that occur approximately every 12 hours during last 2-3 days of the luteal phase (8, 35, 146). The intermittent secretion of PGF has been recently simulated in heifers by intrauterine (IU) infusions of low doses of PGF that mirrored both the concentrations of PGFM pulses and the decline in circulating P4 and CL volume that occur during natural luteolysis (98). A recent study (59) evaluated the response of the bovine CL to four IU infusions of low doses of PGF on the patterns of gene expression in the CL. Interestingly, each of the 4 infused pulses of PGF were followed by an increase in expression of early response genes (FOS, JUN, EGR-1). However, it was only after the second PGF pulse that immune regulators, such as IL1B, IL8, had increased mRNA expression. The mRNA for enzymes involved in steroidogenesis were inhibited at varying times after PGF pulses, with StAR, a key regulator of intracellular transport of cholesterol, inhibited after the second pulse of PGF; whereas CYP11A1, also known as cholesterol side chain cleavage enzyme, only showing significant inhibition following the fourth pulse. Consistent with these patterns, genes involved in the biosynthesis of PGF (PTGS2, PTGFS) increased their expression after the first and second PGF pulses, respectively, consistent with induction of an auto-amplification pathway for PGF production within the CL during luteolysis. Further, mRNA for the gene that metabolizes PGF into PGFM, HPGD, decreased after the third pulse of PGF, perhaps allowing increased activity for PGF within the CL. Thus, numerous specific gene expression pathways are activated in the CL in response to physiological pulses of PGF and these are likely to represent the critical intracellular effector systems that lead to luteolysis.

In contrast, during pregnancy, the CL remains steroidogenically active, as measured by luteal P4 production and circulating P4 concentrations, and structurally intact, as evidenced by luteal volume and cellular histology (51, 146-148). Similar to the local effects of PGF during CL regression, the protective effect of the embryo appears to be mediated by local pathways, as evidenced by elegant vascular anastomoses experiments showing that the uterine venous effluent from the gravid uterine horn contains a small molecule that is transferred to the ipsilateral ovarian artery and this blocks the normal luteolytic process (149, 150). A number of reports indicate that this locally-active, luteoprotective factor is likely to be PGE (10-13, 151). For example, during pregnancy, the bovine or ovine uterus produces much greater amounts of PGE than during a similar time period in non-pregnant animals (152-154). In addition, ovine (128, 155) and bovine (129, 156) embryos also produce PGE during early pregnancy. There are also numerous studies demonstrating that treatment with PGE can inhibit the luteolytic actions of PGF in the ovine and bovine CL (10, 12, 92, 157, 158). In addition, studies have demonstrated that PGE2 (12) or PGE1 (10) can diffuse through the utero-ovarian plexus and thereby could provide a luteoprotective effect on the CL during establishment of pregnancy in sheep.

This study uses our previous model of CL regression by pulsatile IU infusions of low doses of PGF (0.25 mg) but it extends this model by simultaneously infusing low doses of PGE (2 mg) to mimic physiological concentrations of PGE that are secreted into the uterine vein by the uterus during pregnancy (12, 159). To eliminate any confounding effects of PGE2 binding to the FP receptor, PGE1 was utilized as the PGE agonist in this study. Dynamic changes in CL structure and function during the IU treatments were measured by evaluating changes in circulating P4 concentrations and CL volume, using ultrasound. Circulating concentrations of PGFM and PGEM were assessed in order to

know if infusions of PGE1 altered the transport of PGF from the uterus to the uterine vein. In addition, serial CL biopsies were obtained following each PGF pulse to allow quantitative evaluation of gene expression using QT-PCR analysis of each luteal. Thus, this study focused on three specific hypotheses. Our first hypothesis was that IU pulses of low doses of PGF would induce CL regression and a gene expression pattern in the CL that would be typical of luteolysis, as we previously reported (59). Our second hypothesis was IU pulses of low doses of PGE1 would not alter the size or P4 production by the CL but would induce a distinct pattern of gene expression that would be typical of PGE actions. Finally, our third hypothesis was that simultaneous infusion of PGF and PGE1 would maintain the CL, both structurally and functionally, and would produce distinctive gene expression patterns after each PGF pulses that would signify lack of CL regression, through inhibition of specific pathways that are normally activated by physiological PGF pulses.

MATERIALS AND METHODS

2.1 Reagents

The PGF (Lutalyse) and intravaginal P4 inserts (Eazi-Breed CIDR) were purchased from Zoetis, Inc. (Zoetis Animal Health, Kalamazoo, MI). The GnRH (GONAbreed) was a gift from Parnell Veterinary Pharmaceuticals (Parnell US 1 Inc. Leawood, KS). Specific primers and target genes were synthesized by the Biotechnology Center of the University of Wisconsin-Madison. RNeasy Plus Universal Mini Kit was purchased from QIAGEN (Qiagen Inc. Valencia, CA, USA). The iScript cDNA Synthesis Kit (catalog # 1708891) and SsoFast EvaGreen Supermix (catalog # 1725200), were purchased from Bio-Rad (Bio-Rad Laboratories Inc. Hercules, CA). Secondary antibody (Goat anti-Rabbit IgG, catalog ab6702.) was purchased from Abcam (Abcam Inc. Boston, MA). PGFM (catalog # 16670), PGEM ELISA kit (Item N^{o} 514531) and Prostaglandin E1 (Item N^{o} 13010)

were purchased from Cayman (Cayman Chemical Company, Ann Arbor, MI). Primary antibody (Rabbit anti-PGFM) was a generous gift from Dr. W.W. Thatcher, University of Florida.

2.2 Animals: housing and estrus synchronization

This experiment was performed from August 2015 to October 2015, using multiparous nonpregnant dry cows housed at the University of Wisconsin dairy facilities. Holstein cows with normal estrous cycles were used for this experiment. A cow was not used if there was an indication of uterine or ovarian abnormality based on ultrasonic scanning. Cows had ovulation synchronized with a CIDR-synch modified protocol consisting of an initial treatment with GnRH and insertion of a CIDR insert, followed by a PGF treatment on days 6 and 7, followed by CIDR withdrawal on day 8, and 24 hours later (day 9) a second treatment with GnRH. Day of ovulation was determined by ultrasonography and designated Day 1. All procedures used in this experiment were approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences at the University of Wisconsin- Madison.

2.3 Experimental protocols

Cows with a mature CL on day 10 of the estrous cycle were assigned randomly to one of four treatment groups, with equal numbers in each group. All cows received four IU infusions at 6-h intervals followed by a luteal biopsy 30 min after each IU infusion. Cows in saline group (n=5) received IU infusions of 0.1 ml of saline and 0.1 ml of DMSO. Cows in group PGE (n=5) received IU infusions of 0.1 ml of saline and 2 mg of PGE1 diluted in 0.1 ml of DMSO. Cows in group PGF (n=5) received IU infusions of 0.25 mg of PGF diluted in 0.1 ml of saline and 0.1 ml of DMSO. Cows in group PGE+PGF (n=5) received IU infusions of 2 mg of PGE1 in 0.1 ml of DMSO and 0.25 mg of PGF in 0.1

ml of saline (Figure. 1). The infused dose (2 mg) of PGE1 was calculated based on an approximate concentration of 30 ng/ml of PGE that has been reported in the uterine vein of pregnant sheep (12) and a rate of blood flow in the uterine vein of 200 ml/min (159) during a 6-hour period ((30 ng/ml x 200 ml/min) x (360 min) = 2.16 mg every 6 hours). All treatments were infused into the greater curvature of the uterine horn ipsilateral to the CL, using an embryo transfer gun. All cows had an ultrasonography-guided biopsy of the CL 30 minutes after each IU infusion. A total of 25 experimental periods were performed but 5 periods were removed due to either inapropriate biopsies or responses during the protocol. Therefore, 20 experimental periods were analyzed (n = 5/treatment group).

2.4 Ovarian ultrasound imaging

Synchronized cows had ovaries evaluated by transrectal ultrasonography once a day from the day of the second GnRH (Day 0), and on Days 2, 10, 11, 12, and 13 of the estrous cycle (Figure 2). Serial ultrasound videos of the ovary containing the CL were recorded using a B-mode, portable ultrasound fitted with a 7.5 MHz linear-array transducer (Ibex Pro; E. I. Medical Imaging, Loveland, CO) to determine day of ovulation and changes in volume of the luteal tissue on days 10, 11, 12 and 13. The ultrasound settings (focus position, field gain, total gain and frequency) were configured and maintained for all the replicates. Videos of the CL were recorded for 16 s (241 frames) by a single technician. Analyses of ultrasonographic videos were performed using the open-source image processing software, Image J 1.49v (National Institute of Health, Bethesda, MD; http: rsb.info.nih.gov/ij/index.html).

