

UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

**EFEITO DO CLOPROSTENOL SÓDICO SOBRE A
INVOLUÇÃO UTERINA NO PUERPÉRIO DE VACAS
NELORE**

CAROLINA NOGUEIRA DE MORAES

Botucatu, São Paulo

Fevereiro, 2014

UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

**EFEITO DO CLOPROSTENOL SÓDICO SOBRE A
INVOLUÇÃO UTERINA NO PUERPÉRIO DE VACAS
NELORE**

CAROLINA NOGUEIRA DE MORAES

Dissertação apresentada junto ao Programa de
Pós-Graduação em Medicina Veterinária da
Universidade Estadual Paulista (Unesp) para
obtenção do título de Mestre em Biotecnologia
Animal

Orientadora: Prof^a Dr. Eunice Oba

Botucatu, São Paulo

Fevereiro, 2014

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO DE BIBLIOTECA E DOCUMENTAÇÃO - CAMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO - CRB 8/7500

Moraes, Carolina Nogueira de.

Efeito do cloprostenol sódico sobre a involução uterina no puerpério de vacas
Nelore / Carolina Nogueira de Moraes. - Botucatu, 2014

Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de
Medicina Veterinária e Zootecnia

Orientador: Profa. Dra. Eunice Oba

Capes: 50504002

1. Reprodução animal. 2. Bovino. 3. Hormônios progestacionais. 4. Puerpério.

Palavras-chave: bovinos; cloprostenol sódico; involução uterina; puerpério.

Nome da Autora: Carolina Nogueira de Moraes

Título: EFEITO DO CLOPROSTENOL SÓDICO SOBRE A INVOLUÇÃO UTERINA
NO PUERPÉRIO DE VACAS NELORE

COMISSÃO EXAMINADORA

Prof^a. Tit. Eunice Oba
Presidente e Orientadora
Depto. de Reprodução Animal e Radiologia Veterinária
FMVZ – UNESP- Botucatu

Prof^a. Dra Fabiana Ferreira de Souza
Membro
Depto. de Reprodução Animal e Radiologia Veterinária
FMVZ – UNESP- Botucatu

Prof.Dr. Thales Ricardo Rigo Barreiros
Membro
Depto de Veterinária e Produção Animal
Universidade Estadual do Norte do Paraná - Bandeirantes

Data da defesa: 28 de fevereiro de 2014.

DEDICATÓRIA

A Deus, meu grande Salvador, que proporcionou mais esta conquista

À minha família

Ao meu noivo Leandro

AGRADECIMENTOS

Agradeço primeiramente a *Deus*, Pai e grande Salvador por estar sempre presente em minha vida, me guiando e me honrando. Agradeço pela graça recebida e pela oportunidade de realização de mais este sonho. Agradeço pela força dada quando pensei que tudo estava perdido e que nada mais havia sentido. Agradeço e, definitivamente a Ele, sou infinitamente grata.

Agradeço aos meus pais *Enio* e *Salviana* pelo apoio, consideração e compreensão para que eu cumprisse essa etapa. Obrigada queridos porque mesmo sem muitas vezes dimensionar minhas coisas, vocês sempre estiveram ao meu lado. À você minha mãe, que mesmo longe, sempre esteve tão perto, com suas palavras amigas e seus incentivos tão doces. À você Eninho, tão igualzinho a mim, que demonstra seus sentimentos da mesma maneira que eu. Amo vocês incondicionalmente.

Às minhas irmãs *Camila* e *Daniela* pelo suporte e compreensão, broncas e puxões de orelha para que pudesse me tornar quem eu sou. Aos meus cunhados *Joelson* e *Juninho* pelo apoio mesmo sem saberem.

Ao *Arthur*, meu amorzinho, que mora no meu coração. Meu querido gordinho, que acompanha meus estudos de longe e que me faz tanta falta: obrigada “fio”, pelo seu sorriso, suas brincadeiras e seu abraço tão gostoso nas horas tão necessárias.

À *Manuela*, minha “princesaZas” que me traz um sorriso todos os dias, mesmo estando longe. Obrigada gatinha, por fazer meu amor por você somente crescer.

Aos demais familiares pela ajuda e incentivo.

À você querido *Leandro*. Meu noivo tão amado e que em nenhum momento mediu esforços para que eu terminasse o Mestrado e fosse mais longe. Você foi e é meu anjo, braço direito, companheiro, cúmplice e amigo. Certamente, meus dias em Botucatu ficaram mais alegres com você ao meu lado. Amo você.

À *Carla Martins (Carlinha)*, a irmã que Deus permitiu que eu escolhesse. Muito obrigada pela amizade sempre certa e pela ajuda presente no experimento. Juntamente com o Leandro, você possibilitou o cumprimento dessa etapa na minha vida. Parceria sempre é parceria!

À *Fazenda Marino Avaré* e ao seu proprietário, *Sr. Marino* que permitiu que as porteiras fossem abertas a mim, confiando a mim a realização do experimento. Ao *Cristiano (Gato)* que me acompanhou em todas as coletas, sempre ajudando, juntamente com sua família (*Ana, João, Luizinho, Duda, Nanda, Lurdinha e Alê*) que me adotou como parte dos seus. A todos os funcionários da *Fazenda Marino Avaré (Alan, Dona Ilda, Alcides, Karina e Valmir)*, agradeço pelos momentos de ajuda e descontração.

Ao Médico Veterinário *Marcelo Piagentini (Mosquito)* pelo auxílio na parte inicial do experimento.

À minha orientadora Professora *Eunice Oba* pelo exemplo de profissionalismo, amizade e confiança em mim depositada durante o Mestrado.

Ao Professor *Thales Ricardo Rigo Barreiros* por um dia ter me proporcionado a oportunidade de realização do estágio com Reprodução Animal, me mostrando a importância da disciplina, da organização e do compromisso com a Veterinária.

Aos Professores do Departamento de Reprodução Animal: *Frederico Papa, João Ferreira, Cezinande Meira, Sony Bicudo, Denise Lopes, Fernanda Landim, Nereu Prestes, Eunice Oba e Marco Alvarenga* que me acompanharam desde a Residência e que hoje são grandes exemplos de excelência de profissionais. Agradeço em especial ao Prof. João Ferreira pela orientação nas dosagens hormonais ao final do experimento. Agradeço também à Dra. Fabiana Ferreira (*Fabi*) pela amizade, assistência e auxílio nas dosagens hormonais e na interpretação dos dados estatísticos.

Ao Professor *José Buratini Júnior* e ao seu laboratório, em especial à *Paula Lima* pela paciência na explicação e na realização da técnica de rtPCR.

Ao amigo *Mateus Sudano* pela compreensão dos prazos e empenho na realização da análise estatística.

Aos funcionários, *José, Edivaldo, Cristina, Valter, Edilson, Dona Raquel, Camila* (Reprodução Animal) e *José* (Patologia). E ainda, consideravelmente a todos os funcionários e amigos que contribuíram para realização deste trabalho.

À *Universidade Estadual Julio de Mesquita Filho*, campus Botucatu pela infraestrutura e excelente corpo docente e a todos os amigos de Pós-Graduação, em especial a *Carla Martins (Carlinha)*, *Marianne Camargos (Mari)*, *Caroline Destro (Destro)*, *Priscila Guasti (Pri)*, *Caroline Scott (Scott)*, *Rosiara Maziero (Rosi)*, *Cinthia*

Ormond, *Letícia* Crocomo, Eduardo Trevisol (*Du*), *Camila* Ackermann, *Paula* Lima e *Talita* Raposo.

À **FAPESP** pela concessão da bolsa de mestrado (Proc.2011/15330-0), e à **Capes** (Proc./096/2010) pelo suporte financeiro.

Finalmente, aos animais, peças fundamentais neste estudo.

A gratidão seria em vão se não fosse eterna. Muito Obrigada!

"Porque eu bem sei os pensamentos que
tenho a vosso respeito, diz o Senhor;
pensamentos de paz, e não de mal,
para vos dar o fim que esperais."

Jeremias 29:11

LISTA DE ABREVIACÕES

AA – ácido aracdônico
BEN – balanço energético negativo
°C – graus Celsius
CA⁺ - cálcio
CASP-3 – caspase 3
cDNA – DNA complementar
CL- corpo lúteo
cm – centímetros
COX- enzima ciclo-oxigenase
COX-2 – ciclo-oxigenase 2
CYC-A – ciclofilina A
DAB- diaminobenzadina
DAG – diacilglicerol
DNA – ácido desoxirribonucléico
DNase – enzima que degrada o ácido desoxirribonucléico
dNTP – desoxiribonucleotídeo trifosfato
DP1, DP2 – receptor de prostaglandina D₂
DTT – ditioneitol
EDTA – ácido etilenodiamino tetra-acético
eCG – gonadotrofina coriônica equina
EP1,EP2, EP3, EP4 – receptor de prostaglandina E₂
FD – folículo dominante
FP ou PTGFR – receptor completo de prostaglandina F_{2α}
FP_α, FP_β, FP_γ, FP_δ, FP_ε e FP_ζ, PTGFR_ζ e PTGFR_α – isoformas do receptor de prostaglandina F_{2α}
FSH – hormônio folículo estimulante
GAPDH – gliceraldeído-3-fosfato desidrogenase
GnRH – hormônio liberador de gonadotrofinas
GPD – guanosina trifosfato
hCG – gonadotrofina coriônica humana
HE – hematoxilina – eosina

IATF – inseminação artificial em tempo fixo

IGF-1 – fator de crescimento semelhante à insulina tipo 1

IP – receptor de prostaglandina I_2

IP_3 – inositol trifosfato

LH – hormônio luteinizante

M - molar

MHC-I – complexo de histocompatibilidade principal da classe I

mL – mililitro

mm – milímetro

mM – milimolar

mRNA – ácido ribonucleico mensageiro

ng – nanograma

P – nível de significância

pb – pares de bases

pg – picogramas

P4 – progesterona

PGD_2 – prostaglandina D_2

PGE_2 – prostaglandina E_2

$PGF_{2\alpha}$ – prostaglandina $F_{2\alpha}$

PGFM - 13,14-dihidro-15-ceto prostaglandina $F_{2\alpha}$

PGG_2 – prostaglandina G_2

PGH_2 – prostaglandina H_2

PGI_2 – prostaglandina I_2

PIP_2 - fosfatidilinositol difosfato

PKC – proteína C quinase

PLA_2 – fosfolipase A_2

RNA – ácido ribonucleico

RNAse – enzima que degrada o ácido ribonucleico

rpm – rotações por minuto

RT-PCR – reação de transcrição reversa seguida por reação em cadeia de polimerase

S.E.M- erro padrão da média

TP_α e TP_δ – receptor de tromboxano A_2

TRIS – tris(hidroximetil)aminometano

TXA₂ – tromboxano A₂

VE – valerato de estradiol

µg – micrograma

µL – microlitro

µm – micrômetro

% - por cento

= - igual

> - maior

< - menor

LISTA DE TABELAS

CAPÍTULO 2

Tabela 1 -	Sequence of primers and amplification product size of CASP-3, COX-2, CYC-A, FP and GAPDH	61
Tabela 2 -	Analyzed variables and classification on immunohistochemistry of samples from day 1, 14, 28 and 42 postpartum of Nelore cow.....	62
Tabela 3 -	Mediam, minimum and maximum values of score of staining and score of staining intensity of immunohistochemical detection of FP and COX-2 of endometrial samples during postpartum period	62

CAPÍTULO 3

Tabela 1 -	Summarized description of the variables evaluated during the experimental period	90
Tabela 2 -	Mean and stardard error of cervical ostium and cervical discharge evaluation on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cow, independent of treatment	90
Tabela 3 –	Mean and stardard error of degree of uterine involution on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cows independent of treatment	91

LISTA DE FIGURAS

CAPÍTULO 1

- Figura 1 - Via de biossíntese dos prostanóides. Fonte: Adaptado de Barros; Di Stasi, 2012..... 10
- Figura 2 - Estrutura esquemática do receptor acoplado à proteína G. Observam-se as α hélices (TM1, TM2, TM4, TM4, TM5, TM6, TM7) (seta vermelha); as subunidades da proteína G (seta verde); e os domínios intracelular e extracelular (seta preta). Fonte: Adaptado de Barros; Di Stasi, 2012..... 12
- Figura 3 - Receptor acoplado à proteína G e sua forma de ativação. Fonte: Adaptado de Barros; Di Stasi, 2012..... 13

CAPÍTULO 2

- Figura 1 - Timeline. Animals were submitted to treatment (T) with cloprostenol sodium or 0.9% NaCl solution on D1 and D4 (D0= Birth - B). Then it was collected endometrial biopsies (BIO) for evaluation of gene expression (GE) on D1, D7, D14, D28 and D42 and for immunohistochemistry (IHC) performance on D1, D14, D28 and D42..... 57
- Figura 2 - Ratio of FP/CYC-A mRNA abundance measured by real time rtPCR of 24 Nelore cows during postpartum period (D1, D7, D14, D28 and D42). Values are shown as mean values ($\pm$ S.E.M). Uncommon letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package..... 57

- Figura 3 - Ratio of CASP-3/CYC-A mRNA abundance measured by real time rtPCR of 24 Nelore cows during postpartum period (D1, D7, D14, D28 and D42). Values are shown as mean values ($\pm$ S.E.M). Uncommon letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package..... 58
- Figura 4 - Ratio of COX-2/CYC-A mRNA abundance measured by real time rtPCR of 24 Nelore cows during postpartum period (D1, D7, D14, D28 and D42). Values are shown as mean values ($\pm$ S.E.M). Uncommon letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package..... 58
- Figura 5 - Immunohistochemical detection of FP receptor during postpartum of Nelore cows. Note positive cytoplasmic staining (brown) in A and B (arrows). C: negative control by omitting primary antibody. Bar = 50 μ m. D: positive control (bovine corpus luteum). Bar = 100 μ m. ESE: endometrial surface epithelium, STR: stroma, GE: glandular epithelium..... 59
- Figure 6 - Immunohistochemical detection of COX-2 during postpartum of Nelore cows. Note positive cytoplasmic staining (brown) in A and B (arrows). Bar = 50 μ m. C: negative control by omitting primary antibody. D: positive control (bovine lung adenocarcinoma). Bar = 100 μ m. ESE: endometrial surface epithelium, STR: stroma, GE: glandular epithelium..... 60

CAPÍTULO 3

- Figura 1 - Mean and standard error of P4 dosage on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 23 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by the mixed linear

	model (MIXED) procedure with the SAS statistical software package.....	87
Figura 2 -	Mean and standard error of PGFM dosage on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 22 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package.....	87
Figura 3 –	Mean and standard error of diameter of uterine horn (cm) on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package.....	88
Figura 4 –	Mean and standard error of score of fluid accumulation (score 0-3) on days 1, 7, 14, 21, 28, 35 and 42 postpartum on 24 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by mixed linear model (MIXED) procedure with the SAS statistical software package. Score 0- without fluid, 1- less than 1 cm of fluid in the mean of 3 measurements, 2- more than 1 cm of fluid in the mean of 3 measurements , 3 – more than 2 cm in the mean of 3 measurements.....	88
Figura 5 -	Mean and standard error of follicular size (mm) from right ovary on days 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package.....	89

CAPÍTULO 4

- Figura 1 - Photomicrograph of bovine endometrium 1 day postpartum of placebo (A) and treated (B) group. In A note the presence of edema (red arrow) and inflammatory cell (yellow arrow) in stromal portion and epithelial portion (*). In B note the presence of edema (red arrow) in the stromal portion; polymorfonuclear infiltrate (below the epithelium, perivascular, intravascular and at stromal portion) and the presence of blood vessels (white arrow). (Hematoxylin-eosin, 40x magnification) Bar = 100µm..... 118
- Figura 2 - Photomicrograph of bovine endometrium 7 days postpartum of placebo (A) and treated (B) group. In A note the presence of large amounts of polymorfonuclear infiltrate (yellow arrow) at epithelial portion, below the epithelium and in some areas of stromal portion; beyond areas of hemorrhage (white arrow). In B note small areas of edema (red arrow) as well as small areas of hemorrhage (white arrow). Furthermore, in B there is no the presence of polymorphonuclear infiltrates. (Hematoxylin-eosin, 40x magnification) Bar = 100µm..... 119
- Figura 3 - Photomicrograph of bovine endometrium 14 days postpartum of placebo (A) and treated (B) group. In A note the presence of intense polymorphonuclear infiltrate (yellow arrow) across endometrial sample; areas of hemorrhage (green arrow); endometrial epithelium not intact (*) and high cellularity. In B note the presence of polymorphonuclear infiltrate (yellow arrow) and a small vessel (red arrow). (Hematoxylin-eosin, 40x magnification) Bar = 100µm..... 119
- Figura 4 - Photomicrograph of bovine endometrium 28 days postpartum of placebo (A) and treated (B) group. In A note the presence of

endometrial glands (yellow arrow) and arterioles (red arrow). It also can be observed high cellularity, but not polymorphonuclear infiltrate. In B note the presence of endometrial glands (yellow arrow). Also, there is some cellularity and absence of polymorphonuclear infiltrate. (Hematoxylin-eosin, 20 x magnification) Bar = 100µm..... 120

- Figura 5 - Photomicrograph of bovine endometrium 42 days postpartum of placebo (A) and treated (B) group. In A note the tissue without inflammation, it also can be observed only one inflammatory cell (yellow arrow). In B note the presence of endometrial glands (yellow arrows) and endometrial tissue without signs of inflammation. (Hematoxylin-eosin, 40x magnification) Bar = 100µm..... 120
- Figura 6 - Mean and standard error scores of endometrial glands (score 0 to 3) in the postpartum period (D1 to D42) of endometrial histological evaluation of Nelore females treated or not with cloprostenol sodium. Score 0: none, 1: scarce, 2: moderate and 3: heavy. ^{a,b,c} Uncommon letters differ ($P < 0.05$)..... 121
- Figura 7 - Mean and standard error of average of leukocytes at 5 random fields in the postpartum period (D1 to D42) of endometrial histological evaluation of Nelore females. ^{a,b} Uncommon letters differ ($P < 0.05$)..... 122
- Figura 8 - Mean and standard error scores of degree of inflammation (score 0 to 3) in the postpartum period (D1 to D42) of endometrial histological evaluation of Nelore females. Score 0: no inflammation, 1: low inflammation, 2: moderate inflammation, 3: severe inflammation. ^{a,b} Uncommon letters differ ($P < 0.05$)..... 122

SUMÁRIO

	Página
RESUMO.....	xx
ABSTRACT.....	xxi
CAPÍTULO 1	
1 INTRODUÇÃO.....	2
2 REVISÃO DE LITERATURA.....	4
2.1 Fisiologia e classificação do período puerperal.....	4
2.2 Fases do período puerperal.....	4
2.2.1 Eliminação das secundinas fetais ou delivramento.....	4
2.2.2 Involução uterina ou puerpério propriamente dito.....	5
2.3 Avaliação da involução uterina.....	6
2.3.1 Palpação transretal.....	7
2.3.2 Vaginoscopia.....	7
2.3.3 Exame Ultrassonográfico.....	8
2.3.4 Biópsia endometrial.....	8
2.4 PGF _{2α}	9
2.4.1 FP.....	11
2.5 Retorno à ciclicidade.....	14
2.5.1 Desenvolvimento folicular.....	15
2.6 Principais fatores que alteram o retorno à ciclicidade.....	16
2.6.1 Anestro.....	16

2.6.2 Amamentação.....	17
2.6.3 Condição Corporal.....	18
2.7 Estratégias para acelerar o primeiro estro e ovulação pós- parto.....	19
2.7.1 Métodos não hormonais.....	19
2.7.1.1 Manejo de amamentação.....	19
2.7.1.1.1 Desmame precoce.....	19
2.7.1.1.2 Remoção temporária do bezerro.....	20
2.7.1.1.3 Amamentação controlada.....	20
2.7.1.2 Exposição ao touro.....	21
2.7.2 Métodos hormonais.....	22
2.7.2.1 GnRH.....	22
2.7.2.2 Gonadotrofinas.....	23
2.7.2.3 Esteróides.....	23
2.7.2.4 PGF _{2α}	24

CAPÍTULO 2

Artigo Científico nas normas e a ser submetido ao periódico “Reproduction Fertility and Development”: Temporal analysis of mRNA expression of FP receptor, CASP-3 and COX-2 and immunohistochemistry for FP receptor and COX-2 of endometrial tissue from multiparous Nelore (<i>Bos taurus indicus</i>) cows treated with cloprostenol sodium on puerperium.....	26
---	----

CAPÍTULO 3

Artigo Científico nas normas e a ser submetido ao periódico “Journal of Reproduction and Development”: Effect of cloprostenol sodium on uterine involution, progesterone and 13,14-dihydro-15-keto prostaglandin F2 α concentrations of multiparous Nelore (<i>Bos taurus indicus</i>) cows.....	64
---	----

CAPÍTULO 4

Artigo Científico nas normas e submetido ao periódico “Anatomia, Histologia, Embryologia”: Temporal histologic analysis of Nelore (<i>Bos taurus indicus</i>) endometrium of cows treated or not with cloprostenol sodium at postpartum...	93
--	----

CAPÍTULO 5

3 DISCUSSÃO GERAL.....	124
4 CONCLUSÕES GERAIS.....	133
5 BIBLIOGRAFIA.....	134

MORAES, C.N. **Efeito do cloprostenol sódico sobre a involução uterina no puerpério de vacas Nelore**. Botucatu, 2014. 149p. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

RESUMO

Após o parto, alguns eventos fisiológicos ocorrem no útero de vacas para reparar as modificações ocorridas durante a prenhez e o parto. O uso do cloprostenol sódico no período puerperal visa reduzir o período de involução uterina, atuando na contração uterina, auxiliando na redução do tamanho do útero, na limpeza do útero e no retorno da atividade ovariana. Portanto, o objetivo deste trabalho foi avaliar o efeito da aplicação de cloprostenol sódio no pós-parto sobre a involução uterina, dosagem hormonal de progesterona (P4) e 13,14 -di-hidro-15-ceto prostaglandina F2a (PGMF), sobre as variáveis histológicas, de expressão do mRNA do receptor de PGF2 α (FP), caspase - 3 (CASP - 3) e ciclo-oxigenase 2 (COX - 2), e também sobre a expressão proteica de FP e da COX - 2. Para isso, vacas Nelore multíparas (n=24) foram tratadas ou não com cloprostenol sódio nos dias 1 e 4 pós-parto (D0 = parto) e foram feitos exames ultrassonográficos, de palpação transretal, vaginoscopia e coleta de sangue em D1, D7, D14, D21, D28, D35 e D42 pós-parto (D0 = parto). Adicionalmente, biópsias endometriais foram colhidas com uma pinça de biópsia Yomann em D1, D7, D14, D28 e D42 para análise histológica, de expressão do mRNA por RT-PCR em tempo real e imunolocalização por imunohistoquímica. De acordo com os resultados, o cloprostenol sódio exerceu influência ($P < 0,05$) sobre a descarga cervical e expressão de mRNA da CASP-3. Para as variáveis relacionadas à dosagem hormonal, involução uterina, variáveis histológicas (glândulas endometriais e grau de inflamação), foi observado efeito apenas no momento e para as demais, nenhum tipo de influência foi notada. Com base nos resultados, conclui-se que o uso deste protocolo em vacas Nelore multíparas selecionadas geneticamente, com manejo adequado e saúde pode ser desnecessário para reduzir período pós-parto e pode promover despesa adicional. Contudo, novos estudos devem ser conduzidos utilizando o cloprostenol sódico ou outros análogos de PGF2 α em diferentes doses e momentos do pós-parto associados ou não a outros protocolos em zebuínos.

Palavras-chave: cloprostenol sódico, FP, involução uterina, puerpério.

MORAES, C.N. **Effect of cloprostenol sodium on uterine involution at puerperium of Nelore cows.** Botucatu, 2014. 149p. Dissertation (Magister Scientiae) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, São Paulo State University.

ABSTRACT

After calving, some physiological events occur in the uterus of cows to repair the modifications occurred during pregnancy and delivery. The use of cloprostenol sodium at postpartum period aims to reduce the period of uterine involution by acting at uterine contraction, helping in reducing uterine size, at uterine cleaning, and at the resumption of ovary activity. For this, the aim of this work was to evaluate the effect of the application of cloprostenol sodium on postpartum on uterine involution, hormonal dosage of progesterone (P4) and 13,14-dihydro-15-keto prostaglandin F2 α (PGMF), histological variables, mRNA expression of PGF2 α receptor (FP), caspase-3 (CASP-3) and cyclooxygenase 2 (COX-2), and also protein expression of FP and COX-2. For this, multiparous Nelore cows (n=24) were treated or not with cloprostenol sodium on days 1 and 4 postpartum (D0 = birth) and it was done ultrasound, transretal palpation, vaginoscopy exams and blood collection on D1, D7, D14, D21, D28, D35 and 42 postpartum (D0 = birth). Also, endometrial biopsies were collected with a Yomann biopsy instrument at D1, D7, D14, D28 and D42 for histological analysis, mRNA expression by qRT-PCR and immunolocalization by imunohistochemistry. According to the results, cloprostenol sodium had influence ($P < 0,05$) on cervical discharge and integrity and mRNA expression of CASP-3. For the variables related to hormonal dosage, uterine involution, histological variables (endometrial glands and degree of inflammation) there was observed effect only on moment and for the others, any kind of influence was noted. Based on the results, it can be conclude that the use of this protocol in multiparous Nelore cows with good genetic selection, adequate management and health can be unnecessary to reduce postpartum period and can promote additional spending. However, further studies should be conducted using cloprostenol or other analogs of PGF2 α at different doses and moments of postpartum, associated or not with other protocols in zebu.

Key words: cloprostenol sodium, FP, uterine involution, puerperium.

Capítulo 1

1. INTRODUÇÃO

A maior parte do rebanho bovino mundial se concentra nas regiões tropicais, com predominância do *Bos indicus* por sua grande capacidade de adaptação climática e de condições de manejo (BARUSELLI et al., 2004). Neste contexto, de acordo com dados do Departamento de Agricultura dos Estados Unidos (United States Department of Agriculture - USDA), o Brasil possui o segundo maior rebanho mundial e destaca-se na segunda posição em relação à produção de carne (LIVESTOCK, 2013).

A eficiência reprodutiva dos bovinos de corte é determinada pelo número de bezerros nascidos ou vacas servidas no ano, tendo em vista a manutenção da viabilidade econômica do setor (GREGORY, 2009). Portanto, quanto menor for o período puerperal, com a produção de um bezerro/vaca/ano e prenhez dentro de 83 dias pós-parto, mais rentável torna-se o setor (SENGER, 2003; CROWE, 2008).

O puerpério é definido como o período que se inicia imediatamente após o parto e persiste até o restabelecimento da condição fisiológica de uma fêmea não gestante. É caracterizado pela ocorrência de alterações fisiológicas no útero para prepará-lo para o recebimento de uma nova gestação (HAFEZ; HAFEZ, 2004; PRESTES; LANDIM-ALVARENGA, 2006).

A duração do puerpério é variável e pode ser afetada por fatores tais como: (1) produção leiteira, (2) amamentação, (3) nutrição, (4) escore de condição corporal e (5) condições uterinas (BALL; PETERS, 2004). Estes fatores podem prolongar a duração do puerpério e alterar a eficiência reprodutiva por diminuir a rentabilidade, aumentar os dias em aberto, intervalo entre partos, número de serviços/concepção, gastos com a reposição de animais e mão de obra especializada (KAFI; MIRZAEI, 2010).

Em bovinos de corte, tendo em vista a influência principalmente da amamentação e da nutrição na duração do puerpério, a utilização de protocolos hormonais, mudanças de manejo e o acompanhamento da evolução do período puerperal tornam-se estratégias válidas para a antecipação da atividade reprodutiva, visando o alcance de melhores índices reprodutivos.

As prostaglandinas são importantes reguladoras de diversos processos reprodutivos (TITHOF et al., 2007), sendo que a prostaglandina $F_{2\alpha}$

(PGF_{2α}) pode ser utilizada em bovinos na indução do ciclo estral (SHIRASUNA et al., 2012), na indução ao parto, no controle do ciclo estral (BALL; PETERS, 2004), na indução ao aborto (SENGER, 2003) e no período de involução uterina (FERNANDES et al., 2005; FERNANDES et al., 2012).

Os análogos de PGF_{2α}, como o cloprostenol sódico, são amplamente utilizado na reprodução animal e aplicados no período puerperal para indução ou sincronização do ciclo estral (TENHAGEN et al. 2000), para controlar a função luteal e reduzir o período de involução uterina (FERNANDES et al., 2012).

Neste contexto, o objetivo do presente estudo foi avaliar o efeito da administração do cloprostenol sódico sobre o processo de involução uterina de vacas multíparas Nelore (*Bos taurus indicus*) em diferentes momentos do puerpério, mediante avaliação histológica, ultrassonográfica, palpação transretal, vaginoscopia, dosagem hormonal e expressão gênica de Caspase-3 (CASP-3), FP e Ciclo-oxigenase -2 (COX-2), bem como imunolocalização dos últimos marcadores.

2. REVISÃO DE LITERATURA

2.1. Fisiologia e classificação do período puerperal

No período puerperal, uma série de eventos fisiológicos é necessária para que uma nova gestação possa ser estabelecida. Segundo Senger (2003), cinco principais eventos estão envolvidos após o parto, sendo estes: 1) a contração miometrial; 2) a expulsão do lóquio; 3) o reparo endometrial; 4) o retorno das funções ovarianas e por fim 5) a eliminação da contaminação bacteriana do trato reprodutivo. Tais processos devem culminar no crescimento de um folículo ovariano saudável com o desenvolvimento de um oócito competente, demonstração de cio, ovulação, fertilização e adesão uterina por um embrião viável (LEROY et al., 2008).

Uma classificação objetiva foi proposta por Prestes e Landim-Alvarenga (2006) que dividiram o período puerperal em duas etapas: a eliminação das secundinas fetais ou delivramento (1) e o puerpério propriamente dito ou involução uterina (2).

2.2. Fases do período puerperal

2.2.1 Eliminação das secundinas fetais ou delivramento

O delivramento compreende a fase que vai desde o final do parto até a eliminação das membranas fetais e ocorre em virtude do processo de contração do miométrio e perda da aderência placentária (PRESTES; LANDIM-ALVARENGA, 2006).

Imediatamente após o parto, o miométrio inicia uma série de contrações com a finalidade de facilitar a descarga de fluidos e debris do útero, minimizar o risco de hemorragia por compressão dos vasos uterinos e por fim reduzir o tamanho uterino (SENGER, 2003). As contrações que persistem por vários dias com redução gradativa na frequência, amplitude e duração (NOAKES et al., 2001), provocam diminuição no tamanho das fibras musculares, e consequente redução do tamanho uterino (PRESTES; LANDIM-ALVARENGA, 2006).

Células gigantes binucleadas aparecem entre os dias 18 e 20 e são encontradas apenas nas placentas dos ruminantes. Estas células são originadas a partir de células do trofoblasto e constituem cerca de 20% da porção fetal da placenta. Durante o desenvolvimento, essas células migram da porção fetal (córion) para a porção maternal da placenta (epitélio endometrial) e produzem lactógenos placentários, proteína da gestação, progesterona (P4) e estrógeno (SENGER, 2003). O cortisol fetal, responsável pelo início do parto, induz a migração das células gigantes binucleadas para o endométrio, e ao migrarem, degranulam e expressam os antígenos do complexo de histocompatibilidade principal da classe I (MHC-I) (WILTBANK, 2008).

Normalmente, as células do trofoblasto não expressam o MHC-1 antes de 120 dias de gestação. Porém, durante o terço final da gestação, as células da região entre os placentomas expressam o MHC-1. Entretanto, na região das criptas/vilos do placentoma e na porção do epitélio endometrial, não há a expressão do MHC- 1 (DAVIES, 2006). O reconhecimento materno do MHC-1 leva a uma resposta imune-inflamatória que contribui para a separação placentária (DAVIES et al., 2000; DAVIES, 2006).

A separação da placenta ocorre devido à resposta do sistema imune materno e pela produção de fatores ativadores dos neutrófilos dentro do epitélio caruncular. Além do mais, quando há completa maturação da unidade materno-fetal há estímulo para a queda de colágeno dentro do vilo coriônico, auxiliando assim na separação da carúncula e do cotilédone (MCNAUGHTON; MURRAY, 2009).

Em zebuínos, acredita-se que altas concentrações do hormônio relaxina podem contribuir para a rápida liberação da placenta no puerpério, indicando que a maturação da placenta nesses animais é mais precoce quando comparada aos taurinos (MARTINS et al., 2004).

2.2.2. Involução uterina ou puerpério propriamente dito

A involução uterina é um processo fisiológico de preparação do útero para uma nova gestação envolvendo contração física, necrose e liberação das carúnculas, regeneração do endométrio e eliminação bacteriana (GUSTAFSSON et al., 2004; HAFEZ; HAFEZ, 2004; PRESTES; LANDIM-

ALVARENGA, 2006; SHELDON et al., 2008). De acordo com levantamentos bibliográficos realizados por Martins e Borges (2011), o tempo de involução uterina em animais *Bos taurus indicus* pode variar de 15 a 41 dias dependendo da ordem de parição, sistemas de produção e região geográfica dos rebanhos.

A involução uterina ocorre em escala logarítmica decrescente com as principais mudanças (contração uterina e atrofia das miofibrilas) ocorrendo durante os primeiros dias do pós-parto (NOAKES et al., 2001).

Após a liberação do alantocórion, há vasoconstrição e necrose das carúnculas uterinas que após cinco dias do parto começam a perder a organização e a integridade celular e são liberadas até o 12º dia após o parto (SENGER, 2003; SHELDON et al., 2008). A liberação do tecido caruncular que sofreu necrose contribui para a redução considerável do tamanho uterino, uma vez que este tecido corresponde a quase metade do peso uterino (SHELDON et al., 2008).

O lóquio apresenta-se normalmente como um fluido viscoso de coloração amarelada a amarronzada, sem odor, composto por restos de fluidos fetais, tecido caruncular e sangue da ruptura do cordão umbilical. Logo após o parto, o útero abriga cerca de um a dois litros de lóquio, com maior descarga nos primeiros dois a três dias, o que tende a desaparecer ao redor do 14º, 18º dia do pós-parto (SHELDON et al., 2008), entretanto pode ser eliminado por até 30 dias (PRESTES; LANDIM-ALVARENGA, 2006), sendo que a quantidade varia individualmente (NOAKES et al., 2001).

A regeneração da porção epitelial do endométrio ocorre após o parto nas porções menos danificadas, sendo na área intercaruncular aproximadamente no 8º dia e na porção caruncular no 25º dia pós-parto (NOAKES et al., 2001). Contudo, as camadas uterinas mais internas apenas completam a regeneração ao redor dos 42- 56 dias do pós-parto (SHELDON et al., 2008).

2.3. Avaliação da involução uterina

A avaliação da involução uterina no puerpério é importante, pois permite a identificação e tratamento precoce de enfermidades que podem intervir na vida reprodutiva futura dos animais. Tal avaliação pode ser realizada

por meio de exames tais como palpação transretal, exame ultrassonográfico, biópsia uterina e avaliação da secreção cervical.

Com a associação dos exames, a involução uterina pode ser considerada completa quando os cornos uterinos encontram-se simétricos, alcançando o diâmetro mínimo (sem grandes alterações os últimos dois exames consecutivos), não apresentando conteúdo luminal, retornando a tonicidade, consistência e à posição pélvica (NAKAO et al., 1997).

2.3.1 Palpação transretal

A palpação transretal apresenta-se como um método prático, simples e de boa utilidade clínica para a avaliação do sistema reprodutivo durante o puerpério.

Mediante tal exame é possível a avaliação do tempo de retorno do útero ao posicionamento normal na cavidade pélvica, avaliação da recuperação do tamanho e simetria dos cornos, bem como avaliação da consistência uterina (GONZÁLEZ SÁNCHEZ et al., 1999). Também é possível a avaliação de estruturas ovarianas tais como folículos e corpo lúteo (CL) (HANZEN et al., 2000) visando o controle do retorno a ciclicidade.

2.3.2. Vaginoscopia

A avaliação da descarga cervical auxilia na investigação da origem da descarga, uma vez que a mesma pode ser de origem uterina, cervical ou vaginal (WILLIAMS et al., 2005).

A vaginoscopia pode ser adotada como um método diagnóstico auxiliar para a detecção de casos de endometrite, conforme citado por Azawi (2008). Entretanto, vale ressaltar que, a ausência da descarga vulvar não descarta a presença de inflamação uterina. A identificação vaginoscópica da descarga pode ser influenciada pela severidade da infecção, contrações miométriais, conformação perineal, condição de escore corporal, mudanças de postura, exercício e grau de abertura cervical (KASIMANICKAM et al., 2004).

A avaliação da descarga vulvar é feita de acordo com as características de coloração, odor e quantidade. Com relação à coloração, a

descarga pode ser classificada como: 1- translúcida, 2- com flocos de pus, 3- mucopurulento e 4 - purulento. Já com relação ao odor, a descarga pode ser inodora ou fétida (WILLIAMNS et al., 2005).

2.3.3 Exame Ultrassonográfico

O exame ultrassonográfico é um método prático e eficiente de acompanhamento do puerpério, uma vez que permite a avaliação de fluido intrauterino, tamanho cervical (MEIRA et al., 2012), diâmetro (SHELDON et al., 2003) e área do corno uterino (OKANO; TOMIZUKA, 1987), possibilitando assim a verificação do mecanismo de limpeza (KASIMANICKAM et al., 2004) e o diagnóstico de enfermidades puerperais tais como a endometrite subclínica (MEIRA et al., 2012; AZAWI, 2008).

Também, com o exame ultrassonográfico é possível o acompanhamento do desenvolvimento de estruturas ovarianas (CL e/ou folículos) (SHELDON et al., 2003).

2.3.4 Biópsia endometrial

A biópsia endometrial é uma técnica segura que permite o diagnóstico de enfermidades uterinas em vacas (MEIRA et al., 2012; KATAGIRI; TAKAHASHI, 2004), éguas (RODRIGUEZ et al., 2012; OVERBECK et al., 2013), cadelas (CHRISTENSEN et al., 2012; MIR et al., 2013), humanos (GULL et al. 2000; AL-JEFOUT et al., 2009) e búfalos (AZAWI; AL-SADI, 2010), pois permite a análise das diferentes porções do tecido uterino, tais como o epitélio endometrial, a lâmina própria, as glândulas endometriais e os vasos sanguíneos (MEIRA et al., 2012).

Durante o pós-parto, tal técnica também pode ser utilizada para avaliação das funções uterinas (CHAPWANYA et al., 2010) pela realização de uma análise histológica e de abordagens moleculares.

O período de obtenção da biópsia do tecido uterino durante o puerpério de bovinos é diversificado de acordo com a literatura, com relatos de colheitas nos dias 26 e 40 (BONNETT et al., 1991), 15, 30 e 60 (CHAPWANYA et al., 2010), 67 (GOSHEN et al., 2012), 1, 3, 5, 7, 11, 15, 19, 23, 27, 31, 45 e

60 (ARCHBALD et al., 1972) pós-parto. A colheita de material seriada visa a formação de um perfil histopatológico detalhado da involução e reparação uterina durante o pós- parto (BONNETT et al., 1991; OHTANI et al., 1993; CHAPWANYA et al., 2010).

