UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS DE BOTUCATU

DOENÇAS INFLAMATÓRIAS EM VACAS LEITEIRAS: FATORES DE RISCO E ASSOCIAÇÕES COM MANUTENÇÃO DA GESTAÇÃO APÓS TRANSFERÊNCIA DE EMBRIÃO

INGRID NUNES FERREIRA EDELHOFF

Dissertação apresentada ao Programa de Pós-graduação em Zootecnia como parte dos requisitos para obtenção do título de Mestre em Zootecnia.

BOTUCATU – SP
Agosto 2021

UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS DE BOTUCATU

DOENÇAS INFLAMATÓRIAS EM VACAS LEITEIRAS: FATORES DE RISCO E ASSOCIAÇÕES COM MANUTENÇÃO DA GESTAÇÃO APÓS TRANSFERÊNCIA DE EMBRIÃO

INGRID NUNES FERREIRA EDELHOFF

Orientador: Prof. Dr. José Eduardo Portela Santos

Dissertação apresentada ao Programa de Pós-graduação em Zootecnia como parte dos requisitos para obtenção do título de Mestre em Zootecnia.

BOTUCATU – SP
Agosto 2021

FICHA CATALOGRAFICA ELABORADA PELA SECAO TECNICA DE AQUISICAO E TRATAMENTO DA INFORMACAO – SERVICO TECNICO DE BIBLIOTECA E DOCUMENTACAO – UNESO – FCA – LAGEADO – BOTUCATU (SP)

E21d Edelhoff, Ingrid Nunes Ferreira
Doenças inflamatórias em vacas leiteiras: fatores de risco e associações com manutenção da gestação após transferência de embrião / Ingrid Nunes Ferreira Edelhoff. -- Botucatu, 2021
76 p.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Medicina Veterinária e Zootecnia, Botucatu
Orientador: José Eduardo Portela Santos

1. Transferência de embriões. 2. Reprodução Animal. 3. Inflamação. I. Título.

"Permitida a cópia total ou parcial deste documento, desde que citada a fonte"

BIOGRAFIA DO AUTOR

Ingrid Nunes Ferreira Edelhoff, nascida em 21 de fevereiro de 1995, no município de Paraíba do Sul, estado do Rio de Janeiro, filha de Adolf Arno Edelhoff Filho e Denise Nunes Ferreira Edelhoff. No ano de 2013 mudou-se para a cidade de Valença no Estado do Rio de Janeiro onde iniciou seus estudos no curso de medicina veterinária no Centro Universitário de Valença (UNIFAA). Durante a graduação focou seus estudos e estágios na área de reprodução e produção de bovinos leiteiros. No ano de 2017, ao finalizar a graduação em medicina veterinária, mudou-se para Passos, no Estado de Minas Gerais, onde participou de pesquisas e coletas de dados na área de desenvolvimento de programas de transferências de embriões sob a orientação do Prof. Dr. José Eduardo Portela Santos, Prof. Dr. José Luiz Moraes Vasconcelos e Dr. Marcos Henrique Colombo Pereira. Em março de 2019 ingressou no programa de mestrado em zootecnia pela FMVZ-UNESP/Botucatu-SP, onde foi bolsista pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) sob a orientação do Prof. Dr. José Eduardo Portela Santos. Em julho de 2019 mudou-se para a cidade de Gainesville, no Estado da Flórida nos Estados Unidos, onde realizou suas análises de pesquisa no Department of Animal Sciences na University of Florida. Durante o mestrado participou de pesquisas nas áreas de saúde e nutrição de vacas de leite em período de transição.

DEDICATÓRIA

Aos meus pais e irmão, Adolf Arno Edelhoff Filho, Denise Nunes Ferreira Edelhoff e Adolf Arno Edelhoff Neto, pelo amor incondicional e por todo incentivo e esforços que fizeram e fazem para que eu consiga alcançar meus objetivos.

A toda minha família e amigos pelo carinho, amor e por toda ajuda e torcida para meu sucesso.

AGRADECIMENTOS

A Deus, por sempre estar presente em minha vida, me guardando, me dando sabedoria e sempre me mostrando os caminhos onde devo seguir.

Aos Professores Drs. José Eduardo Portela Santos e José Luiz Moraes Vasconcelos pela grande oportunidade e confiança que me foi dada ao ter participado de seus grupos de pesquisas, e pelos ensinamentos e cobranças durante o período de convivência que ajudaram para meu amadurecimento profissional e pessoal.