2.4.1 Luteal volume determination

Videos were analyzed frame by frame to select the cross sectional area in which the CL size was maximal. Images including a central cavity were taken into account. To determine the volume of the CL, electronic calipers were used to trace the perimeter of the entire CL and the perimeter of any central cavity for the CL. A scale of 5 pixels/mm was used to measure the length in each image. The area was used to calculate the radius by the formula: r^2= area/π and the radius was used to calculate the total CL volume (V = $4/3\pi r^3$) minus the volume of the central cavity in cm^3. Values in cm^3 were calculated for each animal and the percentage volume, relative to day 10, was determined for each day.

2.5 Transvaginal ultrasound-guided biopsy of the corpus luteum

Procedures to collect luteal biopsies were done in a similar manner as previously described (160). Cows were given caudal epidural anesthesia using 5 ml of lidocaine hydrochloride (Phoenix Pharmaceutical, Inc., St. Joseph, MO). A 7.5 MHz convex array ultrasound transducer (Aloka SSD 900, Hitachi Aloka Medical, Japan) was adapted with a needle guide to allow a 48 cm, 16-gauge biopsy needle (US Biopsy, Division of Promex Inc, Indianapolis, IN) to be inserted through the needle guide. The transducer face was applied to the wall of the vaginal fornix and the ovary containing the CL was positioned transrectally against the vaginal wall. The needle was then advanced through the vaginal wall and into the CL. The biopsy cutting blade was triggered and this cut off the luteal tissue that was trapped within the specimen notch. After removing the biopsy device, the tissue was inspected to ensure that only luteal tissue was removed from the ovary. Only biopsies that had at least 20 mg of tissue collected were analyzed for this experiment. Biopsies were rinsed with PBS, weighed, and immediately frozen in liquid nitrogen and stored at -80^{o}C for later quantification of specific RNA transcripts.

2.6 Blood sampling and hormonal assays

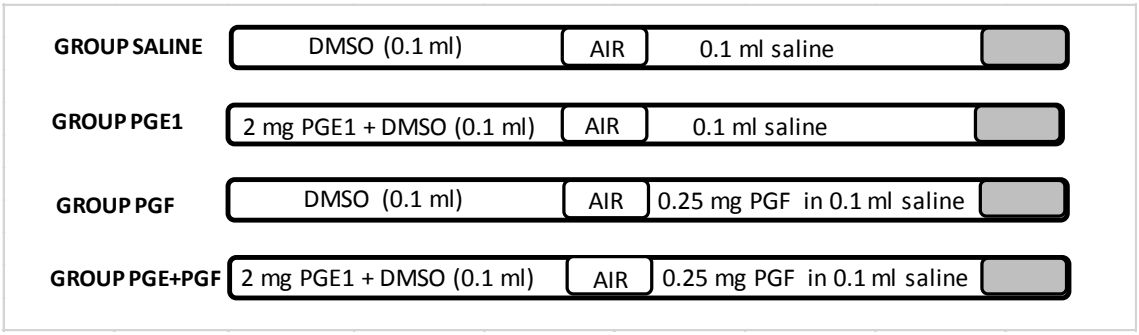
To determine changes in P4 concentrations coccygeal blood was collected just before each IU infusion (Hour 0 – first infusion; Hour 6 – second infusion; Hour 12 – third infusion; Hour 18 – fourth infusion). Thereafter, starting at 24 h after the first infusion, P4 was assayed every 12-h until 72-h (Figure 2). Blood samples were stored on ice, allowed to clot, and centrifuged at 3000 rpm for 20 min. Sera were stored at -20^{o} C until the assay. For P4 determination, samples were analyzed using an antibody-coated tube RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) with intra-assay and inter-assay coefficients of variation (CV) of 3.37% and 7.28%, respectively

In a subset of cows (n=16), additional samples were taken just before and 10 min after the first and second infusions, in order to determine circulating concentrations of PGFM and PGEM. These samples were collected into heparinized tubes, centrifuged at 3000 rpm for 20 min, and stored at -20^{o} C until assayed. The plasma samples were assayed for PGFM by an ELISA assay that was previously developed for use in bovine and equine plasma (161) with some modifications. Briefly, the ELISA plates were coated with 100 µl of secondary antibody (2 µg/ml) in coating buffer (0.05 M sodium carbonate [pH 9.6]) overnight. Standards were prepared by serial dilution (1250 to 4.88 pg/ml) of PGFM in prostaglandin-free (banamine-treated) bovine plasma. Prostaglandin free plasma was obtained from 2 cows treated at 12-h intervals with three intravenous injections of a prostaglandin synthase inhibitor (1.1 mg/kg of flunixin meglumine. Banamine; Intervet International B.V.). Blood was collected in heparinized tubes 1-h after the last injection. Aliquots of 250 µl of standards (B_0, serial dilutions and non-specific binding [NSB]),

quality control samples, and unknown samples were transferred to glass extraction tubes. The pH was adjusted to 3.0 with diluted hydrochloric acid and vortexed immediately. Two ml of diethyl ether were added to all the samples and mixed using a vortex for 3 min The tubes were placed in a bath of dry ice and methanol for at least 1 min. Unfrozen, ether extracts were transferred to new glass culture tubes and dried overnight. On the day of the assay, 250 µl of ELISA assay buffer (0.04 M MOPS, 0.12 M sodium chloride, 0.01 M EDTA, 0.05% Tween 20, 0.005% chlorhexidine digluconate [pH 7.4], 0.1% gelatin) was added to all dried extracts and vortexed for 2 min. The tubes were incubated for 90 min at room temperature and vortexed in the middle and the end of incubation. The ELISA plates were coated with secondary antibody, washed four times with wash buffer, and 100 µl of primary antibody (diluted 1:4000 in ELISA assay buffer) added to all wells, except the NSB wells, which received 100 µl assay buffer. The plate was incubated for 1.5 h at room temperature and washed again four times with wash buffer. Reconstituted extracts of standard, control, and unknown samples in duplicate were transferred (100 µl/well) to the respective wells in duplicate. After incubating the plate for 25 min at room temperature and, without washing the plate, 50 µl of the PGFM-HRP conjugate (161), diluted in assay buffer (1:1000), was added to all wells, and the plate was incubated for 1 h at room temperature. The plate was washed four times, and 125 µl of substrate solution was added with incubated for 20 min at 37^{o} C. To stop the reaction, 50 µl of stop solution (0.5 M H_2SO_4) was added to all wells, and the optical density was measured at a dual wavelength of 450 and 600 nm. A pool of samples collected from 25 pregnant and non-pregnant cows on day 19 of the estrous cycle was used as a quality control in all the assays. The intra-assay and inter-assay coefficients of variation were 9.8% and 10.1%, respectively. The samples collected to determine PGFM concentrations, were also assayed for PGEM using a commercial available kit (Cayman Chemicals) according to

instructions described by the manufacturer. The kit specifications report a 100% cross reactivity with 13,14-dihydro-15-keto Prostaglandin E1 and 13,14-dihydro-15-keto Prostaglandin E2 and a minimum detection limit of 0.39 pg/ml. Intra-assay CV was 6.62%.

Figure 1. Graphic representation of the straws loaded with treatments used in the four experimental groups.



