UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

ROTA DE AÇÃO DA PROSTAGLANDINA $F_{2\alpha}$ ADMINISTRADA VIA SUBMUCOSA VULVAR NA LUTEÓLISE DE BOVINOS

DISSERTAÇÃO DE MESTRADO

Monique Tomazele Rovani

Santa Maria, RS, Brasil

2011

ROTA DE AÇÃO DA PROSTAGLANDINA $F_{2\alpha}$ ADMINISTRADA VIA SUBMUCOSA VULVAR NA LUTEÓLISE DE BOVINOS

Monique Tomazele Rovani

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Fisiopatologia da Reprodução, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Mestre em Medicina Veterinária.

Orientador: Prof. João Francisco Coelho de Oliveira

Santa Maria, RS, Brasil
2011

Universidade Federal de Santa Maria
Centro de Ciências Rurais
Programa de Pós-Graduação em Medicina Veterinária

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

ROTA DE AÇÃO DA PROSTAGLANDINA $F_{2\alpha}$ ADMINISTRADA VIA SUBMUCOSA VULVAR NA LUTEÓLISE DE BOVINOS

elaborada por
Monique Tomazele Rovani

como requisito parcial para obtenção do grau de
Mestre em Medicina Veterinária

COMISSÃO EXAMINADORA:

João Francisco Coelho de Oliveira, Dr.
(Presidente/Orientador)

José Carlos Ferrugem Moraes, Dr. (EMBRAPA)

William Schoenau, Dr. (UFSM)

Santa Maria, 16 de setembro de 2011.

AGRADECIMENTOS

Aos meus orientadores Prof. João Francisco Coelho de Oliveira e Paulo Bayard Dias Gonçalves pela oportunidade de fazer parte de um centro de excelência em pesquisa, pelos ensinamentos, amizade, confiança e apoio;

Ao Laboratório (e família) BioRep por ter me proporcionado, além de muito aprendizado, uma referência de como trabalhar em grupo e aos estagiários e colegas de pós-graduação, que me proporcionaram um ótimo ambiente de amizade e cooperação;

Ao Alfredo Antoniazzi, Bernardo Gasperin, Marcos Barreta e Rogério Ferreira, que me guiaram ao longo da minha vida acadêmica e, com muita paciência, me passaram muitos ensinamentos e aos companheiros de experimentos - Marcos, Rogério, Bernardo e Joabel – amigos que me proporcionaram aprendizado e bons momentos;

Aos colaboradores do laboratório BioRep: Fazenda do Leão, Vinicius de Oliveira e José Manoel Ferreira. Sem eles, não seria possível a realização do presente trabalho;

À CAPES pela concessão da bolsa de estudos e ao CNPq pelo apoio financeiro à pesquisa;

Ao Rogério, exemplo de profissional e pessoa, que me "adotou" por toda ajuda, atenção, confiança, e amizade e a toda sua família, que sempre me receberam muito bem, como se fosse integrante dela;

Aqueles que me motivaram na escolha desta profissão, mesmo sem perceberem: Vô Gusto, tios e primos;

Ao meu namorado Bernardo, que esteve ao meu lado durante este período me incentivando e ajudando, pelo amor, companheirismo e paciência;

Aos meus pais, guerreiros que não mediram esforços para me proporcionar um lar com muito carinho e a melhor educação, pelo amor incondicional; aos meus irmãos, pela amizade e muitas risadas; e a toda a minha família pelo apoio nas horas difíceis e compreensão nos momentos de ausência;

A todos aqueles que passaram pelo meu caminho e deixaram sua marca.

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Medicina Veterinária
Universidade Federal de Santa Maria

ROTA DE AÇÃO DA PROSTAGLANDINA $F_{2\alpha}$ ADMINISTRADA VIA SUBMUCOSA VULVAR NA LUTEÓLISE DE BOVINOS

AUTORA: MONIQUE TOMAZELE ROVANI
ORIENTADOR: JOÃO FRANCISCO COELHO DE OLIVEIRA
Data e Local da Defesa: Santa Maria, 16 de setembro de 2011.

O presente trabalho teve por objetivo verificar se a prostaglandina $F_{2\alpha}$ ($PGF_{2\alpha}$) administrada na submucosa vulvar (IVSM) induz a luteólise ao alcançar o corpo lúteo (CL) diretamente por uma via local, evitando sua metabolização nos pulmões, ou após a absorção e distribuição através da circulação sistêmica. Em um primeiro estudo, o estro de 1937 vacas de corte foi monitorado durante 5 dias (25,6% de estro). No dia 5, as vacas que não apresentaram estro receberam 5mg de dinoprost via IVSM (1/5 da dose padrão; n=1440), resultando em 68,2% de estro nos 5 dias seguintes. Todavia, em outro experimento utilizando a mesma dose, o número de novilhas detectadas em estro após o tratamento IVSM (47,4%, n=97) ou intramuscular (IM; 54,7%, n=95) no dia 5 não diferiu entre os grupos (P>0,05). Com base na concentração sérica de progesterona, os animais tratados com 5mg de dinoprost no dia 5 do ciclo estral não apresentaram luteólise funcional, independentemente da via de administração. Após o tratamento com 5mg dinoprost IM ou IVSM no dia 10 do ciclo, 3/5 e 2/5 animais apresentaram luteólise, respectivamente. Entretanto, a luteólise ocorreu em todos os animais tratados com 25mg de dinoprost, independente do dia do ciclo estral (dia 5 ou 10). A concentração de $PGF_{2\alpha}$ não diferiu no soro das veias uterina e jugular. O mesmo foi observado quanto ao padrão de 13,14-dihidro-15-ceto prostaglandina $F_{2\alpha}$ (metabólito de $PGF_{2\alpha}$; PGFM) sérico ao longo do tempo após a aplicação de 5 mg de dinoprost via IM ou IVSM. Em resumo, a via de administração de $PGF_{2\alpha}$ (IVSM ou IM) resultou em concentrações séricas semelhante de $PGF_{2\alpha}$, PGFM e luteólise. Embora evidências anatômicas permitam sugerir que a $PGF_{2\alpha}$ injetada via IVSM possa ser transportada diretamente aos ovários, a injeção de $PGF_{2\alpha}$ via IVSM atinge a circulação sistêmica antes de chegar ao ovário, e a eficácia de baixas doses de $PGF_{2\alpha}$ é dependente da fase luteal e não da via de administração.

Palavras chave: Bovinos. Corpo lúteo. Luteólise. Prostaglandina $F_{2\alpha}$. Submucosa vulvar.

ABSTRACT

Master's Dissertation
Programa de Pós-Graduação em Medicina Veterinária
Universidade Federal de Santa Maria

ROUTE OF ACTION OF PROSTAGLANDIN $F_{2\alpha}$ AFTER INTRAVULVOSUBMUCOUS INJECTION IN BOVINE LUTEOLYSIS

AUTHOR: MONIQUE TOMAZELE ROVANI
ADVISOR: JOÃO FRANCISCO COELHO DE OLIVEIRA
Date and Place of Defense: Santa Maria, September 16th, 2011.

The aim of this study was to verify if prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administered by intravulvosubmucous (IVSM) injection induces luteolysis by reaching the corpus luteum (CL) directly by a local absorption rote, preventing its metabolism in the lungs, or after absorption and distribution via the systemic circulation. In a first trial, the estrus rate of 1,937 beef cows was monitored for 5 days (25.6% of estrus). At day 5, the cows that did not show estrus received 1/5 of the standard dose of dinoprost IVSM (5 mg; n = 1440) and resulted in 68.2% of estrus in the next 5 days. However, in a second trial, the number of heifers detected in estrus after dinoprost injected via the IVSM (47.4%; n = 97) or intramuscular route (IM; 54.7%; n = 95) at day 5 was not different ($P > 0.05$). Based on serum progesterone concentrations, animals treated with 5 mg dinoprost at day 5 of the estrous cycle do not present functional luteolysis regardless of the administration route. At day 10 of the estrous cycle, luteolysis was variable in cows treated with 5 mg of dinoprost. Nevertheless, luteolysis occurred in all animals treated with 25 mg dinoprost independent of the estrous cycle day. After treatment, the $PGF_{2\alpha}$ concentration did not differ in serum from uterine and jugular veins. This was further confirmed by measuring the concentration of 13,14-dihydro-15-keto-PGF (PGFM) after 5 mg dinoprost injection via the IM or IVSM route. Dinoprost IM- and IVSM-administered resulted in a similar PGFM serum pattern over time, suggesting the same absorption rate for both routes. Although anatomical evidences suggest that $PGF_{2\alpha}$ injected IVSM could be taken direct to the ovaries, avoiding the systemic circulation, the results do not support this hypothesis. In summary, the route of $PGF_{2\alpha}$ administration (IVSM or IM) resulted in similar serum concentrations of $PGF_{2\alpha}$, PGFM, and luteolysis. Taking all the results together, the $PGF_{2\alpha}$ injection via IVSM reached the systemic circulation before reaching the ovary, and the effectiveness of low doses of $PGF_{2\alpha}$ was dependent on luteal phase and not on the route of administration.

Key words: Cattle. Corpus luteum. Intravulvar submucosa. Luteolysis. Prostaglandin $F_{2\alpha}$.

LISTA DE FIGURAS

LISTA DE APÊNDICE

SUMÁRIO

INTRODUÇÃO

A $PGF_{2\alpha}$ é um composto natural que, dentre diversas funções, promove a morte de um corpo lúteo cuja ovulação não tenha resultado em prenhez. Devido ao seu potencial luteolítico, tem sido amplamente utilizada para manipular o ciclo estral de bovinos. Com a finalidade de reduzir os custos nos programas de sincronização, diversos estudos têm sido realizados na busca pela dose mínima efetiva administrada por diversas vias de aplicação. A utilização de vias alternativas de administração (SUÑE et al., 1985; ALVAREZ et al., 1991; COLAZO et al., 2002; COLAZO et al., 2002) já foram descritas, sendo que a administração do fármaco via IVSM resultou em sincronização de estro (SUÑE et al., 1985; HORTA et al., 1986; ALVAREZ et al., 1991; CHOHAN, 1998). Ao longo de muitos anos, nosso grupo sincronizou mais de 64000 vacas de rebanhos comerciais, obtendo-se a média de 79,8% de estro utilizando a via IVSM e o sistema de 10 dias de manejo com uma injeção de $PGF_{2\alpha,}$ descrito por Donaldson et al. (1982).