Conforme citado por Chapwanya et al. (2010), a relutância na utilização da biópsia endometrial ainda pode ser encontrada devido ao equívoco pensamento da necessidade da realização de grande treinamento para a execução da técnica e interpretação dos resultados. Adicionalmente, de acordo com Meira et al. (2012), a técnica de biópsia endometrial é considerada um procedimento invasivo e que pode causar prejuízos ao desempenho reprodutivo. Entretanto, estudos que avaliaram as consequência das biópsias uterinas sob a fertilidade de bovinos, comprovaram que a realização de uma (GOSHEN et al., 2012) ou até mesmo três biópsias (KATAGIRI; TAKAHASHI, 2004; CHAPWANYA et al., 2010) não interferem na taxa de prenhez no ciclo subsequente (CHAPWANYA et al., 2010; GOSHEN et al., 2012) ou nos próximos dois ciclos após a realização da técnica (KATAGIRI; TAKAHASHI, 2004).

2.4 PGF_{2α}

As prostaglandinas pertencem à classe de prostanóides, os quais agrupam os metabólitos do ácido aracdônico produzidos a partir da ação da ciclo-oxigenase (COX) sobre o ácido aracdônico (AA), sendo estes prostanóides as prostaglandinas, tromboxano A e prostaciclina (BARROS; DI STASI, 2012).

Para que ocorra a produção das diferentes prostaglandinas, é necessária a catalização pela enzima fosfolipase A₂ (PLA₂), a qual hidroliza o AA liberado da membrana dos fosfolípídeos das células (TITHOF et al., 2007). A COX, por sua vez, cataliza a oxigenação do AA, dando origem à prostaglandina G₂ (PGG₂) e posteriormente à PGH₂ por ação da hidroperoxidase a qual se torna substrato para a formação dos prostanóides prostaglandina G₂ (PGD₂), prostaglandina E₂ (PGE₂), PGF_{2α}, tromboxano A₂, (TXA₂) e prostaglandina I₂ (PGI₂) (Figura 1) (BARROS; DI STASI, 2012).

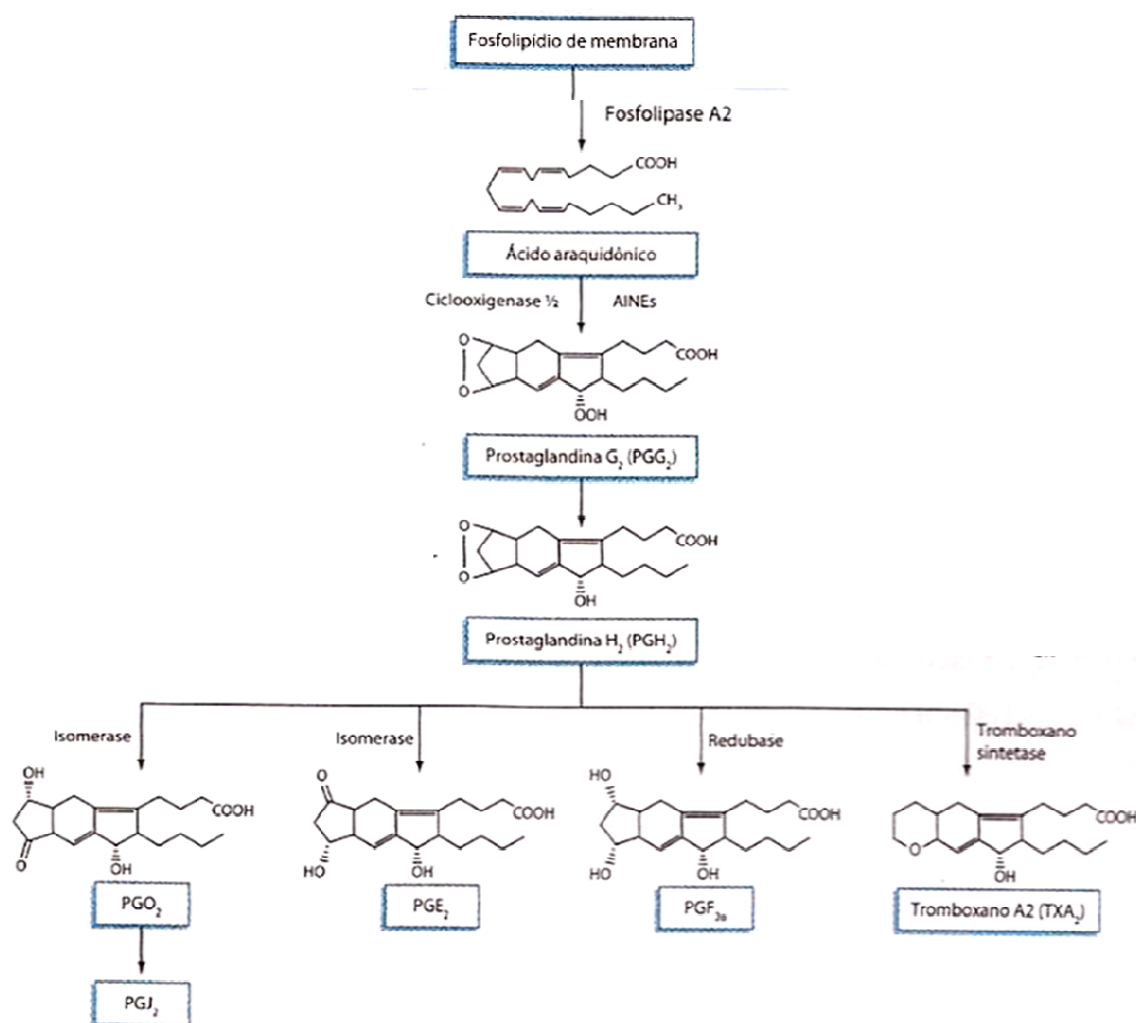


FIGURA 1: Via de biossíntese dos prostanóides. Fonte: Adaptado de Barros; Di Stasi, 2012.

A $\text{PGF}_{2\alpha}$ é metabolizada pelos rins, fígado e pulmões, sendo que aproximadamente 98% é metabolizada em primeira passagem pelos pulmões (GUILBAULT et al., 1984; SHRESTHA et al., 2010, BARROS; DI STASI, 2012). A meia vida de seu metabólito, conhecido como PGFM (13,14-dihidro-15-ceto prostaglandina $\text{F}_{2\alpha}$) é bem maior do que seus precursores (BARROS; DI STASE, 2012), sendo que em estudo, apresentou período de meia vida média de 18 minutos (GUILBAULT et al., 1984). A mensuração da PGFM no sangue apresenta-se como um reflexo da concentração de $\text{PGF}_{2\alpha}$ (SENGER, 2003).

As prostaglandinas são produzidas por todo o corpo e estão presentes em diversas atividades patológicas e fisiológicas, exercendo efeito nas funções reprodutivas durante o parto e durante o ciclo estral, envolvendo

os fenômenos de luteólise e ovulação (BARROS; DI STASI, 2012; SHRESTHA et al., 2012).

Durante o processo de luteólise, a $\text{PGF}_{2\alpha}$ produzida pelo útero é transportada ao ovário ipsilateral por meio do mecanismo vascular de contracorrente, no qual a prostaglandina que se encontra em altas concentrações na veia uterina e vasos linfáticos uterinos é transportada por difusão passiva para a artéria ovariana, uma vez que artéria e veia ficam em íntimo contato (SENGER, 2003).

Dentre os análogos da $\text{PGF}_{2\alpha}$ estão o fenprostalene, dinoprostetromehamina, cloprostenol, dl-cloprostenol e d-cloprostenol e são amplamente utilizados na reprodução de bovinos na sincronização de estro, agindo como agente luteolítico, como terapêutico em casos de endometrite ou no processo de involução uterina prolongada (TENHAGEN et al., 2000; NAKAO et al., 2007; BARROS; DI STASI, 2012; FERNANDES et al., 2012).

A importância da $\text{PGF}_{2\alpha}$ no período puerperal está relacionada com o processo de involução uterina, provocando uma aceleração na involução (FERNANDES et al., 2005; FERNANDES; FIGUEIREDO, 2007, FERNANDES et al., 2012). O útero apresenta-se como fonte primária de produção da $\text{PGF}_{2\alpha}$, sendo o tecido caruncular um sítio ativo de sua síntese e metabolização. A liberação de altas quantidades de $\text{PGF}_{2\alpha}$ pelo tecido endometrial durante o período de pós-parto, favorece a involução uterina por estimular contrações miométriais (GUILBAULT et al., 1984).

Em estudo, foi comprovado que a forma d-cloprostenol apresenta afinidade de ligação aos receptores de $\text{PGF}_{2\alpha}$ similar a forma original de $\text{PGF}_{2\alpha}$, enquanto que a mistura racêmica apresenta uma menor afinidade (Re et al., 1994)

2.4.1 FP

Já foram identificados receptores específicos para todos os prostanóides, sendo eles DP1 e DP2 para PGD_2 , EP1, EP2, EP3, EP4 para PGE_2 , FP para $\text{PGF}_{2\alpha}$, TP_α e TP_δ para TXA_2 e IP para PGI_2 (BARROS; DI STASI, 2012).

O FP é um receptor de contração, que media a liberação do cálcio (Ca^{+}) intracelular, provocando consequentemente a contração muscular, (NARUMIYA et al., 1999) e exerce sua função por meio de receptores acoplados à proteína G (BARROS, DI STASI, 2012).

Os receptores acoplados a proteína G são cadeias polipeptídicas únicas que transpassam sete vezes a membrana plasmática na forma de α hélices, com a porção aminoterminal extracelular e a carboxiterminal intracelular. A proteína G fica adjacente ao receptor e apresenta três subunidades (α , β e γ). Quando o receptor é ativado, a subunidade α da proteína G se desliga de suas outras subunidades e se liga a uma molécula de guanosina trifosfato (GTP) (Figura 2). Por fim, ocorre a ativação da enzima fosfolipase C, a qual hidroliza os fosfolípides fosfatidilinositol difosfato (PIP_2), formando o diacilglicerol (DAG) e o inositol trifosfato (IP_3). O DAG é responsável por ativar a proteína quinase C (PKC) e o IP_3 por promover a liberação de Ca^{+} intracelular por meio da ligação a seus recetores na membrana das organelas (Figura 3) (BARROS; DI STASI, 2012).

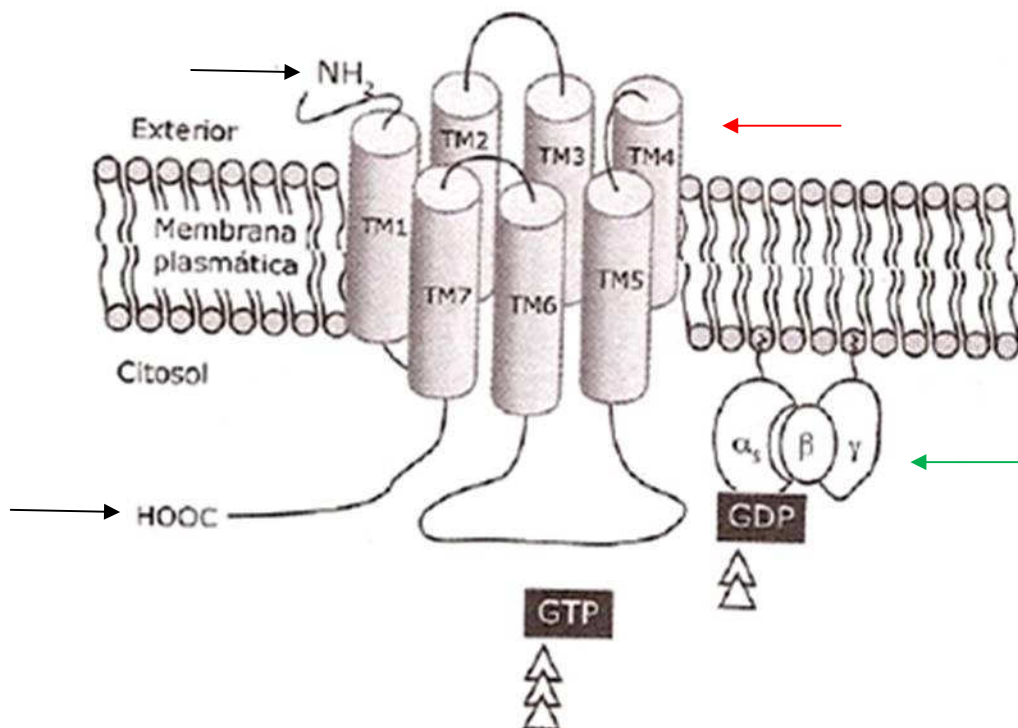


FIGURA 2: Estrutura esquemática do receptor acoplado à proteína G. Observam-se os sete domínios transmembrana em α -hélices (TM1, TM2, TM4, TM4, TM5, TM6, TM7) (seta vermelha); as subunidades da

proteína G (seta verde); e os domínios intracelular e extracelular (seta preta) Fonte: Adaptado de Barros; Di Stasi, 2012.

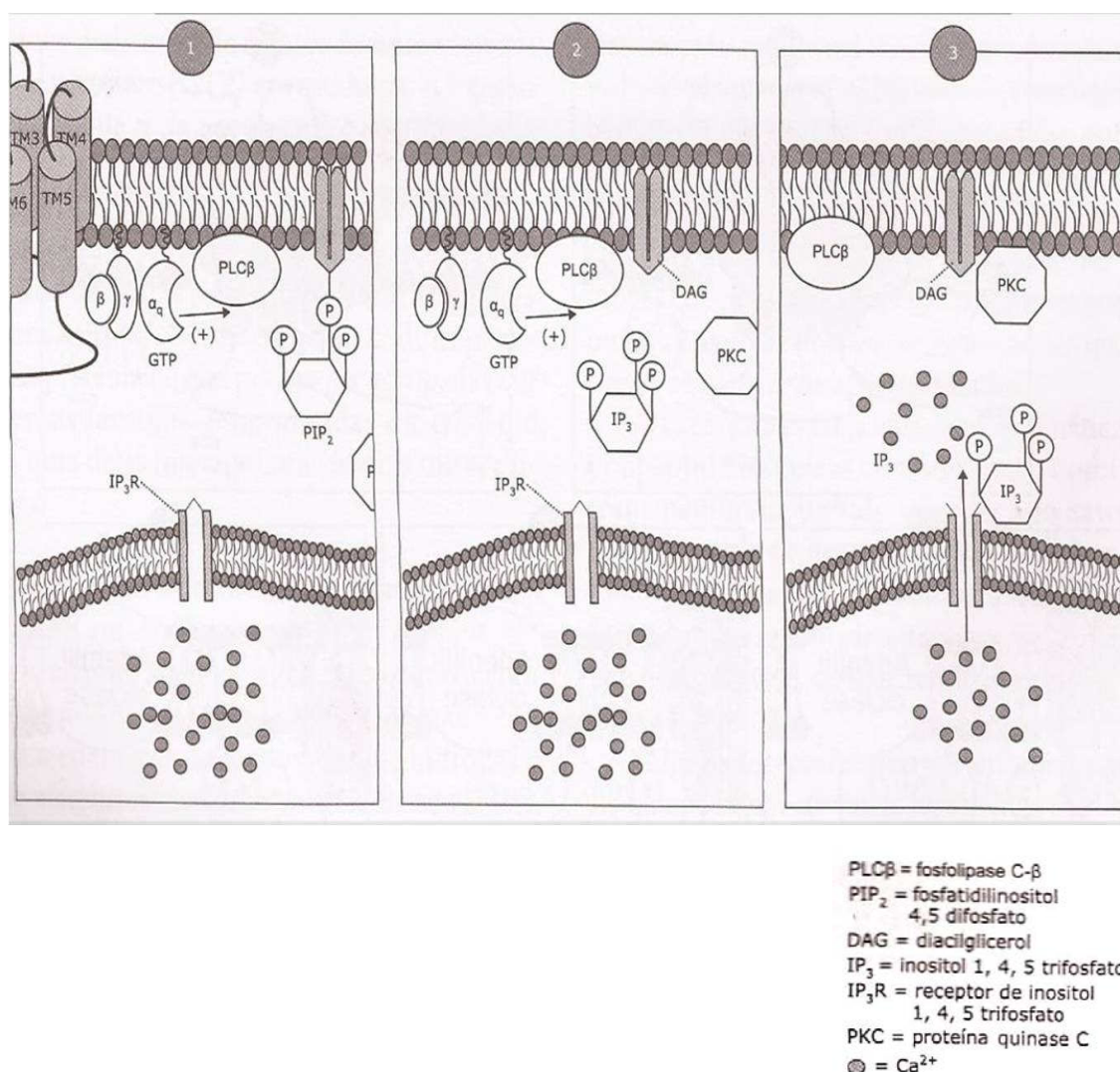


FIGURA 3: Receptor acoplado à proteína G e sua forma de ativação. Fonte: Adaptado de Barros; Di Stasi, 2012.

Portanto, quando a PGF_{2α} se liga a seu receptor, exerce efeito sobre a fosfolipase C, a qual aumenta a formação de DAG e IP₃, ocasionando a ativação de proteínas quinases e aumento do Ca⁺ intracelular (BARROS; DI STASI, 2012).

O FP, em sua forma completa, apresenta sete domínios transmembrana (Sakamoto et al., 1995) e em bovinos é altamente expresso em células luteais, (Sakamoto et al., 1994, Sakamoto et al., 1995; Ishii; Sakamoto, 2001; Weems et al., 2012, Shirasuna et al., 2012) células foliculares, (Bridges;

Fortune, 2007) membrana fetais e tecido uterino durante o período gestacional (Arosh et al., 2004, Wehbrink et al., 2008).

Em ovinos, o FP apresenta duas isoformas (FP_A e FP_B), geradas por *splicing* alternativo do terminal C em apenas um aminoácido (Kristen et al., 1997). A isoforma FP_B apresenta-se idêntica à isoforma FP_A, mas difere nos últimos nove aminoácidos do terminal carboxil, e em FP_B um par de introns está ausente (Pierce et al., 1997).

Do receptor completo de PGF_{2α} no corpo lúteo de bovinos, foram encontradas isoformas, sendo que cada uma carrega uma cauda de carboxil específica causada por *splicing* alternativo. Dentre elas, estão FP_α, FP_β, FP_γ, FP_δ, FP_ε e FP_ζ, sendo que de acordo com a localização do *splicing*, elas podem ser divididas em dois grupos sendo a FP_α, FP_β, FP_γ do tipo 1 e com os sete domínios transmembrana intactos, mas com diferença na porção C terminal. Já os receptores FP_ε e FP_ζ são do tipo 2 e apresentam-se como uma forma truncada, sem o VI domínio transmembrana e sem um segmento intracelular C terminal, fato que dificulta sua atividade (Sakamoto et al., 2002).

Em estudo para avaliação da forma completa do receptor de FP (também denominado PTGFR) e das isoformas PTGFR_ζ e PTGFR_α, Shirasuna et al. (2012) observaram o aumento das três formas estudadas do começo ao meio da fase luteal, sendo que a expressão da forma completa foi três e 20 vezes maior que a expressão da PTGFR_α e PTGFR_ζ, respectivamente.

A existência de *splicing* alternativo pode ocasionar em alterações na função dos receptores no que diz respeito à desensitização e/ ou internalização, mas não afeta a especificidade de ligação (Breyer et al., 2001).

2.5 Retorno à ciclicidade

O escore de condição corporal, a presença do bezerro, do touro e o reestabelecimento da concentração de hormônio luteinizante (LH) tem sido relatados como sendo fatores que influenciam o atraso da primeira ovulação pós-parto (EMERICK et al., 2009).

Mesmo sendo possível a indução da ovulação no pós-parto, os efeitos do balanço energético negativo (BEN) associado à amamentação e à

carência nutricional apresentam-se como os principais fatores determinantes da baixa fertilidade em animais criados extensivamente (MORAES et al., 2009).

Para que ocorra o retorno regular dos ciclos ovarianos é necessária a recuperação do eixo reprodutivo central (hipotálamo e hipófise). Os mecanismos que controlam o reinício da secreção de LH que interferem no desenvolvimento folicular e ovulação incluem principalmente: 1) a recuperação da hipófise dos efeitos da alta concentração de estradiol produzido pela placenta; 2) a condição nutricional e 3) a amamentação (WILLIAMS; AMSTALDEN, 2010).

Como resultado da ausência ou das baixas concentrações de gonadotrofinas, o ovário permanece relativamente em queiscência e a vaca adentra um período de anestro (NOAKES et al., 2001).

2.5.1 Desenvolvimento folicular

A competência reprodutiva do bovino de corte no pós-parto é dependente da interação do eixo hipotálamo-hipófise-ovário. Fisiologicamente, a liberação do hormônio liberador de gonadotrofina (GnRH) pelo hipotálamo induz a secreção de gonadotrofinas (hormônio folículo estimulante (FSH) e LH) pela hipófise. O início do crescimento e desenvolvimento folicular é mediado pelo FSH, mas a maturação final é realizada em virtude da secreção pulsátil de LH a cada uma ou duas horas. Uma vez que o estradiol produzido pelo folículo em desenvolvimento causa uma onda de alta amplitude de LH, a ovulação deste folículo ocorre (HESS et al., 2005).

Após o parto, as concentrações de hormônios esteróides reduzem a níveis basais devido à ausência de crescimento folicular durante a gestação (YAVAS; WALTON, 2000b). Entretanto, há aumento dos níveis plasmáticos de FSH entre o 3º e 5º dia pós-parto, induzindo à emergência da primeira onda folicular pós-parto entre o 7º e 10º dia (CROWE, 2008).

O desenvolvimento do folículo dominante (FD) inicia-se com o recrutamento de um *pool* de folículos dependentes de gonadotrofinas. Deste *pool*, um único folículo com mais de 10 mm de diâmetro é selecionado, torna-se dominante e suprime o crescimento de outros folículos. Em condições

favoráveis o FD ou ovula (na ausência do CL) ou torna-se atrésico (na presença do CL) (ROCHE et al., 1992).

Em bovinos de corte, o folículo da primeira onda pós-parto geralmente não ovula e entra em atresia devido a insuficientes pulsos de LH associado aos níveis nutricionais ou amamentação (CROWE, 2008). A baixa frequência de LH, responsável pela maturação folicular final, ocorre em virtude da redução nos pulsos de GnRH e dos estoques de LH na hipófise, quadro o qual é restabelecido entre o 15º e 30º dia do pós-parto (YAVAS; WALTON, 2000b). A primeira ovulação em bovinos normalmente é silenciosa (sem sinais de estro) e seguida por ciclo curto em 70% dos animais (CROWE, 2008). O ciclo curto ocorre, pois a luteólise é causada pela liberação de $\text{PGF}_{2\alpha}$ pelo endométrio em resposta à liberação de ocitocina tanto pela neurohipófise quanto pelo corpo lúteo (CLARO JUNIOR et al., 2010).

O FD somente irá ovular quando os pulsos de LH forem de 1 pulso/40 min (ROCHE et al., 1992). Animais com boa condição corporal, geralmente ovulam com aproximadamente 30 dias enquanto animais com baixa condição corpórea ovulam entre 70 – 100 dias (CROWE, 2008).

2.6 Principais fatores que alteram o retorno à ciclicidade

2.6.1 Anestro

O anestro pós-parto é o período em que a fêmea não exibe ciclos estrais regulares principalmente em virtude da inadequada liberação de gonadotrofinas (SENGER, 2003; OLIVEIRA et al., 2010).

Durante esse período, apesar do desenvolvimento folicular, há falha na ovulação devido a não maturação folicular. A nutrição e amamentação são os principais agravantes neste período uma vez que exercem influência no eixo hipotalâmico-hipofisário-gonadal (MONTIEL; AHUJA, 2005).

Esse período, considerado o maior causador de infertilidade em bovinos, é visto como um evento fisiológico após o parto, todavia torna-se patológico quando excede o tempo esperado (GREGORY, 2009). É considerado o maior fator negativo que afeta a fertilidade pós-parto por aumentar o intervalo entre o parto e o primeiro cio, reduzir a produção do

bezerro e consequentemente gerar perdas econômicas (YAVAS; WALTON, 2000b; BRAUNER et al., 2009).

Embora grande parte dos quadros de anestro possam se resolver sem nenhum tratamento, prolongados intervalos pós-partos são observados e geram prejuízos econômicos (KOZICKI, 1998).

2.6.2 Amamentação

Sabe-se que a ovulação e o comportamento de estro podem ser atrasados tanto em bovinos de corte quanto bovinos de leite em detrimento da amamentação (BALL; PETERS, 2004).

Por quase meio século acreditou-se que a amamentação representava a principal causa de anovulação no pós-parto. Entretanto, com diversos estudos, ficou evidenciado que animais que amamentavam, animais com denervação do teto, animais mastectomizados e animais que possuíam apenas contato visual com os bezerros não tiveram o anestro pós-parto reduzido (Revisado por WILLIAMS; ALMSTALDEN, 2010). Com isso, ficou comprovado que não apenas o fato da amamentação, mas também a percepção visual, auditiva e olfatória influenciam na produção de GnRH pelo hipotálamo (SENGER, 2003), reduzindo os níveis de LH. Segundo Lamb et al. (1999b), para que a amamentação interfira na ovulação, é necessário que a vaca primeiramente reconheça e crie laços com sua cria e que a amamentação ocorra pelo menos duas vezes ao dia.

Adicionalmente, acredita-se que parte da ação inibitória da amamentação esteja também correlacionada com os opióides endógenos, especialmente às β endorfinas as quais diminuem a pulsatilidade de GnRH e consequentemente de LH (OLIVEIRA et al., 2010).

A duração do anestro lactacional é influenciada pela frequência do número de mamadas. Quando o número de mamadas é de 3 a 20/dia, a amplitude e a frequência do pulso de LH são muito baixas e o animal permanece em anestro. Conforme ocorre a redução do número de mamadas por dia (duas ou menos vezes ao dia), a amplitude e pulso de LH aumentam e a vaca retorna a ciclicidade, ou então, com a remoção dos bezerros, há aumento nos níveis de LH entre dois a três dias (SENGER, 2003).

2.6.3 Condição Corporal

A nutrição é firmemente caracterizada como um fator que interfere na duração do anestro pós-parto. Seus efeitos variam de acordo com a quantidade e qualidade da alimentação, reservas nutricionais e competição de nutrientes para outras funções fisiológicas que não a reprodução (SHORT et al., 1990).

O escore de condição corporal (ECC) – ferramenta que monitora o status nutricional da vaca – embora seja subjetivo, é de fácil realização e não requer equipamentos especializados (BALL; PETERS, 2004). Idealiza-se que seja mantido escores entre 2,75 a 3,00 no parto (escala de 1 a 5) (ROCHE, 2006) com perda de menos de 0,5 unidade de ECC no pós-parto (CROWE, 2008). Isso prevenirá o BEN prolongado e a ocorrência de enfermidades metabólicas (SHRESTHA et al., 2004).

Conforme revisado por Emerick et al. (2009), a manutenção da condição corporal das vacas no pré-parto ainda é considerado como a ferramenta mais econômica para minimizar os custos de restabelecimento corpóreo, isso pois, no pós-parto o animal encontra-se em um processo catabólico, o que exige mais custos para o restabelecimento. Acredita-se que os animais que parem com bom estado de condição corporal apresentam menor intervalo parto-primeira ovulação, menor anestro pós-parto e menor intervalo entre os partos.

Em animais leiteiros, a demanda nutricional aumenta rapidamente após o parto pela necessidade de produção leiteira, resultando em BEN. Em bovinos de corte, o BEN não traz consequências tão marcantes, tendo em vista que a grande quantidade de leite produzida pelos bovinos de leite não é condizente com a realidade produzida pelos bovinos de corte (LAMB, 1999a; BUTLER, 2000).

A má nutrição em bovinos de corte pode atrasar a ovulação do folículo dominante devido à inibição dos pulsos de LH, baixa concentração de glicose, fator de crescimento semelhante à insulina tipo 1 (IGF-1) e insulina e que em conjunto restringem a produção de estrógeno pelo folículo (BUTLER, 2000). Animais mantidos em pastagem de melhor qualidade durante o período pré e pós-parto apresentam melhores concentrações de insulina, glicose, IGF-1

e leptina na circulação durante o período pós-parto, o que foi associado com o retorno a ciclicidade mais precocemente (SAMADI et al., 2013).

2.7 Estratégias para acelerar o primeiro estro e ovulação no pós-parto

Vários métodos hormonais ou não hormonais foram descritos para antecipar o retorno à ciclicidade no pós-parto. Dentre os métodos não hormonais pode-se citar o desmame dos bezerros (completo, temporário ou parcial) e a exposição ao touro; e dentre os métodos hormonais o GnRH, gonadotrofinas (gonadotrofina coriônica equina (eCG), FSH, gonadotrofina coriônica humana (hCG)), esteróides (estrógeno e P4) e prostaglandinas (PGF_{2α}) (YAVAS; WALTON, 2000a; FERNANDES et al., 2012).

Esses métodos podem ser utilizados separadamente ou em conjunto e preferencialmente em animais com condição corpórea superior a três, em uma escala de um a cinco (MORAES et al., 2009).

2.7.1 Métodos não hormonais

2.7.1.1. Manejo de amamentação

Tendo em vista os efeitos negativos da amamentação sobre o pós-parto (WILLIAMS & AMSTALDEN, 2010), podem-se fazer o uso de estratégias tais como desmame precoce; remoção temporária do bezerro ou amamentação controlada, para minimização destes efeitos.

2.7.1.1.1 Desmame precoce

O desmame precoce consiste na separação da vaca e do bezerro com 70 (LOBATO et al., 2000) a 90 dias de idade (VAZ et al., 2010) e tem por vantagem reduzir a demanda energética da vaca, aumentar os índices corpóreos de fêmeas com baixa condição corporal (PELEGRINNI; LOPES, 2011), reduzir o período de espera e aumentar as taxas de prenhez (PENCAI et al., 2011).

Experimentalmente observou-se que as vacas submetidas ao desmame precoce com 100 dias pós-parto apresentaram taxas de prenhez superiores (60%) aos animais submetidos ao desmame convencional (180 dias) (12%) (PELEGRINNI; LOPES, 2011).

Tal manejo pode ser empregado em condições de seca ou quando é mais viável alimentar os bezerros do que as fêmeas em lactação (WILLIAMS; AMSTALDEN, 2010; PENCAI et al., 2011).

2.7.1.1.2 Remoção temporária do bezerro

A remoção temporária do bezerro consiste na separação do bezerro da vaca durante 48, 72 ou 96 horas (MACIEL et al., 2001; SILVEIRA et al., 2010) e apresenta-se como uma técnica de fácil adoção, sem custos, porém que exige grande manejo (SILVEIRA et al., 2010).

De acordo com Williams e Amstalden (2010), a remoção do bezerro por 48 horas somente deve ser realizada quando se puder associar um tratamento de sincronização que induza à ovulação em bovinos anovulatórios. Isso, pois, em estudo Maciel et al. (2001) evidenciaram que somente a realização do desmame durante 96 horas não é suficiente para induzir a ciclicidade estral e elevar os índices de prenhez a taxas satisfatórias, sendo assim importante a associação do tratamento hormonal.

2.7.1.1.3 Amamentação controlada

A amamentação controlada pode ser realizada separando-se os bezerros das mães e permitindo a mamada por períodos controlados (20-30 minutos/dia) com contato visual, auditivo ou não (STAGG et al., 1998).

Apesar disso, animais que não tiveram contato visual e auditivo com suas crias apresentaram primeira ovulação pós-parto mais precocemente que animais com contato visual, uma vez que há aumento na frequência dos pulsos de LH quando houve redução na frequência de mamada e ausência total de contato (STAGG et al., 1998).

Pencai et al. (2011) observaram incidência de folículos mais precocemente (na segunda semana do tratamento) e maior taxa de prenhez

em animais mestiços de corte que sofreram amamentação controlada (mamada uma vez ao dia/30 minutos) e desmame precoce (separação definitiva dos bezerros com 70 dias) do que em animais com cria ao pé constantemente.

2.7.2 Exposição ao touro

Acredita-se que a exposição ao touro exerça efeito no pós-parto por atuar no eixo hipotálamo-hipófise-ovário (FIOL; UNGERFELD, 2012) e induzir uma maior produção de LH (RABASSA et al., 2007). O efeito que o macho exerce sobre a fêmea pode ser mediado pela estimulação genital e produção de ferormônios (FIOL; UNGERFELD, 2012).

Menores intervalos de parição e retorno à atividade cíclica foram observados em vacas expostas ao touro pelo período de 12 horas, a partir do 51º dia pós-parto por um período de 45 dias, em comparação com a não exposição ou exposição por 6 horas. Pressupõe-se que para ocorrer a resposta de uma vaca ao efeito bioestimulatório dos touros, as fêmeas devem ser expostas por um período de 12 horas por dia para captação direta do feromônio (TAUCK et al., 2010).

Em outro estudo, Miller e Ungerfeld (2008) evidenciaram que a troca semanal dos touros durante o período de exposição encurtou o período pós-parto quando comparado com fêmeas que permaneceram com os mesmos touros. Acredita-se que a troca dos touros provoque um maior contato entre os animais, tornando-se um gatilho para a ovulação.

Apesar de apresentar como uma técnica de baixo custo e livre da utilização de hormônios (TAUCK et al., 2010), seus resultados são inconsistentes e variáveis devido a fatores tais como: relação touro/vaca, condição corporal ao parto, período do pós-parto em que ocorre a exposição (YAVAS; WALTON, 2000a), duração da exposição, tipo de manejo e características do macho (FIOL; UNGERFELD, 2012).

2.7.3 Métodos hormonais

O grande desafio do uso da inseminação artificial em tempo fixo (IATF) em bovinos de corte é o anestro pós-parto em animais que amamentam. Frente a isso, com o objetivo de aumentar o uso da inseminação artificial (SCHNEIDER et al., 2010), realiza-se a associação hormonal em animais que apresentem condições metabólicas condizentes com o retorno à ciclicidade (MACIEL et al., 2001).

Entretanto, o atraso no retorno à ciclicidade embora permita a utilização do uso de protocolos hormonais (IATF), pode também ocasionar grandes perdas econômicas pelo não alcance de produção de bezerros anualmente.

2.7.3.1 GnRH

Tendo em vista que a principal causa da não ovulação no pós-parto é a baixa frequência na pulsatilidade de GnRH, sua utilização exógena apresenta-se como uma alternativa para antecipar a ovulação (ROCHE et al., 1992).

O uso do GnRH estimula a liberação de FSH e LH. A resposta da hipófise ao GnRH aumenta no pós-parto, sendo maior por volta do 20º a 30º dia do pós-parto (BALL; PETERS, 2004). Com isso, a utilização de GnRH atua tanto no crescimento folicular quanto na indução da ovulação (RABASSA et al., 2007). Entretanto, os folículos necessitam de dois a três dias de aumento nas concentrações plasmáticas de LH para maturação folicular antes da ovulação. Portanto, o folículo somente ovulará se estiver no correto estágio de desenvolvimento (BALL; PETERS, 2004).

Quando utilizado em associação com a P4, caso administrado juntamente com a inserção de dispositivo intravaginal de P4, o GnRH inicia a emergência da onda folicular e a P4 previne a manifestação de estro e a ovulação. Caso o GnRH seja utilizado após a remoção do dispositivo de P4, o mesmo induz a ovulação (Revisado por WHEATON; LAMB, 2007).

2.7.2.2 Gonadrofinas

A utilização de FSH e eCG possui potencial para estimular o crescimento folicular e a utilização do hCG induz a ovulação folicular no pós-parto. O uso de FSH não proporciona a maturação final do folículo, pois para isso é necessário o aumento na frequência de LH. Por outro lado, com o uso do hCG, pode ocorrer a ovulação de um folículo que não sofreu o processo de maturação final (YAVAS; WALTON, 2000a; ROSSA et al., 2009).

Experimentalmente comprovou-se que animais com menos de 45 dias pós-parto quando submetidos ao tratamento com P4, valerato de estradiol (VE) e eCG apresentaram taxas de concepção maiores em comparação ao grupo sem tratamento com eCG. Isso ocorreu provavelmente, pois no momento da aplicação de eCG o FD encontrava-se em seu 4 ou 5 dia pós-emergência, e o eCG induziu o crescimento final do folículo em virtude do maior número de receptores para o LH e FSH (ROSSA et al., 2009).

Adicionalmente, a administração de hCG 7 dias após a inseminação artificial promoveu a formação de corpos lúteos acessórios, proporcionando maiores concentrações de progesterona até o 33º dia de gestação, e consequentemente melhores índices de prenhez (DAHLEN et al., 2010).

Portanto, para o alcance da ovulação, é necessário que ocorra uma associação hormonal, e não apenas o uso de hormônios isoladamente.

2.7.2.3 Esteróides

O uso de protocolos hormonais tem a finalidade de proporcionar melhor desenvolvimento hormonal e ovulação. Protocolos hormonais existem, tendo como principal base a associação de progestágenos e estrógenos (ANDRIGA et al., 2013; SALES et al., 2012).

A P4 suprime a liberação de LH e a queda da P4 (retirada do implante intravaginal de P4) resulta em um aumento gradual de LH, e na ocorrência da onda pré-ovulatória de LH após 48 horas (BALL; PETERS, 2004). Já o estrógeno pode ser utilizado devido ao seu *feed back* positivo sobre a liberação de FSH e LH (YAVAS; WALTON, 2000a).

2.7.2.4 PGF_{2α}

A prostaglandina e seus análogos são amplamente utilizados no período pós-parto com a finalidade de aceleração o processo de involução uterina por favorecer as contrações miométriais (FERNANDES et al., 2005; FERNANDES et al., 2012).

Também, a ovulação pode ser induzida com aplicações exógenas de PGF_{2α} ou de um de seus análogos durante a fase luteal do ciclo. Com a luteólise, ocorre uma redução nas concentrações de progesterona, seguida por um aumento na secreção de gonadotrofinas e estradiol 17β, ocasionando uma onda pré-ovulatória e eventual ovulação (BALL & PETERS, 2004).

Equinos são mais sensíveis à aplicação da PGF_{2α} que bovinos. Isso, pois a dose recomendada para cavalos é cinco vezes mais baixa que para bovinos, correspondendo a 5 mg de PGF_{2α} para equinos e 25 mg de PGF_{2α} para bovinos (SHRESTHA et al., 2010).

Capítulo 2

**Artigo Científico nas normas e a ser submetido ao periódico
“Reproduction, Fertility and Development”**

Normas para publicação no periódico:

“Reproduction, Fertility and Development”

REPRODUCTION, FERTILITY AND DEVELOPMENT

Instructions to Authors

Reproduction, Fertility and Development is an international journal for the publication of original work, review and comment in the fields of reproductive biology, reproductive technologies and developmental biology.

Submission of a paper implies that the results reported have not been published and are not being considered for publication elsewhere. Abstracts from conferences would not normally be regarded as publications, but where material has been disseminated in report form the Editor should be consulted. The Journal assumes that all authors of a multi-authored paper agree to its submission. The Journal will use its best endeavours to ensure that work published is that of the named authors except where acknowledged and, through its reviewing procedures, that any published results and conclusions are consistent with the primary data. It takes no responsibility for fraud or inaccuracy on the part of the authors. All papers are refereed to international standards.