2.7. RNA Isolation, RT Reaction and Quantitative PCR

Ten milligrams of luteal tissue were minced with a scalpel and homogenized in QIAzol Lysis Reagent with a Bio-gen PRO200 Homogenizer Motor Unit (PRO Scientific, CT, US). Total RNA from each sample was extracted using RNeasy Plus Universal Kit (RNeasy; QIAGEN), according to manufacturer's protocol. Due to technical problems, luteal biopsies after the fourth pulse could not be analyzed and therefore only data from the first three pulses are available. All individually homogenized samples were treated with gDNA Eliminator Solution (QIAGEN, US) to reduce genomic DNA contamination. Concentrations of RNA from each sample was determined by optical density at OD_{260nm}/OD_{280nm} ratio using NanoDrop 2000 spectophometer (Thermo Scientific, DE, US). Total RNA of 1 ug from each sample was reverse transcribed into cDNA using the iScript cDNA Synthesis Kit and diluted (1:5) in deionized water. All samples were stored at -20° C until used. Real-Time qPCR (RT-qPCR) reactions were

conducted with the CFX Connect Real-Time System (Bio-Rad Life Science, Foster City, CA). Reactions were done using SSoFast EvaGreen Supermix, as described previously (162). Thermal cycling was done using the following procedure: 1 cycle at 95° C for 3min and 49 cycles at 95° C for 10 sec, followed by 30 sec at 60° C (annealing temperature). The sequences for primers from target and housekeeping genes were designed using Primer3 from the NCBI (http://www.ncbi.nlm.nih.gov) gene database or were derived from published studies (163). Primer sequences for analyzed genes (FOS, JUN, STAR, LHCGR, PTGS2, PTGFS, HPGD) from samples collected after first, second, and third infusions are shown in Table 1.

2.8 RNA-Seq analysis

To compare the effect of the third IU infusion of PGE1, PGF, and PGE+PGF on gene expression of the CL, luteal biopsies (n=5/treatment) were further analyzed using RNA-seq. The mapping of sequence reads and subsequent assembly of transcripts was performed as previously described (164). Briefly, sequencing reads were mapped to the bovine reference genome UMD3.1 using Tophat (v2.0.13) (165). The resulting alignments were used to reconstruct gene and transcript models using Cufflinks (v2.2.1) (166). The computational tool *cuffmerge* was used for merging together each of the assemblies with the reference bovine annotation file in order to combine known annotated transcripts with novel transcripts. Finally, for each sample, the number of sequencing reads that mapped to each gene was inferred using the python script *htseq-count* (167). Gene expression was evaluated using the *R* package *edgeR* (v.3.14.0). This package combines the use of trimmed mean of M-values (TMM) as the normalization method of the sequencing data, an empirical Bayes approach for estimating genewise negative binomial dispersion values, and finally, an exact test for detecting differentially expressed

genes between the four treatments. A subset of 19 candidate genes (Table 2) were analyzed and compared for differential gene expression among treatments.

Table 1. List of genes analyzed by RT-qPCR (CypA and RPS15 were used as housekeeping genes).

Genes		Sequence of primer (5`-3`)	Accession code / reference	Size(bp)
FOS	Forward	gaatctgaggaggccttcacc	NM182786	103
	Reverse	tcagccttcagctccatgc		
JUN	Forward	agagcggcgcctacggctacag	AF069514	123
	Reverse	gtgaggaggtcggagttcttg		
STAR	Forward	cagcagaagggtgtcatcaga	NM174189	152
	Reverse	gagaggacctggttgatgatg		
LHCGR	Forward	ttgccacatcatcctatt	NM174381	122
	Reverse	ctcgtttgttggcaagtt		
PTGS2	Forward	catgatgttctttgttggcatt	NM_174445	154
	Reverse	gcgaattccaactttccatc		
PTGFS	Forward	gatggccacttcattcctgt	Shirasuna et al. 2010 (163)	189
	Reverse	cacagtgccatctgcaatct		
HPGD	Forward	ggaaagctggacatcttggt	NM001034419	150
	Reverse	gcaaattgcgttcagtctca		
CypA	Forward	caccgtgttcttcgacatcg	NM_178320	521
	Reverse	acagctcaaaagagacgcgg		
RPS15	Forward	cgcgacatgatcattctacc	NM_001024541	489
	Reverse	ttacttgtgggggatgaagc		

Table 2. List of 19 candidate genes analyzed by RNA-seq

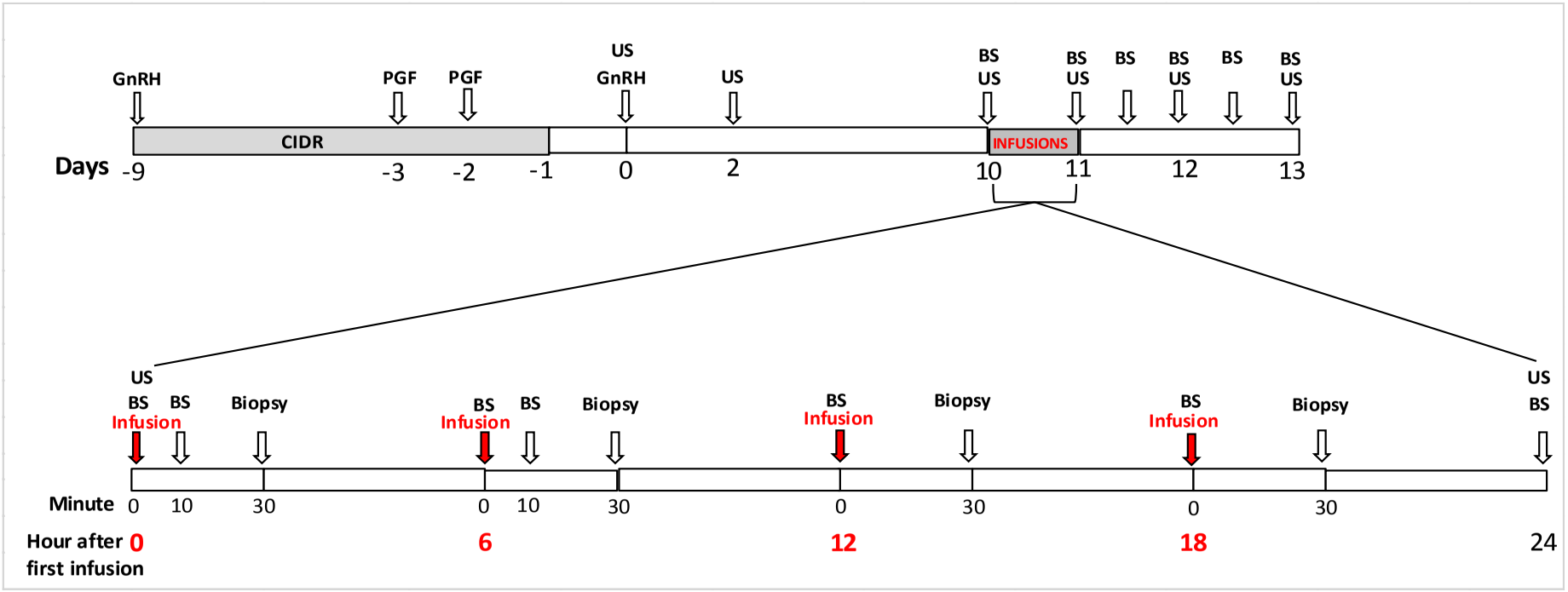
Gene	Definition
FOS	FBJ murine osteosarcoma viral oncogene homolog
JUN	Jun proto-oncogene
EGR1	Early growth response 1
NR4A1	Nuclear receptor subfamily 4, group A, member 1
IL1B	Interleukin 1, beta
IL-8	Interleukin 8
FAS	Fas, TNF receptor superfamily, member 6
FASLG	Fas ligand
STAR	Steroidogenic acute regulatory protein
CYP11A1	Cytochrome P450, family 11, subfamily A, polypeptide 1
NR5A1	Nuclear receptor subfamily 5, group A, member 1
LHCGR	Luteinizing hormone/choriogonadotropin receptor
PTGS2	Prostaglandin-endoperoxide synthase 2
PTGFS	Prostaglandin F synthase
HPGD	Hydroxyprostaglandin dehydrogenase 15-[NAD]
PTGFR	Prostaglandin F receptor
VEGFA	Vascular endothelial growth factor
FGF2	Fibroblast growth factor 2
PTGES	Prostaglandin E synthase

2.9 Statistical Analysis

Differences between variables prior to treatments were calculated by Levence`s test for homogeneity of variance. The data obtained for the response variables concentration of P4 (ng/ml) and luteal volume (cm^3) for each one of the four treatment groups were normalized to 100 percent compared to time 0 (Hour 0 of treatment for P4 concentrations and Day 0 for luteal volume) and expressed as percentage change in each cow relative to

time 0. The values were analyzed for differences between treatments using the Proc Mixed procedure of SAS and differences between means at specific timepoints were assessed using Fisher LSD. Data for PGFM and PGEM concentrations were not normally distributed and therefore were transformed to natural logarithms. Differences between treatments were analyzed by one-way ANOVA. Assumptions of normality and homogeneity of variance were evaluated and transformations (natural logarithm) performed, when appropriate. Mean cycle threshold (Ct) values for the housekeeping genes Cyclophilin A (CypA) and ribosomal protein S15 (RPS15) were averaged by geometric mean and used to normalize the expression of target genes. Validation of these housekeeping genes in bovine luteal tissue was performed in preliminary assays in our lab (unpublished results). The Ct values from biopsies of the saline group were used as the calibrator according to the $2^{-\Delta\Delta Ct}$ methods described by Livak and Schmittgen (168). Differences in fold change concentrations between treatments, for each gene, within a pulse, were analyzed by one-way ANOVA or Kruskal Wallis non-parametric analysis of variance when assumptions of normality and homogeneity of variance were not met. Multiple comparisons between treatments were performed using Fisher's LSD. Data were considered statistically significant when *P* values were lower than 0.05. Data in tables are expressed as the fold change of the target genes compared to controls.