A meia-vida plasmática da $PGF_{2\alpha}$ é muito curta e sua metabolização é realizada principalmente nos pulmões (FERREIRA & VANE, 1967). Algumas evidências sugerem que a $PGF_{2\alpha}$ administrada via IVSM pode atingir o ovário sem sofrer essa metabolização, por evitar a circulação sistêmica. Com base em dados preliminares do nosso grupo (Apêndice A) realizados através de contraste radiográfico, adicionados aos estudos de angioarquitetura do trato reprodutivo, evidencia-se a presença de muitas anastomoses entre os vasos vulvares e útero-ovarianos (GINTHER & DEL CAMPO, 1974; GIOSO et al., 2005). Essas estruturas anatômicas, associadas ao mecanismo de contra-corrente entre a artéria ovariana e a veia útero-ovariana, poderiam ser a rota utilizada pela $PGF_{2\alpha}$ administrada via IVSM para evitar a metabolização pulmonar antes de atingir os ovários. Embora estes dados evidenciem a ligação vascular entre a vulva e o útero, para o nosso conhecimento, não há estudos funcionais demonstrando o mecanismo local utilizado pela $PGF_{2\alpha}$ injetada na via IVSM.

O corpo lúteo bovino é pouco responsivo à aplicação de $PGF_{2\alpha}$ anteriormente ao dia 5 do ciclo estral (HENRICKS et al., 1974). Goravanahally et al. (2009) sugerem que alguns genes podem estar envolvidos nesta aquisição de sensibilidade ao comparar CLs do dia 4 (pouco responsivo à $PGF_{2\alpha}$) e 10 (responsivo à $PGF_{2\alpha}$) do ciclo estral. Estudos utilizando diferentes vias de administração e doses de $PGF_{2\alpha}$ têm avaliado a resposta em manifestação de estro e as concentrações de progesterona, sem considerar a responsividade do corpo lúteo (CL; ALVAREZ et al., 1991; CHOHAN, 1998; COLAZO et al., 2002). Portanto, o objetivo

do presente estudo foi determinar o perfil das concentrações $PGF_{2\alpha}$ e do seu metabólito PGFM nas veias jugular e uterina após a administração de doses reduzidas de $PGF_{2\alpha}$ IM ou IVSM. Adicionalmente, foi avaliado o efeito da dose e via de administração de $PGF_{2\alpha}$ na manifestação de estro e luteólise em períodos de alta e baixa responsividade à $PGF_{2\alpha}$.

REVISÃO BIBLIOGRÁFICA

Corpo Lúteo e Luteólise

O ciclo estral de bovinos tem duração média de 21 dias, correspondendo ao período compreendido entre duas ovulações. Por serem animais poliéstricos, estes intervalos são repetidos regularmente até que uma concepção ou outros fatores ocorram, tais como patologias, determinando um período de anestro (MORAES et al., 2008). Após a ovulação, desencadeada pelo pico pré-ovulatório de LH, inicia-se a formação do corpo lúteo. As células da granulosa e da teca, que no folículo dominante eram responsáveis pela produção de andrógenos e estradiol, respectivamente, passam a produzir progesterona sob o estímulo do hormônio luteinizante (RICHARDS & HEDIN, 1988). A camada celular da granulosa dá origem às células luteais grandes e a teca, às células luteais pequenas (DONALDSON & HANSEL, 1965). O rompimento da membrana basal do folículo faz com que estes tipos celulares se reorganizem, adicionados de células endoteliais e fibroblastos, formando a estrutura do corpo lúteo (MCCRACKEN; CUSTER & LAMSA, 1999; NISWENDER et al., 2000). Essa glândula endócrina transitória, secretora de progesterona e com vida média de 17 dias nos bovinos, é essencial para o estabelecimento e manutenção da gestação (RODGERS; MITCHELL & SIMPSON, 1988). Durante a fase luteínica, a progesterona pode agir diretamente nos receptores bloqueando o sítio de ligação da ocitocina (BISHOP & STORMSHAK, 2006) ou inibindo a síntese de receptores de estrógeno alfa e, consequentemente, receptores de ocitocina (SPENCER & BAZER, 1995).

No caso de insucesso da gestação, é necessário que ocorra a lise do corpo lúteo, permitindo assim que um novo ciclo estral se inicie. A luteólise é caracterizada pela diminuição da secreção de progesterona e posterior perda de tecido luteal, sendo estas fases conhecidas por luteólise funcional e estrutural, respectivamente (NISWENDER et al., 1994). A progesterona, em altas concentrações neste período, inibe seus receptores permitindo a ação do estrógeno, que causa o aumento da expressão de receptores de ocitocina no endométrio (SPENCER & BAZER, 1995) e liberação da mesma pela hipófise posterior (MCCRACKEN; CUSTER & LAMSA, 1999). A ligação da ocitocina em seus receptores provoca a liberação de cinco a oito pulsos de $PGF_{2\alpha}$, ao redor do dia 17 do ciclo estral, sendo absorvida até o corpo lúteo por uma circulação local através do mecanismo de contracorrente (MCCRACKEN et al., 1972; WATHES & LAMMING, 1995). Em bovinos, a $PGF_{2\alpha}$ é o

principal fator luteolítico (WOLFENSON et al., 1985), sendo a presença do útero essencial neste processo, pois quando se realiza a histerectomia há um atraso no período esperado para a luteólise (WILTBANK & CASIDA, 1956; MALVEN & HANSEL, 1964). Também foi demonstrada a produção intra-luteal de $PGF_{2\alpha}$ em várias espécies, sugerindo que as prostaglandinas luteais possam contribuir para uma auto-regulação do corpo lúteo (NISWENDER et al., 2000; DAVIS & RUEDA, 2002). A $PGF_{2\alpha}$ estimula a secreção de ocitocina no CL (FLINT & SHELDRICK, 1982), que potencializa a secreção de $PGF_{2\alpha}$ no útero e CL (SCHALLENBERGER et al., 1984; SHIRASUNA et al., 2007).

Prostaglandina $F_{2\alpha}$

A $PGF_{2\alpha}$ é um hormônio derivado, através de reações enzimáticas, do ácido araquidônico, um ácido graxo presente nas membranas fosfolipídicas (BERGSTROM; CARLSON & WEEKS, 1968). Nos pulmões, a $PGF_{2\alpha}$ é rapidamente transformada no metabólito inativo 13,14-dihidro-15-ceto-$PGF_{2\alpha}$ pela ação da 15-hidroxi-prostaglandina-desidrogenase, sendo em ruminantes 65 a 90% da $PGF_{2\alpha}$ degradada na primeira passagem pelos pulmões (FERREIRA & VANE, 1967; PIPER; VANE & WYLLIE, 1970; MCCRACKEN; CUSTER & LAMSA, 1999). Em bovinos, foram detectados pulsos de $PGF_{2\alpha}$ na veia uterina no momento esperado da luteólise (NANCARROW et al., 1973), bem como a liberação periférica de pulsos de PGFM, sendo este considerado um indicador de liberação de $PGF_{2\alpha}$ na circulação (KINDAHL et al., 1976).

Bovinos e ovinos apresentam um mecanismo de contracorrente, uma circulação local que permite a transferência de $PGF_{2\alpha}$ proveniente do útero aos ovários (MCCRACKEN et al., 1972). Entretanto, a $PGF_{2\alpha}$ demonstra baixo fluxo de difusão simples através das membranas celulares, apesar de ser lipossolúvel, não permitindo concentrações adequadas para que este prostanóide realize sua função biológica (SCHUSTER, 2002). A expressão de proteínas transportadoras de $PGF_{2\alpha}$ no plexo útero-ovariano foi demonstrada no momento esperado para a luteólise em bovinos (BANU et al., 2003). Adicionalmente, a inibição destas proteínas impediu a liberação de $PGF_{2\alpha}$ induzida por ocitocina na veia útero-ovariana, prolongando a duração do CL e a produção de progesterona (BANU et al., 2008). Lee et al. (2010) sugerem que o transporte de $PGF_{2\alpha}$ no plexo útero-ovariano de ruminantes seja controlado por estas proteínas transportadoras de $PGF_{2\alpha}$, também demonstrado através da sua inibição após a liberação endógena ou administração de $PGF_{2\alpha}$ marcada radioativamente.

Há evidências de que a $PGF_{2\alpha}$ proveniente do endométrio inicie uma cascata de produção intra-luteal de $PGF_{2\alpha}$ (PATE, 1988). As células luteais grandes de ovinos expressam ciclooxigenase-2 (COX-2), enzima envolvida na síntese de $PGF_{2\alpha}$, em resposta à $PGF_{2\alpha}$ (TSAI & WILTBANK, 1997). Já em bovinos, a $PGF_{2\alpha}$ diminui a expressão de COX-2 no período inicial do ciclo estral, correspondendo ao período em que o CL não responde ao sinal luteolítico da $PGF_{2\alpha}$, sugerindo a importância deste mecanismo local (TSAI & WILTBANK, 1998). Foi demonstrado que o CL bovino possui a maquinaria necessária para a auto-regulação, tais como os componentes para o metabolismo, transporte e sinalização de $PGF_{2\alpha}$ (AROSH et al., 2004). Através de estudos com microdiálise de CL, foi demonstrado que a $PGF_{2\alpha}$ é liberada do CL na veia ovariana durante a luteólise espontânea (SHIRASUNA et al., 2004). Sugere-se que esta produção intra-luteal de $PGF_{2\alpha}$ complemente a função daquela proveniente do endométrio, causando uma "ampliação do sinal luteolítico" (AROSH et al., 2004; SHIRASUNA et al., 2004).

A $PGF_{2\alpha}$ exerce seus efeitos no CL ao se ligar aos seus receptores acoplados a proteína G (PTGFR; NARUMIYA; SUGIMOTO & USHIKUBI, 1999). Os receptores são altamente expressos em células luteais grandes e têm baixa expressão em células luteais pequenas e endoteliais (SAKAMOTO et al., 1994). A ligação desse hormônio ao seu receptor provoca um declínio na produção luteal de progesterona (MCGUIRE; JUENGEL & NISWENDER, 1994), embora os mecanismos intracelulares envolvidos não sejam bem conhecidos. Acredita-se que isso se deva a apoptose, redução da atividade das enzimas esteroidogênicas, redução na aquisição celular de colesterol ou ainda no transporte através de membranas celulares e mitocôndriais (NISWENDER et al., 2000). A hipótese de que há uma diminuição da expressão de receptores de LH não foi confirmada, já que isto só é observado após a queda na concentração sérica de progesterona (SPICER; IRELAND & ROCHE, 1981).