Ao proprietário da Fazenda Santa Luzia, Mauricio Silveira Coelho, pela oportunidade e confiança que me foi dada. Aos funcionários da fazenda Santa Luzia que foram essenciais para realização do presente estudo, em especial ao Marcos, Rafael e Roberto Júnior que me ajudaram diariamente durante toda coleta de dados.

Aos companheiros de pós-graduação e grupo de pesquisa a toda ajuda que recebi durante as análises laboratoriais do experimento. Por todo convívio, ensinamentos, companheirismo e momentos de distração: Achilles Vieira Neto, Ali Husnain, Anderson Kloster, Jéssica Prim, Karla Ferreira, Mariana Nehme Marinho, Roney Zimpel, Usman Arshad, Vitória Camargo e Victoria Rizzato.

Aos funcionários da seção de pós-graduação da UNESP – Botucatu, e em especial a Cláudia Cristina Moreci pela disposição e prontidão para ajudar.

A Phibro Animal Health e ao Conselho Nacional de Desenvolvimento Científico e Tecnologia (CNPQ) deixando claro meu agradecimento a todos os apoiadores e contribuintes da realização desse trabalho. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001

Muito obrigada a Todos!

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS

CAPÍTULO 1

AGNE	Ácidos graxos não esterificados
ATP	Adenosina trifosfato
BHB	β-hidroxibutirato
CMS	Consumo de matéria seca
CCS	Contagem de células somáticas
DA	Deslocamento de abomaso
DEL	Dias em leite
ECC	Escore de condição corporal
E. coli	*Escherichia coli*
IA	Inseminação artificial
Pp	Pontos percentuais
$PGF_{2\alpha}$	Prostaglandina $F_{2\alpha}$
RP	Retenção de placenta
TE	Transferência de embrião
TGI	Trato gastrointestinal
NK	Natural killers

CAPÍTULO 2

AI	Artificial insemination
BCS	Body condition score
BHB	β-hydroxybutyrate
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
E2	Estradiol
ET	Embryo transfer
LH	Luteinizing hormone
NUTD	Nonuterine diseases
P/ET	Pregnancy per embryo transfer
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PMN	Polymorphonuclear leukocytes
SCH	Subclinical hypocalcemia
tCa	Total Ca
TMR	Total mixed ration
UTD	Uterine diseases

SUMÁRIO

CAPÍTULO 1
CONSIDERAÇÕES INICIAIS

1. Introdução

As melhoras genéticas e de manejo de vacas leiteiras levaram a um constante aumento da produção de leite por vaca nos últimos 50 anos. Contudo, o aumento na produção de leite requer mais nutrientes que devem ser fornecidos pela dieta consumida ou originados de reservas corporais durante períodos de baixo consumo de matéria seca (CMS). Durante o pré-parto, há um declínio no CMS que ocorre em torno das últimas duas semanas de gestação, sendo mais pronunciado na última semana deste período (Hayirli et al., 2002). Por outro lado, após o parto, a vaca aumenta seu CMS na tentativa de compensar os nutrientes necessários para manutenção, atividade, lactogênese e lactopoiese. Porém, esse incremento no CMS não é capaz de suprir as demandas por nutrientes necessários durante as primeiras semanas da nova lactação, resultando em um período transitório de balanço negativo de nutrientes que resulta em aumento na mobilização do tecido adiposo e, consequentemente, levando ao aumento das concentrações de metabólitos lipídicos como ácidos graxos não esterificados (AGNE) e β-hidroxibutirato (BHB) no plasma (Drackley, 1999; Grummer et al., 2004; French, 2006). A diminuição do CMS e o aumento das concentrações de AGNE e BHB estão associados à disfunção imunológica tipicamente observada no final da gestação e primeiras semanas da lactação (Hammon et al., 2006; Moyes et al., 2009), levando assim a uma maior suscetibilidade a doenças e distúrbios durante o período pós-parto.

Doenças que afetam o trato uterino e a glândula mamária, e distúrbios metabólicos são os problemas de saúde mais comuns durante o início da lactação. Entre essas doenças ou distúrbios pode-se mencionar problemas ao parto como a distocia, retenção de placenta, metrite, cetose, mastite, distúrbios digestivos e manqueira (Ospina et al., 2010a). Estas doenças que afetam até 40 a 45% das vacas nas primeiras semanas de lactação causam não só reduções na produção de leite e aumento nos custos de produção, mas também reduzem o desempenho reprodutivo de vacas leiteiras o que, consequentemente, prejudica a sustentabilidade do rebanho (Santos et al., 2010; Hostens et al., 2012; Carvalho et al., 2019).