Scope

Reproduction, Fertility and Development publishes original and significant contributions to the fields of reproduction and developmental biology in humans, domestic animals and wildlife. Papers are encouraged on the scientific aspects of:

- reproductive technologies and cloning
- genetics in reproduction
- gametogenesis
- fertilisation
- early embryonic development
- fetal physiology and maternal–fetal interactions
- maternal reproductive physiology including lactation
- andrology
- reproductive endocrinology, immunology and cell biology
- reproductive behaviour

Critical feature articles that adequately summarise work in a particular area of these fields and indicate fruitful lines of research are also welcomed, as are viewpoint articles,

reviews, short communications and comments on published papers. Comments should be confined to the substance of the paper; the authors of the paper referred to will be offered the opportunity to respond.

Submission of manuscripts

Please submit your manuscript using our Online Submission and Peer Review system OSPREY (<http://publish.csiro.au/osprey>), which can be reached directly through this link or from the icon on the Journal's homepage. Choose Reproduction, Fertility and Development from the drop-down list and, if a first time user, log in via the New User box, or use your existing username and password to log in. Choose 'Submit manuscript' from the menu on the left side of the screen and then follow the steps, providing the information requested under each step.

A covering letter must accompany the submission and should include the name, address, fax and telephone numbers, and email address of the corresponding author. The letter should also contain a statement justifying why the work should be considered for publication in the Journal, and that the manuscript has not been published or simultaneously submitted for publication elsewhere. Suggestions of possible referees are welcome. A completed Licence to Publish form (which you will be asked to download from the website as part of the submission process) should be faxed or mailed to the Journal as soon as possible after submission.

Format of manuscripts

Papers must be typed with double- or 1.5-line spacing throughout and with a margin of at least 3 cm on the left-hand side. Line numbers should be included. All pages of the manuscript must also be numbered consecutively, including those carrying references, tables and figure captions, all of which are to be placed after the text. Illustrations, both line drawings and photographs, are to be numbered as figures in a common sequence, and each must be referred to in the text. Figures that are of the same quality as those to be reproduced in the published paper must be included at the end of the electronic file or submitted as separate electronic files in correct order and must be clearly named and numbered (e.g. Smith et al_Fig1).

Authors are advised to note the style of headings, tables and illustrations exemplified in the latest issues of the Journal. Strict observance of these and the requirements listed under 'Preparation of manuscripts' will shorten the interval between submission and publication.

Poorly prepared or unnecessarily lengthy manuscripts have less prospect of being accepted. Poor quality figures will be returned for correction and will delay acceptance.

Rapid and short communications

The Journal publishes preliminary communications of results that are of special significance or of current and extreme interest. Such papers should yield no more than four pages when printed, including illustrations, tables and references, and should conform with every aspect of the Notice to Authors. An article submitted as a Rapid Communication will be subject to accelerated, but very strict, refereeing and additional assessment by a member of the Editorial Board. The article should be accompanied by a statement explaining why it merits urgent publication.

Review articles

The Journal welcomes review articles and they should be submitted in the same way as research papers. They should be formatted as simply as possible, using no more than three levels of heading and normal or body text style for the main text. Summary diagrams should be used where possible to reduce the amount of description required to introduce a topic. Authors should remember the wide readership of the Journal when preparing their article, and are advised to discuss the review with the Editor or a member of the Editorial Board before submission.

Submission of cover images

Reproduction, Fertility and Development welcomes the submission of suitably eye-catching and high quality images for consideration for the cover of the Journal. Image files must be at least

300 dpi at 150–180 mm wide for maximum quality reproduction. If your paper is accepted, please submit (as Accessory Material) any figures/photographic images that you consider suitable for a cover image, with your production files.

Preparation of manuscripts

General presentation. The work should be presented clearly and concisely in English. The title should reflect the key points of interest in the paper. The names and addresses of all authors should be presented on the first page, together with the full postal address and email address (or fax number) of the corresponding author. Authors of multi-authored papers may wish to assign relative values to their contributions to the work or to indicate that two or more authors contributed equally to the paper. This can be done in a note at the end of

the address field on the paper. The introduction should indicate the reason for the work and include essential background references.

Human and animal experimentation. Papers reporting work with humans or animals must include a reference to the code of practice adopted for the experimentation. It is expected that reported experiments have been performed according to appropriate ethical and legal standards, and that relevant licences have been obtained. Editors will take account of ethical and animal welfare issues and reserve the right not to publish.

Title. The title should be concise and appropriately informative and should contain all keywords necessary to facilitate retrieval by modern searching techniques. Additional keywords not already contained in the title or abstract may be listed beneath the abstract. An abridged title suitable for use as a running head at the top of the printed page and not exceeding 50 letter spaces should also be supplied.

Abstract. The abstract should be fewer than 200 words and should state concisely the scope of the work and give the principal findings. It should be complete enough for direct use by abstracting services. Acronyms and references should be avoided.

References. In the text, references are cited chronologically by author and date and are not numbered. Names of two coauthors are linked by 'and'; for three or more coauthors; the first author's name is followed by 'et al.'. All references cited must be listed alphabetically at the end of the paper; all entries in this list must correspond to references in the text. No editorial responsibility can be taken for the accuracy of the references and authors are requested to check these with special care. Titles must be included for all references as well as first and last page numbers. Papers that have not been accepted for publication may not be included in the list of references and must be cited either as 'unpubl. data' or as a 'pers. comm.'; the use of such citations is discouraged. It is the authors' responsibility to ensure that they have permission to cite material as a personal communication. Titles of periodicals must be abbreviated. Abbreviations should conform to those given in the latest edition of 'Serial Sources for the BIOSIS Data Base' (Bio-Sciences Information Service, Philadelphia, PA). References should be in the following formats:

Chapter in a book

Calderon, I., and Healy, D. (1993). Endocrinology of IVF. In 'Handbook of in vitro Fertilization'. (Eds A. O. Trounson and D.

K. Gardner.) pp. 1–16. (CRC Press: Boca Raton, FL.)

Journal article

Cohen, J., Malter, H., Elsner, C., Kort, H., Massey, J., and Mayer, M. P. (1990). Immunosuppression supports implantation of zona pellucida dissected human embryos. *Fertil. Steril.* 54, 662–665.

Whole book

Cohen, J., Malter, H. E., Talansky, B. E., and Grifo, J. (1992a). 'Micromanipulation of Human Gametes and Embryos.' (Raven Press: New York.)

Conference proceedings

Hayman, P. T., and Collett, I. J. (1996). Estimating soil water: to kick, to stick, to core or computer? In 'Proceedings of the 8th Australian Agronomy Conference, Toowoomba'. (Ed. M. Asghar.) pp. 664–672. (Australian Society of Agronomy: Toowoomba.)

Units. Authors are requested to use the International System of Units (Système International d'Unités) for exact measurements of physical quantities and, where appropriate, elsewhere. Concentrations should be expressed in molar terms where appropriate. The double solidus must not be used in complex groupings of units; the negative index form is preferred.

Mathematical formulae. These should be carefully typed with symbols in correct alignment and adequately spaced. Equations should not be embedded images; use equation editors that result in an editable format. Each formula should be displayed on a single line if possible.

Enzyme nomenclature. The names of enzymes should conform to the recommendations in 'Enzyme Nomenclature 1992' (Academic Press: San Diego, CA, 1992). Where enzymes are referred to only in the course of discussion, or are obtained from commercial sources and are used solely as a reagent, it will be adequate to use the recommended name without the identifying EC number. For enzymes that are more central to the paper, the recommended names should be used throughout and they should be identified by their EC numbers, at the first mention in body of the paper. If there is good reason to use a name, other than the recommended name, at the first mention of the alternative name in the text, it should be identified by the recommended name and EC

number. The Editor should be advised of the reasons for using the alternative name.

Chemical nomenclature. The nomenclature of compounds such as amino acids, carbohydrates, lipids, steroids, vitamins, etc. should follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. Other biologically active compounds, such as metabolic inhibitors, buffers, etc. should be referred to once by their correct chemical name (which is in accordance with IUPAC rules of Chemical Nomenclature) and then by their most widely accepted common name. Where there is no common name, trade names or letter abbreviations of the chemical may be used.

Hormone assays. The validation of biological and binding assays and the statistical treatment of results should conform to the recommendations as set out in the *Journal of Endocrinology*, 1977, 72, 1–4. In particular, the minimum detectable amount of standard in the assay and the procedure for obtaining this calculated value should be given as also should an assessment of intra- and inter-assay precision. If only a few observations are available the dispersion is better indicated by the range. If the distribution is particularly skewed it may be justifiable to give both the standard deviation and the range. No test establishes absolute specificity; this lack of specificity is a particular problem with peptide hormones where reference to more rigorous physicochemical procedures such as GLC–mass spectrometry is not possible. Activity of fractions obtained by column separation should therefore be included whenever possible as this provides a useful index of possible heterogeneity.

Whenever practicable the tests used should be repeated for each novel physiological or pathological situation.

Statistical evaluation of results. The tests used should be described briefly and, if necessary, supported by references. Numbers of individuals, mean values and measures of variability should be stated. It should be made clear whether the standard deviation or the standard error of the mean has been given.

Tables

Tables must be numbered with arabic numerals and each must be accompanied by a title. A headnote containing material relevant to the whole table should start on a new line, as it will be set in a different font. Tables should be

arranged with regard to the dimensions of the printed page (17.5 by 22.5 cm in two 8.5-cm columns) and the number of table columns kept to a minimum. Excessive subdivision of column headings is undesirable and long headings should be avoided by the use of explanatory notes, which should be incorporated into the headnote. The first letter only of headings to rows and vertical columns should be capitalised. The symbol for the unit of measurement should be placed in parentheses beneath the column heading. Prefixes for units should be chosen to avoid an excessive number of digits in the body of the table or scaling factors in the headings. When scaling factors cannot be avoided, the quantity expressed should be the power of 10 by which the value has been multiplied. Footnotes should be kept to a minimum and be reserved for specific items in columns. Horizontal rules should be inserted only above and below column headings and at the foot of the table. Vertical rules must not be used. Each table must be referred to in the text. Only in exceptional circumstances will the presentation of essentially the same data in both tabular and graphical form be permitted; where adequate, the graphical form should be used. Short tables can frequently be incorporated into the text as a sentence or as a brief untitled tabulation.

Line drawings and graphs

Line illustrations prepared using either a draw or chart/graph program should be saved in the following formats: Adobe Illustrator (.ai) (preferred format); encapsulated postscript (.eps); or Excel (.xls). Illustrations created using Powerpoint should be saved in Powerpoint or as Windows metafiles (.wmf); CorelDraw files should be saved as .eps or .ai files; charts created on a Macintosh computer should be saved as .eps, .ps or PICT files; SigmaPlot files should be saved in .eps format (postscript printer driver required). In all cases they should be editable vector graphic files. Avoid using 3D surface area charts because print quality is often poor. Remove colours from all charts and graphs.

Lettering should be in 'sans-serif' type (Helvetica is preferred) with only the first letter of the first word and of any proper names capitalised. The x-height after reduction should be 1.3–1.7 mm (or 8–10 point in Helvetica). Thus for the reduction of graphs to 30, 40 or 50% of original linear dimensions, the initial x-height of lettering would be 5, 4 or 3 mm (c. 30, 22 and 18 pt) respectively. Proportionately smaller sizes of type, symbols, grid marks and curve thickness should be used for lesser reductions. Symbols and grid marks should be the same respective sizes and, after reduction,

curves and axes should not exceed 1.5 point in thickness unless required for clarity. Lines should not be thinner than 0.5 pt, or they may drop out during printing. The following symbols are readily available and should be used: ● ▲ ▼ ◆. The symbols + or × should be avoided in figures.

Explanations of symbols should be given in the caption to the figure. Lettering of graphs should be kept to a minimum as excessive lettering within the frame of a graph makes the lines difficult to decipher. Grid marks should point inwards; legends to axes should state the quantity being measured and be followed by the appropriate units in parentheses. Unsatisfactory artwork will be returned for correction. The Editor may be consulted for further guidance.

Photographs

Digital images should be prepared and photographs scanned at a resolution of at least 300 dpi at final size and saved in greyscale format as .tif or Photoshop (.psd) files. It is preferable for labels to be applied electronically to the scanned images in Photoshop, rather than scanning manually labeled figures. Electronic files of colour figures or photographs should be saved in CMYK colour not in RGB colour, because the CMYK format is required for printing. Authors should note that colours change when converted to CMYK from RGB and when printed from different types of printer; hence, when colour accuracy is important, authors should provide a hard copy that is correct so that colour reproduction during printing can be matched to an accurate original. Note that the journal does not cover the cost of printing colour pages, so please contact the Editor if you wish to publish photographs in colour.

In multi-part figures, images should be arranged in multiple panels on the page so that their dimensions do not exceed 17.5 by 22.5 cm (double column) when printed. Part labels should be lower-case in italic font and enclosed by parentheses. Each image should exclude features not relevant to the paper and be separated from adjacent images by uniform spaces that will be 1–2 mm wide after reduction. A scale bar must be included on all micrographs except scanning electron micrographs where the magnification can be given in the caption. Lettering should be in 'sans-serif' type (Helvetica is preferred) that contrasts with its background and should be 1.5 to 2 mm (10–12 pt) high when printed. Important features to which attention has been drawn in the text should be indicated.

Electronic files for accepted manuscripts

Electronic files of the final versions of both the text and illustrations should be sent when the paper has been accepted for publication. You will be asked to submit production files via OSPREY. Files should be named using the paper number and appropriate identifying information (e.g. RD08001_finaltext; RD07001_Fig1). The text, tables and figure captions should be sent as a single Word file. If you are unable to supply files in Word, please contact the Editorial Office (publishing.rfd@csiro.au) for acceptable alternatives. The figures should be provided in the formats described above.

Page proofs and corrections

Page proofs are sent to the corresponding author for checking prior to publication. At this stage only essential alterations and correction of printer errors may be undertaken. Excessive author alterations may be charged back to the author at \$5 per item.

Reprints

A free PDF reprint will be supplied to the author on publication of the article. Hard copy reprints may also be ordered before publication using the publication charges form, which is sent to the corresponding author with the page proofs.

June 2010

Address for submission enquiries: Editor
Reproduction, Fertility and Development
CSIRO Publishing
PO Box 1139
Collingwood, VIC 3066
Australia
Email: publishing.rfd@csiro.au

Running head: Effect of cloprostenol sodium on gene expression and immunohistochemistry at endometrium of Nelore cows during puerperium

Temporal analysis of mRNA expression of FP receptor, CASP-3 and COX-2 and immunohistochemistry for FP receptor and COX-2 of endometrial tissue from multiparous Nelore (*Bos taurus indicus*) cows treated with cloprostenol sodium on puerperium

Carolina Nogueira de Moraes^{AE}

Leandro Maia^A

Paula Fernanda de Lima^B

Marianne Camargos Dias^A

Talita Mariana Morata Raposo Ferreira^C

Mateus José Sudano^D

José Buratini Júnior^B

Eunice Oba^A

^A Department of Animal Reproduction and Radiology, São Paulo State University – UNESP, Rubião Júnior, Botucatu, São Paulo, 18618-970, Brazil.

^B Department of Pharmacology, Biosciences Institute, São Paulo State University – UNESP, Rubião Júnior, Botucatu, São Paulo, 18618-970, Brazil.

^C Department of Medical Clinic, São Paulo State University – UNESP, Prof. Paulo Donato Castellane, Jaboticabal, São Paulo, 14884-900, Brazil.

^D Laboratory of Genetics and Animal Breeding, Federal University of Pampa - UNIPAMPA, BR 472 - Km 592, Uruguaiana, Rio Grande do Sul, 97500-970, Brazil.

^E Corresponding author. Email: carolnmoraes@hotmail.com

26 Abstract

27 Cloprostenol sodium at postpartum period aims to reduce the period of uterine
28 involution. At the present study it was hypothesised that cloprostenol sodium changes
29 mRNA expression of prostaglandin F2 α receptor (FP), caspase-3 (CASP-3) and
30 cyclooxygenase 2 (COX-2), and also protein expression of FP and COX-2, decreasing
31 the period of uterine involution during puerperium of cows. For this, multiparous
32 Nelore cows (n=24) were treated (n=12) or not (n=12) with cloprostenol sodium on
33 days 1 and 4 postpartum (D0 = birth) and endometrial biopsies were collected with a
34 Yomann biopsy instrument at D1, D7, D14, D28 and D42. The mRNA expression was
35 done with samples from D1, D7, D14, D28 and D42 and protein expression from D1,
36 D14, D28 and D42. Treatment tested had effect on mRNA expression of CASP-3. This
37 study demonstrates that the treatment with cloprostenol sodium is beneficial to a better
38 cellular renewal but in animals with good selection genetic, management and health it
39 can be unnecessary to promote additional changes at molecular level from studied genes
40 in Nelore cows.

41

42

43

44

45

46

47

48

49

50

INTRODUCTION

During the postpartum period, uterus passes for modifications for the restoration of an environment able to maintain a new pregnancy. In cows, the period of 65 days of puerperium is recommended and the conception within 85 to 90 days is desired according to review of Ymer *et al.* (2010). Being like this, the production of one calf/year can be reach with economical gain.

At postpartum, prostaglandin F_{2α} (PGF_{2α}) produced by uterus is associated with a faster involution by stimulus of myometrial contraction (Guilbalt *et al.* 1984; Fernandes and Figueiredo 2007). Cloprostenol sodium is a PGF_{2α} synthetic analogue and is used for estrus synchronization, treatment of diseases and during postpartum period to reduce uterine involution (Tenhagen *et al.* 2000; Nakao *et al.* 2007; Barros and Di Stasi 2012; Fernandes *et al.* 2012).

PGF_{2α} binds to a specific receptor named FP, a G-protein coupled receptor, which is a contractile receptor that mediates the release of intracellular calcium (Ca⁺) promoting muscular contraction (Narumiya *et al.* 1999; Barros and Di Stasi 2012).

FP at its complete form is highly expressed in luteal (Sakamoto *et al.* 1994; Sakamoto *et al.* 1995; Ishii and Sakamoto 2001; Shirasuna *et al.* 2012; Weems *et al.* 2012) and follicular cells (Bridges and Fortune 2007), and also in fetal membranes and in uterine tissue during gestation (Arosh *et al.* 2004; Wehbrink *et al.* 2008).

Throughout estrous mammalian endometrium passes by a series of morphological and functional changes (Arai *et al.* 2013). Also, during postpartum period, a series of morphological changes occur in the endometrial tissue for restoration of histological features that were altered during pregnancy and delivery period.

75 Apoptosis is a physiologic kind of programmed cell death to ensure the balance
 76 between cells that die and proliferated. At this context, caspases constitute a group of
 77 proteases of cysteine that break other proteins when a residue of aspartic acid is
 78 recognized (Siviero *et al.* 2013). These proteases, involved in the process of apoptosis,
 79 are synthesized as zymogens and classified in two classes: the initiators (caspase -2, -8,-
 80 9, and -10) and the executors (caspase - 3, -6 and -7). Initiators are at monomers form
 81 and is activated by dimerization. Executioner caspases exist as dimers and are activated
 82 by cleavage within their interdomain linker by initiator caspase (Broatright and
 83 Salvesen 2003; Kamemori *et al.* 2011; Siviero *et al.* 2013).

84 Caspase- 3 (CASP-3) is present on the terminal event of apoptosis and can be
 85 activated by intrinsic (mitochondrial) or extrinsic (death receptor) pathway (Broatright
 86 and Salvesen 2003). Presence of mRNA expression of CASP-3 was observed on
 87 maternal and fetal placenta of bovine and at uterus during estrous cycle and gestation
 88 (Kamemori *et al.* 2011; Arai *et al.* 2013).

89 Cyclo-oxygenase (COX) is the enzyme present at the process of biosynthesis
 90 of prostaglandins from arachidonic acid (Vane *et al.* 1998) and is present at two forms:
 91 COX-1 and COX-2. The first is a constitutive enzyme and is present in most cells. On
 92 the other way, COX-2 is an inductive enzyme, with its action initiated after gene
 93 induction by stimuli or mediators (Barros and Di Stasi 2012).

94 COX-2 plays an important role at cell growth and on inflammatory process
 95 (Vane *et al.* 1998) once it is thought to participate for prostaglandin production in
 96 inflammatory situations (FitzGerald 2003).

97 Expression of COX is different between species and tissue (Arosh *et al.* 2002),
 98 but it is known that COX-2 is present at bovine uterine tissue at estrous cycle, gestation

99 and postpartum (Fuchs *et al.* 1999; Arosh *et al.* 2002; Schuler *et al.* 2006; Takagi *et al.*
100 2008).

101 At the present work it was hypothesized that cloprostenol sodium has a positive
102 effect in reducing puerperal period to accelerate uterine involution by changing mRNA
103 expression of FP, COX-2 and CASP-3 and protein expression of FP and COX-2. Thus,
104 we evaluated the effect of serial administration of cloprostenol sodium (D1 and D4, D0
105 = birth) during puerperal period of multiparous Nelore cows by reverse transcriptase
106 polymerase chain reaction (qRT-PCR) for gene expression (D1, D7, D14, D28 and D42)
107 and immunohistochemistry for tecdial protein detection (D1, D14, D28 and D42). The
108 confirmation of the hypothesis under study would be beneficial to a better knowledge of
109 molecular biology at postpartum period of beef cattle and to establish strategies to
110 reduce puerperal period and increase profitability.

111

112

113

114

115

116

117

118

119

120

121

122

123

MATERIALS AND METHODS

Ethical aspects

The study was conducted in accordance with ethical guidelines recommended by National Council for Control of Animal Experimentation and College of Animal Experimentation, and was approved by the Institution's Animal Care and Experimentation Ethics Committee on protocol number 50/2012.

Animals

A total of twenty four multiparous Nelore (*Bos taurus indicus*) female with parity order between 2 - 6 (median = 3), aged between 4 -13 years (median = 9), with body condition score between 3 and 4 (classification 0 to 5) and with the calf were used. Animals were housed in paddocks predominantly of *Brachiaria decumbens*, with mineral supplementation (Minersal®, Presidente Venceslau, SP, BR) and water *ad libitum*. In the dry season, animals received supplementary food of corn silage.

Only the animals with no calving assistance, retained fetal membranes, twin pregnancy or postpartum complications were selected. The experiment was conducted in a farm located in Avaré, State of São Paulo, Brazil (latitude: 23°10'S; longitude: 48°93'E; altitude 766 m).

Experimental delineation

The selected animals were randomly divided into two groups: one group was treated with cloprostenol sodium (TG, n = 12) and the other, which was the control group, received 0.9% NaCl solution (CG, n = 12) on D1 and D4 postpartum (D0 = birth). Endometrial biopsies were collected on D1, D7, D14, D28 and D42. Samples were then used for evaluation of gene expression on D1, D7, D14, D28 and D42 and immunohistochemistry on D1, D14, D28 and D42 (Figure 1).

Treatment

The animals were randomly assigned into the treatment and placebo groups (TG and PG, respectively), as parity order. The treated group (TG) received 0,530 mg (2 mL) of cloprostenol sodium (Ciosin®, MSD Animal Health, São Paulo, SP, BR) intramuscularly, in the region of gluteum, on days 1 and 4 postpartum (D0 = birth). The placebo group (PG) received by the same route and period the same volume of 0.9% NaCl solution (Sanobiol®, Pouso Alegre, MG, BR).

Endometrial Biopsy

Endometrial samples were obtained using a Yomann (Hauptner®, Solingen, North Rhine-Westphalia, Germany) biopsy instrument according to a previously described method (Chapwanya *et al.* 2012). Endometrial samples were obtained at D1, D7, D14, D28 and D42 postpartum in the horn previously identified as the largest by rectal palpation on the first evaluation. For collection, external genitalia and perineum

174 were cleaned with water and 2% chlorhexidine degermante (Riohex 2% Clorexidina®,
175 Rioquimica, São José do Rio Preto, SP, BR) and the biopsy instrument covered by a
176 protective sheath was introduced into the vagina. With the left hand in the rectum the
177 biopsy instrument was directed into the cervical canal, and after passing it the sheath
178 was ruptured and the biopsy instrument was introduced in the horn for obtaining the
179 sample.

180 To be sure of the position of the biopsy instrument, carefully, the left hand
181 identified the tip of the biopsy instrument in the horn. Passed it, the jaws of the biopsy
182 instrument were opened and endometrial sample was collected by pressing the uterine
183 wall against the instrument jaws when then it was closed and the tissue was
184 withdrawing.

185 Between each biopsy, the biopsy instrument was cleaned with a brush to
186 remove endometrial residues, washed with MilliQ water (Millipore®, Bedford, MA,
187 USA) and 2% chlorhexidine degermante (Riohex 2% Clorexidina®, Rioquimica, São
188 José do Rio Preto, SP, Brazil) and covered with a new protective sheath. All biopsies
189 were performed by the same operator for standardization of the technique and quality of
190 the collected sample.

191 It was obtained two endometrial fragments of approximately 0,5cm² for
192 performance of immunohistochemistry and qRTPCR. For qRTPCR analysis, tissue was
193 removed from the jaws with the assistance of a 40x12 sterile needle (Descarpack®, São
194 Paulo, SP, BR) and transferred to 2 mL cryotubes DNase and RNase free (TPP AG®,
195 Trasadingen, SH, SWI) previously identified containing 1 mL of RNA later solution
196 (RNAlater® tissue Collection, Ambion, Austin, TX, USA). The tissue was maintained
197 on this solution for 12 hour at 4°C, when then the solution was withdraw and tissue was
198 transferred for a freezer -80°C until RNA extraction according to the manufacturer's

199 recommendations. Another sample was collected for immunohistochemistry and tissue
200 was removed from the jaws by the same way described above and transferred to 27 mL
201 cylindrical bottles (Injeplast®, São Paulo, SP, BR) previously identified containing 3
202 mL of 10% buffered formalin and passed three days, sample was fixed in 70% alcohol.
203 All material was processed according to routine histological methods until paraffin
204 embedding and obtaining histological sections.

205

206 **Reverse transcriptase polymerase chain reaction (qRTPCR)**

207

208 qRTPCR was done to evaluate temporal mRNA expression of FP, CASP-3 and
209 COX-2 from 24 animals at 5 moments of postpartum (1, 7, 14, 28 and 42 days).

210 Total RNA was isolated from tissue with Trizol® solution (Invitrogen,
211 Carlsbad, CA, EUA) according to the manufacturer's recommendations. For this,
212 samples were thawed and emerged on 1 mL Trizol® when then it was scrunched on
213 tissue homogenizer (Polytron UltraTurrax T-25) until complete dissolution.
214 Homogenized samples were incubated with 0,2 mL chloroform at temperature room for
215 3 minutes after shaken for 15 seconds. Samples were centrifuged at 11000 rpm for 15
216 minutes at 2-8°C and after this, aqueous phase (where there is RNA) was transferred for
217 another tube and RNA was precipitated by the adding 0,5 mL isopropyl alcohol.
218 Samples were then incubated for 10 minutes at room temperature and then centrifuged
219 at 11000 rpm for 10 minutes at 2-8°C. Supernatant was removed and RNA pellet was
220 dried and washed with 1 mL 75% ethanol. Samples were centrifuged at 7500 rpm for 5
221 minutes at 2-8°C, the pellet was dried and dissolved in 20 µL of nuclease free water and
222 incubated for 10 minutes at 55-60 °C. Following this, it was checked the concentration

223 of RNA on spectrophotometer (NanoDrop®, Thermo Scientific, Franklin, MA, USA)
224 and total RNA was stored at – 80 °C.

225 Total RNA isolated from bovine endometrium was treated with DNase with
226 the objective of obtaining 1µg of total RNA of each sample, using Amplification
227 Grade® (Invitrogen®, Carlsbad, CA, EUA). For this it was add 1µl buffer DNase, 1µl
228 DNase and RNase free water until complete 10 µL. It was incubated for 15 minutes at
229 room temperature, and then it was add 1µL ethylenediamine tetraacetic acid (EDTA)
230 and also incubated for 10 minutes at 65°C.

231 Reverse transcription was done using commercial SuperScript III™
232 (Invitrogen®, Carlsbad, CA, EUA) protocol for cDNA obtention. For this 8µg of the
233 solution treated with DNase was transferred for a sterile tube and it was add 1µL oligo
234 dT, 1µL dNTP (deoxynucleotide triphosphates) mix. The mixture was incubated for 5
235 minutes at 65° and then for 1,5 minutes on ice. Thus, it was added 4 µL of RT 5X
236 Buffer, 1 µL DTT (dithiothreitol) 0,1M, 1µL of SuperScript III™ (Invitrogen®,
237 Carlsbad, CA, EUA) and 1µL of RNaseOUT™ (Invitrogen®, Carlsbad, CA, EUA).
238 Then, 7µL of the mix was added to each tube.

239 The technique of qRT-PCR was performed with an ABI 7500 thermocycler
240 (Applied Biosystemns, Foster City, CA, USA) at 96-well plates using Power Sybr
241 Green Master Mix® (Applied Biosystemns, Foster City, CA, USA), cDNA and primer
242 (for each housekeeping or target gene) in a 25 µL final volume of PCR mix. The
243 conditions of PCR were of: 95°C for 10 minutes (1 cycle), denaturing at 95°C for 10
244 seconds and then annealing for 1 minute (40 cycles).

245 All samples were run in duplicate and specificity of PCR was evaluated by
246 melting-curve analysis.

247 Amplification of housekeeping genes cyclophilin (CYC-A) and
248 glyceraldehydes-3-phosphate dehydrogenase (GAPDH) (primer sequence at Table 1) was
249 done in order to choose the best and most stable gene to be used as an internal positive
250 control for PCR reaction. The expression was normalized by the expression of
251 housekeeping CYC-A. The relative expression of mRNA from target gene (FP, CASP-3
252 and COX-2) was calculated using $\Delta\Delta C_t$ method with efficiency correction and using
253 one sample as control calibrator (Pfaffl, 2001).

254

255 **Immunohistochemistry**

256

257 It was done immunohistochemistry for temporal immunolocalization of COX-2
258 and FP on 6 animals (3 from each group), at 4 moments (1, 14, 28 and 42 days
259 postpartum) at endometrial tissue.

260 For deparaffinization of histological section of 5 μm thick, sections were
261 maintained in an incubator at 54°C for 24 hours, placed in recipient with xylene at room
262 temperature (RT) for 30 minutes and then maintained in a new solution of xylene for 20
263 minutes. Subsequently, sections were transferred for other recipient to hydration in
264 decreasing alcohol baths (absolute alcohol I, II, III, 95% and 85%) for three minutes in
265 each alcoholic solution. After complete deparaffinization, antigen retrieval was carried
266 out in preheated solution of 10 mM sodium citrate buffer solution (pH 6.0) in system of
267 Pascal pressure (Dako Cytomation®, Glostrup, CPH, DNK). Thus the slides were
268 cooled at the same antigen retrieval solution for 20 minutes at RT. Sections were
269 washed in distilled water baths to block the action of the solution of the antigen
270 retrieval. Subsequently it was conducted endogenous peroxidase blockage with 3%
271 hydrogen peroxide in a methanolic solution (Dinâmica Química Contemporânea Ltd,

Diadema, SP, BR) for 20 minutes. After blocking, sections were washed in distilled water containing buffer TRIS+ HCl solution (pH= 7,4) in dark conditions and incubated in 3% skimmed milk solution for one hour in an incubator at 27 °C to block nonspecific protein. Then primary antibodies for markers COX-2 (1:400, Rabbit anti-cyclooxygenase 2 polyclonal antibody – BS-0732R, Bioss Antibodies®, Woburn, MA, USA) or FP (1:800, FP Receptor polyclonal antibody – 101802, Cayman Chemical®, An Arbor, MI, USA) were incubated for 2 hours at 27°C. Primary antibodies were detected by incubation in HiDef Detection™ HRP Polymer System (Cell Marque®, Rocklin, CA, USA) according to the manufacturer's recommendations. The revelation of the reaction was conducted using the solution of chromogenic substrate DAB (Dako Cytomation, Glostrup, CPH, DNK) for 5 minutes, followed by counterstaining with Harris hematoxylin (Merck®, São Paulo, SP, BR) for 40 seconds and mounted with Permount for evaluation of the reaction by a light microscopy (Leica® Microsystems, Wetzlar, HESS, GER) at 10x and 20x magnification.

Positive control for COX-2 was done using bovine lung adenocarcinoma and for FP was done using a bovine corpus luteum. Negative control for both was done by omitting primary antibody.

All slides for each marker were stained in a single run to avoid variability inter-incubation (Schuler *et al.* 2013).

All sections were analyzed, for intensity of immunostaining with DAB and immunolocalization structure on endometrial surface epithelium, stroma and glandular epithelium. This evaluation was done based on percentage of staining cells and on colour intensity. Classification used for this analysis is shown on Table 2.

Data Analysis

Data were analysed using the mixed linear model (MIXED) procedure with the SAS statistical software package (Version 9.2; SAS Inst. Inc., Cary, NC, USA). Sources of variation in the model included treatment (TG vs PG), moment (day 1, 7, 14, 28 and 42 postpartum), and first-order interactions; these were considered as fixed effect. Animals were considered as random effect. If necessary, logarithmic transformation was used to improve normality. Means were separated using least square difference (LSD). When there was not observed influence of treatment, groups were grouped. All data are reported as untransformed least-squares means $\pm$ s.e.m. For all analyses, $P < 0.05$ was considered to be significant.

RESULTS

Cloprostenol sodium had effect ($P < 0.05$) on mRNA expression of CASP-3. Differences only of moment were observed on mRNA expression of FP, CASP-3 and COX-2 for all studied moments on endometrial samples from Nelore cows at postpartum period. Immunohistochemistry for COX-2 and FP show immunopositive staining for endometrial surface epithelium, stroma, glandular epithelium. Also, cytoplasmatic immunolocalization was identified for all analyzed samples.

mRNA expression of FP

Cloprostenol sodium had no effect ($P = 0.20$) on mRNA expression of FP during the period postpartum, in samples analyzed. It was observed effect of moment

322 ($P < 0.0001$) on mRNA expression of FP, with a progressive increase from D1 to D14
 323 and a decrease from D14, however without significative difference ($P > 0.05$) between
 324 D1 and D42 when it was reached the lowest expression, as shown on Figure 2.

325

326 **mRNA expression of CASP-3**

327

328 Cloprostenol sodium had influence ($P = 0.004$) on mRNA expression of CASP-
 329 3 on samples analyzed with higher expression at treated group ($25,6 \pm 0,35\%$) than at
 330 control group ($17,66 \pm 0,34\%$).

331 It was also observed effect of moment ($P < 0.0001$) on mRNA expression of
 332 CASP-3 with a progressive decrease on its expression from D1 to D42, however
 333 without significative difference from D7 to D28, as shown on Figure 3.

334

335 **mRNA expression of COX-2**

336

337 Cloprostenol sodium had no influence ($P = 0.31$) on mRNA expression of COX-
 338 2 on samples analyzed. It was observed effect of moment ($P < 0.0001$) on mRNA
 339 expression of COX-2 with a progressive increase from D1 to D7 and then a progressive
 340 decrease from D14 to D42 was observed, as shown on Figure 4.

341

342 **Immunodetection of COX-2 and FP protein**

343

344 Two samples ($n=2$) could not be analyzed because of the poor quality of
 345 histological sections and/or tissue loss.

346 COX-2 and FP protein could be detected at endometrial surface epithelium,
347 stroma and glandular epithelium with cytoplasmatic immulocalization, as shown on
348 Figures 5 and 6.

349 For FP, staining intensity varied between animal and moment with weak to
350 strong intensity at endometrial surface epithelium, glandular epithelium and stroma.

351 For COX-2, staining intensity varied between animal and moment with
352 moderate to strong intensity at endometrial surface epithelium, weak to moderate at
353 stroma and weak to strong at glandular epithelium. Also, some inflammatory cells were
354 immunopositive for COX-2 and for FP. Data are shown on Table 3.

355

356 **DISCUSSION**

357

358 At the present study we evaluated the effect of cloprostenol sodium
359 administered on days 1 and 4 on Nelore postpartum cows on temporal mRNA
360 expression of FP, CASP-3 and COX-2 at endometrium. We also investigated by
361 functional assay immunolocalization for FP and COX-2 protein.

362 Comparison between works became quite difficult because most of them are
363 done at gestation or during few hours after delivery. To our knowledge this is the first
364 work that evaluated temporal and tissue-specific expression of FP and COX-2 for a long
365 period (until 42 days) of puerperium of Nelore cows treated with cloprostenol sodium.

366

367 **mRNA expression of FP**

368

369 Works related with mRNA expression at corpus luteum (Sakamoto *et al.* 1994;
370 Sakamoto *et al.* 1995; Ishii and Sakamoto 2001; Shirasuna *et al.* 2012; Weems *et al.*

2012) and at uterine tissues during gestation (Arosh *et al.* 2004; Wehbrink *et al.* 2008) in bovine can be found at literature. However, no works could be found at literature until now related with temporal mRNA expression of FP during puerperium period of beef cattle.

In rats at postpartum, mRNA expression of FP changes between gestation, delivery and at D1 postpartum, with lower levels at postpartum and highest at delivery. This occurs to maintain contraction for uterine involution (Brodt-Eppley and Myatt 1998; Dong and Yallampalli 2000). At the present study, during the postpartum period, level of mRNA expression of FP on D1 is lower than the other days, with exception for D42. PGF_{2α} helps in uterine involution postpartum acting at myometrial contraction (Guilbalt *et al.* 1984) and helping on the elimination of uterine discharge (Lindel *et al.* 1981). Being FP a contractile receptor (Wehbrink *et al.* 2008), the increase from D1 until D14 is desired once at this moment contractions are present and there is reduction of uterine size, fluid accumulation and cervical discharge (unpubl. data). Also, the reduction on mRNA expression of FP by D42 is not an unexpected event, once at this point, contractions are unnecessary.

In bovine, mRNA expression of FP did not change during pregnancy at intercaruncular, caruncular and fetal membranes tissues, with highest expression at caruncular tissue (Arosh *et al.* 2004). However, as observed by the present study, at postpartum period, this fact does not occur once there were observed changes of expression on endometrial tissue during studied moments. This proves that there is a biological difference on mRNA expression of FP during pregnancy and at postpartum period, and at this last when contractions are necessary, this pattern of expression changes.

395 When PGF2 α increase at late gestation in rats, mRNA expression of FP also
 396 increase, leading to more bound between FP receptor and PGF2 α , which may be
 397 associated with the induction of contraction during delivery. However, at 1 day
 398 postpartum it was observed a reduction on its expression (Brodt-Eppley and Myatt
 399 1998). At the present work, concentrations of PGFM (13,14-dihydro-15-keto
 400 prostaglandin F2 α) at the first day postpartum reached its highest value during
 401 experimental period, but it did not occur with mRNA expression of FP, which was low
 402 at this day (unpubl. data).

403 Also, some differences between mRNA expression of FP can be found between
 404 species once at endometrial tissue of pregnant sheep was not detected expression of FP
 405 but at myometrial tissue the expression was present (Ma *et al.* 1999), location where it
 406 was also found expression in humans (Grigsby *et al.* 2006) and rat (Dong and
 407 Yallampalli 2000).

408

409 **mRNA expression of CASP-3**

410

411 During mammal sexual cycle, uterus undergo by a process of apoptosis and
 412 cellular proliferation for the maintenance of a possible pregnancy (revised by Kamemori
 413 *et al.* 2011). This also seems occur at postpartum period, once in the present work
 414 expression of mRNA of CASP-3 was high expressed at first day postpartum, with
 415 reduced expression during all postpartum period. This probably can be observed due a
 416 “cleaning” process that occurs after delivery to eliminate cells that proliferated during
 417 gestation and it is a crucial event to the establishment of a new gestation.