A $PGF_{2\alpha}$ estimula a síntese e a secreção de endotelina 1 (ET-1) nas células endoteliais, que constituem a maior população de células do CL. A ET-1 é uma das mais potentes substâncias vasoconstritoras do organismo, inibindo a produção de progesterona pelo CL. Entre os dias 17 e 21 do ciclo estral, a síntese de ET-1 é aumentada cerca de 30 vezes quando comparada aos dias 5 e 6 do ciclo (GIRSH et al., 1996a; GIRSH et al., 1996b). Recentemente foi demonstrado que a ocitocina produzida no CL pode modular a secreção destas substâncias vasoativas, tanto $PGF_{2\alpha}$ quanto ET-1, sugerindo que estes podem ser mediadores da luteólise espontânea em bovinos (SHIRASUNA et al., 2007).

Um aumento agudo no fluxo sanguíneo do CL é observado a partir do dia 10 do ciclo estral, coincidindo com o aumento da expressão de ET-1 e angiotensina II nos estágios

iniciais da luteólise. A $PGF_{2\alpha}$ estimula a liberação de óxido nítrico, induzindo a vasodilatação das arteríolas do CL e, consequentemente aumentando o aporte sanguíneo antes da luteólise (MIYAMOTO et al., 2005). Todos estes eventos suprimem a produção de progesterona pelas células luteais. Estes autores propuseram ainda que o aumento agudo do fluxo sanguíneo seja um mecanismo chave para o início da luteólise.

Com base neste mecanismo fisiológico, a $PGF_{2\alpha}$ tem sido utilizada no controle do ciclo estral, mais especificamente no controle da duração da fase luteal. Um dos análogos sintéticos mais utilizados é o dinoprost trometamina (Lutalyse, Pfizer Saúde Animal, Brasil), sendo geralmente recomendada a aplicação de 25mg (5ml) por via intramuscular. No entanto, diversos estudos têm sido realizados na busca pela dose mínima efetiva administrada por diversas vias de aplicação, tais como a via subcutânea (COLAZO et al., 2002), via fossa isquioretal (COLAZO et al., 2002) e via submucosa vulvar (ALVAREZ et al., 1991).

A aplicação de doses reduzidas de $PGF_{2\alpha}$ na submucosa vulvar é utilizada em programas de sincronização por diminuir os custos e por apresentar resultados satisfatórios em porcentagem de estro quando comparada à dose recomendada aplicada por via intramuscular (ALVAREZ et al., 1991; CHOHAN, 1998). Entretanto, o mecanismo pelo qual a prostaglandina administrada via submucosa vulvar atinge os ovários ainda não foi demonstrado in vivo.

O CL bovino é pouco responsivo à aplicação de $PGF_{2\alpha}$ anteriormente ao dia 5 do ciclo estral (HENRICKS et al., 1974). Entretanto, a falta de responsividade não se deve à deficiência de seus receptores de alta afinidade (WILTBANK et al., 1995). Além disso, foi demonstrado que a $PGF_{2\alpha}$ atinge o CL neste período, ligando-se aos receptores e desencadeando alguns passos iniciais da cascata, mas que não são suficientes para que a luteólise seja concretizada com apenas uma aplicação de $PGF_{2\alpha}$ (TSAI & WILTBANK, 1998). Algumas evidências sugerem que aplicações seriadas de $PGF_{2\alpha}$ são capazes de causar a luteólise em alguns animais em CLs de baixa responsividade (BEAL; MILVAE & HANSEL, 1980). Goravanahally et al. (2009) sugerem que alguns genes podem estar envolvidos nesta aquisição de sensibilidade ao comparar CLs do dia 4 (pouco responsivo à $PGF_{2\alpha}$) e 10 (responsivo à $PGF_{2\alpha}$) do ciclo estral. Adicionalmente, foi demonstrado que o CL no dia 4 do ciclo estral não apresenta resposta vascular à administração de $PGF_{2\alpha}$, diferente do observado no dia 10 a 12 do ciclo em que há um aumento agudo seguido por uma diminuição do fluxo sanguíneo no CL (ACOSTA et al., 2002).

Anatomia, mecanismo de contracorrente e metabolização da $PGF_{2\alpha}$

Em ovinos e bovinos, é necessária a presença do útero para que ocorra a regressão do CL (WILTBANK & CASIDA, 1956; MALVEN & HANSEL, 1964). Estudos realizados na década de 70 demonstraram que a excisão do corno uterino ipsilateral ao CL impedia a luteólise, evidenciando que um fator proveniente do útero era essencial para o início do processo, posteriormente sendo identificado esse fator como $PGF_{2\alpha}$ (MCCRACKEN et al., 1972; ROWSON; TERVIT & BRAND, 1972; NANCARROW et al., 1973; GINTHER, 1974). Nessas espécies, a artéria ovariana apresenta circunvoluções sobre a veia utero-ovariana (plexo útero-ovariano) permitindo uma relação íntima entre estes vasos (GINTHER & DEL CAMPO, 1974). Adicionalmente, foi descrito que a parede desta artéria se apresenta mais delgada nesta região de contato com a veia utero-ovariana (DEL CAMPO & GINTHER, 1974). Esta disposição anatômica permite que a $PGF_{2\alpha}$ uterina seja transferida do sangue venoso para o arterial através de um mecanismo de contracorrente, atingindo o ovário (MCCRACKEN et al., 1972). Em ovinos, a administração de $PGF_{2\alpha}$ na veia uterina permite sua transferência para a artéria ovariana ispilateral (MCCRACKEN; BAIRD & GODING, 1971). A administração de $PGF_{2\alpha}$ intra-uterina resulta em maiores concentrações de $PGF_{2\alpha}$ na artéria ovariana quando comparado ao plasma da artéria carótida, sustentando a hipótese do mecanismo de contra-corrente (HIXON & HANSEL, 1974). Isso evita que a $PGF_{2\alpha}$ seja absorvida pela circulação sistêmica, sendo metabolizada em grande parte em uma única passagem pelos pulmões (FERREIRA & VANE, 1967; PIPER; VANE & WYLLIE, 1970).

Um plexo venoso evidente situa-se sobre a face ventral do útero e vagina, podendo drenar para várias direções (DYCE; SACK & WENSING, 2004). Através de estudos radiográficos, foi demonstrada a presença de uma proeminente rede de anastomoses entre os ramos da veia vaginal e as veias que drenam a cérvice, corpo e cornos uterinos (GIOSO et al., 2005). A presença desse mecanismo de contracorrente e estruturas vasculares permite formular a hipótese de que fármacos aplicados pela via IVSM possam atingir diretamente a circulação ovariana.

ARTIGO

TRABALHO SUBMETIDO PARA PUBLICAÇÃO:

LUTEOLYSIS AFTER INTRAVULVOSUBMUCOUS INJECTION OF PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE: SYSTEMIC OR LOCAL CIRCULATION?

Monique Tomazele Rovani, Marcos Henrique Barreta, Rogério Ferreira, Bernardo Garziera Gasperin, Alfredo Quites Antoniazzi, Rafael Festugatto, João Francisco Coelho de Oliveira, Paulo Bayard Dias Gonçalves

DOMESTIC ANIMAL ENDOCRINOLOGY, 2011

Luteolysis after intravulvosubmucous injection of prostaglandin $F_{2\alpha}$ in cattle: systemic or local circulation?

M. T. Rovani[a], M. H. Barreta[a], R. Ferreira[a], B. G. Gasperin[a], A. Q. Antoniazzi[b], R. Festugatto[c], J. F. C. Oliveira[a], P. B. D. Gonçalves[a,*]

[a] Laboratory of Biotechnology and Animal Reproduction (BioRep), Federal University of Santa Maria, 97105-900, Santa Maria (RS), Brazil

[b] Animal Reproduction and Biotechnology Laboratory (ARBL), Colorado State University, 80521, Fort Collins (CO), USA

[c] Laboratory of Experimental Surgery, Federal University of Santa Maria, 97105-900, Santa Maria (RS), Brazil

Author emails: mtrovani@gmail.com; barretamh@yahoo.com.br; rferreira.sul@gmail.com; bggasperin@gmail.com; Alfredo.Antoniazzi@colostate.edu; rfestuga@yahoo.com.br; j.francisco.oliveira@gmail.com; bayard@ufsm.br

[*] Corresponding author: Universidade Federal de Santa Maria, Laboratório de Biotecnologia e Reprodução Animal, 97105-900, Santa Maria, RS, Brasil. Telephone: +55 55 3220 8752; Fax: +55 55 3220 8484.
E-mail address: bayard@ufsm.br (P. B. D. Gonçalves)

Abstract

The intravulvosubmucous (IVSM) route has been used to reduce the dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as luteolytic agent in estrus synchronization programs. To validate the effectiveness of $PGF_{2\alpha}$ via IVSM, the estrus rate of 1,937 beef cows was monitored for 5 d (25.6% of estrus). At day 5, the cows that did not show estrus received 5 mg of dinoprost IVSM (1/5 of the standard dose; n = 1440) and resulted in 68.2% of estrus in the next 5 d. In a second trial, the rate of heifers detected in estrus after 5 mg dinoprost via IVSM (47.4%; n = 97) or intramuscular (IM; 54.7%; n = 95) at day 5 was not different ($P > 0.05$). Luteolysis (serum progesterone concentrations below 1 ng/mL) was dependent of the estrous cycle period when cows were treated with 5 mg of dinoprost via IM or IVSM. Luteolysis was observed in 5 out of 10 cows treated at day 10 but none at day 5 of the estrous cycle. Luteolysis occurred in all animals treated with 25 mg dinoprost independent of the estrous cycle period (day 5 or 10). After treatment, the $PGF_{2\alpha}$ concentration did not differ in uterine and jugular veins. This was further confirmed by measuring the concentration of 13,14-dihydro-15-keto-PGF (PGFM) after 5 mg dinoprost injection via the IM or IVSM route. Dinoprost IM- and IVSM-administered resulted in a similar PGFM serum pattern over time, suggesting the same absorption rate into the systemic circulation. Taking all the results together, we concluded that $PGF_{2\alpha}$ injection via IVSM reached the systemic circulation before reaching the ovary, and the effectiveness of low doses of $PGF_{2\alpha}$ was dependent on the luteal phase and not on the route of administration.