Ribeiro e Carvalho (2017) compilou informações de vários estudos e relataram que cerca de um terço das vacas leiteiras tem pelo menos 1 doença clínica (metrite, mastite, problema digestivo, problema respiratório ou manqueira) durante as primeiras 3 semanas de lactação. Vacas com diagnostico de pelo menos uma doença clínica pós parto tem fertilidade reduzida durante a primeira inseminação pós parto que ocorre aproximadamente 8 a 10 semanas após a resolução

clínica do problema de saúde (Santos et al., 2010; Ribeiro et al., 2013). A fertilidade pode diminuir ainda mais com o diagnóstico de várias doenças clínicas e (ou) subclínicas (Ribeiro et al., 2013; 2016), devido a um período anovulatório prolongado, redução da prenhez por inseminação e aumento do risco de perda de prenhez.

A inflamação que caracteriza condições como retenção de placenta, mastite e metrite produzem mediadores pró inflamatórios que afetam negativamente a função uterina, o crescimento folicular, a qualidade do oócito e o desenvolvimento embrionário (Santos et al., 2010; Bradford et al., 2015; Bromfield et al., 2015; Ribeiro et al., 2016). Muitos dos mecanismos subjacentes ao reconhecimento de patógenos microbianos pelo sistema imunológico inato em vertebrados já foram identificados e caracterizados. Esses mecanismos de imunidade inata não são, em grande parte, mediados por células imunes clássicas, como neutrófilos e macrófagos, mas estão evidentes também em células endometriais e ovarianas de mamíferos. Além de afetar o sistema imunológico e resposta inflamatória, micróbios ou moléculas associadas a patógenos interrompem a função endócrina no trato reprodutivo (Sheldon et al. 2002; Sheldom et al., 2009; Herath et al., 2006).

Portanto, uma melhor compreensão dos mecanismos pelos quais doenças e distúrbios do periparto afetam a reprodução bovina e da biologia durante o período de transição pode ajudar a propiciar soluções para reduzir o risco de tais doenças ou, então, minimizar seus impactos ocasionando assim uma transição mais saudável do final gestação para o início da lactação e, consequentemente, reduzir o impacto econômico na produção leiteira.

2. Revisão de Literatura

2.1 Imunidade durante o período de transição

O comprometimento na função imunológica durante o período de transição é um dos fatores de risco para doenças durante o início do pós-parto. Kimura et al. (2002) mostrou que vacas com retenção de placenta (RP) tiveram menor atividade das células natural killers (NK) e quimiotaxia de leucócitos do que vacas sem RP. Na mesma linha de pesquisa, Hammon et al. (2006) mostrou que vacas que eventualmente desenvolveram doença uterina como a metrite e endometrite tiveram menor CMS já antes do parto e comprometimento na função neutrofílica em comparação com vacas que não desenvolveram doença uterina.

Os neutrófilos são células de defesa responsáveis, em grande parte, por respostas inespecíficas também denominadas como imunidade inata. Sua função é afetada negativamente

durante o período de transição por várias razões como desequilíbrios metabólicos e estresse ambiental. Hammon et al. (2006) mostraram que a função neutrofílica diminui à medida que os AGNE plasmáticos aumentam. Além disso, Martinez et al. (2012) mostraram que a fagocitose de patógenos exercida por neutrófilos e sua habilidade de matar patógenos in vitro estava reduzida em vacas com hipocalcemia subclínica em comparação com vacas normocalcêmicas. De fato, indução de hipocalcemia em vacas secas reduziu a atividade neutrofílica in vitro mesmo 48 horas após o restabelecimento das concentrações sanguíneas de cálcio ionizável em vacas leiteiras (Martinez et al., 2014), portanto demonstrando a relação causa-efeito entre hipocalcemia subclínica e a função imune inata em bovinos. Além disso, vacas que desenvolveram metrite tinham neutrófilos com menos glicogênio do que vacas que não desenvolveram metrite (Galvão et al., 2010), portanto sugerindo que neutrófilos de vacas com doença uterina tem menor reserva energética para síntese de adenosina trifosfato (ATP), o que pode limitar sua função uma vez que ao serem ativadas e migrarem para tecidos, se tornam dependentes de glicogenólise e glicólise para síntese de ATP. Estresse ambiental, como o estresse térmico também tem um impacto negativo na função dos neutrófilos. Vacas que passaram por um período de estresse térmico durante o pré-parto tiveram neutrófilos com menor atividade de fagocitose em comparação com aquelas que receberam resfriamento evaporativo durante o pré-parto (do Amaral et al., 2011).