418 Arai *et al.* (2013) in a study during estrous cycle of dairy cow observed that
 419 there was no difference between mRNA expression of CASP-3 throughout the cycle.

420 However, at the present study, it was noted difference on mRNA expression during
421 postpartum period, showing difference between these two physiologic phases. Also,
422 treatment with cloprostenol sodium increased mRNA expression of CASP-3 on treated
423 groups suggesting a positive effect of treatment on inducing apoptosis and promoting a
424 better cellular renewal.

425 It is suggested that low levels of progesterone (P4) and/or estradiol-17 β
426 contributes to induce apoptosis process in bovine endometrium during estrus cycle (Arai
427 *et al.* 2013). Data from the present work corroborates with Arai *et al.* (2013) once at
428 immediate postpartum period, levels of progesterone are low (until D35) and thus this
429 contributed for the high expression of CASP-3 at immediate postpartum with lowest
430 value at D42, corresponding with the highest value of P4 (unpubl. data).

431

432 **mRNA expression of COX-2**

433

434 The mRNA expression of COX-2 was detected in bovine endometrium during
435 estrous cycle, gestation, parturition and until 6 hours postpartum (Fuchs *et al.* 1999;
436 Arosh *et al.* 2002; Arosh *et al.* 2004; Schuller *et al.* 2006; Takagi *et al.* 2008). However,
437 no work describing temporal mRNA expression of COX-2 during puerperal period of
438 Nelore cows could be found at literature.

439 COX-2 is responsible for basal and pulsatile secretion of prostaglandins by the
440 endometrium during the estrous cycle and its expression during follicular phase
441 suggests that prostaglandins are involved in the process of structural luteolysis and
442 uterine tonicity (Arosh *et al.* 2002). Also, during postpartum period there is production
443 of PGF_{2 α} with the objective to contribute for uterine involution by increasing uterine
444 contractions. At the present study, it could be noted that the pattern of expression of

COX-2 and FP are quite similar during postpartum period, proving the association of these two variables. Also, at the present study, mRNA expression of COX-2 decreases from D14 to D42 fact also observed for mRNA expression of FP.

Jischa *et al* (2008) at work with pony did not note significant changes in mRNA expression of COX-2 at 1, 9 and 16 days postpartum. However, at the present study it was observed significant changes among days of study, showing the possibly of difference between species.

Immunodetection of COX-2 and FP protein

Immunostaing for COX-2 was observed at bovine placentome (Schuler *et al.* 2006), fetal membranes (Arosh *et al.* 2002) and at uterus during pregnancy on perinuclear or on cytoplasmic regions (Wehbrink *et al.* 2008). At the present study, immunolocalization of COX-2 was observed at endometrial surface epithelium, stroma and glandular epithelium with citoplasmatic immulocalization, at all moments studied, data that corroborates with other authors (Arosh *et al.* 2004; Wehbrink *et al.* 2008).

At postpartum (6 to 12 hours) the expression of COX-2 protein in bovine endometrium is higher than during gestation (Fuchs *et al.* 1999; Takagi *et al.* 2008). It can be associated with the release of cytokines from cell of immune system that are present at uterus because of the expulsion of fetus and placenta (Fuchs *et al.* 1999). This data corroborates with the present work once at D7 there is a higher degree of inflammation (unpubl. data) and also a high expression of mRNA of COX-2, however protein detection did not varied between analyzed days. However, it is important to remember that the results of mRNA expression are not necessarily correlated with protein expression (Takagi *et al.* 2008).

470 FP is localized at cellular membrane (Naramiya *et al.* 1999) and at cytoplasm
471 (Blesson *et al.* 2012). FP protein was detected in luteal endothelial cells (Shirasuna *et*
472 *al.* 2012) and at cytoplasm of glandular epithelial cells, surface epithelial cells and in
473 stroma of uterus from pregnant cows (Wehbrink *et al.* 2008). At the present study, FP
474 protein was localized at endometrial epithelium, stroma and glandular epithelium with
475 cytoplasmatic immulocalization, confirming data of other authors with rats and bovine
476 (Wehbrinks *et al.* 2008; Blesson *et al.* 2012).

477 No study with immunolocalization of FP thought postpartum period of cows
478 was found at literature. Being FP a contractile receptor, the increase of its amount until
479 delivery seems to help at uterine contractions for delivery (Wehbrink *et al.* 2008). Also,
480 during postpartum period, contractions are desirable to help at uterine clean and at
481 liberation of placenta and lochia.

482 Based on the presented results, it can be concluded that cloprostenol sodium
483 had effect only at CASP-3 mRNA expression. These results not completely prove our
484 initial hypothesis at the present experimental conditions. However, we believe that the
485 use of this protocol is beneficial to a better cellular renewal but in animals with good
486 selection genetic, management and health it can be unnecessary to promote additional
487 changes at molecular level in Nelore cows. We believe that the present work can
488 motivate new researches at puerperium and bring important information on molecular
489 biology of beef cattle in this period.

490

491 **Acknowledgements-** We would like to thank to FAPESP for the scholarship
492 (Proc.2011/15330-0), as well as Capes (Proc./096/2010) for their financial support and to
493 Fazenda Marino Avaré.

494

495

496 **References**

- 497 Arai, M., Yoshioka, S., Tasaki, Y., Okuda, K. (2013). Remodeling of bovine
498 endometrium throughout the estrous cycle. *Anim. Reprod. Sci.* **142**, 1-9.
- 499 Arosh, J.A., Parent, J., Chapdelaine, P., Sirois, J., Fortier, M.A. (2002). Expression of
500 Cyclooxygenase 1 and 2 and prostaglandin E synthase in bovine endometrial tissue
501 during the estrous cycle. *Biol. Reprod.* **67**, 161-169.
- 502 Arosh, J.A., Banu, S.K., Chapdelaine, P., Fortier, M.A. (2004). Temporal and tissue-
503 specific expression of prostaglandin receptors EP2, EP3, EP4, FP, and Cyclooxygenases
504 1 and 2 in uterus and fetal membranes during bovine pregnancy. *Endocrinology* **145**,
505 407-417.
- 506 Barros, C.M., and Di Stasi, L.C. (2012) 'Farmacologia Veterinária.'(Manole: São
507 Paulo).
- 508 Blesson, C.S., Büttner, E., Masironi, B., Sahlin, L. (2012). Prostaglandin receptors EP
509 and FP are regulated by estradiol and progesterone in the uterus of ovariectomized rats.
510 *Reprod. Biol. Endocrin.* **10**, 1-10.
- 511 Boatright ,K.M., and Salvesen, G.Y. (2003). Mechanisms of caspase activation.*Curr.*
512 *Opin. Cell. Biol.* **15**, 725-731.
- 513 Bridges, P.J. and Fortune ,J.E. (2007). Regulation, action and transport of
514 prostaglandins during the periovulatory period in cattle. *Mol. Cell. Endocrinol.* **263**, 1-9.
- 515 Brodt-Eppley, J., and Myatt, L. (1998). Changes in expression of contractile FP and
516 relaxatory EP₂ receptors in pregnant rat myometrium during late gestation, at labor and
517 postpartum. *Biol. Reprod.* **59**, 878-883.
- 518 Chapwanya, A., Meade, K.G., Foley, C., Narciandi, F., Evans, A.C.O., Doherty, M.,
519 Callanan, J.J., O'Farrelly, C. (2012). The postpartum endometrial inflammatory

520 response: a normal physiological event with potential implications for bovine fertility.
521 *Reprod. Fert Develop.* **24**, 1028-1039.

522 Costa, R.P.R., Serrão, P.M., Monteiro, S., Pessa, P., Silva, J.R., Ferrira-Dias, G.
523 (2007).Caspase-3 mediated apoptosis and cell proliferation in the equine endometrium
524 during the oestrous cycle. *Reprod. Fert Develop.* **19**, 925-932.

525 Dong, Y-L., and Yallampalli, C. (2000). Pregnancy and exogenous steroid treatments
526 modulate the expression of relaxant EP₂ and contractile FP receptors in the rat uterus.
527 *Biol. Reprod.* **62**, 533-539.

528 Fernandes, C.A.C., Alves, B.F.L., Oliveira, E.R., Viana, A.J.H.M., Gioso, M.M.,
529 Loyola, Y.C.S. (2012). Efeito de diferentes doses de cloprostenol sódico no período
530 pós-parto de vacas de corte. *Ciênc. Anim. Bras.* **13**, 346-355.

531 Fernandes, C.A.C., and Figueiredo, A.C.S. (2007). Avanços na utilização de
532 prostaglandinas na reprodução de bovinos. *Rev Bras Reprod Anim.* **31**, 406-414.

533 FitzGerald, G.A. (2003). COX-2 and beyond: approaches to prostaglandin inhibition in
534 human disease. *Nat. Rev.* **2**, 879-890.

535 Fuchs, A.R., Rust, W., Fields, M.J. (1999). Accumulation of cyclooxygenases-2 gene
536 transcripts in uterine tissue of pregnant and parturient cows: stimulation by oxytocin.
537 *Biol. Reprod.* **60**, 341-348.

538 Guilbault, L.A., Thatcher, W.W., Drost, M., Hopkins, M. (1984). Source of F series
539 prostaglandins during the early postpartum period in cattle. *Biol. Reprod.* **31**, 879-887.

540 Grigsby, P.L., Sooranna, S.R., Adu-Amankwa, B., Pitzer, B., Brockman, D.E., Johnson,
541 M.R., Myatt, L. (2006). Regional expression of prostaglandin e2 and f2alpha receptors
542 in human myometrium, amnion, and choriodecidua with advancing gestation and labor.
543 *Biol. Reprod.* **75**, 297-305.

- 544 Ishii, Y., and Sakamoto, K. (2001). Suppression of protein kinase C signaling by the
545 novel isoform of bovine PGF_{2α} receptor. *Biochem. Biophys. Res. Commun.* **285**, 1-8.
- 546 Jischa, S., Walter, I., Nowotny, N., Palm, F., Budik, S., Kolodziejek, J., Aurich C.
547 (2008). Uterine involution and endometrial functions in postpartum pony mares.
548 *A.J.V.R.* **69**, 1-10.
- 549 Kamemori, Y., Wakamiya, K., Nishimura R., Hosaka, Y., Ohtani, S., Okuda, K.
550 (2011). Expressions of apoptosis-regulating factors in bovine retained placenta.
551 *Placenta*. **32**, 20-26.
- 552 Lindell, J.O., Kindahl, H., Jansson, L., Edqvist, L.E. Post-partum release of
553 prostaglandin F_{2α} and uterine involution in the cow. *Theriogenology* **17**, 237-245.
- 554 Ma, X.H., Wu, W.X., Nathanielsz, W. (1999). Differential regulation of prostaglandin
555 EP and FP receptors in pregnant sheep myometrium and endometrium during
556 spontaneous term labor. *Biol. Reprod.* **61**, 1281-1286.
- 557 Narumiya, S., Sugimoto, Y., Ushikubi, F. (1999). Prostanoid receptors: structures,
558 properties and functions. *Physiol. Rev.* **79**, 1193-1226.
- 559 Nakao, T., Gamal, A., Osawa, T., Nakada, K., Moriyoshi, M., Kawata, K. (1997)
560 Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained
561 placenta, and effect of fenprostalene on uterine involution and reproductive
562 performance. *J. Vet. Med. Sci.* **59**, 791-794.
- 563 Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time
564 RTPCR. *Nucleic Acids Res.* **29**, 2002-2007.
- 565 Schuler, G., Teichmann, U., Kowalewski, M.P., Hoffmann, B., Madore, E., Fotier,
566 M.A., Klich, K. (2006). Expression of cyclooxygenase-II (COX-2) and 20α-
567 hydroxysteroid dehydrogenase (20α-HSD)/ prostaglandin F-synthase (PGFS) in bovine

568 placentomas: implications for the initiation of parturition in cattle. *Placenta* **27**, 1022-
 569 1029.

570 Shirasuna, K., Akabane, Y., Beindorff, N., Nagai, K., Sasaki, M., Shimizu, T.,
 571 Bollwein, H., Meidan, R., Miyamoto, A. (2012). Expression of prostaglandin F₂ α
 572 (PGF₂ α) receptor and its isoforms in the bovine corpus luteum during the estrous cycle
 573 and PGF₂ α - induced luteolysis. *Domest. Anim. Endocrin.* **43**, 227-238.

574 Sakamoto, K., Ezashi, T., Miwa, K., Okuda-Ashitaka, E., Houtani, T., Sugimoto, T., Ito,
 575 S., Hayaishi, O. (1994). Molecular cloning and expression of a cDNA of bovine
 576 prostaglandin F₂ α receptor. *J. Biol. Chem.* **269**, 3881-3886.

577 Sakamoto, K., Miwa, K., Ezashi, T., Okuda-Ashitaka, E., Okuda, K., Houtani, T.,
 578 Sugimoto, T., Ito, S., Hayaishi, O. (1995). Expression of mRNA encoding the
 579 prostaglandin F₂ α receptor in bovine corpora lutea throughout the oestrous cycle and
 580 pregnancy. *J. Reprod. Fertil.* **103**, 99-105.

581 Siviero, F. (2013). 'Biologia celular bases moleculares e metodologia de pesquisa.'
 582 (Roca : São Paulo).

583 Takagi, M., Yamamoto, D., Ogawa, S., Otoi, T., Ohtani, M., Miyamoto, A. (2008).
 584 Messenger RNA expression of angiotensin-converting enzyme, endothelin,
 585 cyclooxygenase-2 and prostaglandin synthases in bovine placentomas during gestation
 586 and the postpartum period. *Vet. J.* **177**, 398-404.

587 Tenhagen, B.A., Birkelbach, E., Heuwieser, W. (2000). Serum progesterone levels in
 588 post-partum dairy cows after repeated application of prostaglandin F₂ α analogue D (+)
 589 cloprostenol sodium. *J. Vet. Med. A.* **47**, 213-220.

590 Weems, Y.S., Bridges, P.J., Jeoung, M., Arreguin-Arevalo, J.A., Nett, T.M., Vann,
 591 R.C., Ford, S.P., Lewis, A.W., Neyendorff, D.A., Welsh, J.R., Randel, R.D., Weems,
 592 C.W. (2012). In vivo intra-luteal implants of prostaglandin (PG) E1 or E2 (PGE1,

593 PGE₂) prevent luteolysis in cows. II: mRNA for PGF₂α, EP1, EP2, EP3 (A–D),
594 EP3A, EP3B, EP3C, EP3D, and EP4 prostanoid receptors in luteal tissue. *Prostag. Oth.*
595 *Lipid. M.* **97**, 60-65.

596 Wehbrink, D., Hässig, M., Ritter, N., Zerba, H., Bleul, U., Boos, A. (2008).
597 Immunohistochemical demonstration of cyclooxygenase – 2 (COX-2) and prostaglandin
598 receptors EP2 and EP4 expression in bovine intercaruncular uterine wall around term.
599 *Anim. Reprod. Sci.* **106**, 241-254.

600 Vane, J.R., Bakhal, Y.S., Botting, R.M. (1998). Cyclooxygenases 1 and 2. *Annu. Rev.*
601 *Pharmacol. Toxicol.* **38**, 97-120.

602 Ymer, N., Rosnina, Y., Wahid, H., Saharee, A.A., Yap, K.C., Ganesamurthi, P. (2010).
603 Ovarian activity in beef and dairy cows with prolonged postpartum period and heifers
604 that fail to conceive. *Trop. Anim. Health Prod.* **42**, 607-615.

605

606

607

608

609

610

611

612

613

614

615

616

617

List of Figures

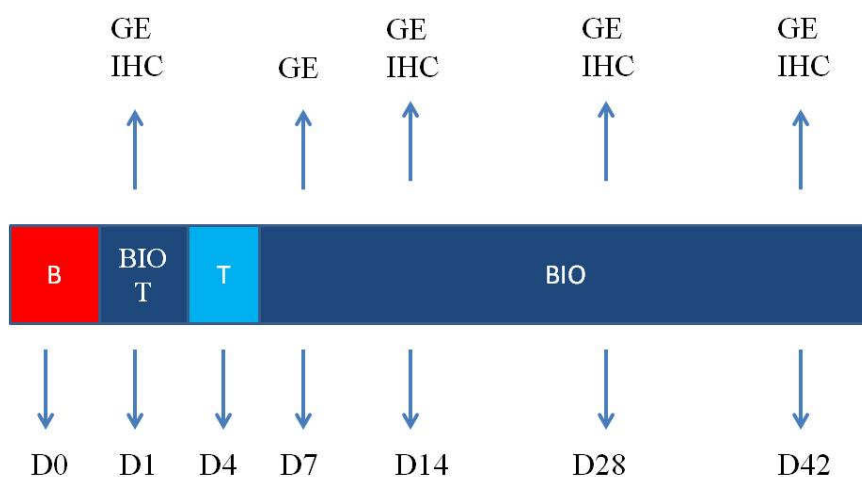


Figure 1: Timeline. Animals were submitted to treatment (T) with cloprostenol sodium or 0.9% NaCl solution on D1 and D4 (D0= Birth - B). Then it was collected endometrial biopsies (BIO) for evaluation of gene expression (GE) on D1, D7, D14, D28 and D42 and for immunohistochemistry (IHC) performance on D1, D14, D28 and D42.

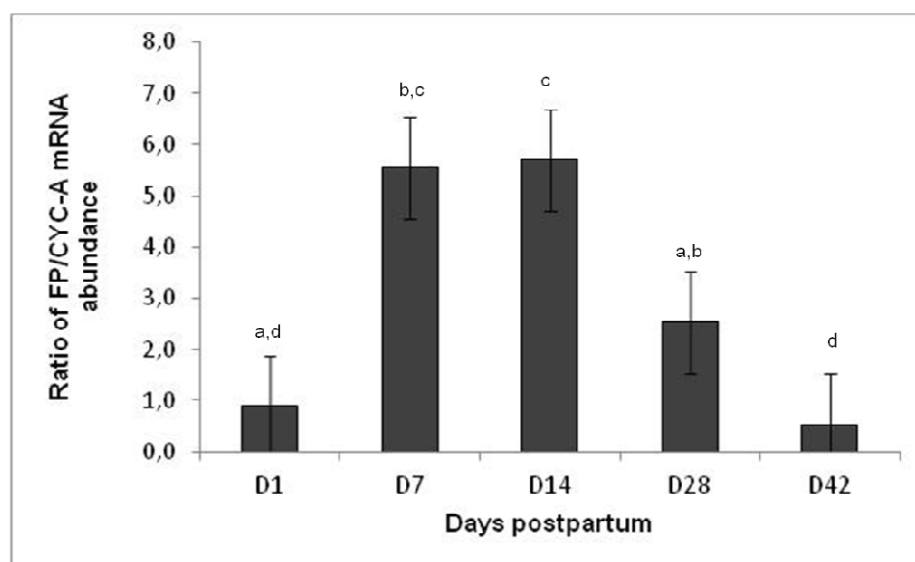


Figure 2: Ratio of FP/CYC-A mRNA abundance measured by real time rtPCR of 24 Nelore cows during postpartum period (D1, D7, D14, D28 and D42). Values are shown as mean values ($\pm$ S.E.M). Uncommon

letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package.

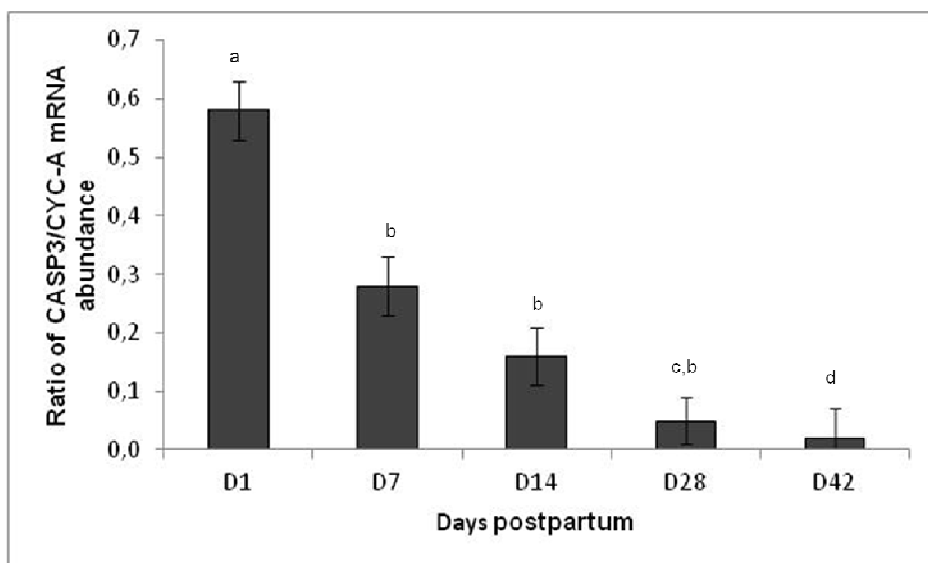


Figure 3: Ratio of CASP-3/CYC-A mRNA abundance measured by real time rtPCR of 24 Nelore cows during postpartum period (D1, D7, D14, D28 and D42). Values are shown as mean values ($\pm$ S.E.M). Uncommon letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package.

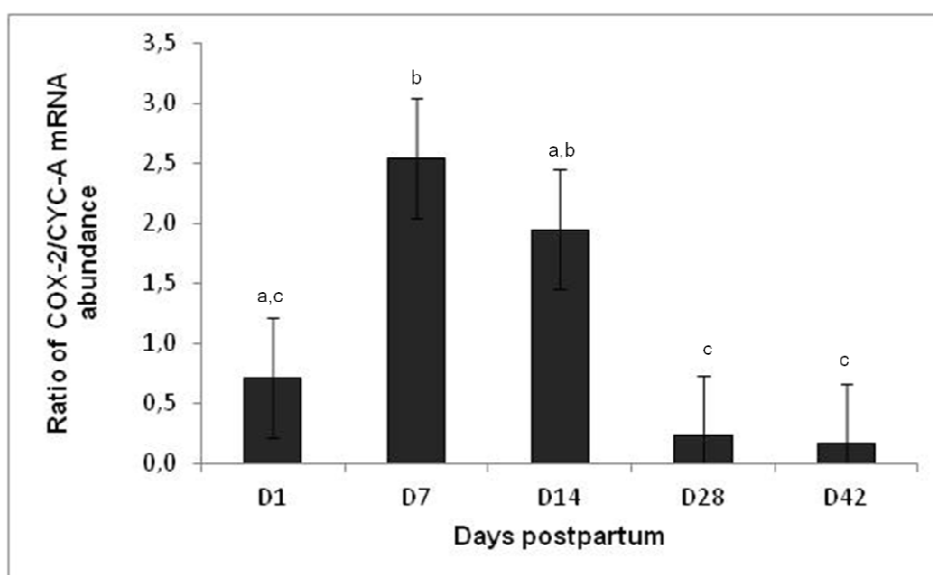


Figure 4: Ratio of COX-2/CYC-A mRNA abundance measured by real time rtPCR of 24 Nelore cows during postpartum period (D1, D7, D14,

D28 and D42). Values are shown as mean values ($\pm$ S.E.M). Uncommon letters differ ($P<0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package.

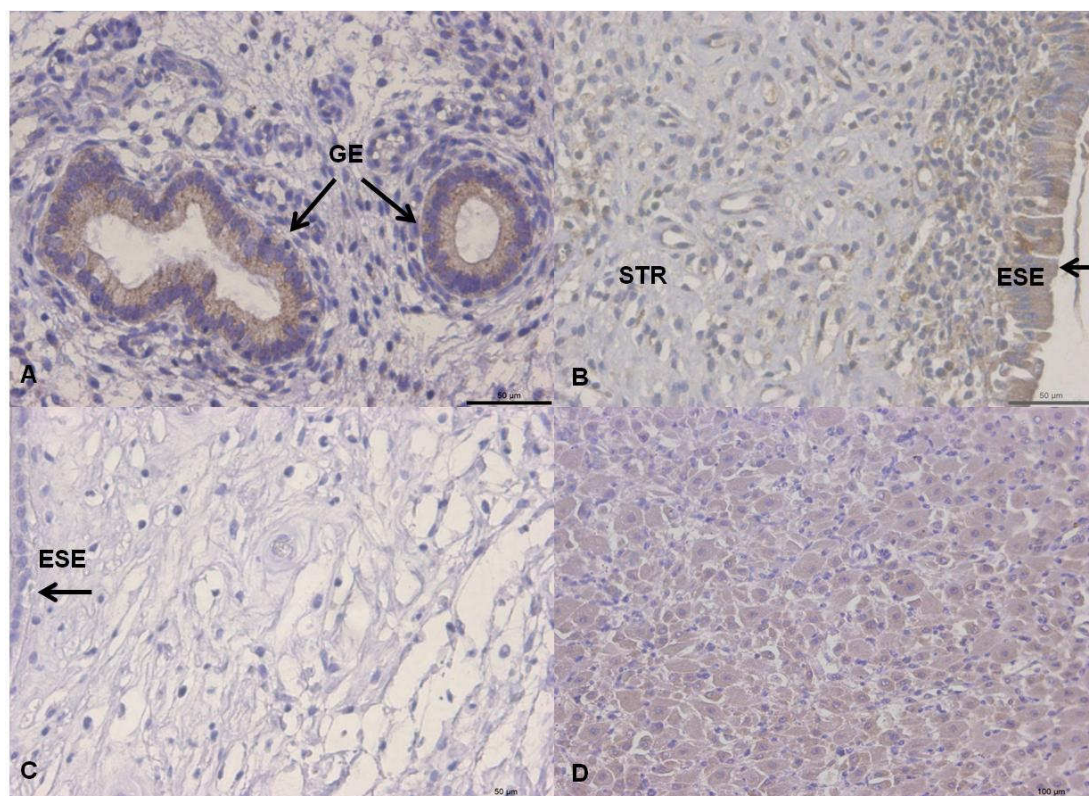


Figure 5: Immunohistochemical detection of FP receptor during postpartum of Nelore cows. Note positive cytoplasmic staining (brown) in A and B (arrows). C: negative control by omitting primary antibody. Bar = 50 μ m. D: positive control (bovine corpus luteum). Bar = 100 μ m. ESE: endometrial surface epithelium, STR: stroma, GE: glandular epithelium.

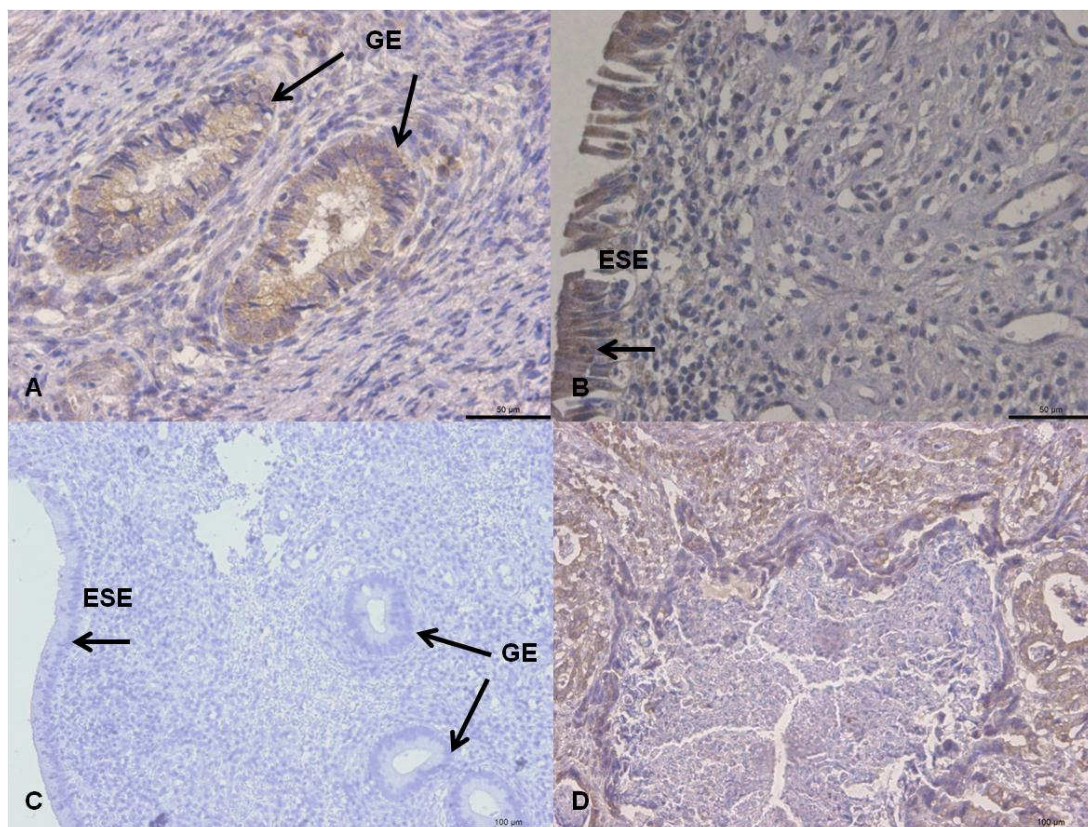


Figure 6: Immunohistochemical detection of COX-2 during postpartum of Nelore cows. Note positive cytoplasmic staining (brown) in A and B (arrows). Bar = 50μm. C: negative control by omitting primary antibody. D: positive control (bovine lung adenocarcinoma). Bar = 100μm. ESE: endometrial surface epithelium, STR: stroma, GE: glandular epithelium.

List of Tables

669

670 Table 1: Sequence of primers and amplification product size of CASP-3, COX-2, CYC-
 671 A, FP and GAPDH.

Gene	Sequence	Product size (bp)
CASP-3	F – AAGCCATGGTGAAGAAGGAA R- GGCAGGCCTGAATAATGAAA	134
COX-2	F – TGCTGAGTTTAAACACGCTCTACCA R - TGAGACCATGTTCCAGTAAGACAGA	125
CYC-A	F – GCCATGGAGCGCTTTGG R - CCACAGTCAGCAATGGTGATCT	65
FP	F – GTAGAGGTGACTTGCACTGTAG R - GTTCTCTGCACCCATCACTTA	96
GADPH	F – GGCGTGAACCACGAGAAGTATAA R - CCCTCCACGATGCCAAAGT	119

672 F: forward; R: reverse; FP, CASP-3: caspase 3, COX-2: cyclooxygenase-2, CYC-A:
 673 cyclophilin-A, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

674

675

676

677

678

679

680

681

682

683

684 Table 2: Analyzed variables and classification on immunohistochemistry of samples
 685 from day 1, 14, 28 and 42 postpartum of Nelore cow.

Analyzed variables	Classification
Endometrial, glandular and stromal score of staining*	0 - 0%
	1 - 25%
	2 - 26% to 50%
	3 - 51% to 75%
	4 - > 75%
Score of colour intensity **	0 - no staining
	1 - weak
	2 – moderate
	3 – strong
Immunolocalization	Citoplasmic
	Nuclear

686 *based on the percentage of staining cells

687 **based on brown intensity

688

689 Table 3: Median, minimum and maximum values of score of staining and score of
 690 staining intensity of immunohistochemical detection of FP and COX-2 of endometrial
 691 samples during postpartum period.

Parameter		Score of staining*	Score of colour intensity*
FP	ESE	4 (0-4)	2 (0-3)
FP	GE	4 (2-4)	2 (1-3)
FP	STR	0 (0-3)	0 (0-2)
COX-2	ESE	4(4)	3 (2-3)
COX-2	GE	4(3-4)	2 (1-3)
COX-2	STR	0 (0-1)	0 (1-2)

692 Median, minimum and maximum scores. ESE- endometrial surface epithelium, GE-
 693 glandular epithelium, STR- stroma. Score of staining: 0 – no staining, 1 – until 25%, 2 –
 694 26 to 50%, 3 – 51 to 75%, 4 – more than 75% of staining. Score of staining intensity: 0
 695 – no staining, 1 – weak, 2 – moderate, 3 – strong.

Capítulo 3

Artigo Científico nas normas e a ser submetido ao periódico “Journal of Reproduction and Development”

Normas para publicação no periódico: “Journal of Reproduction and Development”

INSTRUCTIONS TO AUTHORS

1. Aims and Scope

Journal of Reproduction and Development (JRD) is the official journal of the Society for Reproduction and Development, published bimonthly, and welcomes original articles. The functions of the journal are managed by Editorial Board Members, such as the Editor-in-Chief, Co-Editor-in-Chief, Managing Editors and Editors. All manuscripts are peer-reviewed critically by two or more reviewers. Acceptance is based on scientific content and presentation of the materials. The Editors select reviewers and correspond with authors. Final decisions about acceptance or rejection of manuscripts are made by the Editor-in-Chief and Co-Editor-in-Chief.

2. Manuscript Categories

Manuscripts submitted to JRD must represent original and unpublished materials and should not be under editorial consideration elsewhere. The JRD publishes three types of articles: Original Articles, Technology Reports and Reviews. Original Articles are reports of original, investigative studies that report new findings or concepts in reproductive biology, developmental biology (including development of early embryo and ontogeny of reproductive organs), reproductive engineering, clinical reproduction and any other relevant fields of scientific research on reproduction. Original Articles are full papers including new findings in the above-mentioned research areas. Technology Reports are short reports, in which the emphasis is put more on methodology, such as new techniques or novel approaches to solve reproductive problems. Review articles are usually invited by the Editorial Board Members. Those who would like to contribute Reviews should consult with a member of the Editorial Board in advance.

3. Manuscript Submission Procedures

3.1 Electronic Submission

The entire submission and review process for JRD has been transferred to an online system. Authors should first go to the website of the Society for Reproduction and Development (<http://reproduction.jp/>) and then follow the link to the online submission system. When you submit a manuscript to the JRD through the online system, your manuscript is immediately given a manuscript ID number. Inquiries about submitted manuscripts should be made to the Editorial Office by e-mail at jrd@ipecc-pub.co.jp. Please include the manuscript ID number with inquiry.

3.2 Electronic submission procedures

3.2.1 Materials prepared before submission

- 1) Corresponding author's information (affiliation, address, e-mail address, etc.)
- 2) Title and abstract of the manuscript

- 3) Manuscript files-The file formats listed below are acceptable.

Text: .doc, docx, .rtf, etc.

Tables: .doc, .docx, .rtf, xls, .xlsx, etc.

Figures: .tif, .eps, .jpg, .gif, .psd, .ai, .ppt, .pptx, etc.

Files with the following extensions are not accepted (.exe, .com, .shs, .vbs, .zip).

- 4) Supplementary files

- 5) Names of suggested reviewers and/or not-recommended reviewers, if any.

3.2.2 Electronic manuscript submission

Follow the submission steps indicated on the website.

3.2.3 Supplementary files

Supplementary files will be published online only. The file formats listed below may be used for Supplemental files.

.doc or .docx, MS Word documents; .xls or .xlsx, MS Excel Spreadsheet; .ppt or .pptx, MS PowerPoint presentation; .jpg, JPEG image; .gif, Graphical image; .pdf, Adobe Portable Document Format; .mov Quick Time; .wav, Sound; .txt, Plain ASCII.

3.2.4 Affirmation of Originality and Assignment of Copyright Form

A completed Affirmation of Originality and Assignment of Copyright Form should be signed by all authors and either e-mailed or faxed to the editorial office at +81-3-5978-4068 immediately after you make an electronic submission to the JRD. The Affirmation of Originality and Assignment of Copyright Form can be downloaded through the website of the Society for Reproduction and Development (<http://reproduction.jp/jrd/jrdtop.html>).

4. Manuscript Preparation

4.1 General Format

Authors should prepare a manuscript in standard American English and follow the Instructions to Authors. Manuscripts that do not meet minimum standards in English grammar and syntax or do not follow the Instructions to Authors will immediately be returned to the authors. The manuscripts must be typed with 25-mm margins on both sides using 11-point type in Times New Roman font with 24-26 lines per page on A4-size (210 × 297 mm) or Letter-size (216 × 279 mm) paper. All lines of the text should be numbered serially in the left margin, and the entire manuscript should be paginated. Approximately 3.5 type-written pages correspond to one printed page. Failure to restrict the length of manuscripts as described below can negatively influence the reviewers' and editor's decisions. The total file size the authors are allowed to upload is 50

Mbytes in the online submission system. Should the authors need any assistance for the online submission process, please contact JRD Editorial Office (e-mail: jrd@ipecc-pub.co.jp).

4.1.1 Original Articles

Original Articles are to be arranged in the following order, and the following sections must begin on separate pages: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, References, Tables and Figures (including figure legends on the same page). Original Articles are limited to no more than 7 printed pages. The abstract should be written in a single paragraph of no more than 250 words.

4.1.2 Technology Reports

Technology Reports are succinct reports and prepared in the following order: Title page, Abstract, Text, Methods, Acknowledgments, References, Tables and Figures (including figure legends on the same page). The text should have NO SUBHEADINGS. Printed pages should be no more than 4 pages. The abstract should be written in a single paragraph of up to 150 words. Up to 30 references may be cited.

4.1.3 Reviews

Reviews include a Title page, Abstract, Text, Acknowledgments, References, Tables and Figures (including figure legends on the same page). The text should be written with concise subheadings for easy reading, and the number of printed pages should be no more than 8 pages. The abstract should be written in a single paragraph of no more than 250 words.

4.2 Title page

4.2.1 Titles and subtitles

Titles should be concise, specific, informative and clear and should emphasize the main point of the article. Avoid abbreviations or symbols that are not easily understood by general scientific readers. They should include key words or phrases that are useful for indexing. Subtitles may be used to amplify the title; however, they should be able to stand alone.

4.2.2 Authorship

The authors should be indicated immediately below the title or subtitle. Each author's full name and address with postal code of the institution where the study was performed should be included. Persons who made subsidiary contributions may be listed in the "Acknowledgments".

4.2.3 Running head

A running head not exceeding 40 characters including spaces should be provided on the first page.

4.2.4 Corresponding author

An e-mail address with the name of an author to whom all correspondence regarding the manuscript is to be directed must be provided at the bottom of the title page. The e-mail address will appear on the first page of the published article for readers who may want to request reprints or additional information.

4.3 Abstract

The abstract summarizes the main points of an article: (1) the purpose of the study, (2) basic procedures, (3) main findings and (4) principal conclusions. The abstract should be clearly understandable in itself. Avoid abbreviations or symbols that are not easily understood by general scientific readers.

4.4 Key words

Three to five key words that represent key concepts of the paper should be given at the end of the abstract in alphabetical order.

4.5 Materials and Methods

This section should provide readers with sufficient information to replicate the work. Although it is not necessary to give details of published methodology if appropriately cited, any modifications must be described. Information regarding statistical analysis of the data should be included.

4.6 Results and Discussion

The Results section should briefly present the experimental data in text, tables or figures. Details about the preparation of tables and figures are provided below. The Discussion should focus on the interpretation and significance of the findings with concise objective comments that describe their relation to other work in that area. The Discussion should not reiterate the Results. The Results and Discussion sections may be combined, if necessary.

4.7 Acknowledgments

Authors should acknowledge financial support, gifts of materials, technical help, editorial assistance, etc. in this section.

4.8 References

References should be cited in the text by Arabic numeral in square brackets, e.g. [1, 2–5, 7]. They should be listed in the order of appearance in the text. Articles included in the reference list must appear in publications that are accessible to most scientists. Reference material that has been accepted for publication must be listed as "in press"; the listing must include journal title and anticipated year of publication, or, for books, the publisher and year of publication. The journal names should be abbreviated according to Serial Sources for the BIOSIS Database or Index Medicus. Sources of reference that are "in preparation," "submitted for publication," "personal

communication” or “unpublished data” should not be included in the reference list; they should appear in the text parenthetically immediately following the names of the authors or the source of information.