Key words: cattle; corpus luteum; intravulvar submucosa; luteolysis; prostaglandin $F_{2\alpha}$

1. Introduction

The luteolytic agent prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been extensively used to manipulate the estrous cycle of cattle. Different routes of administration have been tested in an attempt to reduce the dose [1-3], which has resulted in estrus synchronization after intravulvosubmucous (IVSM) injection [1,4-6], despite of disagreement among different studies [3]. In a commercial basis, our group synchronized more than 64,000 cows over many years and we obtained an average of 79.8% of estrus using IVSM treatment of 5 mg of dinoprost (1/5 of the standard dose) and the 10-day, 1-injection management system reported by Donaldson et al. (1982). Some evidences suggest that $PGF_{2\alpha}$ injected via IVSM reaches the ovaries independently of the systemic circulation [1,4-7]. The establishment of reproductive tract angioarchitecture using contrasted radiography demonstrated many anastomoses between vulvar and utero-ovarian vessels [7,8]. These anatomic structures associated to the vascular countercurrent exchange mechanism between the ovarian artery and the utero-ovarian vein could be the route of $PGF_{2\alpha}$ injected via IVSM to avoid systemic metabolism before reaching the ovary. However, to our knowledge, there have been no studies about the local mechanisms of $PGF_{2\alpha}$ given via the IVSM route.

Studies of different routes and doses of $PGF_{2\alpha}$ administration have evaluated estrus response and serum progesterone concentrations without considering the responsiveness of the corpus luteum (CL) after treatment [1,2,5]. Thus, the aims of the present study were the following: (1) to evaluate the effect of dose and administration route of $PGF_{2\alpha}$ on the estrous behavior, (2) to assess the onset of luteolysis in periods of low and high $PGF_{2\alpha}$ responsiveness, and (3) to determine the profile of $PGF_{2\alpha}$ and prostaglandin metabolite concentrations (13,14-dihydro-15-keto-PGF; PGFM) in the uterine and jugular veins after intramuscular (IM) or IVSM administration of a reduced dose of $PGF_{2\alpha}$.

2. Materials and methods

All protocols and procedures were approved by the Institutional Committee for Ethics in Animal Experiments of the Federal University of Santa Maria, RS, Brazil (23081.008212/2010-68).

2.1. Experiment 1. Effect of reduced dose of $PGF_{2\alpha}$ administration IVSM on estrous behavior

A large number of nonpregnant cyclic beef cows were used to validate the efficiency of 5 mg (1/5 of the standard dose) of dinoprost tromethamine (dinoprost; Lutalyse, Pfizer Animal Health, São Paulo, Brazil) via IVSM in an estrus synchronization system. Beef cows (*Bos taurus taurus*; n = 1,937) were synchronized with the 10-day, 1-injection management system [9]. Briefly, estrus detection was performed twice a day, 12 h apart for 5 d, which was considered the control period. In the morning of day 5, a dose of 5 mg of dinoprost (IVSM; using a 21-gauge, 2.5-cm needle) was administered and estrous behavior was observed for an additional 5 d, as previously described above.

2.2. Experiment 2. Intramuscular vs. intravulvosubmucous injection of $PGF_{2\alpha}$

After assessing the efficiency of 1/5 of the standard dose of $PGF_{2\alpha}$ via IVSM, a second experiment was conducted to evaluate the $PGF_{2\alpha}$ injection route (IVSM or IM) on estrous behavior using the low dose of $PGF_{2\alpha}$. Nonpregnant cyclic beef heifers (n = 251) were observed for estrous behavior twice a day for 5 d. The animals not observed in estrus during the first 5 d received 5 mg of dinoprost and were randomly placed in either the IM (n = 95; in

the gluteal muscles using a 21-gauge, 4-cm needle) or the IVSM (n = 97) group. After treatment, estrous behavior was observed and recorded for 5 d.

2.3. Experiment 3. $PGF_{2\alpha}$ injection (IM or IVSM) at low and high corpus luteum sensitivity

Nonpregnant crossbred beef cows (n = 50) were pre-synchronized with progestagen-based protocol and estrus detection was performed twice a day for 5 d. Cows observed in estrus (n = 22) were randomly allocated to receive 25 mg IM, 5 mg IM, or 5 mg IVSM of dinoprost on day 5 (low $PGF_{2\alpha}$ responsiveness; n = 3/group) or 10 (high $PGF_{2\alpha}$ responsiveness; n = 3-5/group) after estrus. Two cows at day 5 and two at day 10 of the estrous cycle were used as control and were not treated. Blood samples for progesterone analysis were collected by caudal venipuncture once a day for 5 d, starting at dinoprost injection. Luteolysis was considered when the serum progesterone concentration was lower than 1 ng/mL at day 4 after treatment.

2.4. Experiment 4. Systemic and uterine vein concentrations of $PGF_{2\alpha}$ and PGFM after IVSM administration

The first experiments indicated that the reduced dose was effective to synchronize estrus and the route of administration did not influence treatment efficacy. Then, this experiment was performed to test the hypothesis that $PGF_{2\alpha}$ administered via IVSM reaches CL through the systemic circulation. Uterine and jugular veins of a crossbred beef cow without reproductive abnormalities were cannulated according to the procedure of Lewis et al. [10]. Briefly, the cow was fasted for 12 h before anesthesia and surgical procedures. A vertical incision in the flank was performed to cannulate a branch of the uterine vein at the

hilus of the left ovary [11]. The free end of a silicone tube (2 mm i.d. and 3.2 mm o.d.) was exteriorized through a small incision in the flank and maintained by infusion of heparinized saline. The jugular vein was also cannulated and maintained by flushing with heparinized saline [10]. Treatments started 12 h after surgery. One milliliter of saline was injected via the IVSM route (control), and after 3 h and 6 h, the animal was treated with 5 mg of dinoprost IVSM. These 3 h intervals were necessary to completely clear $PGF_{2\alpha}$ from the systemic circulation [12]. Blood was collected from the jugular and uterine veins at the same time by two different people immediately before each treatment and after every 5 min in a 40 min period. Additionally, blood samples were collected after 120 min of dinoprost injection to verify the decrease of systemic and ovarian concentrations of $PGF_{2\alpha}$. The samples were assayed for $PGF_{2\alpha}$ as described above.

Prostaglandin metabolite was measured to investigate the route effect on the $PGF_{2\alpha}$ pulmonary metabolism [13]. The jugular vein of a crossbred beef cow with normal estrous cycles was cannulated as described. The cannulated cow was submitted to the following treatments: 25 mg dinoprost IM, 5 mg dinoprost IM, 5 mg dinoprost IVSM, and 1 mL saline IVSM. As stated previously, a 3 h interval was allowed between each treatment. Blood samples were collected at 5 min intervals during a 40 min period and were assayed for PGFM.

2.5. Blood sampling and hormone assays

Blood samples were collected and allowed to clot for 30 min at room temperature before centrifugation at 1,500 X *g* for 10 min at room temperature. Serum was placed into cryogenic vials, frozen, and stored at -80 °C for further analysis. Enzyme immunoassay kits (Cayman Chemical Company, Ann Arbor, MI) were used to quantify PGFM and $PGF_{2\alpha}$ [14]. The intra-assay CV was 7.13% and 2.58% sensitivity was 0.015 and 0.010 ng/mL,

144 respectively, for PGFM and $PGF_{2\alpha}$. Electrochemiluminescence immunoassay (Roche, Brazil)
145 was performed to determine serum progesterone concentrations [15]. The intra- and inter-
146 assay CV were 2.09 % and 1.23 %, respectively.
147
148 *2.6. Statistical analysis*
149
150 All statistical analyses were performed using SAS software package (SAS Institute
151 Inc., Cary, NC, USA). Data are presented as the mean ± SEM, unless otherwise indicated. A
152 probability of $P \leq 0.05$ was considered statistically significant. The frequency of estrus on the
153 different treatments was analyzed using categorical data analysis models (CATMOD
154 procedure) and the differences between groups accessed by contrasts. The assessment of
155 treatment effects on serum progesterone concentration as well as systemic and local levels of
156 $PGF_{2\alpha}$ was performed as repeated measures data and analyzed using mixed models (MIXED
157 procedure) with a repeated measure statement [16]. The model for a repeated measures
158 experiment was: $\gamma_{ijk} = \mu + \alpha_i + \tau_k + (\alpha\tau)_{ik} + \varepsilon_{ijk}$, where γ_{ijk} is the response at time k on animal j
159 in treatment group i, μ is the overall mean, α_i is a fixed effect of treatment i, τ_k is a fixed effect
160 of time k, $(\alpha\tau)_{ik}$ is a fixed interaction effect of treatment i with time k, and ε_{ijk} is random error
161 at time k on animal j in treatment i. Main effects of treatment group, hour, and their
162 interaction were determined. Differences at specific time points were compared between
163 groups using estimates. Compound symmetry was used as a covariance structure. Other
164 continuous data were submitted to ANOVA using the GLM and multicomparison between
165 groups was performed by least square means.
166
167

3. Results

3.1. Experiment 1. Effect of reduced dose of $PGF_{2\alpha}$ administration IVSM on estrous behavior

The estrus detection rate of cows over the first 5 d (control) was 25.6% (497 out of 1,937). The cows that did not show estrus by the morning of day 5 (n = 1440) received 5 mg of dinoprost (1/5 of the standard dose) via IVSM. As a result, a rate of 68.2% of cows showed estrus (983 out of 1440) in the next 5 d (Figure 1A). Considering the cows in the experiment, a total of 73.4% of cows presented estrus. The rate of estrus from days 1 to 6 was about 5% per day (from 4% to 6%), increasing at day 7 to 7.9% and reaching a peak to 19.9% at day 8 (3 d after prostaglandin injection). This distribution of estrus revealed that the cows were cycling at the beginning of the experiment and 5 mg of $PGF_{2\alpha}$ (1/5 of the standard dose) resulted in induction of estrus (Figure 1B).