Figure 2. Representation of the protocol used to synchronize ovulations, timing of IU infusions and collection of samples. Abbreviation key: BS=Blood sample, US= Ultrasound evaluation.



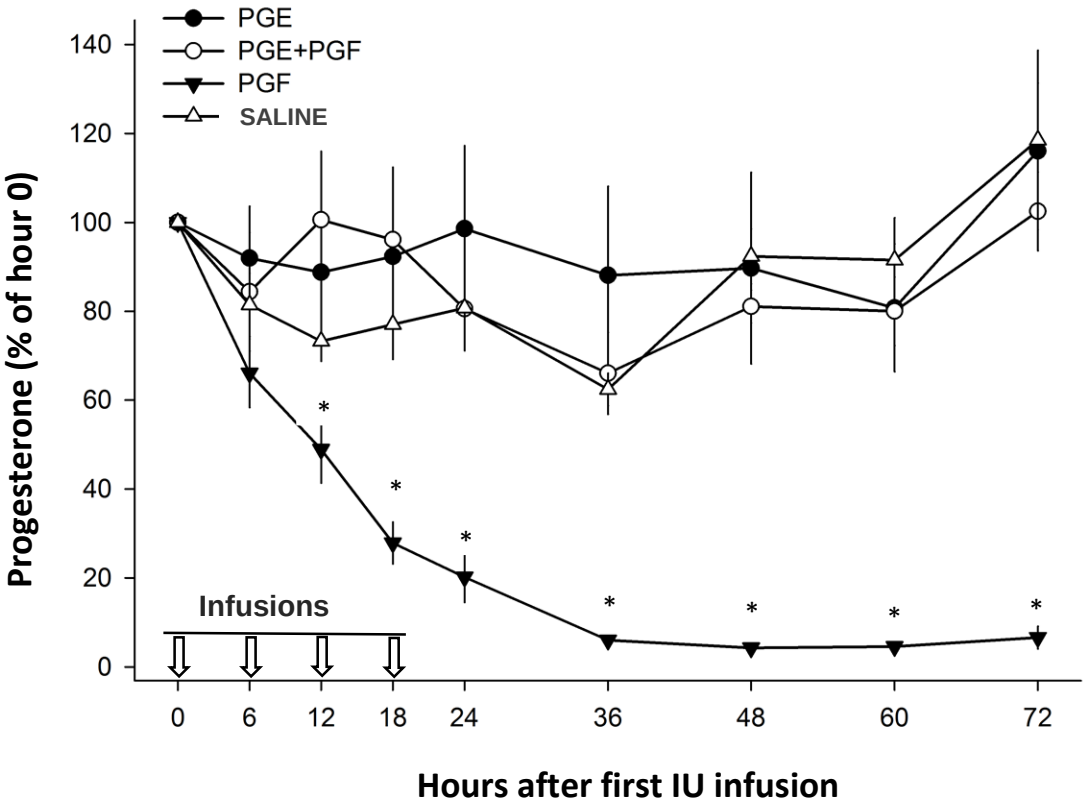
Results

3.1 Concentrations of Circulating P4 after Treatments

Circulating concentrations of P4 for the 4 treatment groups are shown in Figure 3. Circulating P4 concentrations were somewhat variable between the 4 treatment groups prior to treatments, although they were not significantly different ($P = 0.21$): saline – 5.0 ± 0.6, PGF– 7.1 ± 0.9, PGE– 6.0 ± 1.5, PGE+PGF– 4.15 ± 0.4 ng/ml (mean ± SEM). To normalize for variation in pretreatment circulating P4, the P4 value for each cow was standardized, using the pretreatment P4 as 100%, and all subsequent circulating P4 concentrations in that cow were calculated as a percentage of this pretreatment value.

There was a significant effect of hour ($P < 0.0001$), treatment ($P < 0.0001$) and an hour by treatment interaction ($P < 0.0001$) for circulating P4 concentrations (Figure 3). The source of these effects was the PGF group. There were no differences between groups or within hours in circulating P4 concentrations during the experimental period (0-72 h) for control, PGE, or PGE+PGF groups. In contrast, the PGF-treated group was different from each of the other three groups ($P < 0.0001$) during the entire experimental period, starting at 12 h after the first infusion of PGF and at all subsequent time points ($P < 0.05$). For example, at 12 h after treatment there were clear differences between the PGF group and the saline group ($P = 0.0314$), the PGE+PGF group ($P = 0.0008$) and the PGE group ($P = 0.0042$). At all subsequent times the PGF group maintained this difference when compared to each of the other treatments ($P < 0.0001$), whereas the other three groups were similar at all of the evaluated times ($P > 0.05$; Figure 3). Figure 5 shows individual profiles of circulating P4 concentrations from treatments.

Figure 3. Effect of IU Saline, PGF, PGE and PGE+PGF treatments (n = 5 / treatment) on circulating P4 concentrations in cows. Data are presented as means ± SEM. Asterisks indicates significant decrease in plasma P4 concentrations (*P* < 0.05).



3.2 Luteal Volume

There was some variability in luteal volume between the 4 treatment groups prior to treatments, although there was no significant difference between treatments ($P = 0.45$): saline – 8.33 ± 0.50, PGF – 11.62 ± 1.05, PGE – 7.82 ± 1.08, PGE+PGF – 9.67 ± 1.27 cm^3 (mean ± SEM). To normalize for variation in pretreatment luteal volume, the value in cm^3 for each cow was standardized, using the pretreatment volume as 100%, and all

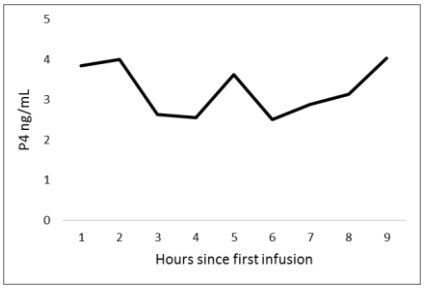
subsequent values for volume in that cow were calculated as a percentage of this pretreatment value (Figure 4).

Similar to circulating P4, the luteal volume was different due to the effect of treatments ($P = 0.0002$), time (Day, $P = 0.0018$) and the treatment by Day interaction ($P < 0.0001$). Again, the only source of significance was the PGF treatment group, which was different from the PGE group ($P < 0.0001$), PGE+PGF group ($P = 0.0004$), and the saline group ($P < 0.0001$). The three other groups were not different from each other. The decrease in luteal volume between the PGF group and the other three groups began to be evident from Day 1 onwards and the differences increased until Day 3 (Figure 4). No differences were detected between any of the treatments PGE, PGE+PGF, and saline for any of the days during which luteal volume was evaluated. Figure 5 shows individual measurments of luteal volume from treatments.

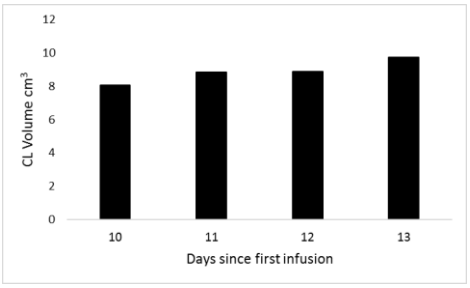
Figure 4. Effect of IU Saline, PGF, PGE and PGE+PGF treatments (n = 5 / treatment) on luteal volume in cows. Data are presented as means ± SEM. Days 0, 1, 2 and 3 after first infusion, corresponds to days 10, 11, 12 and 13 of the estrous cycle. Columns with different letters (a, b) indicate statistical difference among treatments within a day ($P < 0.05$).