Sample references

(1) Journal article

1. **Lee VH, Fields PA.** Rabbit relaxin: The influence of pregnancy and ovariectomy during pregnancy on the plasma profile. *Biol Reprod* 1991; 45: 209-214.

(2) Book chapter

2. **Desjardins C, Lopez MJ.** Sensory and nonsensory modulations of testis function. In: Steinberger A, Steinberger E (eds.), *Testicular Development, Structure and Function*. New York: Raven Press; 1980: 381-388.

(3) Abstracts in published proceedings

3. **Yanagawa T, Shirasu K, Uemura T, Minaguchi H.** Effects of chronic administration of GnRH analog to the pituitary and the ovary. In: Program of the 8th International Congress of Endocrinology; 1988; Kyoto, Japan. Abstract 189.

(4) Supplements

4. **Bravo PW, Stabenfeldt GH, Fowler ME.** Ovarian follicular activity in llamas. *Biol Reprod* 1989; 40 (Suppl 1): 51 (abstract 11).

(5) References in non-English languages

The title and the journal name for references published in Japanese or in any other non-English language should be translated into English with the author's consent if necessary, followed by a description of the original language in parenthesis, e.g., (In Japanese), at the end.

5. **Ushijima H, Tsunoda Y, Eto T, Imai H.** *In vitro* development of bovine reconstituted eggs after fusion with a blastomere from 8-cell to blastocyst stage embryos. *Jpn J Anim Reprod* 1991; 37: 15-19 (In Japanese).

4.9 Figures and Tables

In the initial submission, figures should have enough resolution for review purposes. Please make sure that the figures will be printed with a horizontal diagonal width of 8.4 cm (one column) or 17.5 cm (double column) and a vertical length less than 22 cm. They should be cited in the text in consecutive numerical order. Titles and legends of figures should be written on the pages where the respective figures appear.

Tables without vertical lines are preferred. Any explanation essential to the understanding of the table should be given as a footnote designated by symbols in order, such as a), b), c).

4.10 Abbreviations

Abbreviations in the body of the paper should be used

after having been initially explained. Common recognizable abbreviations, such as M, mM, μ M, C for Celsius, N, m, cm, mm, μ m, nm, pm, cm², l, ml, μ l, kg, g, mg, μ g, ng, pg, h, min, sec, msec, rpm, Hz, GBq, MBq, kBq, cpm, dpm, ppm, J, lx, etc., can be used without definition. The metric system of weights and measures and the 24-hour clock to express time (e.g. 0500 h or 1900 h) should be used.

Make sure that the nomenclature and/or abbreviations of genes or proteins for the particular organisms you deal with in your manuscript are correct. For genes or DNA, use italicized letters (e.g., *Sry*, *SRY* or *lacZ*). For proteins, use Roman letters with at least the first letter capitalized (e.g., Hprt, HPRT, Gpi, GPI or Gal). Note that in names of restriction enzymes, only the first 3-4 letters are italicized (*EcoRI*, *EcoR124/3*, *HindIII* or *StySBI*). Use lower case k for 'kilo', such as in kDa or kb.

The nomenclature and abbreviations will be checked by the Editorial Board members, if necessary.

4.11 Statistical Analysis

Statistical analysis of the data must be done by appropriate methods, which should be indicated in the text.

4.12 Ethics

Investigators should follow the highest standards for the humane care and use of animals in research. The minimum number of animals that meets rigorous scientific and statistical standards should be used. Research animals shall be properly housed and fed, and their surroundings shall be kept in a sanitary condition. The animals shall receive appropriate anesthetics, analgesics, tranquilizers or care to minimize pain and discomfort during preoperative, operative and postoperative procedures. All experiments using animals should be done under the approval of an institutional animal experiment committee and this should be stated and documented in the text. For experiments on human subjects, informed consent must be obtained from the patients, and the experiments must be approved by an institutional human research committee. This must be stated and documented in the text.

4.13 Publication after Acceptance and Electronic Editing

Upon acceptance, authors are requested to supply all text data (including tables) and original figures for publication by the editorial office. Text and table data are preferred in MS WORD or MS EXCEL format. The resolution of each figure for publication should be sufficiently high (at least 300 dpi), and color figures should be saved in CMYK mode. For figures, commonly used formats, such as EPS, GIF, JPG and TIFF, are acceptable. Figures in MS WORD format are not acceptable for publication.

All manuscripts from authors of whose mother tongue is not English will be subject to text editing. The results of text editing will be sent from the editorial office by e-mail, and authors are requested to supply the revised final version of

manuscript files as well as figure and table files by e-mail. If no corrections are made, the files uploaded to the online submission system will be used for printing.

4.14 Proofs and Reprints

The first proofreading is the authors' responsibility. An order form and price list for reprints will be sent to the authors with the first proof.

4.15 Publication charges

4.15.1 Page charge

The page charge is 10,000 JPY per printed page with no color figures. A manuscript will be subject to this charge if the Editorial Board considers that it needs English editing (15,000 JPY for 7 printed pages as a rough estimate).

4.15.2 Color charge and reprints

For color printing, 80,000 JPY will be charged per printed page containing color figures. The Journal of Reproduction and Development offers a 50% discount for the color charge, when the color figures are judged essentially by the editors and the first or corresponding author is a member of the Society for Reproduction and Development. Our editorial policy requires the print version of an article to be identical to the online version. Upon submission of a manuscript with color figures, the authors shall agree to pay the color charge when the paper is accepted for publication.

Reprinting will cost 10,000 JPY per 50 reprints at minimum.

4.16 Notice about photocopying

In order to photocopy any work from this publication, you or your organization must obtain permission from the following organization, which has been delegated for copyright clearance by the copyright owner of this publication.

<Japan and any country other than the
USA> Japan Academic Association for
Copyright Clearance, Inc. (JAACC)
6-41 Akasaka 9-chome,
Minato-ku, Tokyo 107-0052, Japan
TEL: +81-3-3475-5618 FAX: +81-3-3475-5619
E-mail: jaacc@mtd.biglobe.ne.jp

<USA>
Copyright Clearance Center, Inc.
222 Rosewood Drive, Danvers, MA 01923,
USA Phone: +1-978-750-8400 FAX: +1-978-
6468600

Title: Effect of cloprostenol sodium on uterine involution, progesterone and 13,14-dihydro-15-keto prostaglandin F2 α concentrations of multiparous Nelore (*Bos taurus indicus*) cows

Subtitle: Effect of cloprostenol sodium on uterine involution and hormonal dosage at puerperium of cows

Carolina Nogueira de Moraes¹

Leandro Maia¹

Fabiana Ferreira de Souza¹

Carla Martins de Queiroz¹

Mateus José Sudano²

João Carlos Pinheiro Ferreira¹

Eunice Oba¹

¹ São Paulo State University – UNESP, Rubião Júnior s/n, CEP: 18618-970, Botucatu, São Paulo, Brazil.

² Federal University of Pampa - UNIPAMPA, BR 472 – Km 592 - CEP: 97500-970, Uruguaiana – Rio Grande do Sul , Brazil

Running head: Effect of prostaglandin on postpartum of Nelore

Corresponding Author

Carolina Nogueira de Moraes

carolnmoraes@hotmail.com

Abstract

This study aimed to evaluate the effects of cloprostenol sodium at uterine involution and at hormonal dosage of P4 and PGFM on multiparous Nelore cows treated or not with cloprostenol sodium on days 1 and 4 postpartum (D0 = birth), as well as test the hypothesis that treatment promotes faster uterine involution and return to normal cyclical activity postpartum. For this, it was done ultrasound, transretal palpation, vaginoscopy exams and determination of concentration of P4 and PGFM on days 1, 7, 14, 28, 35 and 42 postpartum (D0 = birth). Vaginoscopy evaluated cervical ostium (score 1 to 3) and cervical discharge (score 1 to 5), transrectal palpation evaluated uterine involution (score 1 to 3) and ultrasound evaluated the diameter of follicles, size of uterine horn and intrauterine fluid accumulation (score 0 to 3). Cloprostenol sodium influenced at cervical discharge ($P= 0.034$), and on the other variables it was observed influence of moment ($P> 0.05$). Based on the results, we believe that the use of this protocol in animals with good selection genetic, management and health can be unnecessary and promote additional spending.

Key words: bovine, cloprostenol sodium, hormonal dosage, postpartum, uterine involution

Introduction

During the postpartum period, uterus passes for modifications such as myometrial contraction for uterine cleaning of microbial contamination, uterine and cervical involution, resumption of ovarian cyclicity and endometrial repair [1] for the restoration of an environment able to maintain a new pregnancy. In cows, the period of 65 days of postpartum is recommended and the conception within 85 to 90 days is desired according to review [2]. Being like this, the production of one calf/year can be reach with economical gain.

The monitoring of postpartum uterine involution can be performed by rectal palpation, endometrial biopsies, ultrasonographic and vaginoscopy exams [3,4,5]. Transrectal exam can result in many variations between examiners and endometrial biopsies do not bring information at the time of realization. Ultrasonographic exam has the advantage that allows us to access information about uterine diseases and involution at the moment when it is done.

After delivery the concentrations of steroidal hormones reduce to basal levels because of the absence of follicular growth during gestation [6]. With 5 to 7 days postpartum, there is an increase on the concentration of FSH and the first follicular wave postpartum starts at 7 to 10 days postpartum [7]. However, the resumption of ovarian cycle in beef cattle can be delay because of some factors such as nutrition and presence of calf [8]. Beef cattle with good corporal condition usually ovulates the dominant follicle with 30 days postpartum when the pulses of LH are at the order of 1pulse/40 minutes [7,9].

Prostaglandin $F2\alpha$ ($PGF2\alpha$) is produced in the uterus and helps in uterine involution postpartum acting at myometrial contraction [10] and its metabolite 13,14-dihydro-15-keto prostaglandin $F2\alpha$ (PGFM), is highly correlated with the period of uterine involution [11].

Analogues of $PGF2\alpha$ such as cloprostenol sodium are used in postpartum period to induce or synchronize oestrus [12], to control luteal function and to reduce the period of uterine involution [13]. This work starts from the hypothesis that cloprostenol sodium has a positive effect in

1 reducing the postpartum period to accelerate uterine involution and allow rapid return to normal
2 cyclical activity postpartum. Thus, we evaluated the effect of serial administration of cloprostenol
3 sodium (D1 and D4, D0 = birth) on the process of ovary activity and uterine involution at different
4 times (D1, D7, D14, D21, D28, D35 and D42) of postpartum of multiparous Nelore cows by hormone
5 dosage, vaginoscopy, transrectal palpation, and ultrasound exams. The confirmation of the hypothesis
6 under study would be beneficial to reduce the interval between calving and increase production to a
7 calf/cow/year, at low cost, fact desirable in beef cattle.

Materials and Methods

Ethical aspects

The study was conducted in accordance with ethical guidelines recommended by National Council for Control of Animal Experimentation and College of Animal Experimentation, and was approved by the Institution's Animal Care and Experimentation Ethics Committee on protocol number 50/2012.

Animals

A total of twenty four multiparous Nelore (*Bos taurus indicus*) cows with parity order between 2-6 (median= 3) and aged between 4 -13 years (median =9) were used. Animals had body condition score between 3 and 4 (from 1 to 5) and were *ad libitum* suckling one calf. Animals were housed in paddocks predominantly of *Brachiaria decumbens*, with mineral supplementation (Minersal®, Presidente Venceslau, SP, BR) and water *ad libitum*.

Animals with no calving assistance, retained fetal membranes, twin pregnancy or puerperal complications were selected to the realization of this experiment wich was conducted in a farm located in Avaré, State of São Paulo, Brazil (latitude: 23°10'S; longitude: 48°93'E; altitude 766 m).

Treatment

The animals were randomly assigned into the treatment and placebo groups (TG and PG, respectively), according to parity order. Treated group (TG) received 0,530 mg (2 mL) of cloprostenol sodium (Ciosin®, MSD Animal Health, São Paulo, SP, BR) intramuscularly, on days 1 and 4 postpartum (D0 = birth). Placebo group (PG) received by the same route and period 2 mL of 0.9% NaCl solution (Sanobiol®, Pouso Alegre, MG, BR).

Progesterone and PGFM assays

Blood plasma was obtained by coccygeal venipuncture, after local cleaning with water, 2% chlorhexidine degermante (Riohex 2% Clorexidina®, Rioquímica, São José do Rio Preto, SP, BR) and alcohol iodized, in heparinized tubes on days 1, 7, 14, 21, 28, 35 and 42 postpartum (considering D0 = day of parity).

Immediately after collection, the blood was centrifuged (Centrifuge Fanem, Bangalore, KA, IN) at 2,500 xg for 10 minutes to obtain the plasma, which was stored in plastic tubes previously identified and stored at -20° C until performing the hormone assays for determining the concentrations of progesterone (P4) and PGFM.

The concentration of P4 was measured using the Coat-A -Coat radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA) and PGFM concentration was evaluated using the DetectX 13,14- dihydro ® kit 15 - keto - prostaglandin F2a (PGFM) enzyme immunoassay kit (Assays Arbor, Michigan, USA), according to the manufacturer's recommendations.

The value of P4 > 1ng/mL was used as cut-of value to indicate of ovulation and presence of corpus luteum [14].

Vaginoscopy

The vaginoscopy was performed on days 1, 7, 14, 21, 28, 35 and 42 postpartum (considering D0 = day of parity) for evaluation of cervical ostium and cervical discharge. Therefore, the vulva was cleaned with water and 2% chlorhexidine degermante (Riohex 2% Chlorhexidine ®, São José do Rio Preto, SP, BR) and a tubular speculum was introduced in the vulva until cervical ostium for the evaluation.

The cervical ostium was evaluated for the presence of inflammation and classified as: 1 - rosea (cervical ostium without inflammation), 2 - intermediate (cervical ostium with small reddish areas) and 3 - red (cervical ostium with inflammation). The evaluation of cervical discharge was performed based on the aspect and the discharge was classified as: 1 - translucent or absent; 2 - red, 3

– mucopurulent (50% of clear mucus and 50% of pus flakes), 4 - chocolaty and 5 - purulent (> 50% pus and bad smell). The variables are shown summarized in Table 1.

Transrectal palpation

Transrectal evaluation was performed on days 1, 7, 14, 21, 28, 35 and 42 postpartum (considering D0 = day of parity) to identify the degree of uterine involution.

For this, uterine involution was ranked 1-3, with grade 1, uterus little involuted, with all of its mass in the abdominal cavity, with large asymmetry, not being possible its entire palpation, grade 2, uterus with some part of its mass in the abdominal cavity, with reasonable asymmetry, being possible the palpation of its entire length and grade 3, uterus with almost all its totality in the pelvic cavity, showing little asymmetry or symmetry. The variables are shown summarized in Table 1.

Ultrasound exam

Ultrasound exams were performed on days 1, 7, 14, 21, 28, 35 and 42 postpartum (considering D0 = day of parity). The exam was done with the ultrasound portable equipment (Aloka S500; Aloka, Tokyo, JP) using a linear transducer of 7,5 MHz on the uterine horn previously identified as pregnant by rectal palpation on D1 (according to the difference of size). For the analysis of the involution it was used the following variables: 1 - measurement of the diameter of previously uterine horn (mean of 3 measurements) and 2 - intrauterine fluid accumulation (mean of 3 measurements) according to other study [15] with modifications, classified in: 0 – without fluid; 1- less than 1 cm of fluid in the mean of 3 measurements; 2- more than 1 cm of fluid in the mean of 3 measurements and 3 – more than 2 cm in the mean of 3 measurements. Also, it was done the measurement of the largest follicle in each ovary from D7. The variables are shown summarized on Table 1.

Uterine involution was considered complete when there was observed a minimal uterine diameter, uterine fluid accumulation and degree of uterine involution was observed, without difference

between two successive examinations.

Data analysis

Data were analysed using the mixed linear model (MIXED) procedure with the SAS statistical software package (Version 9.2; SAS Inst. Inc., Cary, NC, USA). Sources of variation in the model included treatment (TG vs PG), moment (day 1, 7, 14, 21, 28, 35 and 42 postpartum), and first-order interactions; these were considered as fixed effect. Animals were considered as random effect. If necessary, logarithmic transformation was used to improve normality. Means were separated using least square difference (LSD). All data are reported as untransformed least-squares means $\pm$ s.e.m. For all analyses, $P < 0.05$ was considered to be significant.

Results

Based on the results of the present study, cloprostenol sodium show effect ($P = 0.03$) only on variable cervical discharge. Additionally, regardless of treatment, differences ($P < 0.05$) were observed in function of time (moment) in the variables studied: concentration of P4, concentration of PGFM, uterine diameter, follicles size, degree of uterine involution, intrauterine fluid, cervical ostium and cervical discharge.

It was considered D1 as the day of minimal degree of uterine involution and D42 as maximal degree of uterine involution and because of it, comparisons were mainly done among these days.

Progesterone and PGFM dosage

Animals with high concentrations of P4 ($n=1$) and PGFM ($n=2$) during all experimental period were omitted from hormonal analysis to avoid incorrect data analysis. It was observed difference between the moments ($P < 0.05$) but there was no influence of treatment ($P > 0.05$) on the concentration of P4. It was also observed difference between the moments ($P < 0.05$) with a tendency of influence of treatment x moment ($P = 0.07$) for concentration of PGFM.

Concentrations of P4 on D42 differed from the other studied moments (D1, D7, D14, D21, D28 and D35) ($P = 0.00$), with an increase and with its highest concentration value on D42, as shown on Figure 1.

Concentration of PGFM differed significantly ($P < 0.00$) between D1 to D42 with a general decrease on its value and with a progressive decrease from D7 to D28, as shown on Figure 2.

Cervical ostium and cervical discharge

Regarding cervical discharge and cervical ostium it was observed influence of moment ($P < 0.0001$) for both and influence of treatment ($P = 0.03$) for the variable cervical discharge. Data are shown on Table 2. The mean scores of the cervical ostium observed in D1 differed ($P < 0.05$), from the

other studied moments (D14, D21, D28, D35 and D42), except for D7 with a reduction in the degree of inflammation as indicated by scores. The mean score of the cervical discharge changes from D1 to D21 ($P < 0.05$) (from a translucent or absent to a red secretion), however with no difference ($p > 0.05$) between D1, D28, D35 and D42.

Also treatment had influence on cervical discharge ($P = 0.03$) being that treated animals were closer (1.38 ± 0.089) to score 1 than control animal (1.64 ± 0.082).

Uterine diameter, fluid accumulation and follicular size

Concerning uterine diameter, fluid accumulation and follicular size there was influence of moment ($P < 0.00$) but not of treatment ($P > 0.05$). However, it is noteworthy that follicular analysis of left ovary shows no influence of moment ($P = 0.12$) or treatment ($P = 0.53$) and thus presented results are from the right ovary. The results are shown on Figures 3, 4 and 5.

It can be observed a progressive and significant reduction in uterine diameter from D1 to D42 ($P < 0.05$) with decreased values observed until D21, however, without differences ($P > 0.05$), between D35 and D42, as shown on Figure 3.

The accumulation of intrauterine fluid decreased ($P < 0.05$) between the D1 to D42, with a lower average score from the D21 as shown on Figure 4.

Follicular diameter differed ($P = 0.00$) on D7 from D42, with a progressive increase between D7 to D21, maintaining constant from the D21, as shown on Figure 5.

Degree of uterine involution

Regarding degree uterine involution there was influence of moment ($P < 0.00$) but not of treatment. Uterine involution progressed significantly ($P < 0.05$) from D1 to D21, with mean values of desirable score (3 - uterus in the pelvic cavity) from this moment, as shown in Table 3.

Discussion

The present study was conducted with the objective to evaluate the effects of the application of cloprostenol sodium on days 1 and 4 of Nelore postpartum cows to investigate its effect on hormonal dosage of P4 and PGFM, on ovary activity and on uterine involution by evaluations using vaginoscopy, transrectal palpation and ultrasound exams at days 1, 7, 14, 21, 28, 35 and 42 postpartum. Cloprostenol sodium had effect on the variable cervical discharge however, for the other variables it was observed only effect of moment. Although it could not prove our initial hypothesis that cloprostenol sodium accelerate uterine involution and allow rapid return to normal cyclical activity postpartum at the present experimental conditions, we believe that the use of this protocol in animals with good selection genetic, management and health can be unnecessary and promote additional spending.

As cited by other author [2], when serum progesterone level is of 1 ng/mL, it is indicative of luteal activity whereas when it is less than 1 ng/mL for at least 14 days it is indicative of cessation of ovarian cycle. The dominant follicle postpartum in beef cattle with good corporal condition will only ovulate when the pulses of LH were at the order of 1 pulse/40 minutes and it usually occurs around 30 days postpartum [7]. At the present study, animals had low levels of P4 (<1ng/mL) from D1 to D28. Although at D35 the level of P4 is more than 1 ng/mL, significant difference was observed only at D42. Based on this, it can be considerate that animals were at anestrous until day 35 and the resumption of ovary activity occurred between days 35 and 42 (mean 38,5 days). This period was different comparing with works in which it was observed 30,9 [16] or 21 [14] days for dairy cow and of 30 [17] or 46,1 days for beef cow [18].

Although differences of anestrous length can be observed between works, this data is supported by the fact that beef cattle usually do not ovulate follicles from first or second wave, different from dairy cow which can ovulate 15 days earlier [7]. Also individual variation can be found regarding day of ovulation, fact that explain a standard error higher on day 42, once after treatment with PGF2 α not all cows undergo ovulation, showing that the onset of estrous and ovulation

can vary among animals [12]. There is a positive correlation between time of uterine involution and the concentration and magnitude of PGFM, once the decrease of PGFM is associated positively with the decrease on diameter of uterine horns. In animals with normal postpartum, the duration of elevated concentration of PGFM is longer than in animals with abnormal postpartum and these animals with normal postpartum have a shorter period of uterine involution [16, 19-21].

At the present study, the period of lowest levels of PGFM is followed by levels of $P_4 > 1\text{ng/mL}$. Also, high values of PGFM with decreased values until D21, are correlated with the period of greater uterine involution and follicular growth. These data corroborate with other authors [16] who also found a positive correlation of long duration and release of PGFM with a shorter time of uterine involution and first ovulation.

At the present study, the highest concentration of PGFM was observed on D1 and the lowest on D28. Other authors [16] found the highest concentration on day 3 and lowest on day 21 postpartum in dairy cows and Del Vecchio et al [17] found the highest concentration of PGFM on day 10 postpartum, with decreased on the concentration on day 20 and 30 postpartum. It is important to emphasize that at the present work it was done one blood collection for week and it could be of great interest to analyze the variations if more than one collection was done, considering the pulsatility and half-life of PGFM.

Vaginal mucus, which can be accessed for evaluation of uterine inflammation and diseases, [4,14,22] varies individually and as more odorless and colorless, better [23]. The characteristic purulent, mucopurulent and fetid vaginal mucus on days 21 and 28 was considerate to reflect the presence of specific bacteria in uterus [4]. On study, the evaluation of mucus on day 28 postpartum in primiparous cow did not show signs of uterine infection [14]. At the present work, this was observed in all moments once no animal show any sign of uterine infection and cervical discharge was near from desirable score of 1 (mucus transparent or absent) from D28.

The effect of treatment on cervical discharge, with a better score for treated group, can be justified because of the increase of uterine contraction favoring uterine discharge [11] and cleaning of

uterine environment. Also, significant reduction of inflammation observed at cervical ostium can be associated with the process of recovery and cervical involution after delivery. At the present study, after one week of delivery was possible to scan follicle with on average 6.22 ± 0.77 mm. Other authors [18] found follicle of 5-10 mm only 2 weeks postpartum of beef cattle. Differences on time of follicle access can be due different methodologies used and different breed.

From D28 to D35 there was a decrease on follicular size with and increase on P4 concentrations, fact also observed by other authors [18] with increase in P4 and a decrease on follicular size possibly caused by follicular luteinization. According to work [24], follicles acquire ovulatory capacity with about 10 mm. However, as already cited above, beef cattle usually do not ovulate first follicles which can reach the diameter of ovulatory follicles.

The decrease on fluid accumulation is an expected and desirable event once it indicates uterine cleaning by contraction and a diminished risk of uterine infections. At the present study it could be observed that by 14 days postpartum all animals are closer to the desirable score of 0, which indicates no fluid. Little quantity of fluid intrauterine is normal and by ultrasound exams it can be observed as non-echogenics areas, once high quantities and echogenics areas are indicative of pathological process (Revised by [25]). The presence of intrauterine fluid accumulation in the horn until 15 days postpartum was also reported by other authors [3].

Based on the parameters to considerate uterine involution complete, it can be assumed that it occurred between days 35 and 42 (on average 38,5 days), once all parameters were at minimal values on these days. This period of uterine involution differs to works with dairy cow with, for example, periods of 23,6 days [16], 29,6 days [11] in multiparous cow and 49 days [14] in primiparous cow. However, it is important to remember that the time for complete uterine involution is quite vague and depend on the exams used [14] and criteria for evaluation, once the exams used can vary from interpretation and are subjective. There is related improvement of reproductive performance by faster uterine involution, reduced numbers of days until artificial insemination and service period in beef cattle treated with analogues of prostaglandin such as cloprostenol or a racemic mixture of D,L-

1 cloprostenol, although some resistance for its use in beef cattle can be found because of the difficult of
2 management [13,26]. At the present study, no influence of treatment was observed on uterine
3 involution, fact also observed in other works with dairy cow using PGF2 α analogue (tromethamine
4 dinaprost) 48 hours after calving [27] and with beef cattle with continuous administration of PGF2 α
5 for 11 days postpartum [28].

6 Another work was conducted by other authos [29] with dairy cows to determinate the
7 efficacy of GnRH and PGF2 α on hastening uterine involution, estrual activity and follicular dynamics.
8 Animals were treated with NaCl, NaCl + PGF2 α analogue (tromethamine dinaprost) and GNRH +
9 PGF2 α on days 15 and 25 postpartum. However, no influence of treatment was observed. The authors
10 suggest that good management including feed, health and wellness management may negate the use of
11 hormonal therapies on early postpartum period. At the present study, this hypothesis can be affirmed
12 once animal were at appropriate management and no influence of treatment was found.

13 A faster uterine involution and difference on hormonal dosage between treated and control
14 group may not have been observed at the present study due the type of analogue of PGF2 α used as
15 well as the period when the analogue cloprostenol sodium was administrated, suggesting that maybe
16 an administration on prolonged intervals or at late puerperium could be of interest. Also, it is possible
17 to observe that comparison between works became difficult, once there are a lot of variables such as
18 breed, different dose, type of analogues, time of application, geographic location, animal selection,
19 type of management and evaluation that may influence on the parameters studied.

20
21
22
23
24
25

Acknowledgements- We would like to thank to FAPESP for the scholarship (Proc.2011/15330-0), as well as Capes (Proc./096/2010) for their financial support and to Fazenda Marino Avaré.

References

- 1- **Senger PL.** Pathways to pregnancy and parturition. 2.ed. Estados Unidos: Current Conceptions, 2003.
- 2- **Ymer N, Rosnina Y, Wahid H, Saharee AA, Yap KC, Ganesamurthi P.** Ovarian activity in beef and dairy cows with prolonged postpartum period and heifers that fail to conceive. *Trop Anim Health Prod* 2010; 42: 607-615.
- 3- **Okano A, Tomizuka T.** Ultrasonic observation of postpartum uterine involution in the cow. *Theriogenology* 1987; 27: 369-376.
- 4- **Williams EJ, Fischer DP, Pfeiffer DU, England GCW, Noakes DE, Dobson H, Sheldon IM.** Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 2005; 63: 102-117.
- 5- **Chapwanya A, Meade KG, Foley C, Narciandi F, Evans ACO, Doherty M, Callanan JJ, O'Farrelly C.** The postpartum endometrial inflammatory response: a normal physiological event with potential implications for bovine fertility. *Reprod. Fert. Develop* 2012; 24: 1028-1039.
- 6- **Yavas Y, Walton JS.** Induction of ovulation in postpartum suckled beef cows: a review. *Theriogenology* 2000a; 54: 1-23.
- 7- **Crowe MA.** Resumption of ovarian cyclicity in post-partum beef and dairy cows. *Reprod Domest Anim* 2008; 43: 20-28.
- 8- **Yavas Y, Walton JS.** Postpartum acyclicity in suckled beef cows: A review. *Theriogenology* 2000b; 54: 25-55.
- 9- **Roche JF, Crowe MA, Boland MP.** Postpartum anoestrus in dairy and beef cows. *Anim Reprod Sci* 1992; 28: 371-378.
- 10- **Guilbault LA, Thatcher WW, Drost M, Hopkins M.** Source of F series prostaglandins during the early postpartum period in cattle. *Biol Reprod* 1984; 31: 879-887.
- 11- **Lindell JO, Kindahl H, Jansson L, Edqvist LE.** Post-partum release of prostaglandin F2 α and uterine involution in the cow. *Theriogenology* 1982; 17: 237-245.

- 1 **12- Tenhagen BA, Birkelbach E, Heuwieser W.** Serum progesterone levels in post-partum dairy
2 cows after repeated application of prostaglandin F2 α analogue D (+) cloprostenol sodium. *Vet Med A*
3 2000; 47: 213-220.
- 4 **13- Fernandes CAC, Alves BFL, Oliveira, ER, Viana JHM, Gioso MM, Loyola YCS.** Efeito de
5 diferentes doses de cloprostenol sódico no período pós-parto de vacas de corte. *Ciênc Anim Bras* 2012;
6 13: 346-355.
- 7 **14- Scully S, Maillo V., Duffy P, Kelly AK, Crowe MA, Rizos D, Lonergan P.** The effect of
8 lactation on postpartum uterine involution in Holstein dairy cows. *Reprod Domest Anim* 2013; 48:
9 888-892.
- 10 **15- Krueger L, Koerte J, Tsousis G, Herzog K, Flachwsky G, Bollwein H.** Transrectal Doppler
11 sonography of uterine blood flow during the first 12 weeks after parturition in healthy dairy cows.
12 *Anim Reprod Sci* 2009; 114: 23-31.
- 13 **16- Madej A, Kindahl H, Woyno W, Edqvist LE, Stupunick R.** Blood levels of 15-keto-13,14-
14 dihydroprostaglandin f2 α during the postpartum period in primiparous cows. *Theriogenology* 1984;
15 21: 279-287.
- 16 **17- Del Vecchio RP, Chase CC, Bastidas PJR, Randel RD.** Oxytocin-induced changes in plasma
17 13,14 dihydro-15-keto prostaglandin F2 alpha concentrations on days 10, 20 and 30 postpartum in the
18 bovine. *J Anim Sci* 1990; 68: 4261-4266.
- 19 **18- Dimmick MA, Gimenez T, Spitzer JC.** Ovarian endocrine activity and development of ovarian
20 follicles during the postpartum interval in beef cows. *Anim Reprod Sci* 1991; 24: 173-183.
- 21 **19- Wischaral A, Verreschi ITN, Lima SB, Hayashi LF, Barnabe RC.** Pre-parturition profile of
22 steroids and prostaglandin in cows with or without foetal membrane retention. *Anim Reprod Sci* 2001;
23 67: 181-188.
- 24 **20- Mateus L, Lopes da Costa L, Diniz P, Ziecik AJ.** Relationship between endotoxin and
25 prostaglandin (PGE2 and PGFM) concentrations and ovarian function in dairy cows with puerperal
26 endometritis. *Anim Reprod Sci* 2003; 76: 143-154.

- 1 **21- Nakao T, Gamal A, Osawa T, Nakada K, Moriyoshi M, Kawata K.** Postpartum plasma PGF
2 metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on
3 uterine involution and reproductive performance. *J Vet Med Sci* 1997; 59: 791-794.
- 4 **22- Marques Júnior AP, Martins TM, Borges, AM.** Abordagem diagnóstica e de tratamento da
5 infecção uterina em vacas. *Rev Bras Reprod Anim* 2011; 35: 293-298.
- 6 **23- Noakes DE, Parkin-On TJ, England GCW.** Arthur's Veterinary reproduction and obstetrics.8
7 ed. London: Saunders; 2001.
- 8 **24- Sartori R, Fricker PM, Ferreira JCP, Ginther OJ, Wiltbank MC.** Follicular deviation and
9 acquisition of ovulatory capacity in bovine follicles. *Biol Reprod* 2001; 65: 1403-1409.
- 10 **25- Martins TM, Borges AM.** Avaliação uterina em vacas durante o puerpério. *Rev. Bras. Reprod.*
11 *Anim* 2011; 35: 433-443.
- 12 **26- Fernandes CAC, Oliveira ER, Vasconcelos TD.** Efeitos do cloprostenol sódico no pós-parto de
13 vacas de corte. In: XVII Congresso Brasileiro de Reprodução Animal; 2005; Goiás, Brasil. p.351.
- 14 **27- Tian W, Noakes DE.** Effects of four hormone treatments after calving on uterine and cervical
15 involution and ovarian activity in cows. *Vet Rec* 1991;15: 566-569.
- 16 **28- Guilbault LA, Villeveuve P, Dufour JJ.** Failure of exogenous prostaglandin F2 α to enhance
17 uterine involution in beef cows. *Can J Anim Sci* 1988; 68: 669-676.
- 18 **29- Tucker AL, Sanchez HJ, Tucker WB, Williams A, Fuquay JW, Willard ST, Ryan PL.** Effects
19 of early postpartum GnRH and prostaglandin F2 α administration on reproductive activity and
20 ovulation synchronization in lactating dairy cows. *J Anim Vet Adv* 2001; 10: 900-908.

List of Figures and Legends

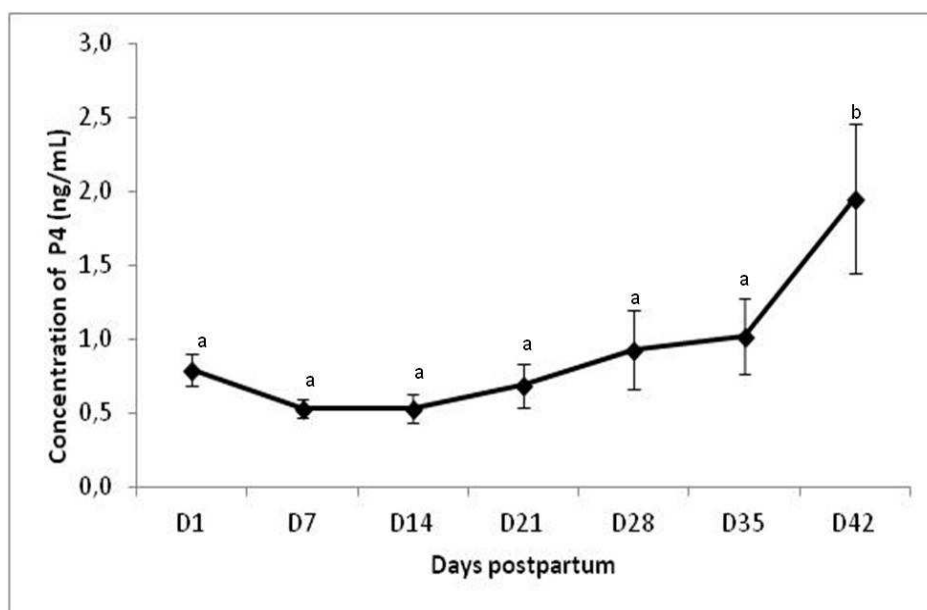


Figure 1: Mean and standard error of P4 dosage on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 23 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package.

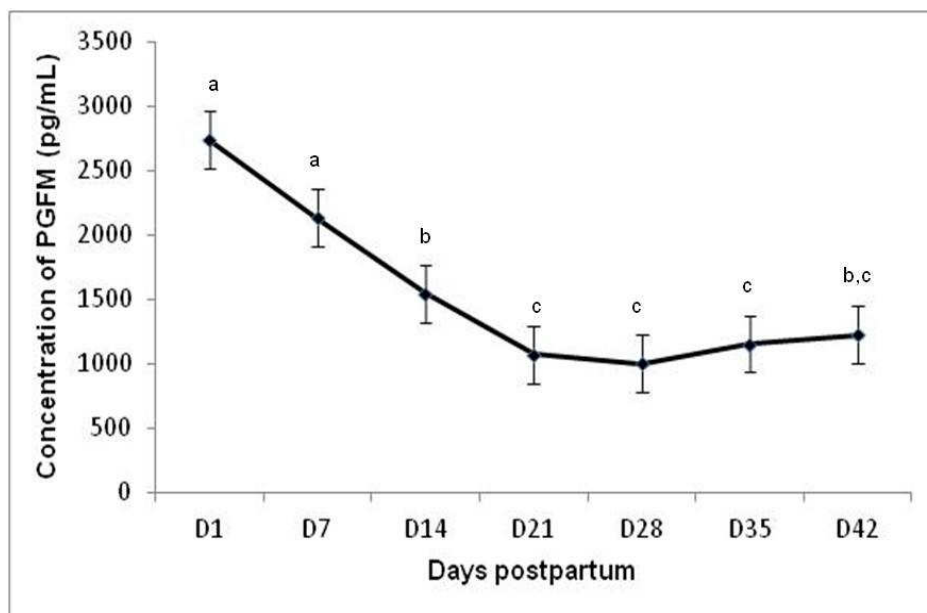


Figure 2: Mean and standard error of PGFM dosage on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 22 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package.

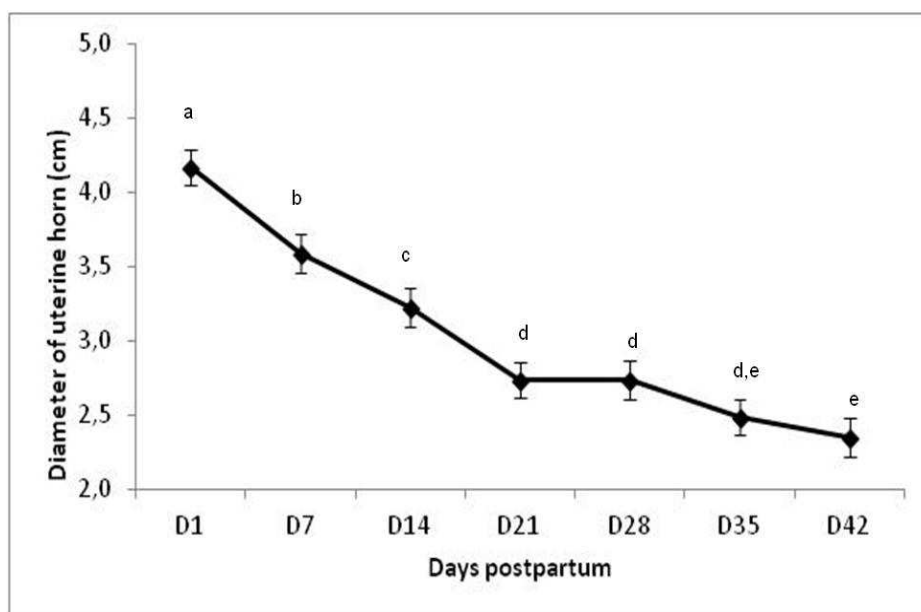


Figure 3: Mean and standard error of diameter of uterine horn (cm) on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments by mixed linear model (MIXED) procedure with the SAS statistical software package.