(Insert Figure 1)

3.2. Experiment 2. Intramuscular vs. intravulvosubmucous injection of $PGF_{2\alpha}$

Once the reduced dose of $PGF_{2\alpha}$ via IVSM was efficient to induce estrous behavior during the luteal phase, two different routes (IM and IVSM) of $PGF_{2\alpha}$ administration were assessed. Before treatment, heifers were observed for estrous behavior during 5 d, totalizing 23.5% of estrus (4.7% per day as expected in a cyclic herd). The animals without detectable estrous signs in the first 5 d were treated with 5 mg of dinoprost via IM (n = 95) or IVSM (n = 97), which resulted, respectively, in 54.7% and 47.4% of estrus during the 5 d after $PGF_{2\alpha}$ (Figure 2; $P > 0.05$). Combining the results of both routes (IM and IVSM), the estrus rate 3 d

192 after 5 mg of dinoprost was 16%, as expected after $PGF_{2\alpha}$ treatment in the 10-day, 1-injection
193 management system.
194 (Insert Figure 2)
195 *3.3. Experiment 3. $PGF_{2\alpha}$ injection (IM or IVSM) at low and high corpus luteum sensitivity*
196
197 Prostaglandin $F_{2\alpha}$ was injected in 22 beef cows in different doses (0, 5, and 25 mg),
198 routes (IM or IVSM), and days of the estrous cycle (day 5 or 10). The pattern of serum
199 progesterone concentration differed in accordance with the dose or day of the estrous cycle
200 but was not affected by administration route. It is important to note that serum progesterone
201 concentration decreased one day after $PGF_{2\alpha}$ administration independently of the dose, route,
202 or day of the cycle. However, a sharp decline in serum progesterone concentration was
203 observed only in the 25-mg dose, with less than 1 ng/mL 24 h after treatment. The data
204 obtained from animals that received 5 mg of dinoprost by IM or IVSM (n = 3/group) at day 5
205 of the estrous cycle (low $PGF_{2\alpha}$ responsiveness period) were not shown because the CL did
206 not undergo luteolysis (4 d after treatment had serum progesterone concentrations $\geq$ 5 ng/mL).
207 Animals treated at day 10 of the estrous cycle resulted in lower progesterone levels
208 than those of the control group ($P < 0.05$). However, luteolysis (serum progesterone
209 concentrations below 1 ng/mL) was observed in only 3 out of 5 cows (IM) and 2 out of 5
210 cows (IVSM) treated with 5 mg (Figure 3). The serum progesterone concentrations were not
211 different in cows that did not have luteolysis and those of the control group 4 d after treatment
212 (data not shown). All cows that received 25 mg IM (standard dose; n=4) at day 5 and day 10
213 underwent luteolysis.
214 (Insert Figure 3)

3.4. Experiment 4. Systemic and uterine vein concentrations of PGF2α and PGFM after IVSM administration

Concentrations of $PGF_{2\alpha}$ were not different between jugular and uterine veins after saline or $PGF_{2\alpha}$ (5 mg) injections via IVSM (Figure 4A). However, as expected, concentrations of $PGF_{2\alpha}$ were greater in the jugular and uterine veins after dinoprost treatment via the IVSM group (from 0.392 to 1.163 ng/mL) than the saline control group (under 0.120 ng/mL). In blood samples collected after 120 min of dinoprost treatment to make sure that the systemic and ovarian $PGF_{2\alpha}$ levels had dropped, serum $PGF_{2\alpha}$ levels were 0.133 and 0.226 ng/mL, respectively. We also demonstrated that the concentration of the $PGF_{2\alpha}$ metabolite (PGFM) had a similar pattern after 5 mg of dinoprost via IM or IVSM (Figure 4B). However, the PGFM pattern was greater with 25 mg IM injection when compared to the other treatments. Our hypothesis that dinoprost injected IVSM reaches CL through the systemic circulation was confirmed.

(Insert Figure 4)

4. Discussion

The most important findings in our study were: (1) 1/5 of the standard dose of $PGF_{2\alpha}$ was effective to synchronize estrous behavior in a large number of animals when injected in cows beyond day 5 of the estrous cycle by either the IM or IVSM routes; (2) the effectiveness of $PGF_{2\alpha}$ to induce luteolysis was dependent on the dose and phase of development of CL, regardless of the route; (3) $PGF_{2\alpha}$ concentrations in the jugular and uterine veins were not different after IVSM injection of 5 mg of dinoprost; and (4) $PGF_{2\alpha}$ metabolite (PGFM) concentration over time was not route-dependent, but was rather dose-dependent.

A large number of cows (n = 1,937) were synchronized with $PGF_{2\alpha}$ via IVSM (5 mg dinoprost) using the 10-day, 1-injection management system [9]. This synchronization system is a good model to study the route of $PGF_{2\alpha}$ injection. Animals were monitored twice a day for 5 d to determine their estrous cyclicity. In the first 5 d, a rate of 5.1% of the cows showed estrous behavior, confirming that a high number of the cows were cycling. In the morning of day 5 of estrus detection, dinoprost (5 mg) was administered IVSM and a peak of estrous behavior was observed 3 d after $PGF_{2\alpha}$ injection. This estrus profile validated the luteolytic efficiency of the reduced dose of $PGF_{2\alpha}$. Low doses of $PGF_{2\alpha}$ and alternative routes of administration have been extensively used by veterinary practitioners mainly with the purpose of reducing costs [2,3,6,17,18]. With this aim, IVSM has been the route of choice when using low-dose $PGF_{2\alpha}$ as a luteolytic agent, mainly in the estrus synchronization system in cattle [1,4-6,19]. Our group synchronized more than 64,000 cows over many years and we obtained an average of 79.8% of estrus using IVSM treatment of 5 mg of dinoprost and the 10-day, 1-injection management system reported by Donaldson et al. (1982).

The local vascular countercurrent of $PGF_{2\alpha}$ from the uterine venous blood to the ovarian arterial blood is well established [20-24]. Recently, it has been demonstrated that the transport of $PGF_{2\alpha}$ from the uterus to the ovary is regulated by $PGF_{2\alpha}$ transporter protein-mediated mechanisms at the time of luteolysis [25]. Thus, the IVSM route has been used based on the hypothesis that $PGF_{2\alpha}$ would reach the ovary, avoiding pulmonary clearance and, consequently, a low dose of $PGF_{2\alpha}$ would be enough to induce luteolysis. However, in the second experiment, we demonstrated that 5 mg of $PGF_{2\alpha}$ (1/5 of the standard dose) administered IM or IVSM in heifers resulted in similar rates of estrous behavior, which agrees with Alvarez et al. [1]. The distribution pattern of estrus was similar to the first experiment. The heifers were also cyclic (4.7% of estrus per day in the first 5 d) and the number of animals in estrus peaked at day 8, indicating that the 5 mg of dinoprost via the IM and IVSM

route induced luteolysis at the same rate. This finding provides indirect evidence that $PGF_{2\alpha}$ given via IVSM is absorbed and reached the ovary through the systemic circulation instead of any local communication system.

For the past 30 years, there has been controversy about the rationale of using reduced doses of $PGF_{2\alpha}$ via the IVSM route [1,3,5]. A low dose of $PGF_{2\alpha}$ is required when it is infused intrauterine [17,26] or injected into the uterine wall [18]. Furthermore, a low dose of $PGF_{2\alpha}$ via IVSM was as efficient as the IM recommended dose to induce labor in swine [27]. The stage of the CL might be related to the effectiveness of 5 mg of dinoprost via IVSM to induce luteolysis. For this reason, we tested the hypothesis that the CL during early formation was less sensitive to the low dose of $PGF_{2\alpha}$. Cows treated with reduced doses at a lower CL sensitivity period (day 5 of the estrous cycle) did not show functional luteolysis regardless of administration route (IM or IVSM). These results may explain the absence of luteolysis induction when low doses of $PGF_{2\alpha}$ were administered subcutaneously or IM at day 5 or 7 of the estrous cycle [2,28]. However, in this study, luteolysis occurred in 5 out of 10 cows treated with 5 mg (IVSM or IM) at day 10 of the estrous cycle. In contrast, all animals, regardless of luteal phase (day 5 or 10 of estrous cycle), responded to 25 mg of dinoprost injection. It is important to note that cows treated at day 5 with 5 mg IM or IVSM showed an average of 6.4 ± 0.3 ng/mL of serum progesterone concentration 4 d after treatment, which is normally observed at day 9 of the estrous cycle [29]. Consequently, the age of the CL has to be considered as an important factor to influence the effectiveness of a reduced dose of $PGF_{2\alpha}$ [1,2,5].

The blood obtained simultaneously from the jugular and uterine veins was used to determine the systemic and ovarian circulatory pathway of $PGF_{2\alpha}$ [10,20]. The surgery for uterine vein cannulation was extremely invasive. In view of animal welfare, only one animal was cannulated. With the same approach, Hixon and Hansel [20] demonstrated that $PGF_{2\alpha}$

290 administered intrauterine are transferred from the uterus to the ovarian artery before reaching
291 the systemic circulation in cattle. Despite of the countercurrent mechanism and the vascular
292 anatomy between the vulva and the uterus, our results suggested that there were no local
293 transfer of $PGF_{2\alpha}$ from IVSM to the ovaries. Therefore, we tested the hypothesis that $PGF_{2\alpha}$
294 injected IVSM reaches CL through the systemic circulation. The concentrations of $PGF_{2\alpha}$
295 were not different in both jugular and uterine veins over time when dinoprost was IVSM
296 injected.

297 The pattern of PGFM in the jugular vein (an indicator of $PGF_{2\alpha}$ release into the
298 circulation [13]) was similar over time after treatment with 5 mg of dinoprost either via IM or
299 IVSM. These results provide further evidence that $PGF_{2\alpha}$ reaches the circulatory system
300 before the ovary and support the absence of a route (IM or IVSM) effect. The goal of this
301 experiment was to confirm results from $PGF_{2\alpha}$ experiment using a less invasive approach.
302 Despite only one cow was used, all results from the current study (estrous behavior, $PGF_{2\alpha}$
303 and PGFM levels) are in agreement and clearly demonstrated that $PGF_{2\alpha}$ reached the systemic
304 circulation before reaching the ovary after IVSM.

305 In summary, this is the first study that investigated the $PGF_{2\alpha}$ pathway (systemic or
306 local circulation) after IVSM $PGF_{2\alpha}$ treatment. We also demonstrated with a large number of
307 animals that the dose of $PGF_{2\alpha}$ could be reduced when using the 10-day, 1-injection
308 management system. The IVSM route could be useful to avoid muscle tissue damage caused
309 by dinoprost [30] when applied in commercial systems. Furthermore, the reflux of low
310 volume (1 mL) is more likely in the IM route when compared to the IVSM route. However,
311 the IVSM is not as practical as IM administration in a large number of animals. In conclusion,
312 $PGF_{2\alpha}$ injected via IVSM reached the systemic circulation before reaching the ovary. These
313 data also demonstrated that the luteolytic effectiveness of reduced doses of dinoprost is

mainly dependent on the luteal phase and is not affected by IVSM or IM administration routes.

Acknowledgements

We are grateful to Fazenda Bertolini and Fazenda Santa Rita for providing the animals and facilities. We thank Dr. J.F. Suñe, Dr. J.L.B. Macedo and Dr. J.C.F. Moraes for their assistance in the estrus synchronization study. This project was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) from Brazil.