Figure 5. Individual P4 profiles and luteal volume from cows treated with IU infusions. A) Saline, B) PGF, C) PGE and D) PGE+PGF.

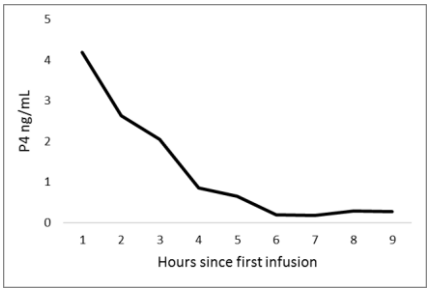
A) Cow 2234



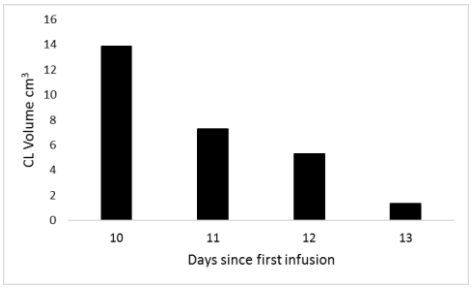
Cow 2234



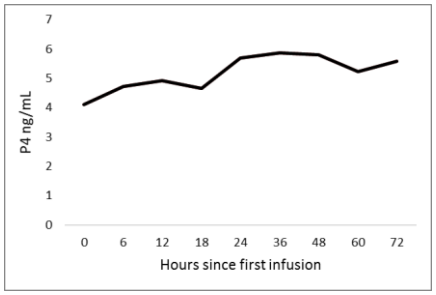
B) Cow 2135



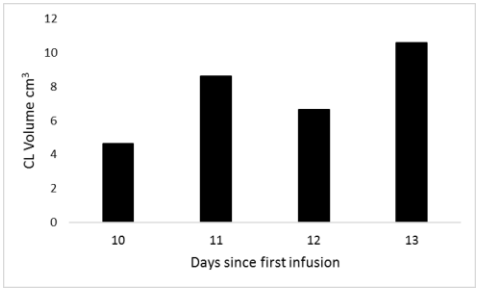
Cow 2135



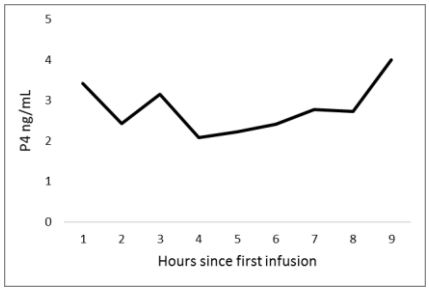
C) Cow 7223



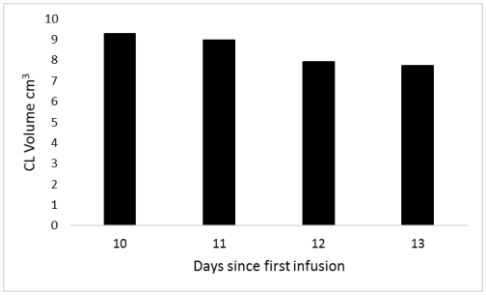
Cow 7223



D) Cow 7475



Cow 7475



3.3 Concentrations of Circulating PGFM

Circulating PGFM concentrations were low and did not differ among treatments prior to IU infusions. Concentrations of PGFM in the saline group were similar before and after the first two infusions (Figure 6). Cows in the PGE group showed low concentrations before and 10 min after the two pulses. However, there was a small but significant ($P = 0.015$) increase in PGFM in pulse 2 (3.1 ± 1.7 pg/ml at min 0 vs 11.1 ± 2.7 pg/ml at min 10). Concentrations of PGFM increased dramatically at 10 min after infusion of PGF for either the first (6.5 ± 1.9 pg/ml at min 0 vs 97.1 ± 34.6 pg/ml at min 10; $P < 0.001$) and second (5.7 ± 1.9 pg/ml at min 0 vs 86.8 ± 30.6 pg/ml at min 10; $P = 0.062$) IU infusions of PGF. Similarly, cows in the group PGE+PGF had increased concentrations of PGFM after pulse 1 (5.0 ± 2.4 pg/ml for min 0 vs 139.1 ± 54.5 pg/ml for min 10; $P = 0.006$) and pulse 2 (4.3 ± 2.2 pg/ml for min 0 vs 132.9 ± 61.1 pg/ml for min 10; $P = 0.037$). At 10 min after IU infusions, cows enrolled in groups saline and PGE, had lower concentrations of PGFM than cows in the groups PGF and PGE+PGF during pulse 1 ($P = 0.001$) and pulse 2 ($P = 0.001$).

3.4 Concentrations of Circulating PGEM

Circulating concentrations of PGEM were low and did not vary among treatments before IU infusions (Figure 7). Cows in the saline group showed low concentrations of PGEM before and 10 minutes after first and second pulse. Nevertheless, there was a slight increase ($P = 0.05$) after the first infusion (32.6 ± 6 pg/ml at min 0 vs 83.1 ± 10.1 pg/ml at min 10). Concentrations of PGEM in the PGE group increased significantly after the first (55.5 ± 12.5 pg/ml at min 0 vs 323.2 ± 92.5 pg/ml at min 10; $P = 0.029$) and second (50.1 ± 11.2 pg/ml at min 0 vs 417.4 ± 98.4 pg/ml at min 10; $P = 0.01$) IU infusions. In

cows that received IU infusions of PGF, concentrations of PGEM did not increase after pulse 1 (P = 0.83) or pulse 2 (P = 0.79). In contrast, cows receiving IU infusions of PGE+PGF, showed a marked increase in PGEM levels after pulse 1 (49.9 ± 7 pg/ml for min 0 vs 476.9 ± 118.5 pg/ml for min 10; $P < 0.001$) and pulse 2 (47.1 ± 4.3 pg/ml for min 0 vs 434.1 ± 61.2 pg/ml for min 10; $P < 0.001$).

Figure 6. Effect of IU Saline, PGF, PGE and PGE+PGF treatments (n = 4 / treatment) on circulating PGFM concentrations in cows . Data are presented as means ± SEM. Min 0 and Min 10 corresponds to moments of sampling before and 10 minutes after intrauterine infusions in each pulse. Asterisks indicate significant differences ($P < 0.05$) between min 0 and min 10 within a pulse.