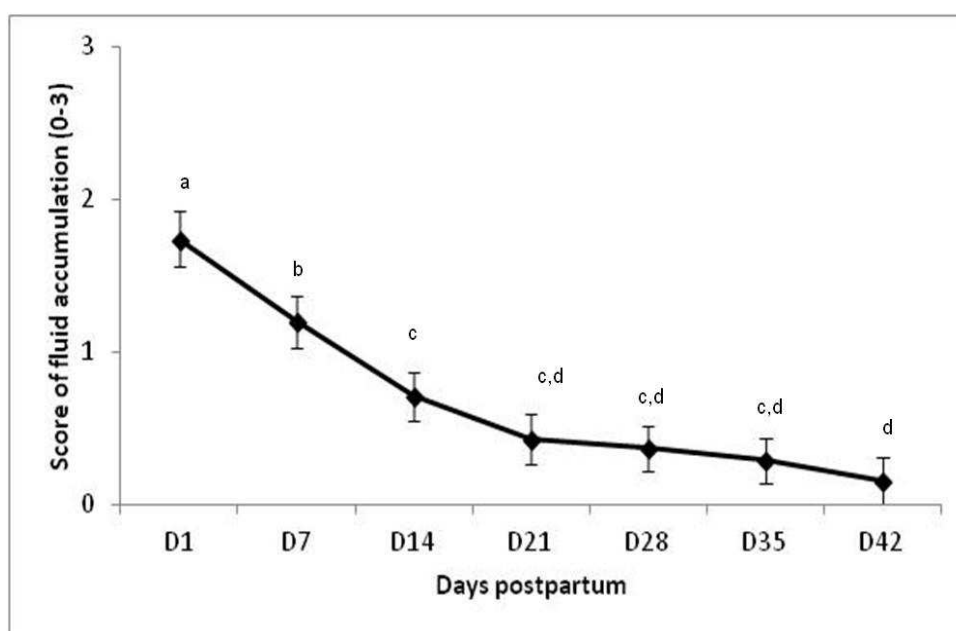


Figure 4: Mean and standard error of score of fluid accumulation (score 0-3) on days 1, 7, 14, 21, 28, 35 and 42 postpartum on 24 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by mixed linear model (MIXED) procedure with the SAS statistical software package. Score 0- without fluid, 1-

less than 1 cm of fluid in the mean of 3 measurements, 2- more than 1 cm of fluid in the mean of 3 measurements , 3 – more than 2 cm in the mean of 3 measurements.

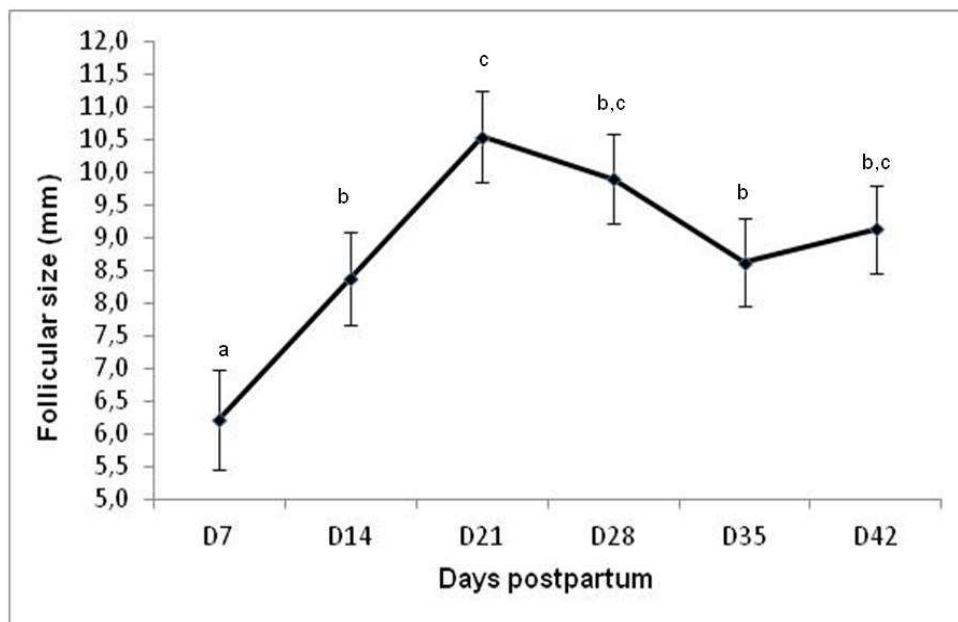


Figure 5: Mean and standard error of follicular size (mm) from right ovary on days 7, 14, 21, 28, 35 e 42 postpartum of 24 Nelore cows. Uncommon letters differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package.

List of Tables and Legends

Table 1: Summarized description of the variables evaluated during the experimental period.

Variables Analyzed	Scores	Description
Diameter of gravid uterine horn	-	Mean of three measurements
Intrauterine fluid accumulation	0 to 3	Mean of three measurements. Vary from without fluid to more than 2 cm
Uterine involution by palpation	1 to 3	According to the position of uterus (abdominal cavity or pelvic cavity)
Cervical ostium	1 to 3	According to the degree of inflammation
Cervical discharge	1 to 5	According to the characteristic of the mucus
Follicle		Measurement of the largest follicle in each ovary

Table 2: Mean and standard error of cervical ostium and cervical discharge evaluation on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cow, independent of treatment.

Moments (day)	Cervical ostium (Score 1 -3)	Cervical discharge (Score 1-5)
1	1.61±0.098 ^a	1.11±0.17 ^a
7	1.42±0.098 ^{a,b}	2.18±0.17 ^b
14	1.22±0.098 ^{b,c}	2.25±0.17 ^b
21	1.11±0.098 ^c	1.79±0.17 ^b
28	1.07±0.098 ^c	1.14±0.17 ^a
35	1.18±0.098 ^c	1.11±0.17 ^a
42	1.06±0.098 ^c	1.00±0.17 ^a

^{a,b}, Uncommon letters in the same column differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package. Score of cervical ostium: 1- rosea, 2- intermediate, 3- red. Score of cervical discharge: 1- translucent or absent, 2- red, 3- mucopurelent, 4- chocolaty, 5- purulent.

Table 3: Mean and standard error of degree of uterine involution on days 1, 7, 14, 21, 28, 35 and 42 postpartum of 24 Nelore cows independent of treatment.

Moments (day)	Degree of uterine involution (Score 1-3)
1	1.03±0.69 ^a
7	1.58±0.69 ^b
14	2.37±0.69 ^c
21	2.81±0.69 ^d
28	2.92±0.69 ^{d,e}
35	3.00±0.69 ^e
42	3.00±0.69 ^e

^{a,b,c,d,e} Uncommon letters in the same column differ ($P < 0.05$) between moments, by the mixed linear model (MIXED) procedure with the SAS statistical software package. Score 1 - uterus little involuted, at abdominal cavity, 2 - uterus with some part of its mass in the abdominal cavity, with reasonable asymmetry, 3 - uterus with almost all its totality in the pelvic cavity.

Capítulo 4

Artigo Científico nas normas e submetido ao periódico “Anatomia, Histologia, Embryologia”

Normas para publicação no periódico:

“Anatomia, Histologia, Embryologia”

NIH Public Access Mandate

For those interested in the Wiley-Blackwell policy on the NIH Public Access Mandate, [please visit our policy statement](#)

Author Guidelines

Content of Author Guidelines: 1. General, 2. Submission of Manuscripts, 3. Ethical Guidelines, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance.

Relevant Documents: [Colour Work Agreement Form](#)

Useful Websites: [Articles published in Anatomia, Histologia, Embryologia](#), [Author Services](#), [Blackwell Publishing's Ethical Guidelines](#), [Guidelines for Figures](#).

1. GENERAL

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *Anatomia, Histologia, Embryologia*. Authors are encouraged to visit [Wiley-Blackwell Author Services](#) for further information on the preparation and submission of articles and figures.

2. SUBMISSION OF MANUSCRIPTS

All papers must be submitted electronically to <http://mc.manuscriptcentral.com/ahe>

The use of an online submission and peer review site speeds up the decision-making process, enables immediate distribution and allows authors to track the status of their own manuscripts. If assistance is needed (or if for some reason online submission is not possible), the Editorial Office can be contacted and will readily provide any help users need to upload their manuscripts.

Editorial Office: Iduna Haus

E-mail: iduna-haus@ethz.ch

2.1. Online Submission

To submit a manuscript, please follow the instructions below.

Getting Started

1. Launch your web browser (Internet Explorer 6 or higher, Netscape 7 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's ScholarOne Manuscripts

homepage

(<http://mc.manuscriptcentral.com/ahe>).

2. Log-in or click the “Create Account” option if you are a first-time user of Manuscript Central.

3. If you are creating a new account.
- After clicking on “Create Account”, enter your name and e-mail information and click “Next”. Your e-mail information is very important.
- Enter your institution and address information as appropriate, and then click “Next.”
- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your area of expertise. Click “Finish”.

4. If you have an account, but have forgotten your log in details, go to Password Help on the journals online submission system (<http://mc.manuscriptcentral.com/ahe>) and enter your e-mail address. The system will send you an automatic user ID and a new temporary password.

5. Log-in and select “Author Center.”

Submitting Your Manuscript

5. After you have logged in, click the “Submit a Manuscript” link in the menu bar.

6. Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.

7. Click the “Next” button on each screen to save your work and advance to the next screen.

8. You are required to upload your files.

- Click on the “Browse” button and locate the file on your computer.

- Select the designation of each file in the drop down next to the Browse button.

- When you have selected all files you wish to upload, click the “Upload Files” button.

9. Review your submission (in PDF format) before sending to the Journal. Click the “Submit” button when you are finished reviewing.

You may suspend a submission at any phase before clicking the “Submit” button and save it to submit later. After submission, you will receive a confirmation e-mail. You can also access Manuscript Central any time to check the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

Manuscripts should be uploaded as Word (.doc, docx) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS

files are suitable for printing. The files will be automatically converted to a PDF document on upload and will be used for the review process. The text file must contain the entire manuscript including title page, abstract, text, references, tables, and figure legends, but *no* embedded figures. Figure tags should be included in the file. Manuscripts should be formatted as described in the Author Guidelines below. Revised manuscripts must be uploaded within 2 months of authors being notified of conditional acceptance pending satisfactory revision.

3. ETHICAL GUIDELINES

Anatomia, Histologia, Embryologia adheres to the ethical guidelines below for publication and research.

3.1. Authorship and Acknowledgements

Authorship: Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal. ALL named authors must have made an active contribution to the conception and design and/or analysis and interpretation of the data and/or the drafting of the paper and ALL must have critically reviewed its content and have approved the final version submitted for publication. Participation solely in the acquisition of funding or the collection of data does not justify authorship and, except in the case of complex large-scale or multi-centre research, the number of authors should not exceed six.

Anatomia, Histologia, Embryologia adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Acknowledgements: Under

Acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Suppliers of

materials should be named and their location (town, state/county, country) included.

3.2. Ethical Approvals

Experimental Subjects: In experiments where animals were used, authors are requested to give full details about the animals (source, breeding, pathogen-free, conventional conditions, etc.) and their maintenance (standard diet, environment, etc.). When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

3.3 Conflict of Interest and Source of Funding

Conflict of Interest: Authors are required to disclose any possible conflict of interest.

These include financial (for example patent, ownership, stock ownership, consultancies, speaker's fee). Author's conflict of interest (or information specifying the absence of conflicts of interest) will be published under a separate heading entitled 'Conflict of interest'.

Anatomia, Histologia, Embryologia requires that sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. As of 1st March 2007, this information will be a requirement for all manuscripts submitted to the Journal and will be published in a highlighted box on the title page of the article. Please include this information under the separate headings of 'Source of Funding' and 'Conflict of Interest' at the end of your manuscript.

If the author does not include a conflict of interest statement in the manuscript then the following statement will be included by default: "No conflicts of interest have been declared".

Source of Funding: Authors are required to specify the source of funding for their research when submitting a paper. Suppliers of materials should be named and their location (town, state/county, country) included. The information will be disclosed in the published article.

3.4 Appeal of Decision

The decision on a paper is final and cannot be appealed.

3.5 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

3.6 Copyright

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

For authors signing the copyright transfer agreement

If the OnlineOpen option is not selected the corresponding author will be presented with the copyright transfer agreement (CTA) to sign. The terms and conditions of the CTA can be previewed in the samples associated with the Copyright FAQs below:
CTA Terms and Conditions:
http://authorservices.wiley.com/bauthor/faqs_copyright.asp

For authors choosing OnlineOpen

If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):

Creative Commons Attribution License OAA
Creative Commons Attribution Non-Commercial License OAA
Creative Commons Attribution Non-Commercial -NoDerivs License OAA

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services:

http://authorservices.wiley.com/bauthor/faqs_copyright.asp and visit <http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html>.

If you select the OnlineOpen option and your research is funded by The Wellcome Trust and members of the Research Councils UK (RCUK) you will be given the opportunity to publish

your article under a CC-BY license supporting you in complying with Wellcome Trust and Research Councils UK requirements. For more information on this policy and the Journal's compliant self-archiving policy please visit: <http://www.wiley.com/go/funderstatement>.

4. OnlineOpen

OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to non-subscribers upon publication via Wiley InterScience, as well as deposited in the funding agency's preferred archive. For the full list of terms and conditions, see <http://olabout.wiley.com/WileyCDA/Section/id-406241.html>.

Any authors wishing to send their paper OnlineOpen will be required to complete the payment form available from our website at: https://authorservices.wiley.com/bauthor/onlineopen_order.asp. Prior to acceptance there is no requirement to inform an Editorial Office that you intend to publish your paper OnlineOpen if you do not wish to. All OnlineOpen articles are treated in the same way as any other article. They go through the journal's standard peer-review process and will be accepted or rejected based on their own merit.

5. MANUSCRIPT TYPES ACCEPTED

Original Articles: Original papers should not exceed 30 DIN A4 typewritten pages including figures, tables and references. Summaries of original papers should not exceed 1200 characters.

Short Communications: Short communications should not exceed 5 DIN A4 typewritten pages including figures, tables and references. Summaries of short communications should not exceed 600 characters. Short communications may be given priority for publication.

Reviews: Due to their nature, reviews are not limited in length per se, however editors reserve the right to have them shortened for brevity during review.

6. MANUSCRIPT FORMAT AND STRUCTURE

6.1. Format

Language: The language of publication is English. Authors for whom English is a second language must have their manuscript professionally edited by an English speaking person before submission to make sure the English is of high quality. It is preferred that manuscripts are professionally edited. A list of independent suppliers of editing services can be found at

http://authorservices.wiley.com/bauthor/english_language.asp All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Abbreviations, Symbols and Nomenclature: All measurement specifications must follow the S.I. system. Solution concentrations are to be given in mol/l. All other concentrations are to be given in % (volume or weight). Abbreviations of biological, chemical or other terms should only be employed when it is certain they are internationally known. The full name must be stated in brackets when an abbreviation is used for the first time in a paper.

Scientific Names: All biological, chemical, medical or other names are to be given according to the most recent international nomenclature. In the case of substances or reagents obtained commercially, the name and address of the manufacturer or supplier (as a footnote) should be given the first time the product is mentioned. Products with a trademark should be marked with ©. Names of bacteria should be in accordance with the latest edition of Bergey's Manual of Determinative Bacteriology (The Williams and Wilkins Co., Baltimore). Viruses should be classified and named as recommended by the International Committee on the Nomenclature of Viruses.

Font: Names of micro-organisms and zoological or botanical names should be given in italics.

6.2. Structure

All manuscripts submitted to *Anatomia*, *Histologia*, *Embryologia* should include: Summary, Introduction, Materials and Methods, Results and Discussion, and References.

Title Page: The following information should be given on page one of the manuscript: the name of the institution where the work was carried out, the title of the manuscript, the name(s) of the author(s), the address(es) of the author(s), the e-mail address of the corresponding author, and the number of figures and tables accompanying the manuscript.

Summary: Summaries of original articles should not exceed 1200 characters, and those of short communications should not exceed 600 characters.

Optimizing Your Summary for Search Engines

Many students and researchers looking for information online will use search engines such as Google, Yahoo or similar. By optimizing your article for search engines, you will increase the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in another work. We have compiled [these guidelines](#) to enable you to maximize the web-friendliness of the most public part of your article.

6.3. References

Each original paper should have a list of references which should be restricted to a minimum. The references should be listed alphabetically according to the name of the first author.

Every reference should be structured as follows: author or author's surname and initials of the given name; year of publication (without brackets); title of paper; name of the journal; volume number (bold); page numbers, e.g.:

Gothe, R., Y. Gold and A. Kraiss, 1986: On the subspecific validity of *Rhipicephalus evertsi* mimeticus DÖnitz, 1910. J. Vet. Med. B 33, 47-59.

Books or separate publications should be quoted as follows:

Boch, J. and R. Supperer, 1977: Veterinary Medical Parasitology. Paul Parey Scientific Publishers, Berlin.

Reference to the quoted literature in the text should be given by putting the name(s) of the author(s) in brackets, with the year of publication, e.g.: (Thein and Härtl, 1986).

The editor and publisher recommend that citation of online published papers and other material should be done via a DOI (digital object identifier), which all reputable online published material should have - see www.doi.org/ for more information. If an author cites anything which does not have a DOI they run the risk of the cited material not being traceable.

We recommend the use of a tool such as

[Reference Manager](#) for reference management and formatting.

Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

6.4. Tables, Figures and Figure Legends

The number and size of illustrations and tables should be kept to the minimum necessary.

Tables: Tables should be created using the table function.

Figures: Please note that figures will generally be reduced to fit within the column-width or the print area. This means that numbering and lettering must still be readable when reduced (e.g. maps) and that the scale might not correspond with the original (microscopic pictures), thereby invalidating references to scale in the text. These figures should include a bar the size of which is defined in the caption of the photograph concerned. If a figure is to be cropped, please mark the lines on a photocopy or tracing paper. Printouts should be made with a laserprinter at the highest resolution (> 600 dpi). If artwork is to be scanned, line drawings should only be contour drawings without halftones (shades of grey). Please do not use patterns; rough hatching is possible.

Graphs with an x and y axis should not be enclosed in frames; only 2-dimensional representations. Do not forget the labels and units.

Preparation of Electronic Figures for Publication

Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of at least 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). Please submit the data for figures in black and white or submit a Colour Work Agreement Form (see Colour Charges below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: line art: >600 dpi; halftones (including gel photographs):

>300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley-Blackwell's guidelines for figures: <http://authorservices.wiley.com/bauthor/illustration.asp>

Check your electronic artwork before submitting it: <http://authorservices.wiley.com/bauthor/eachecklist.asp>

Permissions: If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publisher.

Colour Charges: It is the policy of the *Anatomia*, *Histologia*, *Embryologia* for authors to pay the full cost for the reproduction of their colour artwork. However, in the event that an author is not able to cover the costs of reproducing colour figures in colour in the printed version of the journal, *Anatomia*, *Histologia*, *Embryologia* offers authors the opportunity to reproduce colour figures in colour for free in the online version of the article (but they will still appear in black and white in the print version). Questions concerning colour figures should be directed to the Editorial Office, Iduna Haus (iduna-haus@ethz.ch). If an author wishes to take advantage of this free colour-on-the-web service, they should liaise with the Editorial Office (iduna-haus@ethz.ch) to ensure that the appropriate documentation is completed for the Publisher. Therefore, please note that if there is colour artwork in your manuscript when it is accepted for publication, Wiley-Blackwell Publishing require you to complete and return a colour work agreement form before your paper can be published. This form can be downloaded from the Author Guidelines [here](#). Please return the Colour Work Agreement Form to the address listed on the form.

Figure Legends: Legends for the figures should give a precise description of the content and should not be repeated within the figure.

Note to NIH Grantees: Pursuant to NIH mandate, Wiley-Blackwell will post the accepted version of contributions authored by NIH grant-holders to PubMed Central upon acceptance. This accepted version will be made publicly available 12 months after publication. For further information, see www.wiley.com/go/nihmandate

7. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

7.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website. A working e-mail address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site.

Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following website: www.adobe.com/products/acrobat/readstep2.html.

This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs.

Proofs must be returned to the Production Editor within three days of receipt. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in their work, including changes made by the copy editor.

7.2 Early View (Publication Prior to Print)

Anatomia, Histologia, Embryologia is covered by Wiley-Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page

numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

7.3 Author Services

Online production tracking is available for your article through Wiley-Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript. Visit <http://authorservices.wiley.com/bauthor/> for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more.

7.4 Author Material Archive Policy

Please note that unless specifically requested, Blackwell Publishing will dispose of all hardcopy or electronic material submitted two months after publication. If you require the return of any material submitted, please inform the editorial office or production editor as soon as possible.

7.5 Offprints and Extra Copies

Free access to the final PDF offprint or your article will be available via author services only. Please therefore sign up for author services if you would like to access your article PDF offprint and enjoy the many other benefits the service offers.

Additional paper offprints may be ordered online. Please click on this [link](#), fill in the necessary details and ensure that you type information in all of the required fields. If you have queries about offprints please e-mail offprint@cosprinters.com

Last update: February 2014

São Paulo State University – UNESP- Botucatu, São Paulo, Brazil

Temporal histologic analysis of endometrium from Nelore (*Bos taurus indicus*) treated or not with cloprostenol sodium at postpartum

Carolina Nogueira de Moraes^{a,*}, Leandro Maia^a, Carla Martins de Queiroz^a, Marcelo Piagentini^a, Mateus José Sudano^b, Eunice Oba^a

^a São Paulo State University – UNESP, Rubião Júnior s/n, CEP: 18618-970, Botucatu, São Paulo, Brazil.

^b Federal University of Pampa - UNIPAMPA, BR 472 - Km 592 - CEP: 97500-970, Uruguaiana – Rio Grande do Sul , Brazil.

Corresponding Author *

carolnmoraes@hotmail.com

Telephone/Fax number: 55(14) 3880-2119

Number of Figures: 08

SUMMARY

After calving, physiological events occur in the uterus of cows to repair the modifications occurred during pregnancy and delivery and the use of cloprostenol sodium aims to reduce the period of uterine involution. This study aimed to promote a temporal histological description of endometrial samples from multiparous Nelore cows treated or not with cloprostenol sodium on days 1 and 4 postpartum, as well as test the hypothesis that treatment promotes changes at histological variables studied. For this, endometrial sample was collected on days 1, 7, 14, 28, and 42 postpartum using a Yomann biopsy instrument. A semiquantitative analysis was performed for the presence/absence of myometrial and endometrial tissue and its integrity, number of endometrial glands (classification 0 to 3), edema, hemorrhage, vascular congestion and type of epithelial cell. A quantitative analysis was performed (classification 0 to 3) in accordance to the average number of leukocytes. Cloprostenol sodium had no influence on variables studied ($P > 0.05$). For number of endometrial glands and degree of inflammation there was influence of moment ($P < 0.05$) and for the others, no type of influence was observed ($P > 0.05$). It can be concluded that cloprostenol sodium did not alter histology of uterine though postpartum period which could reduce the period of uterine involution. Uterine histological analysis by serial biopsies are an important tool not only for a better understanding and evaluation of physiological events involved in the process of uterine involution, but also for the identification and monitoring of pathological process that may compromise reproductive life.

1. Introduction

In the postpartum period, a series of morphological changes occur in the endometrial tissue for restoration of histological features that were altered during pregnancy and delivery period.

The obtaining of serial samples allows the formation of a detailed profiling on tissue expression and repair during the postpartum period (Bonnett et al. 1991; Ohtani et al. 1993; Chapwanya et al. 2010). In the researches, there is variation in the period of obtaining bovines' endometrial biopsies, with reports of collections on days such as 26 and 40 (Bonnett et al. 1991), 15, 30, 60 (Chapwanya et al. 2010), 67 (Goshen et al., 2012), 1, 3, 5, 7, 11, 15, 19, 23, 27, 31, 45 and 60 (Archbald et al. 1972) postpartum.

With the technique of endometrial biopsy it is possible to obtain endometrial sample with high quality and significant value (Chapwanya et al. 2010). Endometrial samples can be used for histological evaluation allowing the monitoring of inflammation and uterine involution in cattle (Chapwanya et al. 2012, Bonnet and Martin, 1995, Bonnet et al. 1990, Archbald et al. 1972), and also for the diagnosis of diseases (Madoz et al. 2014, Meira et al. 2012, Herath et al. 2009, Katagiri and Takahashi, 2004, Holt et al. 1989).

Although ultrasound exams and rectal palpation may direct evidence of uterine diseases (Snider et al. 2011), through endometrial samples a histopathological evaluation of uterine layers (Bonnett et al. 1991; Ohtani et al. 1993) and uterine health is possible (Snider et al. 2011). Also, the use of various techniques and equipment of biopsies are described in the literature for obtaining samples (Archbald et al. 1972; Holt et al. 1989; Bonnett et al. 1991; Ohtani et al. 1993; Chapwanya et al. 2012).

Prostaglandin F_{2α} (PGF_{2α}) is produced in the uterus and helps in uterine involution postpartum acting in the myometrial contraction (Guilbault et al. 1984). Analogues of PGF_{2α} such as cloprostenol sodium are used in postpartum period to induce or synchronize oestrus (Tenhagen et al. 2000), to control luteal function and to reduce the period of uterine involution (Fernandes et al. 2012).

Works related to histological analysis in the postpartum focus on dairy cattle and in some periods of postpartum (Bonnet et al. 1990; Bonnett et al. 1991; Bonnet and Martin, 1995; Chapwanya et al. 2009; Meira et al. 2012), with few reports in beef cattle (Chapwanya et al. 2012) at these conditions and various moments. Based on this, the objective of this work was to perform a histological analysis of endometrial biopsies at different times (5 moments) in the postpartum of Nelore females treated or not with cloprostenol sodium on days 1 and 4 postpartum (D0 = birth) as well as test the hypothesis that the treatment promotes changes at histological variables studied.

2. Materials and Methods

2.1 Ethical aspects

The study was conducted in accordance with ethical guidelines recommended by National Council for Control of Animal Experimentation and College of Animal Experimentation, and was approved by the Institution's Animal Care and Experimentation Ethics Committee on protocol number 50/2012.

2.2 Animals

A total of twenty four multiparous Nelore (*Bos taurus indicus*) female with parity order between 2 - 6 (median = 3) and aged between 4 -13 years (median = 9)

were used. Animals were housed in paddocks predominantly of *Brachiaria decumbens*, with mineral supplementation (Minersal®, Presidente Venceslau, SP, BR) and water ad libitum. In the dry season, animals received supplementary food of corn silage.

Only the animals with no calving assistance, retained fetal membranes, twin pregnancy or postpartum complications were selected. The experiment was conducted in a farm located in Avaré, State of São Paulo, Brazil (latitude: 23°10'S; longitude: 48°93'E; altitude 766 m).

2.3 Treatment

The animals were randomly assigned into the treatment and placebo groups (GT and GP, respectively), as parity order. The treated group (GT) received 0,530 mg (2 mL) of cloprostenol sodium (Ciosin®, MSD Animal Health, São Paulo, SP, Brazil) intramuscularly, in the region of gluteus, on days 1 and 4 postpartum (D0 = birth). The placebo group (GP) received by the same route and period the same volume of 0.9% NaCl solution (Sanobiol®, Pouso Alegre, MG, Brazil).

2.4 Endometrial biopsy

Endometrial biopsies were obtained using a Yomann (Hauptner®, Solingen, North Rhine-Westphalia, Germany) biopsy instrument following a previously described method (Chapwanya et al. 2012). Endometrial samples were taken at 1, 7, 14, 28 and 42 days postpartum at the previously gravid horn, on median region, after uterine bifurcation. For this, the external genitalia and perineum were cleaned with water and 2% chlorhexidine degermante (Riohex 2% Clorexidina®, Rioquímica, São José do Rio Preto, SP, Brazil). After this, the biopsy instrument covered by a protective sheath was introduced into the vagina. With the left hand in the rectum the instrument was directed

into the cervical canal, after passing it the sheath was ruptured and the biopsy instrument was introduced in the horn.

To be sure of the position of the biopsy instrument, with a lot of care, the left hand identified the tip of the biopsy instrument in the horn. Done this, the jaws of the biopsy instrument were opened and the sample was collected by pressing the uterine wall against the instrument jaws when then it was closed and the tissue was withdrawing. All biopsies were performed by the same operator for standardization of the technique and quality of the collected sample thus reducing the chances of variation.

After obtaining endometrial fragment of approximately 0,5 cm², it was removed from the jaws with the assistance of a 40x12 sterile needle (Descarpac®[®], São Paulo, SP, Brazil) and transferred to a 27 mL cylindrical bottles (Injeplast®[®], São Paulo, SP, Brazil) previously identified containing 3 mL of buffered formalin and it was fixed in 70% alcohol past three days. All material was processed according to routine histological methods until paraffin embedding and obtaining histological sections, which were subsequently stained with hematoxylin-eosin.

Between each biopsy, the biopsy instrument was cleaned with a brush to remove possible endometrial residues, washed with MilliQ water (Millipore®[®], Bedford, MA, USA) and 2% chlorhexidine degermante (Riohex 2% Clorexidina®[®], Rioquímica, São José do Rio Preto, SP, Brazil) and covered with a new protective sheath (WalMur®[®], Porto Alegre, RS, Brazil).

2.5 Histological evaluation of endometrial samples

Histological analysis were made semi-quantitatively and quantitatively and performed by a single person who was blind to the treatment.

First, the samples were analyzed semi-quantitatively in bifocal microscope at 200x and 400x magnification, as already described Bonnett et al. (1991) and Chapwanya et al. (2012) with some modifications. In each sample it was analyzed the presence or absence of myometrium and/ or endometrium and its integrity and the concentration of endometrial glands, (according to the total number of glands at the tissue) classified in: 0: none (0 glands); 1: scarce (1 - 3 glands); 2: moderate (4 - 6 glands) and 3: heavy (> 7 glands). It was also observed the edema in the stroma, areas of hemorrhage and vascular congestion and the cell type present in the endometrial epithelium and classified in: 1: columnar or 2: cuboid.

Following this, it was done a quantitative analysis as classification model already used Chapwanya et al. (2012), with modifications for histological samples. Such analysis was performed at higher magnifications to count the number of leukocytes in 5 random fields of the sample (high-power field (hpf), 400x magnification). Based on the number of leukocytes found, it was done the mean, evaluated the presence and degree of inflammation and classified in: 0 no inflammation (0-25 cells per hpf); 1: low inflammation (26-50 cells per hpf); 2: moderate inflammation (51-75 cells per hpf) and 3: severe inflammation (> 75 cells per hpf).

2.6 Data analysis

Data were analysed using the mixed linear model (MIXED) procedure with the SAS statistical software package (Version 9.2; SAS Inst. Inc., Cary, NC, USA). Sources of variation in the model included treatment (GT vs GP), moment (day 1, 7, 14, 28 and 42 postpartum), and first-order interactions; these were considered as fixed effect. Animals were considered as random effect. If necessary, logarithmic

transformation was used to improve normality. Means were separated using least square difference (LSD). All data are reported as untransformed least-squares means $\pm$ s.e.m.

Endometrium, myometrium and epithelial integrity data were analysed using the logistic regression (PROC LOGISTIC) in SAS.

Sources of variation in the model included treatment (GT vs GP), moment (day 1, 7, 14, 28 and 42 postpartum) and first-order interactions. All factors, except animals, were considered to be fixed effects. In the absence of significant interactions, only main effects are presented. Data are reported as percentages. For all analyses, $P < 0.05$ was considered to be significant.

3. Results

It was performed endometrial biopsies from 24 cows at 5 moments and analyzed a total of 110 samples. Considering the largest horn at the palpation per rectum as the gravid horn from the anterior pregnancy, biopsies were performed at the right horn on 67% and at the left horn on 33% of the animals.

Cloprostenol sodium had no influence on the variables, but at the number of endometrial glands and degree of inflammation there was influence of moment and for the others variables, no type of influence was observed.

Figures 1, 2, 3, 4 and 5 show bovine endometrium at different days postpartum (1, 7, 14, 28 and 42), with emphasis on edema, areas of polymorfonuclear infiltrate and blood vessels.

3.1 Presence of endometrium and myometrium

Regarding the analysis of presence of endometrial tissue on samples, no effect of moment or treatment ($P > 0.05$) was observed. In this category, 89.9% of samples from placebo and 97.1% of samples from treated group were with endometrial tissue. Furthermore, regarding the presence of myometrial tissue, no effect of moment or treatment was observed ($P > 0.05$), and 8.82% of samples from placebo and 9.51% samples from treated group were with myometrial tissue.

3.2 Epithelial integrity

Regarding epithelial integrity it was not observed effect of treatment or moment ($P > 0.05$), and at placebo group 52.89% of analyzed samples were with intact epithelium, whereas 78.8% of samples of treated group were with intact epithelium.

3.3 Score of endometrial glands

With respect to endometrial glands there was observed effect of moment ($P < 0.001$). Score of endometrial gland showed an increase on the number of endometrial gland from D1 to D42. From all analyzed biopsies, 70% of samples had glands (scarce, moderate or heavy quantity) in some moment of postpartum and 30% had no glands. Data is shown on Figure 6.

3.4 Degrees of inflammation

The average number of leukocytes counts had influence of moment ($P = 0.01$) with significant increase on the average of leukocytes count from D7 to D14, with posterior progressive decrease on its values. Data are shown on Figure 7.

Based on the average number of leukocyte of five fields randomly and according to the classification already described by Chapwanya et al. (2012) with modifications, there was observed effect of moment ($P= 0.0001$) on degree of inflammation (from 0, with no inflammation to 3 with severe inflammation). The score of inflammation decreased from D7 to D42. It was also observed an increase on score of inflammation from D1 to D7. Data are shown on Figure 8.

Also, among inflammatory cells found in the analysis, there was almost complete predominance of polymorphonuclear cells and only a few isolated mononuclear cells were observed in the stromal and epithelial tissue portion.

3.5 Other findings

The edema was observed more markedly in the first day postpartum with reduction over the remaining days of endometrial analysis. Already at 14 days postpartum most samples showed no areas of edema, and at 42 days, no sample had areas of edema.

From the analyzed samples, 35% presented vascular congestion and 65% did not. Also, 48% of biopsies had areas of hemorrhage below the epithelial tissue or at stromal portion and 53% do not have this parameter. From the 84 biopsies that had epithelial tissue, 57% had columnar epithelial cell and 43% had cuboid cell.

4. Discussion

In general, there are few studies in the literature with complete description of uterine histology during uterine involution in postpartum of beef cattle. Furthermore, comparison of histological data of endometrial tissue in beef cattle and dairy cattle often

becomes difficult because the stress faced by them after the period of parturition is distinct (Chapwanya et al. 2012). Also, we could observe that most of the studies in the literature are about dairy cattle (Bonnet et al. 1990; Bonnet et al. 1991; Bonnet and Martin, 1995).

Treatment of animals in the postpartum with cloprostenol sodium is used aiming to short the period of uterine involution by increasing uterine contractions and defense. The use of prostaglandin analogues in beef cattle becomes valid once the major goal of this sector is producing the highest number of calves /year. However, until now, some reluctance can be found with the use of prostaglandin in beef cattle because of the difficult management. In the present study, animals of optimal body condition and appropriate health, feeding and reproductive management were used and the treatment performed with cloprostenol sodium had no influence on the variables studied.

In a study, no significant differences in histological analysis in dairy animals on day 26 postpartum in animals treated with prostaglandin was observed, but at 46 days postpartum this was different and prostaglandin presented effect on the variable histological inflammation, diameter and number of the endometrial glands (Bonnet et al. 1990). This fact was not observed at the present work, probably because prostaglandin at the dose and moment which were used was not efficient in promote uterine contraction and reduction of inflammation.

Once endometrial biopsies were performed by the same operator in all moments and animals using the same equipment of biopsy, obtaining of homogeneous samples with satisfactory quality was possible, allowing the analysis of the proposed variables. Only in the samples that there was no epithelial tissue, the epithelial cell type was not evaluated and on the unreadable samples (3.6%), no analysis was made.

At the present work, most endometrial biopsies (67%) were performed at the right horn, corresponding to the previously pregnant, data that corroborates with previous study (Bonnet et al. 1991) which emphasize that in cattle there is an increased activity of the right ovary.

As in a previous work (Bonnet et al. 1991), most samples showed endometrial epithelium with columnar cells. Additionally, in a work with dogs (Orfanou et al. 2009), all samples showed the columnar interplacental endometrium. However, it was reported epithelial cells of the type cuboid on caruncular portions in bovine's endometrial biopsies (Archbald et al. 1972).

At the present study, the findings of hemorrhage can be attributed to the technique of endometrial biopsy, which possibly caused rupture of small blood vessels. Additionally, the results obtained on the subjective analysis of presence of endometrium and myometrium and integrity of epithelial tissue may have varied and suffered influences on the processing of histological section and biopsy technique itself.

Endometrial glands are embryologically derived from uterine epithelial invaginations and therefore there are morphological similarities between them (Archbald et al. 1972). The concentration of endometrial glands is correlated with the cyclical activity of the animals. In a study, a higher concentration of endometrial glands was observed on day 40 postpartum, compared with day 26 postpartum. This was possibly due to a higher amount of cyclic animals on day 40 (Bonnet et al. 1991). In mares at day 0 postpartum, it was observed dilated and sparse endometrial glands (Gomez-Cuetara et al. 1995). At the present study, it was observed significant differences between the analyzed moments and at D42 it was also observed a higher concentration of endometrial glands when compared with D1, D7 and D14 postpartum.

This can also be attributed to cyclicity of animals, once at 42 days postpartum; animals had luteal tissue (personal communications).

There is a diffuse edema in the endometrial stroma separating collagen fibers and other extracellular matrix constituents. In study conducted by Bügner (1981) it was observed that in Nelore animals, around 10 days postpartum there was a marked edema, and it was absent 20 days postpartum. In the present study, 14 days postpartum the edema in the stromal portion of the endometrium was already reduced, and with 28 and 42 days most animals showed no edema. Moreover, on the first day postpartum all animals had edema, a fact also confirmed by Gomez-Cuetara et al. (1995).

After calving, it is possible to observe an inflammatory response from endometrium with leukocyte infiltration, fact that is expected to be found at postpartum. In healthy animals, after such acute inflammatory response, endometrial tissue is restored (Chapwanya et al. 2012). With 14 days postpartum, most animals (8/22) presented a great amount of infiltrated leukocytes (> 75 cells per hpf), however this value and also the average of leukocytes decreased significantly from this day. These data corroborate with reports of Chapwanya et al. (2012), whose report an increased inflammatory response in the first days postpartum.

Similarly, in dairy cows it was found a significantly higher amount of segmented cells on day 26 than on day 40 postpartum at epithelial and luminal portion, which can be correlated with an acute inflammatory process in earlier postpartum (Bonnet et al. 1991). This further observation of polymorphonuclear cells demonstrates the health and absence of serious diseases at the herd once the presence of a large number of lymphocytes is attributed to chronic conditions (Ferreira et al. 2002). The presence almost predominant of neutrophils on analyzed samples corroborates with

these observation of other authors once no animal show any sign of chronic or acute uterine disease.