References

[1] Alvarez RH, Meirelles CF, Ambrosano GMB, Oliveira JV, Pozzi JR. The use of lower doses of the prostaglandin analogue, cloprostenol, for oestrus synchronization in heifers. Anim Reprod Sci 1991;25:93-96.

[2] Colazo MG, Martínez MF, Kastelic JP, Mapletoft RJ. Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. Anim Reprod Sci 2002;72:47-62.

[3] Colazo MG, Martinez MF, Kastelic JP, Mapletoft RJ, Carruthers TD. The ischiorectal fossa: An alternative route for the administration of prostaglandin in cattle. Can Vet J 2002;43:535-541.

[4] Suñe J, Gonçalves P, Moraes J, Macedo J. Inseminação artificial em vacas de corte durante dez dias com emprego de minidose de prostaglandina. Rev Bras Reprod Anim 1985;9:141-145.

[5] Chohan KR. Estrus synchronization with lower dose of pgf2α and subsequent fertility in subestrous buffalo. Theriogenology 1998;50:1101-1108.

[6] Horta AE, Costa CM, Silva R, Rios Vasques MI. Possibility of reducing the luteolytic dose of cloprostenol in cyclic dairy cows. Theriogenology 1986;25:291-301.

[7] Gioso MM, Costa EP, Fernandes CAC, Paula TAR, Guimarães JD. Angioarquitetura venosa do órgão genital da fêmea bovina. Arq Bras Med Vet Zootec 2005;57:715-719.

[8] Ginther OJ, Del Campo CH. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Cattle. Am J Vet Res 1974;35:193-203.

[9] Donaldson LE, Glaphin SP, Green GA. Comparison of two dose rates and two management systems for synchronization of estrus in cattle. Am J Vet Res 1982;43:1873-1875.

[10] Lewis GS, Wilson L, Jr., Wilks JW, Pexton JE, Fogwell RL, Ford SP, Butcher RL, Thayne WV, Inskeep EK. Pgf2{alpha} and its metabolites in uterine and jugular venous plasma and endometrium of ewes during pregnancy. J Anim Sci 1977;45:320-327.

[11] Turner AS, McIlwraith CW. Bovine gastro-intestinal surgery. In: Turner AS, McIlwraith CW eds. Techniques in large animal surgery. Philadelphia, PA: Lea & Febiger, 1989: 261-267.

[12] Ferreira SH, Vane JR. Prostaglandins: Their disappearance from and release into the circulation. Nature 1967;216:868-873.

[13] Kindahl H, Edqvist LE, Granstrom E, Bane A. The release of prostaglandin f2alpha as reflected by 15-keto-13,14-dihydroprostaglandin f2alpha in the peripheral circulation during normal luteolysis in heifers. Prostaglandins 1976;11:871-878.

[14] Del Vecchio RP, Maxey KM, Lewis GS. A quantitative solid-phase enzymeimmunoassay for 13,14-dihydro-15-keto-prostaglandin f2[alpha] in plasma. Prostaglandins 1992;43:321-330.

[15] Bossaert P, Leroy JLMR, De Vliegher S, Opsomer G. Interrelations between glucose-induced insulin response, metabolic indicators, and time of first ovulation in high-yielding dairy cows. J Dairy Sci 2008;91:3363-3371.

[16] Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using sas procedures. J Anim Sci 1998;76:1216-1231.

[17] Louis TM, Hafs HD, Morrow DA. Intrauterine administration of prostaglandin f2{alpha} in cows: Progesterone, estrogen, lh, estrus and ovulation. J Anim Sci 1974;38:347-353.

[18] Inskeep EK. Potential uses of prostaglandins in control of reproductive cycles of domestic animals. J Anim Sci 1973;36:1149-1157.

[19] Ono H, Fukui Y, Terawaki Y, Ohboshi K, Yamazaki D. An intravulvosubmucous injection of prostaglandin f2α in anoestrous cows. Anim Reprod Sci 1982;5:1-5.

[20] Hixon JE, Hansel W. Evidence for preferential transfer of prostaglandin f2α to the ovarian artery following intrauterine administration in cattle. Biol Reprod 1974;11:543-552.

[21] Barrett S, Blockey MAd, Brown JM, Cumming IA, Goding JR, Mole BJ, Obst JM. Initiation of the oestrous cycle in the ewe by infusions of pgf2a to the autotransplanted ovary. J Reprod Fertil 1971;24:136-137.

[22] McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K, Samuelsson B. Prostaglandin f 2 identified as a luteolytic hormone in sheep. Nat New Biol 1972;238:129-134.

[23] Land RB, Baird DT, Scaramuzzi RJ. Dynamic studies of prostaglandin f-2 alpha in the utero-ovarian circulation of the sheep. J Reprod Fertil 1976;47:209-214.

[24] McCracken JA, Schramm W, Barcikowski B, Wilson L, Jr. The identification of prostaglandin f2 alpha as a uterine luteolytic hormone and the hormonal control of its synthesis. Acta Vet Scand Suppl 1981;77:71-88.

[25] Lee J, McCracken JA, Banu SK, Rodriguez R, Nithy TK, Arosh JA. Transport of prostaglandin f(2alpha) pulses from the uterus to the ovary at the time of luteolysis in ruminants is regulated by prostaglandin transporter-mediated mechanisms. Endocrinology 2010;151:3326-3335.

[26] Tervit HR, Rowson LEA, Brand A. Synchronization of oestrus in cattle using a prostaglandin f2{alpha} analogue (ici 79939). J Reprod Fertil 1973;34:179-181.

[27] Friendship RM, Templeton CL, Deckert AE. An evaluation of vulvomucosal injections of prostaglandins for induction of parturition in swine. Can Vet J 1990;31:433-436.

[28] Berardinelli JG, Adair R. Effect of prostaglandin f2[alpha] dosage and stage of estrous cycle on the estrous response and corpus luteum function in beef heifers. Theriogenology 1989;32:301-314.

[29] Stabenfeldt GH, Ewing LL, McDonald LE. Peripheral plasma progesterone levels during the bovine oestrous cycle. J Reprod Fertil 1969;19:433-442.

[30] Fajt VR, Wagner SA, Pederson LL, Norby B. The effect of intramuscular injection of dinoprost or gnrh in dairy cows on beef quality. J Anim Sci 2011;89:1939-1943.

Figure captions

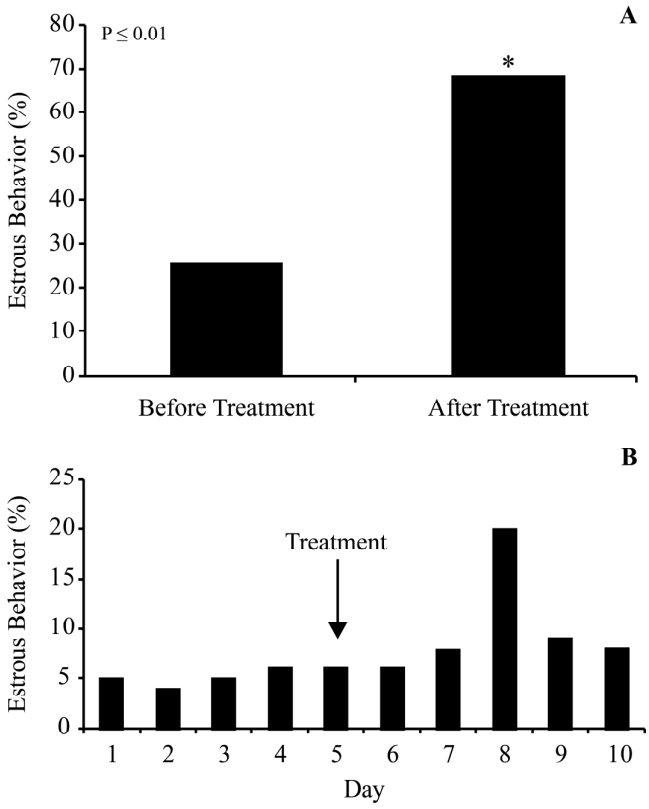
Fig. 1. (A) Rate of estrous behavior before (n = 1,937) and after treatment with 5 mg of dinoprost tromethamine via the intravulvosubmucous (IVSM) route at day 5, using the 10-day, 1-injection management system. Only cows that were not detected (n = 1440) in estrus were treated at day 5. (B) Distribution of estrous behavior of cyclic beef cows (n = 1,937) before and after injection with 5 mg of dinoprost via IVSM at day 5. Asterisk (*) indicates difference ($P \leq 0.01$) and arrow (↓) indicates the day of treatment.

Fig. 2. Rate of estrous behavior of cyclic beef heifers before (control; n = 241) and after treatment with 5 mg of dinoprost tromethamine via the intravulvosubmucous (IVSM) or intramuscular (IM) route at day 5 using the 10-day, 1-injection management system. Only heifers that were not detected in estrus were treated with 5 mg of dinoprost via IM (n = 95) or IVSM (n = 97) at day 5. Asterisk (*) indicates difference ($P \leq 0.01$).

Fig. 3. Mean serum progesterone concentration (ng/mL) after administration of dinoprost tromethamine on day 10 of the estrous cycle via the intravulvosubmucous (IVSM) or intramuscular (IM) route. Animals of the control group were at the same stage of the estrous cycle of treated animals, but were not submitted to any treatment. Asterisk (*) indicates difference from the control group ($P \leq 0.05$).

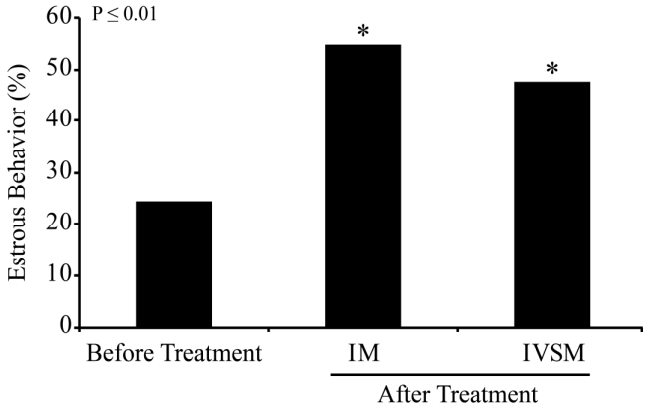
Fig. 4. (A) Mean serum prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations (ng/mL) in the uterine and jugular veins after treatment with 5 mg of $PGF_{2\alpha}$ (dinoprost tromethamine) or saline via the intravulvosubmucous (IVSM) route. Before treatment, the cow was injected IVSM with 1 mL of saline (control) to evaluate systemic and ovarian concentrations of $PGF_{2\alpha}$ for 40 minutes. (B) Serum 13,14-dihydro-15-keto-PGF (PGFM) concentrations (ng/mL) in the jugular vein

433 after injection of saline intramuscularly (IM; control); 5 mg of dinoprost IM (5 mg IM); 5 mg
434 of dinoprost IVSM (5 mg IVSM); or 25 mg of dinoprost IM (25 mg IM).