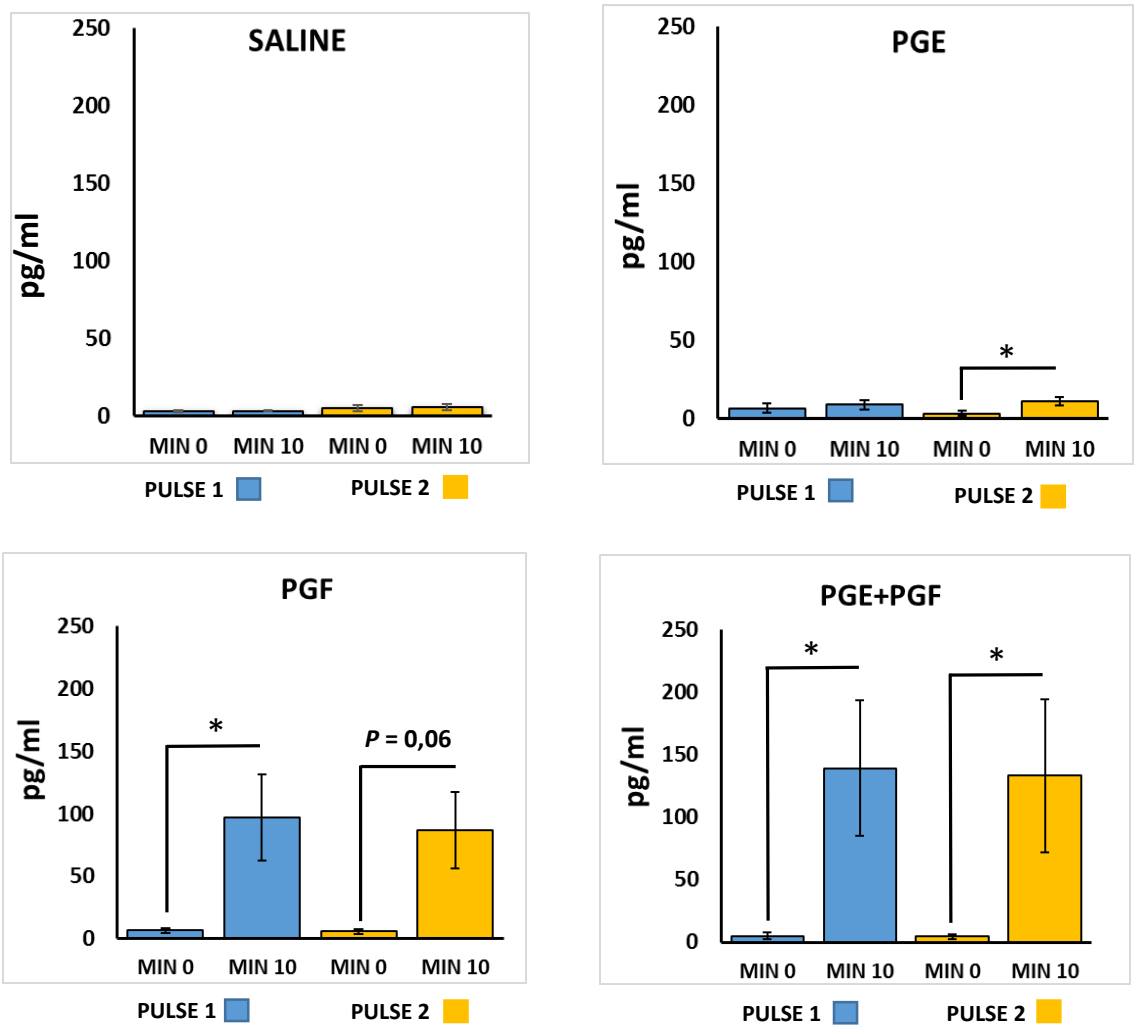
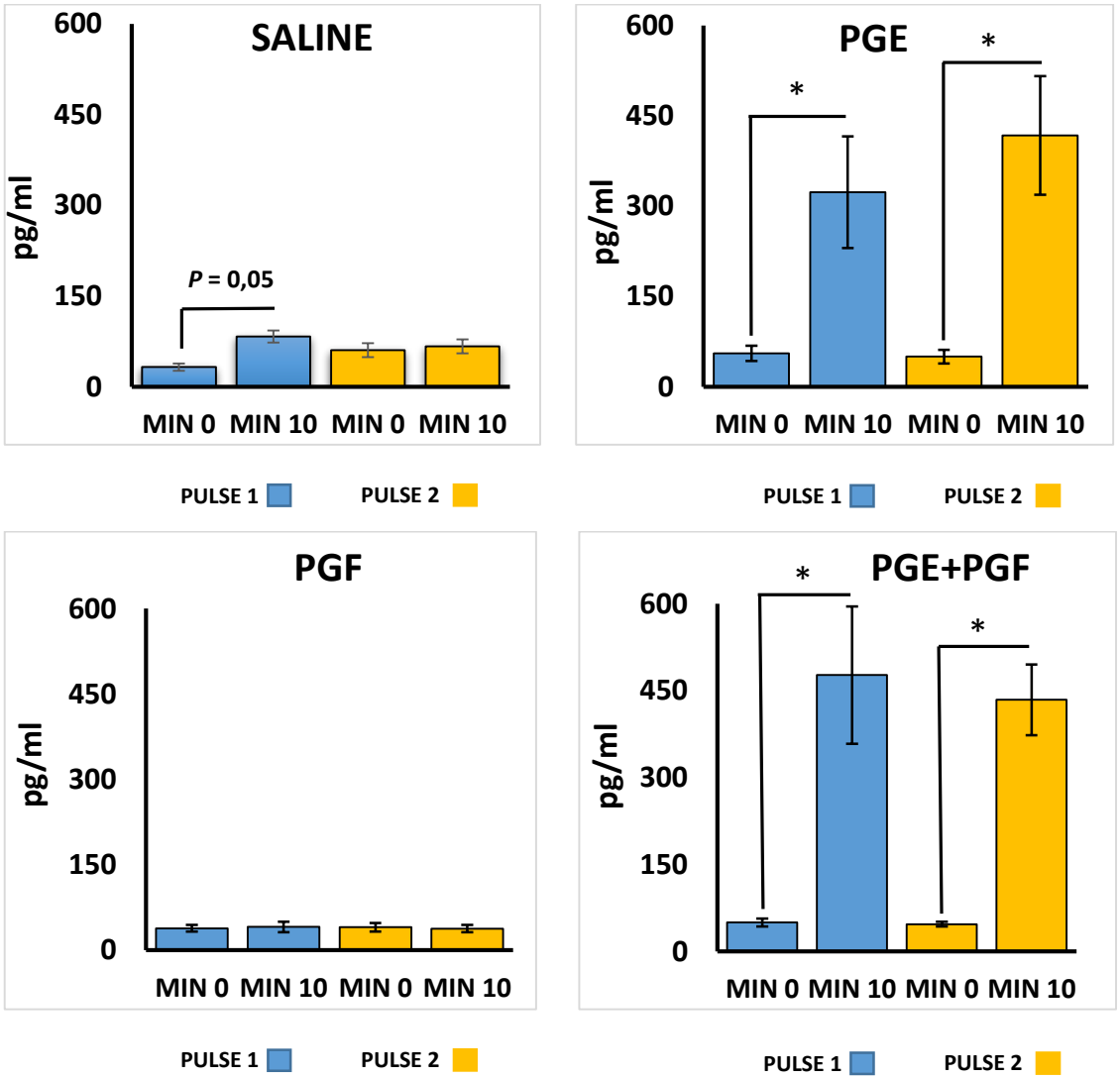


Figure 7. Effect of intrauterine Saline, PGF, PGE and PGE+PGF treatments (n = 4 / treatment) on circulating PGEM concentrations in cows. Data are presented as means ± SEM. Min 0 and Min 10 corresponds to moments of sampling before and 10 minutes after intrauterine infusions in each pulse. Asterisks indicate significant differences ($P < 0.05$) between min 0 and min 10 within a pulse.



3.5 Gene expression in the CL (Quantitative PCR).

The steady state concentrations of mRNA for early response genes (*JUN* and *FOS*) are shown in Table 3. After the first pulse, concentrations of FOS were increased ($P < 0.0001$) by PGF and PGE+PGF, although FOS mRNA was greater for PGF than PGE+PGF. After the second and third pulses, FOS mRNA was generally greater in CL from cows treated with PGF or PGE+PGF than the other two treatments. For JUN, there was an increase in mRNA for CL from cows treated with PGF and PGE+PGF after the first pulse but not after the second or third pulse compared to Saline or PGE-treated cows. There were no detectable differences between treatments for StAR or LHCGR mRNA after any of the treatments or for any of the 3 pulses (Table 4). Expression of PTGS2 was increased ($P < 0.05$) at the first biopsy in CL from cows treated with PGF and PGE+PGF compared to Saline and PGE-treated cows (Table 5). The second and third biopsy were not different between treatments for PTGS2. There were no differences between treatments for PTGFS or HPGD mRNA at any of the three pulses.

Table 3. Effect of IU Saline, PGF, PGE and PGE+PGF treatments on mRNA concentrations for Immeadiate early genes FOS (FBJ murine osteosarcoma viral oncogene homolog) and JUN (jun proto-oncogene). Data are shown as fold changes ± SEM. Columns with different letters (a, b, c) at each biopsy indicate differences; *P* < 0.05. First, second and third, indicate biopsy times.

		Treatments				
Gene	Pulse	SALINE	PGE	PGF	PGE+PGF	*P*-value
FOS	1st	0.8 ± 0.3^{a}	1.3 ± 0.3^{a}	17.8 ± 2.1^{b}	10.4 ± 1.5^{c}	<0.0001
	2nd	0.7 ± 0.3^{ab}	0.5 ± 0.1^{a}	2.68 ± 1^{bc}	3.3 ± 1.2^{c}	0.0402
	3rd	1.6 ± 0.2^{a}	1.9 ± 0.4^{a}	6.8 ± 1.5^{b}	4.4 ± 1.7^{ab}	0.0342
JUN	1st	1.6 ± 0.1^{a}	1.6 ± 0.4^{a}	3.7 ± 0.7^{b}	3.3 ± 0.5^{b}	0.0214
	2nd	0.7 ± 0.1	0.5 ± 0.06	1.37 ±0.4	2 ±0.9	0.2679
	3rd	1.3 ± 0.2	1.3 ±0.2	2.9 ± 0.5	2.68 ± 0.8	0.1185

Table 4. Effect of IU Saline, PGF, PGE and PGE+PGF treatments on mRNA concentrations for steroidogenic genes STAR (steroidogenic acute regulatory protein) and LHCGR (luteinizing hormone/choriogonadotropin receptor). Data are shown as fold changes ± SEM. Columns with different letters (a, b, c) at each biopsy indicate differences; *P* < 0.05. First, second and third, indicate biopsy times.