In a histological analysis of cattle's uterus performed 1 day postpartum and then twice weekly until 30 days postpartum, it was observed an increase in the number of leukocytes over days postpartum (Eduvie et al. 1984). Also in endometrial biopsies of mare, from the second to fifth days postpartum, it was observed an inflammatory reaction characterized by the presence of phagocytic cells and neutrophils (Gomez-Cuetara et al. 1995). But in dogs it was evident that soon after delivery no inflammatory infiltrate was observed in the myometrial portion, however with the passing of days postpartum, the inflammation is intense in this endometrial layer (Orfanou et al. 2009).

At the present work dose and time of application may not have been sufficient to promote changes on endometrial morphology to promote an increase on leukocyte infiltration and a faster restoration of endometrial tissue.

5. Conclusion

The use of cloprostenol sodium on postpartum of Nelore females did not alter histological variables and did not cause significant modifications on endometrial tissue that could impair on a faster uterine involution at the conditions in which this study was performed. Although the hypothesis had not been proved, we believe that the knowledge of histology from endometrial tissue during the postpartum period is very important for the evaluation of uterine involution process once it allows the monitoring the resolution of edema and reduction of leukocyte infiltrates mainly. Moreover the as earlier as possible the identification of pathological processes that may compromise reproductive life of the animal where done, more economical gains will be reach with the treatment or replacement of the animal.

Acknowledgement

We would like to thank to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the scholarship (Proc.2011/15330-0), as well as Capes (Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior) (Proc./096/2010) for their financial support and to Fazenda Marino Avaré.

References

- Archbald, L.F., R.H. Schultz, M.L. Fahning, H.J. Kurtz, and R. Zemjanis, 1972: A sequential histological study of the post-partum bovine uterus. *J. Reprod. Fertil.* **29**, 133-136.
- Bonnet, B.N., W.G. Etherington, S.W. Martin, and W.H. Johnson, 1990: The effect of prostaglandin administration to holstein-friesian cows at day 26 postpartum on clinical findings, and histological and bacteriological results of endometrial biopsies at day 40. *Theriogenology*. **33**, 877-890.
- Bonnett, B.N., R.B. Miller, W.G. Etherington, S.W. Martin, and W.H. Johnson, 1991: Endometrial biopsy in Holstein-Friesian dairy cows I. technique, histological criteria and results. *Can. J. Vet. Res.* **55**, 155-161.
- Bonnet, B.N., and S.W.Martin, 1995: Path analysis of peripartum and postpartum events, rectal palpation findings, endometrial biopsy results and reproductive performance in Holstein-Friesian dairy cow. *Prev. Vet. Med.* **21**, 279-288.
- Bügner, M. Avaliação pós-parto do sistema genital de vacas da raça Nelore. 1981: 29f. Dissertação (Mestrado em Medicina Veterinária) - Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 1981.
- Chapwanya, A., K.G. Meade, M.L. Doherty, J.J. Callanan, J.F. Mee, and C. O'Farrelly, 2009: Histopathological and molecular evaluation of Holstein-Friesian cows postpartum: Toward an improved understanding of uterine innate immunity. *Theriogenology* **71**, 1396-1407.
- Chapwanya, A., K.G. Meade, F. Narciandi, P. Stanley, J.F. Mee, M.L. Doherty, J.J. Callanan, and C. O'Farrelly, 2010: Endometrial biopsy: a valuable clinical and research tool in bovine reproduction. *Theriogenology* **73**, 988-994.

- Chapwanya, A., K.G. Meade, C. Foley, F. Narciandi, A.C.O. Evans, M. Doherty, J.J. Callanan, and C. O'Farrelly, 2012: The postpartum endometrial inflammatory response: a normal physiological event with potential implications for bovine fertility. *Reprod. Fert. Dev.* **24**, 1028-1039.
- Eduvie, L.O., D.I.K. Osori, P.B. Adoo, and C.O. Njoku, 1984: Bacteriological investigation of the postpartum uterus: relationship to involution and histopathological findings. *Theriogenology* **21**, 733-745.
- Fernandes, C.A.C., B.F.L. Alves, E.R. Oliveira, J.H.M. Viana, M.M. Gioso, and Y.C.S. Loyola, 2012: Efeito de diferentes doses de cloprostenol sódico no período pós-parto de vacas de corte. *Ciênc. Anim. Bras.* **13**, 346-355.
- Ferreira, C.M., L. Vasques, F. Neis, M. Tartin, V. Visioly, and C.F. Eurico, 2002: Biópsia endometrial em vacas *Bos indicus* em regime extensivo de criação com problemas de fertilidade. *Ensaio e Ci.* **6**, 13-33.
- Goshen, T., N. Galon, A. Arazi, and N.Y. Shpigel, 2012: The effect of uterine biopsy on reproductive performance of dairy cattle: a case- control study. *Israel J. Vet. Med.* **67**, 34-38.
- Gomez-Cuetara, C., J.M. Flores, J. Sanchez, A. Rodrigues, and M.A. Sanchez, 1995: Histological changes in the uterus during postpartum in the mare. *Anat. Histol. Embryol.* **24**, 19-23.
- Guilbault, L.A., P. Villeveuve, and J.J. Dufour, 1988: Failure of exogenous prostaglandin F_{2α} to enhance uterine involution in beef cows. *Can. J. Anim. Sci.* **68**, 669-676.
- Herath, S., S.T. Lilly, N.R. Santos, R.O. Gilbert, L. Goetze, C.E. Bryant, J.O. White, J. Cronin, and I.M. Sheldon, 2009: Expression of genes associated with immunity in the

endometrium of cattle with disparate postpartum uterine disease and fertility. *Reprod. Biol. Endocrin.* **7**, 1-13.

Holt, L.C., W.D. Whittier, F.C. Gwazdauskas, W.E. Vinson, and P.S. Sponenberg, 1989: Involution, pathology and histology of the uterus in dairy cattle with retained placenta and uterine discharge following GnRH. *Anim. Reprod. Sci.* **21**, 11-23.

Katagiri S, and Y. Takahashi, 2004: Changes in EGF concentrations during estrous cycle in bovine endometrium and their alterations in repeat breeder cows. *Theriogenology* **62**, 103-112.

Madoz, L.V., M.J. Giuliadori, A.L. Migliorisi, M. Jaurequiberry, R.L. de la Sota, 2014: Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. *J. Dairy Sci.* **97**, 195-201.

Meira, E.B.J.R., L.C. Henriques, L.R. Sá, and L. Gregory, 2012: Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. *J. Dairy Sci.* **12**, 6969-6973.

Ohtani, S., K. Okuda, K. Nishimura, and S. Mohri, 1993: Histological changes in bovine endometrium during the estrous cycle. *Theriogenology* **39**, 1033-1042.

Orfanou, D.C., H.N. Ververidis, A. Pourlis, I.A. Fragkou, A.N. Kokoli, I.A. Taitzoglou, A. Tzora, C.M. Nerou, L. Athanasiou, and G.C. Fthenakis, 2009: Post-partum involution of the canine uterus – gross anatomical and histological features. *Reprod. Domest. Anim.* **44**, 152-155.

Snider, T.A., C. Sepoy, and G.R. Holyoak, 2011: Equine endometrial biopsy reviewed: observation, interpretation and application of histopathologic data. *Theriogenology* **75**, 1567-1581.

Tenhagen, B.A., E. Birkelbach, and W. Heuwieser, 2000: Serum progesterone levels in post-partum dairy cows after repeated application of prostaglandin F2 α analogue D (+) cloprostenol sodium. *J. Vet. Med. A.* **47**, 213-220.

List of legends

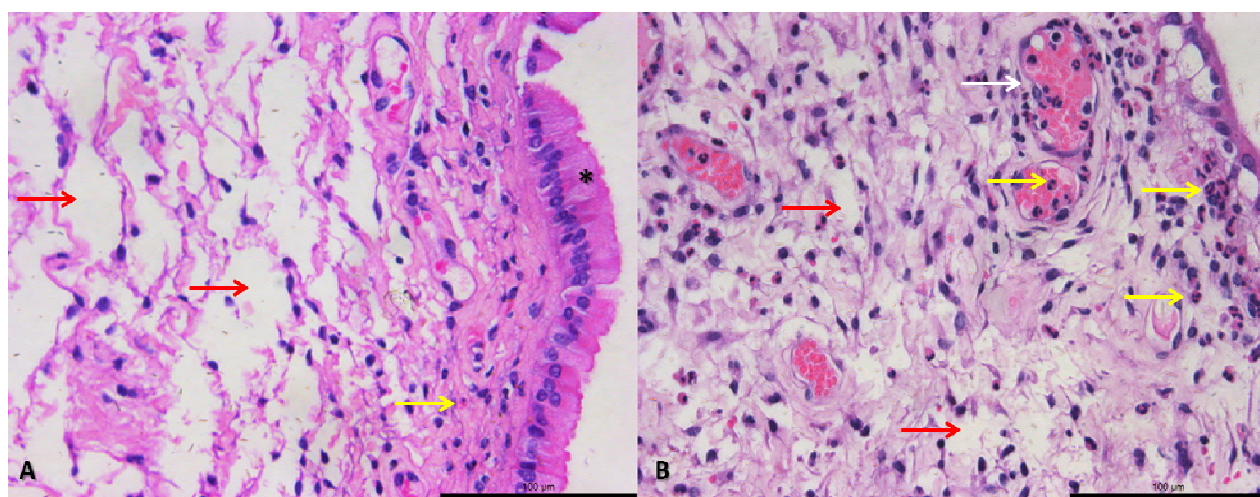


Fig 1 Photomicrograph of bovine endometrium 1 day postpartum of placebo (A) and treated (B) group. In A note the presence of edema (red arrow) and inflammatory cell (yellow arrow) in stromal portion and epithelial portion (*). In B note the presence of edema (red arrow) in the stromal portion; polymorphonuclear infiltrate (below the epithelium, perivascular, intravascular and at stromal portion) and the presence of blood vessels (white arrow). (Hematoxylin-eosin, 40x magnification, Bar = 100µm).

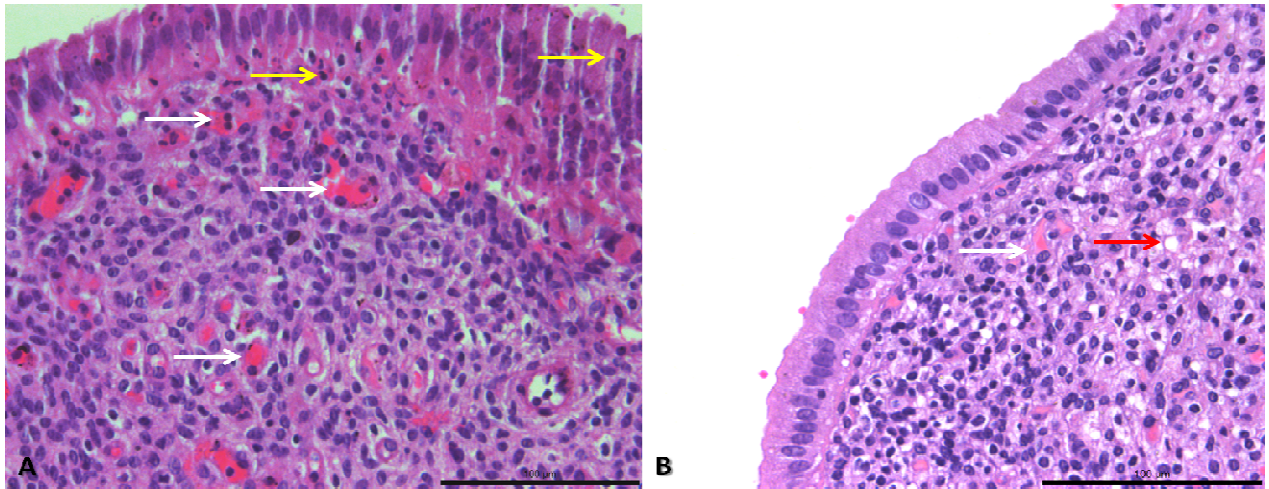


Fig 2 Photomicrograph of bovine endometrium 7 days postpartum of placebo (A) and treated (B) group. In A note the presence of large amounts of polymorphonuclear infiltrate (yellow arrow) at epithelial portion, below the epithelium and in some areas of stromal portion; beyond areas of hemorrhage (white arrow). In B note small areas of edema (red arrow) as well as small areas of hemorrhage (white arrow). Furthermore, in B there is no the presence of polymorphonuclear infiltrates. (Hematoxylin-eosin, 40x magnification, Bar = 100µm).

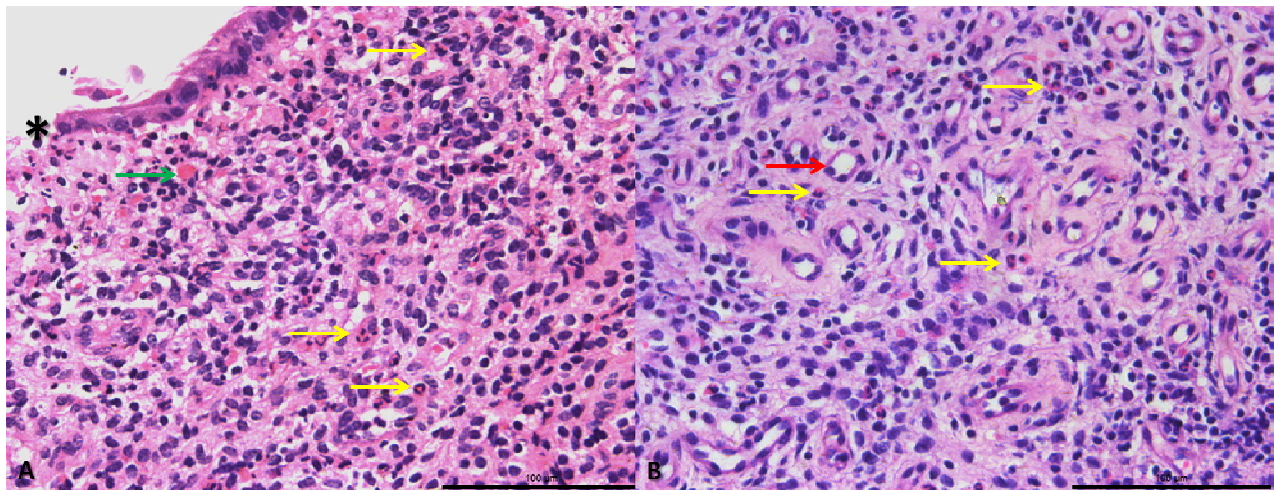


Fig 3 Photomicrograph of bovine endometrium 14 days postpartum of placebo (A) and treated (B) group. In A note the presence of intense polymorphonuclear infiltrate (yellow arrow) across endometrial sample; areas of hemorrhage (green arrow); endometrial epithelium not intact (*) and

high cellularity. In B note the presence of polymorphonuclear infiltrate (yellow arrow) and a small vessel (red arrow). (Hematoxylin-eosin, 40x magnification, Bar = 100µm).

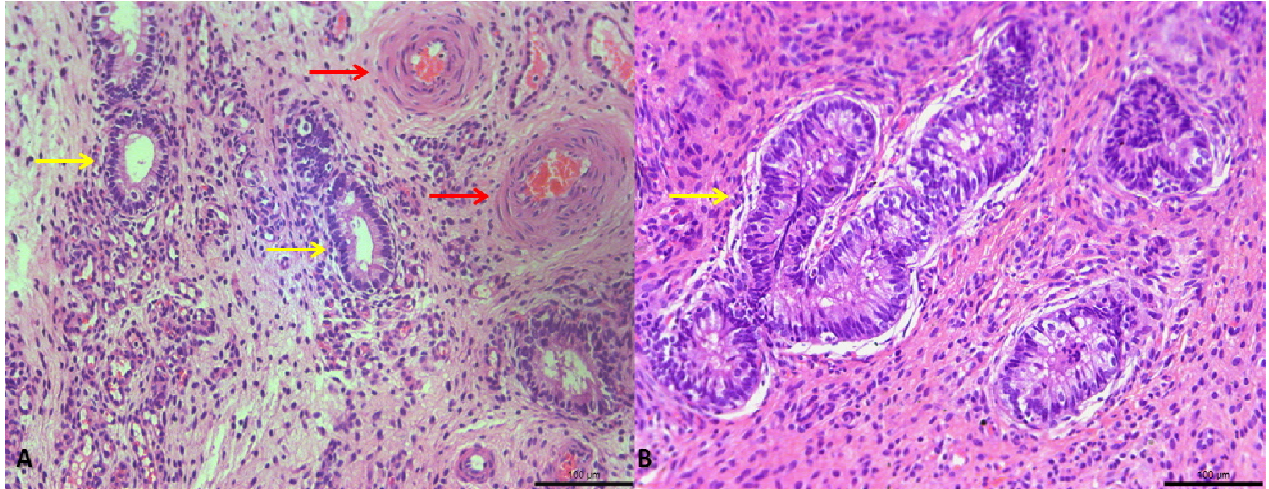


Fig 4 Photomicrograph of bovine endometrium 28 days postpartum of placebo (A) and treated (B) group. In A note the presence of endometrial glands (yellow arrow) and arterioles (red arrow). It also can be observed high cellularity, but not polymorphonuclear infiltrate. In B note the presence of endometrial glands (yellow arrow). Also, there is some cellularity and absence of polymorphonuclear infiltrate. (Hematoxylin-eosin, 20 x magnification, Bar = 100µm).

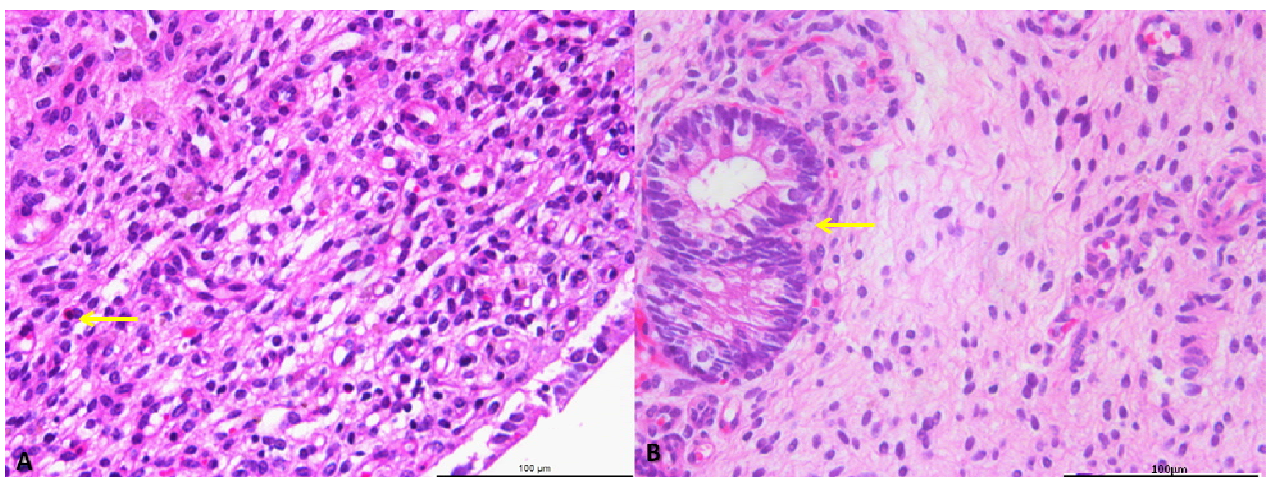


Fig 5 Photomicrograph of bovine endometrium 42 days postpartum of placebo (A) and treated (B) group. In A note the tissue without inflammation, it also can be observed only one

inflammatory cell (yellow arrow). In B note the presence of endometrial glands (yellow arrows) and endometrial tissue without signs of inflammation. (Hematoxylin-eosin, 40x magnification, Bar = 100µm).

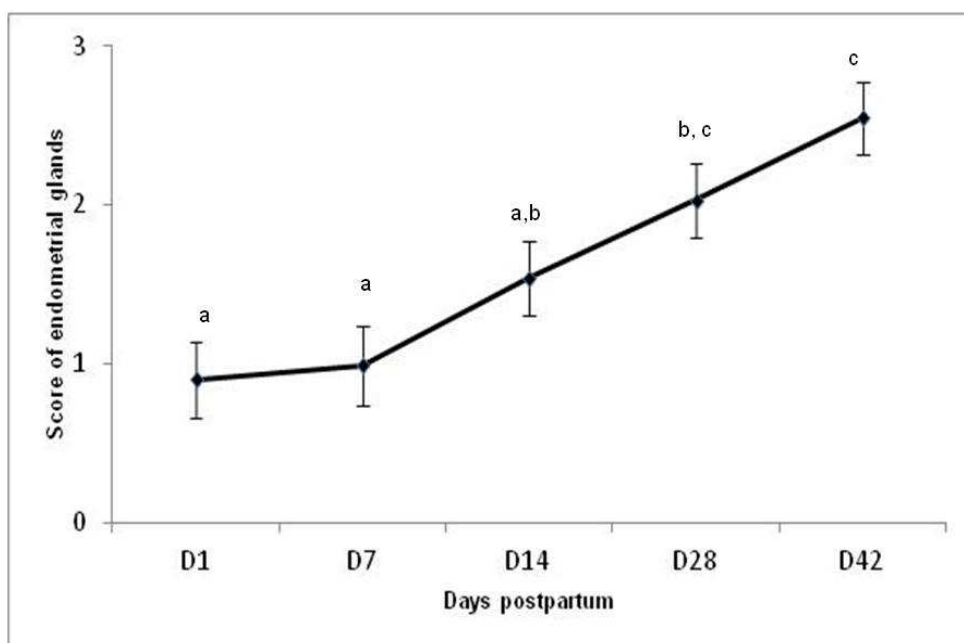


Fig 6 Mean and standard error scores of endometrial glands (score 0 to 3) in the postpartum period (D1 to D42) of endometrial histological evaluation of Nelore females treated or not with cloprostenol sodium. Score 0:none, 1: scarce, 2: moderate and 3: heavy. ^{a,b,c} Uncommon letters differ ($P < 0.05$).

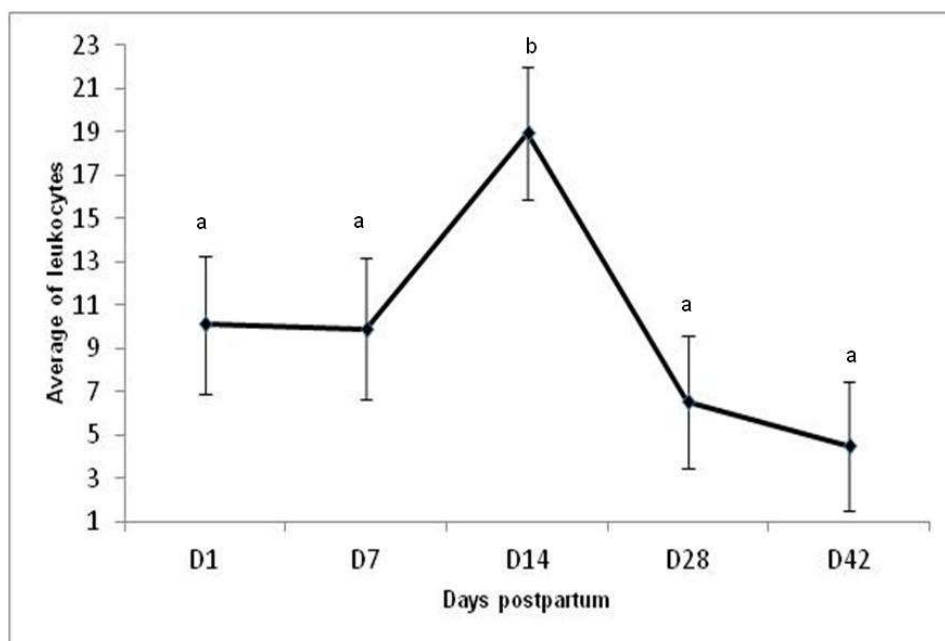


Fig 7 Mean and standard error of average of leukocytes at 5 random fields in the postpartum period (D1 to D42) of endometrial histological evaluation of Nelore females. ^{a,b} Uncommon letters differ ($P < 0.05$).

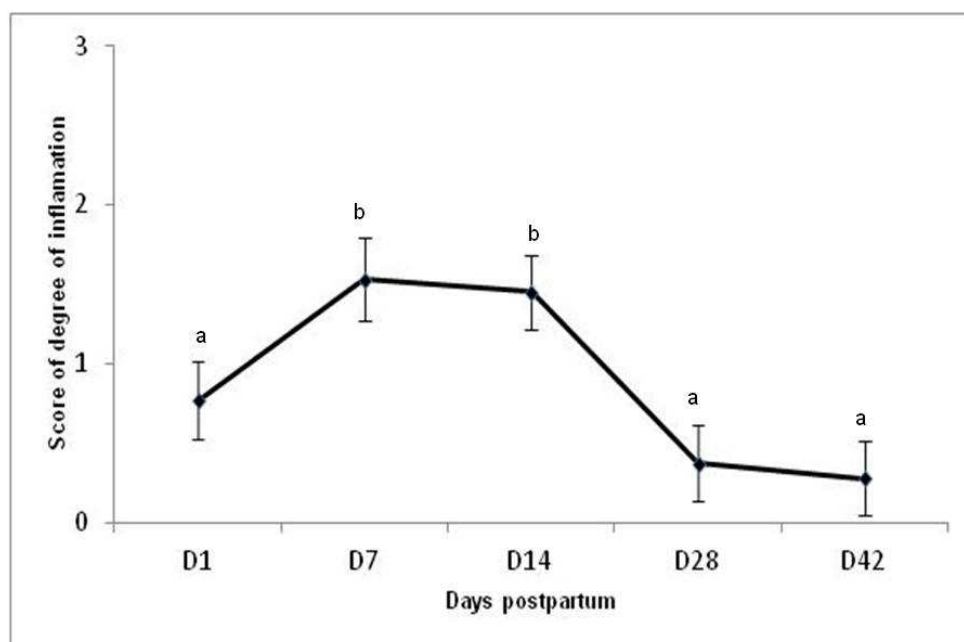


Fig 8 Mean and standard error scores of degree of inflammation (score 0 to 3) in the postpartum period (D1 to D42) of endometrial histological evaluation of Nelore females. Score 0: no inflammation, 1: low

inflammation, 2: moderate inflammation, 3: severe inflammation. ^{a,b}

Uncommon letters differ ($P < 0.05$).

Capítulo 5

3 DISCUSSÃO GERAL

A produção de um bezerro/vaca/ano na pecuária de corte é visto como o grande objetivo para tornar o setor economicamente viável e mais rentável. O uso de protocolos hormonais, como os análogos da $\text{PGF}_{2\alpha}$, durante o período puerperal visa um menor período de involução uterina por meio de sua atuação na contração uterina e retorno à atividade cíclica ovariana precocemente. Neste contexto, o conhecimento dos processos fisiológicos e biológicos que estão envolvidos durante o período puerperal apresenta-se sempre válido para a adequação de tais protocolos, visando tanto a otimização dos resultados quanto a maximização da atividade pecuária.

A avaliação da ação do cloprostenol sódico administrado nos dias 1 e 4 pós-parto (D0 = parto) sobre os padrões hormonais, de involução uterina, histológicos, de imunolocalização, de imunopostividade e de quantificação gênica foram feitos mediante exames hormonais, de vaginoscopia, palpação transretal, ultrassonografia, biópsia uterina, histologia, imunohistoquímica e avaliação gênica em diferentes momentos do pós-parto.

Trabalhos direcionados à expressão gênica do mRNA do FP no corpo lúteo (SAKAMOTO et al., 1994; SAKAMOTO et al., 1995; ISHII e SAKAMOTO 2001; SHIRASUNA et al., 2012; WEEMS et al., 2012) e tecidos uterinos durante o período gestacional (AROSH et al., 2004; WEHBRINK et al., 2008) em bovinos podem ser encontrados na literatura. Entretanto, nenhum trabalho relacionado à expressão gênica temporal do FP durante o período puerperal de bovinos de corte pôde ser encontrado.

Em ratos, a expressão gênica de FP é variável entre os períodos gestacional, de parição e primeiro dia pós-parto, com menores valores no pós-parto e maiores durante o parto (BRODT-EPPLEY e MYATT 1998; DONG e YALLAMPALLI, 2000). No presente estudo durante o período puerperal a expressão de FP no D1 foi menor que nos outros dias, com exceção do D42. A $\text{PGF}_{2\alpha}$ auxilia na involução uterina durante o pós-parto por agir nas contrações miométriais (GIULBALT et al., 1984) e auxiliar na descarga uterina (LINDEL et al., 1981). Sendo o FP um receptor contrátil (WEHBRINK et al., 2008), seu aumento do D1 ao D14 é desejável, uma vez que neste momento as contrações estão presentes e há a redução do tamanho uterino, acúmulo de

fluido e descarga cervical. Adicionalmente, a redução na expressão aos 42 dias pós-parto não é um evento inesperado, uma vez que neste momento, as contrações tornam-se desnecessárias.

Em bovinos, a expressão gênica de FP não se altera durante a gestação nas membranas fetais, tecidos intercaruncular e caruncular com maior expressão neste último (AROSH et al., 2004). Entretanto, conforme observado no presente estudo, este fato não ocorre no período puerperal uma vez que foram observadas mudanças na expressão gênica durante os períodos estudados, mostrando diferenças biológicas durante os dois momentos.

Quando a $\text{PGF}_{2\alpha}$ aumenta durante o período gestacional final em ratos, a expressão gênica de FP também aumenta, proporcionando uma maior ligação entre a $\text{PGF}_{2\alpha}$ e seu receptor, fato que pode estar relacionado com as contrações durante o parto. Entretanto, no primeiro dia pós-parto, é observada uma redução na expressão (BRODT-EPPLEY e MYATT 1998). No presente estudo, as concentrações de PGFM no primeiro dia pós-parto atingiram os valores máximos durante o período experimental, mas o mesmo não ocorreu com a expressão gênica do FP, a qual foi baixa neste dia.

Também, algumas diferenças entre espécies na expressão gênica de FP podem ser encontradas uma vez que no tecido endometrial de ovelhas gestante não foi detectada expressão de FP, mas no tecido miometrial sim (MA et al., 1999), local onde também observou-se expressão em humanos (GRIGSBY et al., 2006) e ratos (DONG e YALLAMPALLI, 2000).

O FP é localizado na membrana celular (NARAMIYA et al., 1999) e no citoplasma (BLESSON et al., 2012). A proteína FP foi detectada nas células endoteliais luteais (SHIRASUNA et al., 2012) e no citoplasma de glândulas epiteliais, superfície de células epiteliais e estroma do útero de vacas gestantes (WEHBRINK et al., 2008). No presente estudo, a proteína do FP foi localizada nas células epiteliais do endométrio, epitélio glandular e estroma uterinos, com imunolocalização citoplasmática, confirmando dados de outros autores com ratos e bovinos (WEHBRINKS et al., 2008; BLESSON et al., 2012).

Nenhum estudo com a imunolocalização e imunomarcagem temporal de FP durante o período puerperal de bovinos foi encontrado na literatura consultada. Devido a característica de contratilidade, o aumento até o parto parece auxiliar nas contrações uterinas para a expulsão fetal (WEHBRINKS et

al., 2008). Igualmente, durante o período puerperal, as contrações são desejáveis para auxiliar na limpeza uterina e liberação da placenta e lóquio.

Durante o ciclo sexual de mamíferos, o útero passa pelo processo de apoptose e proliferação celular para a manutenção da próxima gestação (Revisado por Kamemori et al., 2011). Tal fato também parece ocorrer no período pós-parto visto que, no presente estudo, a expressão gênica de CASP-3 foi alta no primeiro dia pós-parto, com reduzida expressão durante o restante do período de avaliações. Isto ocorreu provavelmente devido ao processo de limpeza que ocorre após o parto para eliminação das células que se proliferaram durante a gestação, sendo um evento crucial para o estabelecimento de uma nova gestação.

Arai et al. (2013) estudaram o ciclo estral de fêmeas bovinas leiteiras e observaram que não há diferença na expressão gênica de CASP-3 durante esta fase. Todavia, no presente estudo, foram notadas diferenças na expressão durante o período pós-parto, evidenciando diferenças entre estas duas fases fisiológicas. Adicionalmente, o tratamento com cloprostenol sódico aumentou tal expressão nos animais do grupo tratado, revelando um efeito positivo do tratamento para indução da apoptose e promoção de uma melhor renovação celular.

É sugerido que baixas concentrações de P4 e/ou estradiol-17 β contribuam na indução do processo de apoptose no endométrio bovino durante o ciclo estral (ARAI et al., 2013). Os dados do presente estudo corroboram com os destes autores, uma vez que no pós-parto imediato o nível de P4 foi baixo (até o D35), contribuindo para uma alta expressão de CASP-3 neste período, com menor valor em D42, correspondendo com o maior valor de P4.

A expressão gênica de COX-2 foi detectada no endométrio bovino durante o ciclo estral, gestação, parto e até 6 horas pós-parto (FUCHS et al., 1999; AROSH et al., 2002; AROSH et al., 2004; SCHULLER et al., 2006; TAKAGI et al., 2008). Entretanto, os trabalhos não relatam a expressão temporal desta enzima durante período de pós-parto em vacas Nelore.

A COX-2 é responsável pela secreção pulsátil e basal de prostaglandinas pelo endométrio durante diferentes dias do ciclo estral (AROSH et al., 2002). De semelhante forma durante o período pós-parto, há produção de $\text{PGF}_{2\alpha}$ com a finalidade de contribuir na involução uterina por meio

das contrações. No presente estudo, pôde-se notar que os padrões de expressão de FP e COX-2 foram semelhantes durante o período pós-parto, comprovando a associação destas duas variáveis.

Jischa et al (2008) em trabalho com pôneis, não notaram mudanças significativas na expressão de COX-2 nos dias 1, 9 e 16 pós-parto. Entretanto, pelo presente estudo, foram verificadas diferenças nos dias estudados, mostrando uma possível distinção entre espécies.

A imunomarcagem para COX-2 foi relatada em placentomas bovinos (SCHULER et al., 2006), membranas fetais durante a gestação (AROSH et al., 2002) e no útero durante a gestação nas regiões perinuclear e citoplasmática (WEHBRINK et al., 2008). No presente estudo, também a COX-2 apresentou marcação positiva no epitélio endometrial, estroma e glândulas endometriais, com imunolocalização citoplasmática, em todos os momentos estudados, dados que corroboram com os de outros autores (AROSH et al., 2004; WEHBRINK et al., 2008).

No período pós-parto (de 6 a 12 horas) a expressão proteica de COX-2 no endométrio bovino é mais alta do que durante a gestação (FUCHS et al., 1999; TAKAGI et al., 2008). Isto pode ser associado com a liberação de citocinas de células do sistema imune que estão presentes no útero devido à liberação do feto e da placenta (FUCHS et al., 1999). Estes dados corroboram com os do presente trabalho, uma vez que no D7 foi observado um maior grau de inflamação e também uma maior expressão gênica de COX-2, contudo, a detecção proteica não variou entre os dias de análise. É importante ressaltar que os resultados de expressão gênica não são necessariamente correlacionados com a expressão proteica (TAKAGI et al., 2008).

Conforme citação de Ymer et al (2012), quando a concentração plasmática de P4 é de 1ng/mL, tem-se o indicativo da presença de atividade luteal, enquanto que, concentrações menores que 1ng/mL por pelo menos 14 dias, tem-se indicativo de cessamento da atividade ovariana. Adicionalmente o folículo dominante de bovinos de corte com boa condição corpórea no pós-parto somente irá ovular normalmente aos 30 dias pós-parto (CROWE, 2008). No presente estudo, os animais apresentaram baixos níveis plasmáticos de P4 (<1ng/mL) até D28, e apesar de ter sido observado nível plasmático de P4 maior que 1ng/mL já no D35, houve diferença significativa somente uma

semana após. Com base nos dados, pode ser considerado que os animais se encontravam em anestro até o D35 e que retorno à atividade ovariana ocorreu entre D35 e D42 (média 38,5 dias). Este período difere de outros trabalhos, nos quais foram observados 30,9 (MADEJ et al., 1984) ou 21 (SCULLY et al., 2013) dias em bovinos de leite e 30 (DEL VECCHIO et al., 1990) ou 46,1 dias em bovinos de corte (DIMMICK, 1991).

Ressalta-se que, embora o período de duração do anestro seja variável entre os trabalhos e entre os animais, tais dados são suportados pelo fato que nem todos os animais ovulam o folículo da primeira ou segunda onda (CROWE, 2008). Além do mais, variações individuais podem ser encontradas com relação ao dia da ovulação, fato que explica o grande erro padrão aos 42 dias no presente estudo, uma vez que, após o tratamento com a PGF2 α nem todos os animais ovulam (TENHAGEN et al., 2000).

Há uma correlação positiva com o tempo de involução uterina e as concentrações e magnitudes de PGFM, sendo que a redução da PGFM é associada positivamente com a redução do diâmetro dos cornos uterinos. Animais com pós-parto normal apresentam uma elevada concentração de PGFM por períodos mais longos que animais com problemas puerperais (MADEJ et al., 1984; WISCHARAL et al., 2001; MATEUS et al., 2003; NAKAO et al., 2007).

No presente estudo, o tempo de menores níveis plasmáticos de PGFM é seguido por níveis plasmáticos de P4 maiores que 1ng/mL. Também, altos valores com regressão até o D21 estão relacionados com o período de maior involução uterina e crescimento folicular. Estes dados corroboram com os de Madej et al. (1984) os quais também encontraram correlação entre a liberação de PGFM, o encurtamento do tempo de involução uterina e a primeira ovulação.

A característica do muco vaginal pode ser utilizada para avaliação de enfermidades uterina (WILLIAMS et al., 2005; SCULLY et al., 2013, MARQUES JÚNIOR et al., 2011), varia individualmente e quanto mais inodoro e incolor for, melhor o prognóstico reprodutivo (NOAKES et al., 2001). No presente estudo, nenhum animal demonstrou sinais de infecção uterina, visto que durante todo o período experimental, os animais encontravam-se próximos do escore desejável (1 – muco translúcido ou ausente).

O efeito do tratamento sobre a descarga cervical, com um melhor escore para nos animais do grupo tratado, pode ser justificada pelo aumento nas contrações uterinas, favorecendo a descarga uterina (LINDEL et al., 1981) e limpeza do ambiente uterino. Adicionalmente, a redução da inflamação observada no óstio cervical está associada ao processo de recuperação e involução cervical após o parto.

No presente estudo, com uma semana pós-parto foi possível a mensuração folicular, com média de $6,22 \pm 0,77$ mm. Do D28 ao D35 houve uma diminuição no crescimento folicular e também nas concentrações de P4, fato também observado por Dimmick et al. (1999) e possivelmente relacionado a luteinização folicular.

Pequenas quantidades de fluido intrauterino pôde ser identificada na ultrassonografia, apresentando-se como áreas anecóicas (Revisado por Martins e Borges, 2011). A diminuição no acúmulo de fluido intrauterino reduz os riscos de infecções e é um evento esperado e desejável uma vez que é indicativo da limpeza uterina pelas contrações. Aos 14 dias do presente estudo todos os animais encontravam-se sem fluido intrauterino, indicando uma limpeza uterina eficiente.

Com base nos parâmetros utilizados para considerar a involução uterina completa, pode ser concluído que a mesma ocorreu entre D35 e D42 (média 38,5 dias), uma vez que todos os parâmetros foram os mínimos nestes dias. Este período de involução difere de trabalhos com bovinos de leite nos quais se observaram período de involução de 23,6 (MADEJ et al., 1984), 29,6 dias (LINDEL et al., 1981) em fêmeas multíparas e 49 dias (SCULLY et al., 2013) em fêmeas primíparas. Entretanto, vale ressaltar que o tempo de completa involução uterina é um pouco vago, dependente dos exames utilizados (SCULLY et al., 2013) e critério de avaliação, uma vez que os exames utilizados podem variar na forma de interpretação e são subjetivos.