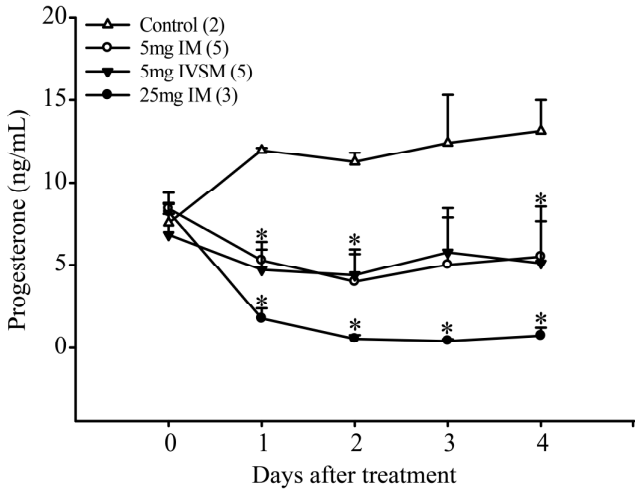
435

436 Figure 1



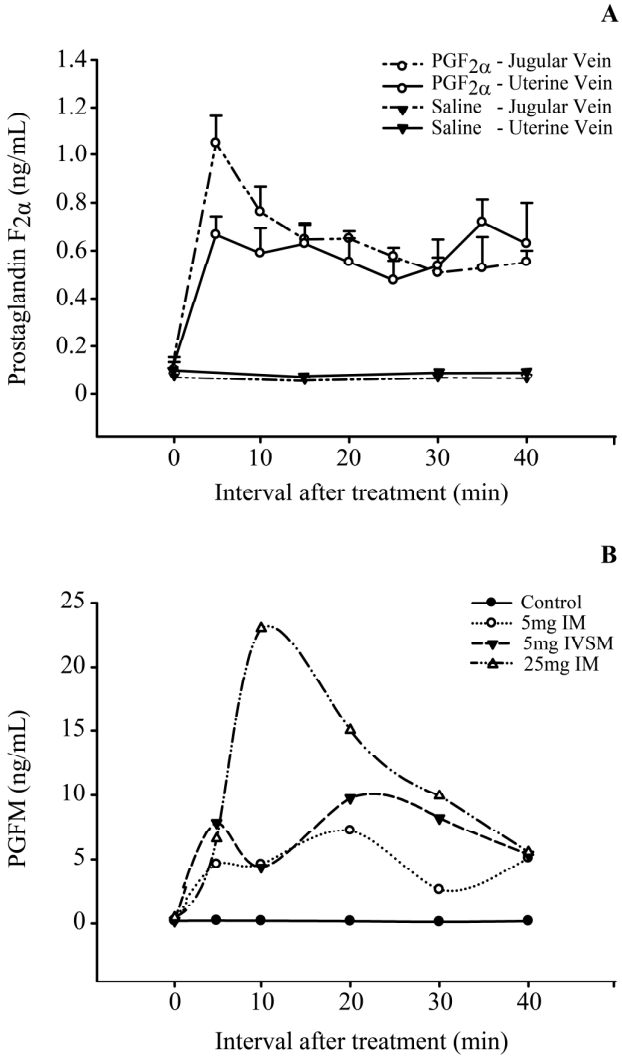
437

438 Figure 2



439

440 Figure 3



441

442 Figure 4.

CONCLUSÃO

Em resumo, verificou-se que não houve diferença nos índices de estro após 5mg IM ou IVSM; os animais tratados com 5mg no dia 5 do ciclo estral não apresentaram luteólise funcional, independente da via; e 2/5 (IVSM) e 3/5 (IM) vacas tratadas com 5mg no dia 10 demonstraram luteólise. Adicionalmente, a concentração de $PGF_{2\alpha}$ não diferiu entre as veias uterina e jugular após 5mg IVSM e a aplicação de 5mg via IVSM ou IM resultou em mesmo padrão sérico de concentração de PGFM.

Coletivamente, os resultados do presente estudo nos permitem concluir que a $PGF_{2\alpha}$ administrada via IVSM não atinge o corpo lúteo diretamente por uma via local e que a eficácia luteolítica é dependente da fase luteal e da dose, mas independe da via de administração (IVSM ou IM).

REFERÊNCIAS

ACOSTA, T.J. et al. Local Changes in Blood Flow Within the Early and Midcycle Corpus Luteum after Prostaglandin F2α Injection in the Cow. **Biology of Reproduction**, v.66, n.3, p.651-658, 2002.

ALVAREZ, R.H. et al. The use of lower doses of the prostaglandin analogue, cloprostenol, for oestrus synchronization in heifers. **Animal reproduction science**, v.25, n.2, p.93-96, 1991.

AROSH, J.A. et al. Prostaglandin Biosynthesis, Transport, and Signaling in Corpus Luteum: A Basis for Autoregulation of Luteal Function. **Endocrinology**, v.145, n.5, p.2551-2560, 2004.

BANU, S.K. et al. Molecular cloning and spatio-temporal expression of the prostaglandin transporter: A basis for the action of prostaglandins in the bovine reproductive system. **Proceedings of the National Academy of Sciences**, v.100, n.20, p.11747-11752, 2003.

_____. Molecular Cloning and Characterization of Prostaglandin (PG) Transporter in Ovine Endometrium: Role for Multiple Cell Signaling Pathways in Transport of PGF2α. **Endocrinology**, v.149, n.1, p.219-231, 2008.

BEAL, W.E.; MILVAE, R.A.; HANSEL, W. Oestrous cycle length and plasma progesterone concentrations following administration of prostaglandin F-2α early in the bovine oestrous cycle. **Journal of Reproduction and Fertility**, v.59, n.2, p.393-396, 1980.

BERGSTROM, S.; CARLSON, L.A.; WEEKS, J.R. The prostaglandins: a family of biologically active lipids. **Pharmacological Reviews**, v.20, n.1, p.1-48, 1968.

BISHOP, C.V.; STORMSHAK, F. Nongenomic Action of Progesterone Inhibits Oxytocin-Induced Phosphoinositide Hydrolysis and Prostaglandin F2α Secretion in the Ovine Endometrium. **Endocrinology**, v.147, n.2, p.937-942, 2006.

CHOHAN, K.R. Estrus synchronization with lower dose of PGF2α and subsequent fertility in subestrous buffalo. **Theriogenology**, v.50, n.7, p.1101-1108, 1998.

COLAZO, M.G. et al. Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. **Animal reproduction science**, v.72, n.1-2, p.47-62, 2002.

_____. The ischiorectal fossa: an alternative route for the administration of prostaglandin in cattle. **Canadian Veterinary Journal**, v.43, n.7, p.535-541, 2002.

DAVIS, J.S.; RUEDA, B.R. The corpus luteum: an ovarian structure with maternal instincts and suicidal tendencies. **Frontiers in Bioscience**, v.7, p.d1949-1978, 2002.

DEL CAMPO, C.H.; GINTHER, O.J. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: histologic structure of uteroovarian vein and ovarian artery in sheep. **American Journal of Veterinary Research**, v.35, n.3, p.397-399, 1974.

DONALDSON, L.; HANSEL, W. Histological Study of Bovine Corpora Lutea. **Journal of Dairy Science**, v.48, n.7, p.905-909, 1965.

DONALDSON, L.E.; GLAPHIN, S.P.; GREEN, G.A. Comparison of two dose rates and two management systems for synchronization of estrus in cattle. **American Journal of Veterinary Research**, v.43, n.10, p.1873-1875, 1982.

DYCE, K.M.; SACK, W.O.; WENSING, C.J.G. Pelve e Órgãos Reprodutores das Fêmeas Ruminantes. In: D. K.M.; S. W.O*., et al*. **Tratado de Anatomia Veterinária**. 3. ed. Rio de Janeiro: Guanabara Koogan, 2004. Pelve e Órgãos Reprodutores das Fêmeas Ruminantes, p.669-689.

FERREIRA, S.H.; VANE, J.R. Prostaglandins: their disappearance from and release into the circulation. **Nature**, v.216, n.5118, p.868-873, 1967.

FLINT, A.P.F.; SHELDRICK, E.L. Ovarian secretion of oxytocin is stimulated by prostaglandin. **Nature**, v.297, n.5867, p.587-588, 1982.

GINTHER, O.J. Internal regulation of physiological processes through local venoarterial pathways: a review. **Journal of Animal Science**, v.39, n.3, p.550-564, 1974.

GINTHER, O.J.; DEL CAMPO, C.H. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: cattle. **American Journal of Veterinary Research**, v.35, n.2, p.193-203, 1974.

GIOSO, M.M. et al. Angioarquitetura venosa do órgão genital da fêmea bovina. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.57, p.715-719, 2005.

GIRSH, E. et al. Effect of endothelin-1 on bovine luteal cell function: role in prostaglandin F2alpha-induced antisteroidogenic action. **Endocrinology**, v.137, n.4, p.1306-1312, 1996a.

_____. Regulation of endothelin-1 expression in the bovine corpus luteum: elevation by prostaglandin F 2 alpha. **Endocrinology**, v.137, n.12, p.5191-5196, 1996b.

GORAVANAHALLY, M.P. et al. Differential Gene Expression in the Bovine Corpus Luteum During Transition from Early Phase to Midphase and Its Potential Role in Acquisition of Luteolytic Sensitivity to Prostaglandin F2 Alpha. **Biology of Reproduction**, v.80, n.5, p.980-988, 2009.

HENRICKS, D.M. et al. The effect of prostaglandin F2alpha during various stages of the oestrous cycle of beef heifers. **Journal of Reproduction and Fertility**, v.41, n.1, p.113-120, 1974.

HIXON, J.E.; HANSEL, W. Evidence for Preferential Transfer of Prostaglandin F2α to the Ovarian Artery Following Intrauterine Administration in Cattle. **Biology of Reproduction**, v.11, n.5, p.543-552, 1974.

HORTA, A.E. et al. Possibility of reducing the luteolytic dose of cloprostenol in cyclic dairy cows. **Theriogenology**, v.25, n.2, p.291-301, 1986.