		Treatments				
Gene	Pulse	SALINE	PGE	PGF	PGE+PGF	*P*-value
STAR	1st	1.5 ± 0.4	1.7 ± 0.1	1.6 ± 0.2	2 ± 0.5	0.8444
	2nd	0.8 ± 0.1	1.3 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.0981
	3rd	1.3 ± 0.5	1.3 ± 0.4	0.5 ± 0.1	1 ± 0.2	0.4535
LHCGR	1st	0.5 ± 0.1	0.5 ± 0.1	1.6 ± 0.7	1.6 ± 0.7	0.5768
	2nd	1.1 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.2611
	3rd	1.1 ± 0.1	1.8 ± 0.8	2 ± 0.5	3.2 ± 1.3	0.6478

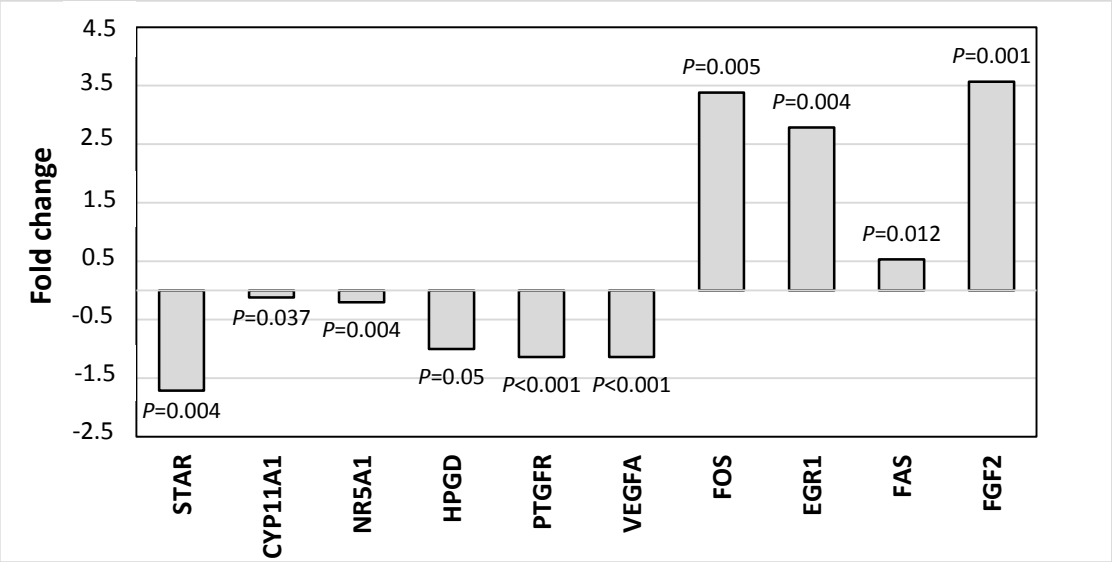
Table 5. Effect of IU Saline, PGF, PGE and PGE+PGF treatments on mRNA concentrations for prostaglandin related genes PTGS2 (prostaglandin-endoperoxide synthase 2), PTGFS (prostaglandin F synthase) and HPGD (hydroxyprostaglandin dehydrogenase 15-[NAD]). Data are shown as fold changes ± SEM. Columns with different letters (a, b, c) at each biopsy indicate differences; P < 0.05. First, second and third, indicate biopsy times.

		Treatments				
Gene	Pulse	SALINE	PGE	PGF	PGE+PGF	*P*-value
PTGS2	1st	0.6 ± 0.1^{ab}	0.6 ± 0.1^{a}	1.7 ± 0.4^{b}	1.9 ± 0.7^{b}	0.0477
	2nd	0.8 ± 0.1	0.8 ± 0.2	2.9 ± 1.4	1.1 ± 0.1	0.6554
	3rd	1.3 ± 0.2	0.8 ± 0.3	2.2 ± 0.8	2 ± 0.4	0.2197
PTGFS	1st	0.4 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.9228
	2nd	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	0.6064
	3rd	1 ± 0.4	0	1.9 ± 0.4	0.2 ± 0.2	0.4857
HPGD	1st	0.9 ± 0.3	0.5 ± 0.3	0.3 ± 0.1	0.3 ± 0.2	0.4501
	2nd	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2737
	3rd	2.6 ± 0.9	1.7 ± 0.5	1.6 ± 0.3	1.7 ± 0.3	0.705

3.6 RNA-seq

Three comparisons were made in order to determine changes in gene expression among treatments after the third infusion. 1. Saline vs PGF: More than 200 genes were differentially expressed between these treatments. To gain insight into the processes that could be regulated differentially between Saline and PGF, 19 candidate genes (Table 2) were analyzed separately. Ten out of the nineteen candidate genes showed differential expression ($P \leq 0.05$); some genes STAR, CYP11A1, NR5A1, HPGD, PTGFR, and VEGFA showed greater expression in saline than PGF treatment, while other genes FOS, EGR1, FAS, and FGF2 showed greater expression in PGF than saline treatment. Figure 8 displays the fold change and the *P*-value for candidate genes differentialy expressed. Nine candidate genes (JUN, NR4A1, IL1B, IL-8, FASLG, LHCGR, PTGS2, PTGFS and PTGES) did not show differential expression between groups. 2. Saline vs PGE: Only the immediate early gene NR4A1 had greater expression (fold change = 3.24; $P = 0.005$) in saline group. 3. Saline vs PGE+PGF: The immediate early genes NR4A1 (fold change = 4.0; $P = 0.002$), EGR1 (fold change = 4.0; $P < 0.001$) and FOS (fold change = 2.56; $P = 0.01$) showed greater expression in the PGE+PGF treated group.

Figure 8. Fold changes of differentially expressed genes measured by RNA-Seq. Bars above the X-axis denote genes with higher expression in the PGF treatment while bars below the X-axis denote genes with higher expression in the saline treatment. The *P*-values for differential expression compared to the saline controls are shown for each gene.



4. DISCUSSION

This study used a model of IU infusions of PGF to simulate the pulsatile secretion of PGF that characterizes luteolysis in ruminants. The dose of 0.25 mg/infusion was used in this experiment, based on a previous report in Holstein heifers (98) and on a preliminary study that we performed in Holstein dry cows (unpublished results) that demonstrated that this dose was consistently luteolytic. This dose was lower than the dose of 0.5 mg of PGF that was utilized in our previous study (66), however intervals between infusions (6-h) and the interval between infusions and biopsies (0.5 h) were the same. The previous study found that 4 IU pulses were needed to induce complete regression of the CL in all cows, and this study observed a similar complete CL regression in cows treated with four IU doses of 0.25 mg. Extremely low doses of PGF are sufficient to regress the

CL when is administered in the uterine horn ipsilateral to the CL due to the local transport of prostaglandins through the utero-ovarian pathway (96, 98, 169). The same model of IU infusions was used to treat the cows with PGE1, in order to mimic endometrial production of high amounts of PGE during pregnancy. The preferred use of PGE1 instead of PGE2 as a luteoprotective agent, was based on previous experiments in which the use of PGE2 was associated with a cross-reaction with FP receptors (69, 91). The infused dose (2 mg) of PGE1 was calculated based on an approximate concentration of 30 ng/ml of PGE that has been reported in the uterine vein of pregnant sheep (12) and a rate of blood flow in the uterine vein of 200 ml/min (159) during a 6-h period ((30 ng/ml x 200 ml/min) x (360 min) = 2.16 mg every 6 hours).