O uso do cloprostenol sódico no período de pós-parto é relacionado com um período de involução uterina mais rápida, reduzidos números de dias até a inseminação artificial e período de serviço em bovinos de corte. Entretanto, relutância ainda pode ser encontrada para o uso de tal protocolo em bovinos de corte devido a dificuldades de manejo (FERNANDES et al., 2005; FERNANDES et al., 2012).

No presente estudo, não foi observada influência do tratamento sobre a involução uterina, fato também relatado por outros autores utilizando bovinos de leite e um análogo de PGF2 α (trometamina dinaprost) por 48 horas e também em bovinos de corte com a administração contínua de PGF2 α por 11 dias no pós-parto (GUILBALT et al., 1988; TIAN e NOAKES, 1991).

Outro trabalho com bovinos de leite determinou que o tratamento com NaCl, NaCl + PGF2 α (trometamina dinaprost) ou GnRH + PGF2 α nos dias 15 e 25 pós-parto não influenciou na aceleração da involução uterina, atividade cíclica e dinâmica folicular (TUCKER et al., 2001). Ainda, os autores sugerem que as boas práticas de manejo alimentar e sanitário podem fazer com que o uso de terapias hormonais seja desnecessário no período pós-parto. Tal hipótese pôde ser confirmada neste estudo, uma vez que os animais foram mantidos em excelente manejo sanitário e alimentar e não houve influência do tratamento.

Com relação à avaliação histológica do endométrio bovino, estudos descrevem a morfologia do tecido uterino durante o período puerperal em bovinos de leite, porém, em bovinos de corte, tais descrições são escassas (BONNET et al., 1990; BONNET et al., 1991; BONNET; MARTIN., 1995; CHAPWANYA et al., 2012).

No presente estudo, a realização de biópsias endometriais foi realizada visando uma análise descritiva do remodelamento e arquitetura do tecido endometrial durante o pós-parto. Neste contexto, grande parte das biópsias endometriais foi realizada na porção mediana do corno uterino direito, previamente identificado como gravídico, o que de acordo com Bonnet et al. (1991) é indicativo de uma maior atividade do ovário direito.

Na avaliação histológica do tecido endometrial de vacas Nelore tratadas ou não com cloprostenol sódico, não foi observada influência do tratamento nas variáveis estudadas. Os achados de hemorragia podem ser atribuídos a rupturas de vasos sanguíneos e a presença de tecido endometrial ou miometrial pode sofrer influência do processamento histológico ou da própria técnica de biópsia.

Em estudo, diferenças nas avaliações histológicas não foram observadas no dia 26 pós-parto, entretanto no dia 46 houve efeito do tratamento na inflamação, diâmetro e número de glândulas endometriais

(BONNET et al., 1990). Este fato, entretanto, não foi observado no presente trabalho e pode ser justificado devido à dose e momento de aplicação do cloprostenol sódico, uma vez que os mesmos podem ter sido incapazes de aumentar as contrações endometriais e reduzir a inflamação.

A concentração de glândulas endometriais nos cortes histológicos pode ser correlacionada com a atividade cíclica dos animais (BONNET et al., 1991). Tal afirmação foi comprovada neste estudo, uma vez que foi encontrada a presença de uma maior concentração de glândulas endometriais observadas no último dia de análise (D42), período no qual os animais possuíam tecido luteal, conforme avaliação por meio da concentração de P4.

O edema observado no estroma do tecido endometrial está presente no primeiro dia pós-parto (GOMEZ-CUETARA et al., 1995) com uma marcada apresentação no 10º dia pós-parto e remissão aos 20 dias pós-parto (BÜGNER, 1981). Isto também foi evidenciado pelo neste estudo, podendo-se observar redução no espaço entre as fibras colágenas aos 14 dias e aos 28 e 42 dias, a maior parte dos animais não apresentava mais áreas de edema.

A presença de células inflamatórias no tecido endometrial frente a uma resposta inflamatória é comumente observada durante o período puerperal e, após tal resposta, há a restauração do tecido endometrial (CHAPWANYA et al., 2012). Adicionalmente, a presença de polimorfonucleares é correlacionada com ausência de problemas uterinos nos animais e é indicativo de bom estado de saúde (FERREIRA et al., 2002). No presente estudo, aos 14 dias pós-parto observou-se uma grande quantidade de infiltrado leucocitário, predominantemente de neutrófilos, com então redução a partir deste dia. Tal dado está de acordo com os relatos de Chapwanya et al. (2012), os quais citam uma maior resposta inflamatória nos primeiros dias pós-parto e com os de Ferreira et al (2002), evidenciando a sanidade dos animais utilizados neste estudo, uma vez que nenhum deles apresentou sinais de enfermidade crônica ou aguda.

Ressalta-se que a realização da biopsia endometrial não acarretou nenhum tipo de alteração reprodutiva ou de saúde nos animais, evidenciando que a realização de tal técnica é segura e indicada para a realização de colheitas de amostras endometriais para a detecção de enfermidades uterinas, bem como para o acompanhamento de todo o processo de involução uterina.

4 CONCLUSÕES GERAIS

De acordo com os objetivos propostos, e nas presentes condições experimentais, pode-se concluir que:

1- A administração do cloprostenol sódico na dose de 0,530 mg em duas aplicações nos dias 1 e 4 pós-parto (D0 = parto) em vacas multíparas Nelore apresentou efeito sobre a descarga cervical e expressão gênica de CASP-3 no período puerperal em estudo.

2- A biópsia endometrial realizada nos animais não apresentou nenhum efeito adverso sobre a saúde dos animais utilizados.

3- A administração do cloprostenol sódico na dose de 0,530 mg em duas aplicações nos dias 1 e 4 pós-parto (D0 = parto) em fêmeas multíparas Nelore não acelerou o tempo de involução uterina e retorno à atividade ovariana.

4- As variáveis relacionadas à dosagem hormonal, involução uterina, variáveis histológicas (glândulas endometriais e grau de inflamação) e de expressão gênica apresentam um comportamento dinâmico ao longo do período puerperal, se modificando ao longo do tempo, independente do tratamento.

5 BIBLIOGRAFIA

AL-JEFOUT, M.; DEZARNUALDS, G.; COOPER, M.; TOKUSHIGE, N.; LUSCOMBE, G.M.; MARKHAM, R.; FRASER, I.S. Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study. **Human Reproduction**, v.24, n.12, p.3019-3024, 2009.

ANDRIGA, M.F.A.; VAN EERDENBURG, F.J.; FERNÁNDEZ, E.; GARCIA, S.; CAVESTANY, D. Comparison between two progesterone sources and two oestradiol formulations in a Heatsynch protocol for postpartum cycling dairy cows in pasture. **Journal of Veterinary Science**, v.14, n.2, p.161-166, 2013.

ARAI, M.; YOSHIOKA, S.; TASAKI, Y.; OKUDA, K. Remodeling of bovine endometrium throughout the estrous cycle. **Animal Reproduction Science**, v.142, n.1-2, p. 1-9, 2013.

ARCHBALD, L.F.; SCHULTZ, R.H.; FAHNING, M.L.; KURTZ, H.J.; ZEMJANIS, R. A sequential histological study of the *post-partum* bovine uterus. **Journal of Reproduction and Fertility**, v.29, n.1, p.133-136, 1972.

AROSH, J.A.; PARENT, J.; CHAPDELAINE, P.; SIROIS, J.; FORTIER, M.A. Expression of Cyclooxygenase 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. **Biology of Reproduction**, v.67, n.1, p. 161-169, 2002.

AROSH, J.A.; BANU, S.K.; CHAPDELAINE, P.; FORTIER, M.A. Temporal and tissue-specific expression of prostaglandin receptors EP2, EP3, EP4, FP, and Cyclooxygenases 1 and 2 in uterus and fetal membranes during bovine pregnancy. **Endocrinology**, v.145, n.1, p.407-417, 2004.

AZAWI, O.I.; AL-SADI, H.I. Uterine leiomyoma in a buffalo: a case report. **Buffalo Bulletin**, v.29, n.2, p.80-82, 2010.

AZAWI, O.I. Postpartum uterine infection in cattle. **Animal Reproduction Science**, v.105, n.3-4, p.187-208, 2008.

BALL, P.J.H.; PETERS, A.R. **Reproduction in Cattle**. 3. ed. Grã Bretanha: Blackwell, 2004.

BARROS, C.M.; DI STASI, L.C. **Farmacologia Veterinária**. 1 ed. Barueri: Manole, 2012.

BARUSELI, P.S.; REIS, E.L.; MARQUES, M.O.; NASSER, L.F.; BÓ, G.A. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. **Animal Reproduction Science**, v.82–83, p.479–486, 2004.

BLESSON, C.S.; BÜTTNER, E.; MASIRONI, B.; SAHLIN, L. Prostaglandin receptors EP and FP are regulated by estradiol and progesterone in the uterus of ovariectomized rats. **Reproduction. Biology and. Endocrinology**. v.10, n.3, p.1-10, 2012.

BONNETT, B.N.; MILLER, R.B.; ETHERINGTON, W.G.; MARTIN, S.W.; JOHNSON, W.H. Endometrial biopsy in Holstein-Friesian dairy cows I. technique, histological criteria and results. **Canadian Journal of Veterinary Research**, v.55, n.2, p.155-161, 1991.

BRAUNER, C.C.; PIMENTEL, M.A.; LEMES, J.S.; PIMENTEL, C.A.; MORAES, J.C.F. Desempenho reprodutivo pós-parto de vacas de corte submetidas a indução/sincronização de cio. **Revista Brasileira de Medicina Veterinária**, v.38, n.1, p.99-103, 2009.

BREYER, R.M.; BAGDASSARIAN, C.K.; MYERS, S.A.; BREYER, M.D. Prostanoid receptors: subtypes and signaling. **Annual Review of Pharmacology and Toxicology**, v.41, p.661-690, 2001.

BRIDGES, P.J.; FORTUNE, J.E. Regulation, action and transport of prostaglandins during the periovulatory period in cattle. **Molecular and Cellular Endocrinology**, v.263, n. 1- 2, p.1-9, 2007.

BRODT-EPPLEY, J.; MYATT, L. Changes in expression of contractile FP and relaxatory EP₂ receptors in pregnant rat myometrium during late gestation, at labor and postpartum. **Biology of Reproduction**, v.59, n.4, 878-883, 1998.

BOATRIGHT, K.M.; SALVESEN, G.Y Mechanisms of caspase activation. **Current Opinion in Cell Biology**, v.15, p.725-731, 2003.

BUTLER, W.R. Nutritional interactions with reproductive performance in dairy cattle. **Animal Reproduction Science**, v.60-61, p.449-57, 2000.

CHAPWANYA, A.; MEADE, K.G.; NARCIANDI, F.; STANLEY, P.; MEE, J.F.; DOHERTY, M.L.; CALLANAN, J.J.; O'FARRELLY, C. Endometrial biopsy: a valuable clinical and research tool in bovine reproduction. **Theriogenology**, v.73, n.7, p.988-994, 2010.

CHRISTENSEN, B.W.; SCHLAFFER, D.H.; AGNEW, D.W.; WANG, C.; KOZLOWSKI, C.; ASA, C.S. Diagnostic value of transcervical endometrial biopsies in domestic dogs compared with full-thickness uterine sections. **Reproduction in Domestic Animals**, v.47, n. s6, p.342-346, 2012.

CLARO JUNIOR, I.; SÁ FIHO, O.G.; ROGRIGES JUNIOR, M.M.; VASCONCELOS, J.L.M. Efeito da frequência de amamentação sobre a taxa de ovulação após tratamento com desmame temporário + GnRH, sobre a incidência de luteólise prematura no primeiro ciclo estral pós-parto e sobre o desempenho da progênie de vacas Nelore. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.62, n.2, p.281-288, 2010.

CROWE, M.A. Resumption of ovarian cyclicity in post-partum beef and dairy cows. **Reproduction Domestic Animal**, v.43, n.s5, p.20-28, 2008.

DAHLEN, C.R.; BIRD, S.L.; MARTEL, C.A.; OLSON, K.C.; STEVENSON, J.S.; LAMB, G.C. Administration of human chorionic gonadotropin 7 days after fixed-time artificial insemination of suckled beef cows. **Journal of Animal Science**, v.88, n.7, p.2337–2345, 2010.

DAVIES, C.J.; FISHER, P.J.; SCHLAFER, D.H. Temporal and regional regulation of major histocompatibility complex class I expression at the bovine uterine/placental interface. **Placenta**, v.21, n.2-3, p.94-202, 2000.

DAVIES, C.J. Why is the fetal allograft not rejected? **Journal of Animal Science**, v.85, n.13, p.32-33, 2006.

DEL VECCHIO, R.P.; CHASE, C.C.; BASTIDAS, P.J.R.; RANDEL, R.D. Oxytocin-induced changes in plasma 13,14 dihydro-15-keto prostaglandin F₂ alpha concentrations on days 10, 20 and 30 postpartum in the bovine. **Journal of Animal Science**, v.68, p. 4261-4266, 1990.

DIMMICK, M.A.; GIMENEZ, T.; SPITZER, J.C. Ovarian endocrine activity and development of ovarian follicles during the postpartum interval in beef cows. **Animal Reproduction Science**, v.24, n.3-4, p.173-183, 1991.

DONG, Y-L.; YALLAMPALLI, C. Pregnancy and exogenous steroid treatments modulate the expression of relaxant EP₂ and contractile FP receptors in the rat uterus. **Biology of Reproduction**, v. 62, n. 3, p.533-539, 2000.

EMERICK, L. L.; DIAS, J.C.; GONÇALVES, P.E.M.; MARTINS, J.A.M.; SOUZA, F.A.; VALE FILHO, V.R.; ANDRADE, V.J. Retorno da atividade ovariana luteal cíclica de vacas de corte no pós-parto: uma revisão. **Revista Brasileira de Reprodução Animal**, v.33, n.4, p.203-213, 2009.

FERNANDES, C.A.C.; OLIVEIRA, E.R.; VASCONCELOS, T.D. Efeitos do cloprostenol sódico no pós-parto de vacas de corte. In: XVII Congresso Brasileiro de Reprodução Animal, 2005, Goiânia – GO. **Anais...2005**, v.1, p.351.

FERNANDES, C.A.C.; FIGUEIREDO, A.C.S. Avanços na utilização de prostaglandinas na reprodução de bovinos. **Revista Brasileira de Reprodução Animal**, v.31, n.3, p.406-414, 2007.

FERNANDES, C.A.C.; ALVES, B.F.L.; OLIVEIRA, E.R.; VIANA, J.H.M.; GIOSO, M.M.; LOYOLA, Y.C.S. Efeito de diferentes doses de cloprostenol sódico no período pós-parto de vacas de corte. **Ciência Animal Brasileira**, v.13, n.3, p.346-355, 2012.

FIOL, C.; UNGERFELD, R. Biostimulation in cattle: stimulation pathways and mechanisms of response. **Tropical and Subtropical Agroecosystems**, v.15, p.29 – 45, 2012.

FUCHS, A.R.; RUST, W.; FIELDS, M.J. Accumulation of cyclooxygenases-2 gene transcripts in uterine tissue of pregnant and parturient cows: stimulation by oxytocin. **Biology of .Reproduction**, v. 60, n. 2, p. 341-348, 1999.

GUILBAULT, L.A; THATCHER, W.W.; DROST, M.; HOPKINS, M. Source of F series prostaglandins during the early postpartum period in cattle. **Biology of Reproduction**, v.31, n.5, p.879-887, 1984.

GONZÁLEZ SÁNCHEZ, J.P.; BIANCHINI SOBRINHO, E.; GONÇALVES, A.A.M. Involução uterina em um rebanho Gir leiteiro segundo o período pós-parto e o número de parições. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.51, n.4, p.345-351, 1999.

GOSHEN, T.; GALON, N.; ARAZI, A.; SHPIGEL, N.Y. The effect of uterine biopsy on reproductive performance of dairy cattle: a case- control study. **Israel Journal of Veterinary Medicine**, v.67, n.1, p.34-38, 2012.

GREGORY, J.W. **Fisiopatologia do anestro pós-parto em bovinos de corte**. 2009. 41p. Monografia (Trabalho de conclusão de curso) Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, 2009.

GRIGSBY, P.L.; SOORANNA, S.R.; ADU-AMANKWA, B.; PITZER, B.; BROCKMAN, D.E.; JOHNSON, M.R.; MYATT, L. Regional expression of prostaglandin e2 and f2alpha receptors in human myometrium, amnion, and choriondecidua with advancing gestation and labor. **Biology of Reproduction**, v. 75, n.2, p. 297-305.

GULL, B.; CARLSSON, S.; KARLSSON, B.; YLÖSTALO, P.; MILSON, I.; GRANBERG, S. Transvaginal ultrasonography of the endometrium in women with postmenopausal bleeding: Is it always necessary to perform an endometrial biopsy? **American Journal of Obstetrics and Gynecology**, v.182, n.3, p.509-515, 2000.

GUSTAFSSON, H.; KORNMATITSUK, B.; KÖNIGSSON, K.; KINDAHL, H. Peripartum and early post partum in the cow – physiology and pathology. IN: Proceedings of the WBC Congress, 23.; 2004, Québec. **Anais...** Canadá: Proceedings of the WBC Congress, 2004. p.2-6.

HAFEZ, E.S.E.; HAFEZ, B. **Reprodução Animal**. 7 ed.; São Paulo: Manole, 2004.

HANZEN, C.H. Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation *Per Rectum*. **The Veterinary Journal**, v.159, n.2, p.161-170, 2000.

HESS, B.W.; LAKE, S.L.; SCHOLLJEGERDES, T.R.; WESTON, T.R.; NAYUGIHUGU, V.; MOLLE, J.D.C.; MOSS, G.E. Nutritional controls of beef cow reproduction. **Journal of Animal Science**, v.83, n.13, p.90–106, 2005.

ISHII Y, SAKAMITO K. Suppression of protein kinase C signaling by the novel isoform for bovine PGF(2 α) receptor. **Biochemistry Biophysic Research Communications**, v.285, n.1, p.1-8, 2001.

JISCHA, S.; WALTER, I.; NOWOTNY, N.; PALM, F.; BUDIK, S.; KOLODZIEJEK, J.; AURICH C. Uterine involution and endometrial functions in

postpartum pony mares. **American Journal of Veterinary Research**, v.69, n.11, p.1-10, 2008.

KAFI, M.; MIRZAEI, A. Effects of first postpartum progesterone rise, metabolites, milk yield, and body condition score on the subsequent ovarian activity and fertility in lactating Holstein dairy cows. **Tropical Animal Health and Production**, v.43, n.4, p.761-767, 2010.

KAMEMORI, Y.; WAKAMIYA, K.; NISHIMURA, R.; HOSAKA, Y.; OHTANI, S.; OKUDA, K. Expressions of apoptosis-regulating factors in bovine retained placenta. **Placenta**, v.32, n.1, p.20-26, 2011.

KASIMANICKAM, R.; DUFFIELD, T.F.; FOSTER, R.A.; GARTLEY, C.J.; LESLIE, K.E.; WALTON, J.S.; JOHNSON, W.H. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. **Theriogenology**, v.62, n.1-2, p.9-23, 2004.

KATAGIRI, S.; TAKAHASHI, Y. Changes in EGF concentrations during estrous cycle in bovine endometrium and their alterations in repeat breeder cows. **Theriogenology**, v.62, n.1-2, p.103-112, 2004.

KRISTEN LP, THOMAS JP, PATRICIA BH, DANIEL WG, DAVIS FW, JHON WR. Cloning of a carboxyl terminal isoform of the prostanoïd FP receptor. **Journal of Biology Chemistry**, v.272, p.883-887, 1997.

KOZICKI, L.E. Aspectos fisiológicos e patológicos do puerpério em bovinos. **Archives of Veterinary Science**, v.3, n.1, p.9-19, 1998.

LAMB, G.C. Influence of nutrition on reproduction in the beef cow herd, 1999a. Saint Paul, University of Minnesota, Disponível em <http://www.extension.umn.edu/beef/components/publications/bcmu48.pdf>. Acesso em: 12 agosto, 2013.

LAMB, G.C.; MILLER, B.L.; LYNCH, J.M.; THOMPSON, K.E.; HELDT, J.S.; LÖEST, C.A.; GRIEGER, D.M.; STEVENSON, J.S. Twice daily suckling but not milking with calf presence prolongs postpartum anovulation. **Journal of Animal Science**, v.77, n.8, p.2207–2218, 1999b.

LEROY, J.L.M.R.; VANHOLDER, T.; VAN KNEGSEL, A.T.; GARCIA-ISPIERTO, I.; BOLS, P.E. Nutrient Prioritization in Dairy Cows Early Postpartum: Mismatch between metabolims and fertility? **Reproduction Domestic Animal**, v.43, n.2, p.96-193, 2008.

LINDELL, J.O. et al. Post-partum release of prostaglandin f2 α and uterine involution in the cow. **Theriogenology**, v.17, n.3, p.237-245, 1981.

LIVESTOCK. In: ESTADOS UNIDOS. Department of Agriculture. PSD : production, supply and distribution online. Reports. Washington, D.C.: United States Department of Agriculture - USDA, 2013. Disponível em: <TTP://www.fas.usda.gov/psdonline>. Acesso em: agosto. 2013.

LOBATO, J.F.P.; MÜLLER, A.; NETO, O.A.P.; OSÓRIO, E.B. Efeitos da idade à desmama dos bezerros sobre o desempenho reprodutivo de vacas de corte primíparas. **Revista Brasileira de Medicina Veterinária e Zootecnia**, v.29, n.5, p.2013-2018, 2000.

MA, X.H.; WU, W.X.; NATHANIELSZ, W. Differential regulation of prostaglandin EP and FP receptors in pregnant sheep myometrium and endometrium during spontaneous term labor. **Biology of Reproduction**, v. 61, n.5, p. 1281-1286, 1999.

MACIEL, M.N.; NEVES, J.P.; GONÇALVES, P.B.D.; OLIVEIRA, J.F.; BORTOLOTO, E.B.; STRANIERI, P. Programa hormonal associado ao desmame temporário, na indução de ovulação em vacas de corte durante o pós-parto. **Ciência Rural**, v.31, n.3, p.473-478, 2001.

MADEJ, A.; KINDAHL, H.; WOYNO, W.; EDQVIST, L.E.; STUPUNICK, R. Blood levels of 15-keto-13,14-dihydroprostaglandin f2 α during the postpartum period in primiparous cows. **Theriogenology**, v.21, n.2, p.279-287, 1984.

MARQUES JÚNIOR, A.P.; MARTINS, T.M.; BORGES, A.M. Abordagem diagnóstica e de tratamento da infecção uterina em vacas. **Revista Brasileira de Reprodução Animal**, v.35, n.2, p.293-298, 2011.

MARTINS, T.M.; BORGES, A.M. Avaliação uterina em vacas durante o puerpério. **Revista Brasileira de Reprodução Animal**, v.35, n.4, p.433-443, 2011.

MARTINS, V.M.V.; MARQUES JUNIOR, A.P.; VASCONCELOS, A.C.; MARTINS, E.; SANTOS, R.L.; LIMA, F.P.C. Maturação e expulsão placentária em vacas das raças Holandesa e Nelore. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.56, n.2, p.157-167, 2004.

MATEUS, L.; LOPES DA COSTA, L.; DINIZ, P.; ZIECIK, A.J. Relationship between endotoxin and prostaglandin (PGE2 and PGFM) concentrations and ovarian function in dairy cows with puerperal endometritis. **Animal Reproduction Science**, v. 76, n.3-4, p.143-154, 2003.

MCNAUGHTON, A.P.; MURRAY R.D. Structure and function of the bovine fetomaternal unit in relation to the causes of retained fetal membranes. **Veterinary Record**, v.165, n.21, p.615-622, 2009.

MEIRA, E.B.J.R.; HENRIQUES, L.C.; SÁ, L.R.; GREGORY, L. Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. **Journal of Dairy Science**, v.95, n.12, p.6969–6973, 2012.

MILLER, V.; UNGERFELD, R. Weekly bull exchange shortens postpartum anestrus in suckled beef cows. **Theriogenology**, v.69, n.8, p. 913-917, 2008.

MIR, F.; FONTAINE, E.; ALBARIC, O.; GREER, M.; VANNIER, F.; SCHLAFER, D.H.; FONTBONNE, A. Findings in uterine biopsies obtained by laparotomy from bitches with unexplained infertility or pregnancy loss: An observational study. **Theriogenology**, v.79, n.2, p.312-322, 2013.

MONTIEL, F.; AHUJA, C. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. **Animal Reproduction Science**, v.85, n.1-2, p.1-26, 2005.

MORAES, J.C.F.; SOUZA, C.J.H.; JAUME, C.M. Requerimentos para uso de tratamentos hormonais e desmame temporário no controle da reprodução de vacas de corte com cria ao pé. **Acta Scientiae Veterinariae**, v.37, n.2, p.113-118, 2009.

NAKAO, T.; GAMAL A.; OSAWA, T.; NAKADA, K.; MORIYOSHI, M.; KAWATA, K. Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on uterine involution and reproductive performance. **Journal of Veterinary Medicine Science**, v.59, n.9, p.791-794, 1997.

NARUMIYA, S.; SUGIMOTO, Y.; USHIKUBI, F. Prostanoid receptors: structures, properties and functions. **Physiological Reviews**, v.79, n.4, p.1193-1226, 1999.

NOAKES, D.E.; PARKIN-ON, T.J.; ENGLAND, G.C.W. **Arthur's Veterinary reproduction and obstetrics**. 8 ed. Londres: Saunders; 2001.

OHTANI, S.; OKUDA, K.; NISHIMURA, K.; MOHRI, S. Histological changes in bovine endometrium during the estrous cycle. **Theriogenology**, v.39, n.5, p.1033-1042, 1993.

OKANO, A.; TOMIZUKA, T. Ultrasonic observation of postpartum uterine involution in the cow. **Theriogenology**, v.27, n.2, p.369-376, 1987.

OLIVEIRA, J.F.C.; GONÇALVES, P.B.D.; FERREIRA, R.; GASPERIN, B.; SIQUEIRA, L.C. Controle sobre GnRH durante o anestro pós-parto em bovinos. **Ciência Rural**, v.40, n.12, p.2623-2631, 2010.

OVERBECK, W.; JÄGER, K. SCHOON, H.A.; WITTE, T.S. Comparison of cytological and histological examinations in different locations of the equine uterus - an in vitro study. **Theriogenology**, v.79, n.9, p.1262-1268, 2013.

PELEGRINNI, C.B.; LOPES, L.F.D. Comportamento reprodutivo de vacas de corte manejadas em pastagem natural com duas cargas animais e duas idades à desmama. **Revista Brasileira de Zootecnia**, v.40, n.11, p.2606-2612, 2011.

PENCAI, F.W.; KOZICKI, L.E.; DA COSTA, C.E.M.P.; DA SILVA, N.L.; MOLLETA, J.L.; MOTTA, J.B.O. Indução ao estro pós puerperal em bovinos mestiços de corte mediante o emprego de diferentes protocolos de amamentação. **Veterinária e Zootecnia**, v.18, n.1, p.53-62, 2011.

PIERCE, K.L.; BAILEY, T.J.; HOYER, P.B, GIL, D.W.; WOODWARD, D.F.; REGAN, J.W. Cloning of a carboxyl-terminal isoform of the prostanoid FP receptor. **The Journal of Biological Chemistry**, v.272, n.2, p.883-887, 1997.

PRESTES, N.C.; LANDIM-ALVARENGA, F.C. **Obstetrícia Veterinária**. 1. ed. Rio de Janeiro: Guanabara Koogan, 2006.

RABASSA, V.R.; PFEIFER, L.F.M.; SCHNEIDER, A.; LUZ, E.M.; COSTA, E.R.M.; CORRÊA, M.N. Anestro pós-parto em bovinos: mecanismos fisiológicos e alternativas hormonais visando reduzir este período – uma revisão. **Revista da FZVA Uruguaiana**, v.14, n.1, p.139-161, 2007.

RE,G.; BALDINO, P.; NOVELLI, A.; VALLISNERI, A.; GIRALDI, C. Specific binding of dl-cloprostenol and d-lcloprostenol to PGF₂ alpha receptors in bovine corpus luteum and myometrial cell membrane. **Journal of Veterinary Pharmacology and Therapeutics**, v.17, n.6, p.455-458, 1994.

ROCHE, J.F.; CROWE, M.A.; BOLAND, M.P. Postpartum anoestrus in dairy and beef cows. **Animal Reproduction Science**, v.28, n.1-4, p.371-378, 1992.

RODRIGUES, J.S.; HAN, S.; NIELSEN, S.; PEARSON, L.K.; GAY, J.M.; TIBARY, A. Consequences of Intrauterine enrofloxacin infusion on mare endometrium. **Journal of Equine Veterinary Science**, v.32, n.2, p.106-111, 2012.

ROSSA, L.A.F.; BERTAN, C.M.; ALMEIDA, A.B.; GASPAR, P.S.; MAZZA, P.H.; BINELLI, M.; BARUSELLI, P.S.; MADUREIRA, E.H. Efeito do eCG ou benzoato de estradiol associado ao norgestomet na taxa de concepção de vacas de corte submetidas à IATF no pós-parto. **Brazilian Journal of Veterinary Research and Animal Science**, v. 46, n.3, p. 199-206, 2009.

SAKAMOTO, K.; EZASHI, T.; MIWA, K.; OKUDA-ASHITAKA, E.; HOUTANI, T.; SUGIMOTO, T.; ITO, S.; HAYAISHI, O. Molecular cloning and expression of cDNA of the bovine prostaglandin $F_{2\alpha}$ receptor. **The Journal of Biological Chemistry**, v.269, n.5, p.3881- 3886, 1994.

SAKAMOTO, K.; MIWA, K.; EZASHI, T.; OKUDA-ASHITAKA, E.; OKUDA, K.; HOUTANI, T.; SUGIOTO, T.; ITO, S.; HAYAISHI, O. Expression of mRNA encoding the prostaglandin $F_{2\alpha}$ receptor in bovine corpora lutea throughout the oestrous cycle and pregnancy. **Journal of Reproduction and Fertility**, v.103, p. 99-105, 1995.

SAKAMOTO, K.; ISHII, Y.; ONODERA, T.; SUGANO, T. Cloning and characterization of the novel isoforms for PGF 2 alpha receptor in the bovine corpus luteum. **DNA sequence**, v.13, n.5, p.307-311, 2002.

SALES, J.N.S.; CARVALHO, J.B.; CREPALDI, G.A; CIPRIANO, R.S.; JACOMINI, J.O.; MAIO, J.R.; SOUZA, J.C.; NOGUEIRA, G.P.; BARUSELLI, P.S. Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in *Bos indicus* cows submitted to a timed artificial insemination protocol. **Theriogenology**, v.78, n.3, p.510-516, 2012.

SAMADI, F.; PHILLIPS, N.J.; BLACHE, D.; MARTIN, G.B.; D'OCCHIO, M.J. Interrelationships of nutrition, metabolic hormones and resumption of ovulation in multiparous suckled beef cows on subtropical pasture. **Animal Reproduction Science**, v.137, n.3-4, p.137-144, 2013.

SCULLY, S.; MAILLO, V.; DUFFY, P.; KELLY, A.K.; CROWE, M.A.; RIZOS, D.; LONERGAN, P. The effect of lactation on postpartum uterine involution in Holstein dairy cows. **Reproduction in Domestic Animals**, v.48, n.6, p.888-892, 2013.

SCHNEIDER, A.; PFEIFER, L.F.M.; SCHIMITT, E.; BIANCHI, I.; VIEIRA, M.B.; XAVIER, E.G.; DEL PINTO, F.A.B.; CORRÊA, M.N. The use of insulin to improve fertility of timed-inseminated postpartum suckled beef cows. **Pesquisa Agropecuária Brasileira**, v.45, n.10, p.1219-1221, 2010.

SENGER, P.L. **Pathways to pregnancy and parturition**. 2.ed. Estados Unidos: Current Conceptions, 2003.

SHELDON, I.M.; WILLIAMS, E.J.; MILLER, A.N.; NASH, D.M.; HERATH, S. Uterine diseases in cattle after parturition. **The Veterinary Journal**, v.176, n.1, p.115–121, 2008.

SHELDON, I.M.; NOAKES, D.E.; RYCROFT, A.N.; DOBSON, H. The effect of intrauterine administration of estradiol on postpartum uterine involution in cattle. **Theriogenology**, v.59, n.5-6, p.1357–1371, 2003.

SHIRASUNA, K.; AKABANE, Y.; BEINDORFF, N.; NAGAI, K.; SASAKI, M.; SHIMIZU, T.; BOLLWEIN, H.; MEIDAN, R.; MIYAMOTO, A. Expression of prostaglandin $F_{2\alpha}$ receptor and its isoforms in the bovine corpus luteum during the estrous cycle and $PGF_{2\alpha}$ - induced luteolysis. **Domestic Animal Endocrinology**, v.43, n.3, p.227-238, 2012.

SHORT, R.E.; BELLOWES, R.A.; STAIGMILLER, R.B.; BERARDINELLI, J.G.; CUSTER, E.E. Physiological mechanisms controlling anestrus and infertility in

postpartum beef cattle. **Journal of Animal Science**, v.68, n.3, p.799-816, 1990.

SHRESTHA, H.T. BEG, M.O.; BURNETTE, R.R.; GINTHER, O.J. Plasma clearance and half-life of prostaglandin F2alpha: a comparison between mares and heifers. **Biology of Reproduction**, v.87, n.2, p.1-6, 2012.

SILVEIRA, A.P.; COSTA, M.Z.; FILHO, L.R.A.G.; CASTILHO, C. Efeito do período pós-parto sobre a taxa de prenhez de vacas de corte submetidas à IATF (inseminação artificial em tempo fixo). **Colloquium Agrariae**, v.6, n.2, p. 40-44, 2010.

STAGG, K.; SPICER, L.J.; SREENAN, J.M.; ROCHE, J.F.; DISKIN, M.G. Effect of calf isolation on follicular wave dynamics, gonadotropin and metabolic hormone changes, and interval to first ovulation in beef cows fed either of two energy levels postpartum. **Biology of Reproduction**, v.59, n.4, p.777-783, 1998.

TAKAGI, M.; YAMAMOTO, D.; OGAWA, S.; OTOI, T.; OHTANI, M.; MIYAMOTO, A. Messenger RNA expression of angiotensin-converting enzyme, endothelin, cyclooxygenase-2 and prostaglandin synthases in bovine placentomas during gestation and the postpartum period. **The Veterinary Journal**, v.177, p.398-4004, 2008.

TAUCK, S.A.; OLSEN, J.R.; WILKINSON, J.R.C.; BERARDINELLI, J.G. Duration of daily bull exposure on resumption of ovulatory activity in postpartum, primiparous, suckled, beef cows. **Animal Reproduction Science**, v.118, n.1, p.13–18, 2010.

TENHAGEN, B.A.; BIRKELBACH, E.; HEUWIESER, W. Serum progesterone levels in post-partum dairy cows after repeated application of prostaglandin F2α analogue D (+) cloprostenol sodium. **Journal of Veterinary Medicine Series A**, v.47, n.4, p.213-220, 2000.

TIAN, W.; NOAKES, D.E. Effects of four hormone treatments after calving on uterine and cervical involution and ovarian activity in cows. **Veterinary Record**, v.128, n.24, p.566-569, 1991.

TITHOF, P.K.; ROBERTS M.P.; GUAN, W.; GODKIN, J.D. Distinct phospholipase A2 enzymes regulate prostaglandin E2 and F2 α production by bovine endometrial epithelial cells. **Reproduction Biology and Endocrinology**, v.5, n.16, p.1-12, 2007.

TUCKER, A.L.; SANCHEZ, H.J.; TUCKER, W.B.; WILLIAMS, A.; FUQUAY, J.W.; WILLARD, S.T.; RYAN, P.L. Effects of early postpartum GnRH and prostaglandin F2 α administration on reproductive activity and ovulation synchronization in lactating dairy cows. **Journal of Animal and Veterinary Advances**. v.10, n.7, p.900-908, 2011.

VAZ, R.Z.; LOBATO, J.F.P.; RESTLE, J. Influence of weaning age on the reproductive efficiency of primiparous cow. **Revista Brasileira de Zootecnia**, v.39, n.2, p.299-307, 2010.

WEEMS, Y.S.; BRIDGES, P.J.; JEOUNG, M.; ARREGUIN-AREVALO, J.A.; NETT, T.M.; VANN, R.C.; FORD, S.P.; LEWIS, A.W, NEUENDORFF, D.A.; WELSH JR, T.H.; RANDEL, R.D.; WEEMS, C.W. *In vitro* intra-luteal implants of prostaglandin (PG) E1 or E2 (PGE1,, PGE2) prevent luteolysis in cows. II: mRNA for PGF2 α , EP1, EP2, EP3 (A-D), EP3A, EP3B, EP3C, EP3D, and EP4 prostanoid receptors in luteal tissue. **Prostaglandins and Other Lipid Mediators**, v. 97, p.60-65, 2012.

WEHBRINK, D.; HÄSSIG, M.; RITTER, N.; ZERBA, H.; BLEUL, U.; BOOS, A. Immunohistochemical demonstration of ciclooxigenase – 2 (COX-2) and prostaglandin receptors EP2 and FP expression in bovine intercaruncular uterine wall around term. **Animal Reproduction Science**, v.106, p.241-254, 2008.

WHEATON, J.E.; LAMB, G.C. Induction of cyclicity in postpartum anestrous beef cows using progesterone, GnRH and estradiol cypionate (ECP). **Animal Reproduction Science**, v.102, n.3-4, p.208–216, 2007.

WILLIAMS, G.L.; AMSTALDEN, M. Understanding postpartum anestrus and puberty in the beef female. Proceedings, Applied Reproductive Strategies in Beef Cattle. Disponível em: http://www.appliedreprostrategies.com/2010/January/pdfs/Gary_Williams.pdf.

Acesso em : 12 de agosto de 2013.

WILLIAM, E.J.; FISCHER, D.P.; PFEIFFER, D.U.; ENGLAND, G.C.; NOAKES, D.E.; DOBSON, H.; SHELDON, M. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. **Theriogenology**, v.63, n.1, p.102–117, 2005.

WILTBANK, M.C. Prevention and treatment of retained placenta. IN: Intermountain Nutrition Conference Dairy Nutritional Strategies to Meet Economic and Environmental Challenges, 10.; 2008, Salt Lake City. **Anais...** Logan : Utah State University 2008. p.81-92.

WISCHARAL, A.; VERRESCHI, I.T.N.; LIMA, S.B.; HAYASHI, L.F.; BARNABE, R.C. Pre-parturition profile of steroids and prostaglandin in cows with or without foetal membrane retention. **Animal Reproduction Science**, v. 67, p. 181-188, 2001.

YAVAS, Y.; WALTON, J.S. Induction of ovulation in postpartum suckled beef cows: a review. **Theriogenology**, v.54, n.1, p.1-23, 2000a.

YAVAS, Y.; WALTON, J.S. Postpartum acyclicity in suckled beef cows: a review. **Theriogenology**, v.54, n.1, p.25-55, 2000b.