KINDAHL, H. et al. The release of prostaglandin F2alpha as reflected by 15-keto-13,14-dihydroprostaglandin F2alpha in the peripheral circulation during normal luteolysis in heifers. **Prostaglandins**, v.11, n.5, p.871-878, 1976.

LEE, J. et al. Transport of prostaglandin F(2alpha) pulses from the uterus to the ovary at the time of luteolysis in ruminants is regulated by prostaglandin transporter-mediated mechanisms. **Endocrinology**, v.151, n.7, p.3326-3335, 2010.

MALVEN, P.V.; HANSEL, W. Ovarian Function in Dairy Heifers Following Hysterectomy. **Journal of Dairy Science**, v.47, n.12, p.1388-1393, 1964.

MCCRACKEN, J.A.; BAIRD, D.T.; GODING, J.R. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. **Recent Progress in Hormone Research**, v.27, p.537-582 passim, 1971.

MCCRACKEN, J.A. et al. Prostaglandin F 2 identified as a luteolytic hormone in sheep. **Nature: New Biology**, v.238, n.83, p.129-134, 1972.

MCCRACKEN, J.A.; CUSTER, E.E.; LAMSA, J.C. Luteolysis: A Neuroendocrine-Mediated Event. **Physiological Reviews**, v.79, n.2, p.263-323, 1999.

MCGUIRE, W.J.; JUENGEL, J.L.; NISWENDER, G.D. Protein kinase C second messenger system mediates the antisteroidogenic effects of prostaglandin F2 alpha in the ovine corpus luteum in vivo. **Biol Reprod**, v.51, n.4, p.800-806, 1994.

MIYAMOTO, A. et al. Blood flow: A key regulatory component of corpus luteum function in the cow. **Domestic animal endocrinology**, v.29, n.2, p.329-339, 2005.

MORAES, J.C.F. et al. Controle do estro e ovulação em ruminantes In: P. B. D. Gonçalves; J. R. Figueiredo*, et al*. **Biotécnicas Aplicadas à Reprodução Animal**. 2. ed. São Paulo: Roca, 2008. Controle do estro e ovulação em ruminantes p.33-56.

NANCARROW, C.D. et al. Hormonal changes around oestrus in the cow. **Journal of Reproduction and Fertility**, v.32, n.3 Suppl, p.S320-S321, 1973.

NARUMIYA, S.; SUGIMOTO, Y.; USHIKUBI, F. Prostanoid Receptors: Structures, Properties, and Functions. **Physiological Reviews**, v.79, n.4, p.1193-1226, 1999.

NISWENDER, G.D. et al. Luteal function: the estrous cycle and early pregnancy. **Biology of Reproduction**, v.50, n.2, p.239-247, 1994.

_____. Mechanisms Controlling the Function and Life Span of the Corpus Luteum. **Physiological Reviews**, v.80, n.1, p.1-29, 2000.

PATE, J.L. Regulation of prostaglandin synthesis by progesterone in the bovine corpus luteum. **Prostaglandins**, v.36, n.3, p.303-315, 1988.

PIPER, P.J.; VANE, J.R.; WYLLIE, J.H. Inactivation of Prostaglandins by the Lungs. **Nature**, v.225, n.5233, p.600-604, 1970.

RICHARDS, J.S.; HEDIN, L. Molecular aspects of hormone action in ovarian follicular development, ovulation, and luteinization. **Annu Rev Physiol**, v.50, p.441-463, 1988.

RODGERS, R.J.; MITCHELL, M.D.; SIMPSON, E.R. Secretion of progesterone and prostaglandins by cells of bovine corpora lutea from three stages of the luteal phase. **Journal of Endocrinology**, v.118, n.1, p.121-126, 1988.

ROWSON, L.E.A.; TERVIT, R.; BRAND, A. The use of prostaglandins for synchronization of oestrus in cattle. **Journal of Reproduction and Fertility**, v.29, n.3_Suppl, p.S145, 1972.

SAKAMOTO, K. et al. Molecular cloning and expression of a cDNA of the bovine prostaglandin F2 alpha receptor. **Journal of Biological Chemistry**, v.269, n.5, p.3881-3886, 1994.

SCHALLENBERGER, E. et al. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. **Journal of Reproduction and Fertility**, v.71, n.2, p.493-501, 1984.

SCHUSTER, V.L. Prostaglandin transport. **Prostaglandins & Other Lipid Mediators**, v.68-69, p.633-647, 2002.

SHIRASUNA, K. et al. Real-time dynamics of prostaglandin F2{alpha} release from uterus and corpus luteum during spontaneous luteolysis in the cow. **Reproduction**, v.128, n.2, p.189-195, 2004.

_____. Positive Association, in Local Release, of Luteal Oxytocin with Endothelin 1 and Prostaglandin F2alpha During Spontaneous Luteolysis in the Cow: A Possible Intermediatory Role for Luteolytic Cascade Within the Corpus Luteum. **Biology of Reproduction**, v.76, n.6, p.965-970, 2007.

SPENCER, T.E.; BAZER, F.W. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. **Biology of Reproduction**, v.53, n.6, p.1527-1543, 1995.

SPICER, L.J.; IRELAND, J.J.; ROCHE, J.F. Changes in serum LH, progesterone, and specific binding of 125I-hCG to luteal cells during regression and development of bovine corpora lutea. **Biology of Reproduction**, v.25, n.4, p.832-841, 1981.

SUÑE, J. et al. Inseminação Artificial Em Vacas de Corte Durante Dez Dias Com Emprego de Minidose de Prostaglandina. **Revista Brasileira de Reprodução Animal**, v.9, n.3, p.141-145, 1985.

TSAI, S.J.; WILTBANK, M.C. Prostaglandin F2alpha induces expression of prostaglandin G/H synthase-2 in the ovine corpus luteum: a potential positive feedback loop during luteolysis. **Biology of Reproduction**, v.57, n.5, p.1016-1022, 1997.

_____. Prostaglandin F2alpha regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. **Biology of Reproduction**, v.58, n.2, p.346-352, 1998.

WATHES, D.C.; LAMMING, G.E. The oxytocin receptor, luteolysis and the maintenance of pregnancy. **Journal of Reproduction and Fertility. Supplement**, v.49, p.53-67, 1995.

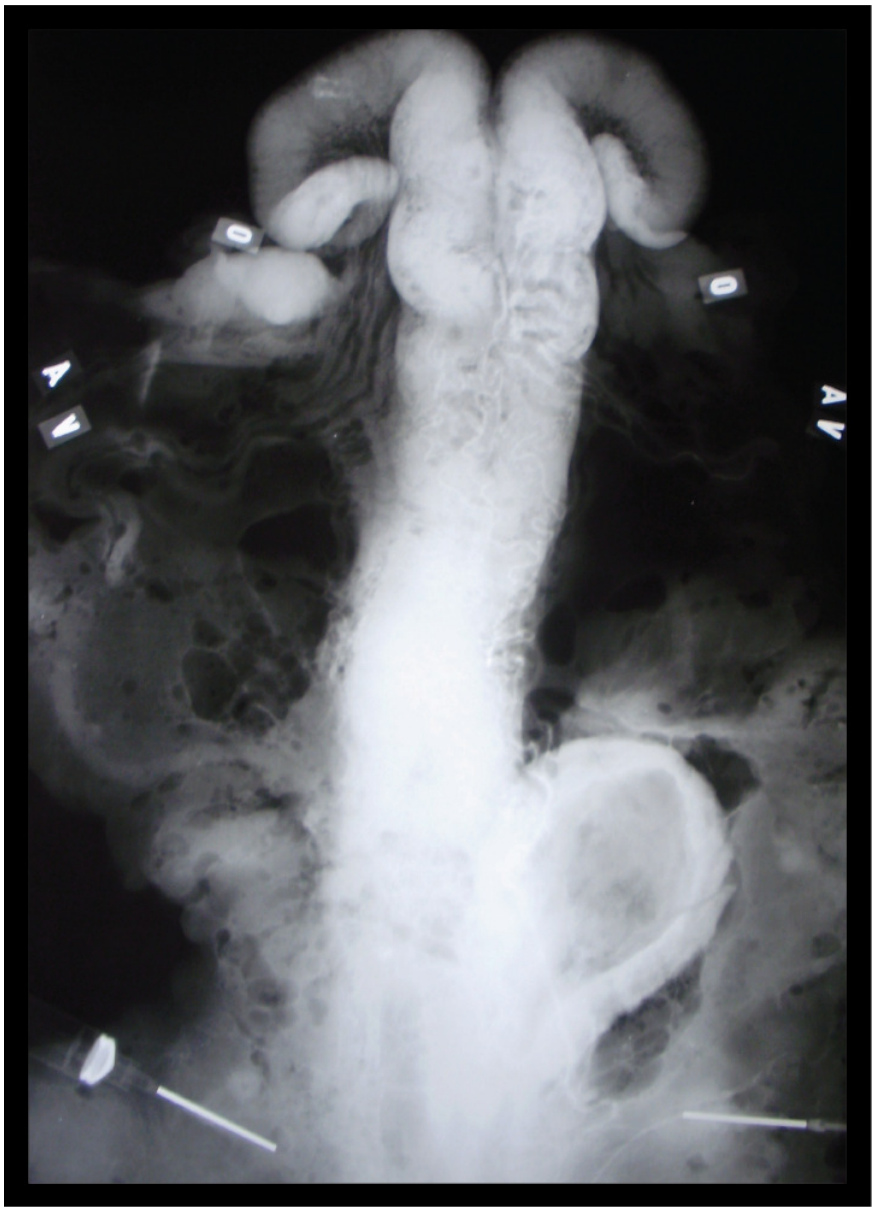
WILTBANK, J.N.; CASIDA, L.E. Alteration of Ovarian Activity by Hysterectomy. **Journal of Animal Science**, v.15, n.1, p.134-140, 1956.

WILTBANK, M.C. et al. Prostaglandin F2 alpha receptors in the early bovine corpus luteum. **Biology of Reproduction**, v.52, n.1, p.74-78, 1995.

WOLFENSON, D. et al. Characteristics of prostaglandin F measurements in the ovarian circulation during the oestrous cycle and early pregnancy in the cow. **Journal of Reproduction and Fertility**, v.75, n.2, p.491-499, 1985.

APÊNDICE

Apêndice A – Raio-X do trato reprodutivo bovino após injeção de contraste na Veia Vaginal



A: artéria ovariana; V: veia uterina; O: ovário.