Blood samples before and 10 minutes after the IU infusions, allowed us to monitor circulating concentrations of PGFM and PGEM after IU treatment with PGF or PGE (8, 87). The interval of 10 minutes between infusions and sampling for PGFM, has been recommended in cattle to detect maximum concentrations after IU infusions with doses of PGF ranging from 0.25 to 4 mg (98). As expected, IU infusions of saline and PGE1 did not induce a considerable increase in PGFM concentrations after first and second pulses. In contrast, infusions of PGF and PGE+PGF resulted in an immediate and large increase in circulating PGFM concentrations at 10 minutes after infusion. Collectively, these results reflect not only the rapid absorption rate of PGF from the uterus but also that simultaneous infusion of PGE1 apparently did not prevent the absorption and transport of PGF from the uterus to the uterine vein. Maximum mean (139 ± 54.5 pg/ml) or individual (288 ± 61 pg/ml) concentrations of PGFM from this experiment, fall within the physiological concentrations for natural PGFM pulses observed in other studies conducted in non pregnant cows (170) or heifers (8, 146) during the luteolytic period. The peak of the PGFM pulse at 10 min after a 0.25 mg IU injection or infusion of PGF has

been reported to be either greater than or less than the peak concentration during a natural PGFM pulse (98). Thus, our pulsatile pattern and dose of IU PGF mimicked the pulsatile pattern that is characteristic of luteolysis.

Our first hypothesis was supported since we observed a consistent and profound decrease in concentrations of P4 that was significant by 12-h after the first infusion of PGF or 6-h after the second PGF treatment. The decrease in circulating P4 coincided with a mean loss of 43.6% in the luteal volume 24-h after the first PGF infusion (Figure 3) with subsequent decreases over the next 2 days resulting in complete CL regression by 72-h after the first PGF treatment. Treatment with PGF produced a rapid change in gene expression in the CL as evidenced by an increase in the steady-state concentrations of mRNA for the early response genes, FOS and JUN, by thirty minutes after the first PGF treatment or first treatment with PGE+PGF (Table 1). Previous studies have reported an increase in expression of these genes in bovine luteal tissue in response to an IU (59) or systemic (110) treatment with PGF. The initial actions of PGF in the CL are mediated by the binding of PGF to specific G-protein-coupled receptors, termed FP receptors (171, 172), that induce a rapid increase in concentrations of free intracellular calcium (142), and activation of protein kinase C (PKC) and mitogen-activated protein (MAP) kinase (1). Activation of multiple intracellular signal transduction pathways, induce the transcription of these early response genes (109, 173). Previous studies have shown that both supraphysiologic (110, 174) and low doses of IU PGF (59), provoke a rapid increase in the expression of these genes. By the second and third infusions, expression of early response genes is less profound but still significant for FOS (59), similar to the results observed in our study in the PGF-treated cows (Table 2). By the third pulse, the steady-state concentrations of mRNA for FOS and JUN are similar among treatments based on either PCR or RNA-seq analysis. The reduced magnitude in expression of FOS and JUN

in PGF-infused cows after the first infusion may be related to down-regulation of PGF receptors by the PGF pulses although, this effects was observed in both this experiment using 0.25 mg of PGF and in the previous experiment using 0.5 mg of PGF. Treatment with PGF dramatically reduces steady-state mRNA concentrations for PTGFR after a single (175) or sequential (59) treatments with PGF. Consistent with these results, we observed a dramatic decrease in mRNA for PTGFR in this experiment, based on the RNA-seq analysis after the third PGF treatment. Other genes that have been observed to be changed by PGF treatment in previous studies (59, 176, 177) were also altered in this experiment including: decreased mRNA for genes involved in steroidogenesis (STAR and NR5A1), angiogenesis (VEGFA), and prostaglandin metabolism (HPGD) and increased mRNA for FAS and FGF2.

Our second hypothesis was also somewhat supported, since treatment with physiological doses of IU PGE1 did not alter circulating P4 concentrations or the volume of the CL. To our knowledge, this is the first experiment using IU infusions of PGE1 in cows, as a model to study protective effects of PGE on the CL. As determined by peripheral blood samples collected before and 10 minutes after infusions, circulating concentrations of PGEM showed a rapid increase in cows treated with PGE and PGE+PGF after IU infusions (Figure 7). This indicates that PGE1 was absorbed from the uterine lumen, transported through the uterine vein to the systemic circulation, and metabolized to the PGE metabolite in the lungs or other part of the circulatory route. An elegant experiment conducted in ewes (12), reported that PGE2 production was 32.3-fold greater in pregnant compared to cycling ewes (Day 15) with efficient transport of PGE2 from the uterine lumen to the uterine vein (92.1% efficiency on Day 16 of pregnancy), and from the uterine vein to the ovarian artery (12.2% transported). Although the scope of this experiment did not allow determination of the precise efficiency of PGE1 transport

from the uterus to the uterine vein, it does show that high PGE1 amounts exit the uterus and are subsequently detected as PGE metabolite in the circulation. The PGEM concentrations (~400 pg/ml) were ~4-fold greater than PGFM concentrations (~100 pg/ml) reflecting the 8-fold greater amounts of PGE1 that were infused compared to PGF. We also expected efficient transport of PGE1 from the uterine vein to the ovarian artery and that we would be able to then detect a pattern of gene expression in the CL that would be distinguishing for PGE action in the CL. A previous study in pregnant ewes found distinctive changes in proteins involved in the production (PTGES), metabolism (PGDH), and signaling (PTGER) of PGE2 (12), and we anticipated that IU treatment with PGE might regulate these same genes. Somewhat surprisingly in our study, luteal mRNA from PGE-treated cows did not exhibit a distinctive pattern of gene expression in the CL, at least for the limited number of genes evaluated in this study . It is possible that diminished changes in PGE-induced gene expression are a result of insufficient concentrations of PGE1 reaching the CL possibly due to inefficiency in transport of PGE1 from the uterine vein to the ovarian artery.

The third and most important hypothesis of the present study related to whether IU infusion of low doses of PGE1 could block the luteolytic effects of IU infusions of low doses of PGF. Thus, both the pulses of PGF and pulses of PGE were chosen to fall within a physiological concentration and pattern that may be present during normal bovine pregnancy. The major finding of the study, at this time, is the dramatic and complete inhibition of both the functional and structural effects of PGF pulses by simultaneous infusion of PGE. This is consistent with our third hypothesis that physiological concentrations of PGE can block the effect of physiological concentrations of PGF. These results are consistent with a great deal of other research that has demonstrated an inhibition of PGF action by simultaneous treatment with PGE (10, 12, 92, 157). Results

from this experiment indicate efficient transport of PGE and PGF from the uterus to the uterine vein, based on circulating PGFM and PGEM after IU infusions. In this regard, PGF, in the absence of PGE1, is being efficiently transported to the ovary through the utero-ovarian plexus since there was a clear luteolytic response in cows treated with pulses of only PGF. However, the luteolytic and most of the transcriptomic changes that were differentially regulated by PGF in our study and typically regulated by PGF in other studies (59, 177) were prevented by simultaneous infusions of PGE+PGF. The expression of some PGF-induced early response genes, such as Fos and EGR1, were not prevented by PGE1 infusion. One important transcription factor that was initially induced by PGF, JUN was not induced in cows treated simultaneously with PGE1 and PGF by third pulse. Previous studies have associated lack of PGF-induced expression of JUN with lack of regression of the porcine CL, even though other members of the activating protein-1 (AP-1) transcription factors, such as FOS, were induced by PGF in CL that subsequently did not regress (178). Transcription in response to AP-1 activation requires heterodimers of the fos and jun family and JUN has a unique role since it is the only member to have both a DNA binding domain and a basic leucine-zipper region that allows heterdimerization with fos family members (179, 180). Thus, lack of induction of JUN may produce a lack of response to induction of other AP-1 transcription factors such as FOS. Alternatively, it seems possible that IU infusion of PGE1 blocked the transport of PGF from the uterine vein to the ovarian artery by the time of the the third treatment and this action prevented luteolysis. It is not possible to distinguish these two distinct mechanisms or other potential mechanisms for PGE-induced inhibition of PGF action from the present experimental results. In summary, the results of the present study, indicate that PGE excerts its luteoprotective effect even when is delivered through IU infusions. This model mimicked the natural production and liberation of prostaglandins during early pregnancy in cows,

suggesting a primary role for PGE during early pregnancy. These results are likely to have important implications for the underlying mechanisms involved in the rescue of the CL in cows.

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Orientador: João Carlos Pinheiro Ferreira
Coorientador: Milo C Wiltbank
Capes: 50504002

1. Corpo lúteo. 2. Expressão gênica. 3. Ruminante. 4. Vaca. 5. Prostaglandinas. 6. Luteólise.

Palavras-chave: Corpo luteo; Luteólise; Prostaglandina E1.