2.2 Problemas ao parto

Vacas com distocia, gêmeos ou natimortos são classificadas como tendo problemas ao parto. Meijering (1984) definiu a distocia como um parto difícil devido a um impedimento da passagem fetal pelo canal do parto, que exige assistência. Foi relatado que partos não assistidos duravam 45,2 ± 24,5 minutos, portanto, eventos de parto que duram mais de 70 minutos após a presença do saco amniótico podem ser considerados distócico (Schuenemann et al., 2011). Os Estados Unidos é um dos países com maior prevalência de distocia em gado leiteiro (22,6% em primíparas e 13,7% em primíparas e multíparas) em comparação com outros países (1,5% para 6,6%) (Mee, 2008). Natimorto é definido como o nascimento de um bezerro morto ou de um bezerro que morre entre 24h e 48h após o nascimento (Bicalho et al., 2007b). A incidência de gêmeos varia de 2,2 a 6,9% com média de 4,9% (Silva Del Río et al., 2007) e 3,3% de bezerros natimortos (Vergara et al., 2014).

Alguns estudos demonstraram a associação entre problemas ao parto e produção de leite (Dematawewa e Berger, 1997; Atashi et al. 2012). Vacas com distocia tiveram uma média de 135 kg de leite em 305 dias de lactação menor do que vacas sem distocia (Atashi et al. 2012). Além disso, foi relatado que vacas primíparas que tiveram gêmeos produziram 1,2 kg/d menos leite do que vacas primíparas que tiveram apenas um bezerro, enquanto vacas multíparas que tiveram gêmeos produziram 0,8 kg/d menor produção de leite do que multíparas que tiveram apenas bezerro único (Bicalho et al., 2007b). Outro ponto que também afeta a produção de leite são natimortos; vacas com bezerros natimortos produziram 544 kg menos leite em 305 dias de lactação comparadas com vacas que tiveram bezerros nascidos vivos (Mahnani et al., 2018).

Os problemas ao parto também afetam negativamente a fertilidade. O intervalo entre o parto e prenhez, também conhecido como período de serviço, e número de inseminações por concepção são afetados negativamente pela distocia. Dematawewa e Berger (1997) observaram que o parto gemelar aumentou o intervalo entre o parto e a prenhez em aproximadamente 45 dias e diminuiu o risco de prenhez em 22% comparado com vacas que pariram apenas um bezerro (Bicalho et al., 2007b). Natimortos aumentaram o período de serviço em aproximadamente 15 dias e o número de inseminações por prenhez em 0,22 (Mahnani et al., 2018).

2.3 Retenção de Placenta

A RP é definida como a falha na expulsão das membranas fetais nas horas seguintes ao parto. Normalmente, a expulsão das membranas fetais ocorre dentro de 12 horas após o parto (Attupuram et al., 2016). O período considerado para diagnosticar RP varia na literatura, e alguns pesquisadores consideram uma vaca com RP se a expulsão não ocorrer dentro das primeiras 12 horas, enquanto outros consideram 24 horas ou até mesmo 48 horas do parto (Fourichon et al., 2000). De maneira geral, muitos dos dados epidemiológicos sobre RP são coletados em fazendas onde o diagnóstico é feito apenas uma vez diariamente. Ou seja, se a vaca parir hoje, ela será observada para o diagnóstico de RP apenas amanhã. Isso faz com que o diagnóstico de RP da maior parte dos dados obtidos de rebanhos comerciais seja de um período de até 24 h.

A incidência de RP relatada na literatura é de cerca de 8,6% em vacas leiteiras (Kelton et al., 1998); no entanto, essa incidência pode variar entre os estudos devido a diferentes definições de RP e às características do rebanho e dos animais avaliados. A RP afeta tanto o desempenho produtivo, assim como o risco de outras doenças no início da lactação. Vacas que desenvolveram

RP por mais de 12 horas produziram 237 kg de leite a menos nos primeiros 100 dias em lactação (DEL) do que vacas que eliminaram a placenta antes das primeiras 12 h do parto (van Werven et al., 1992). Além disso, foi relatado que RP aumenta os dias para o primeiro serviço e com uma taxa de concepção 4 a 10% menor (Fourichon et al., 2000), aumenta o período de serviço em vacas primíparas em 10 dias e reduz a porcentagem de primíparas gestantes aos 150 DEL (Goshen e Shpigel, 2006).

A RP pode ser causada por vários fatores, como desequilíbrios hormonais durante o pré-parto, alterações celulares e um quadro inflamatório. De maneira geral, o ponto fundamental que faz com que as membranas fetais não sejam expelidas prontamente após o parto é a inabilidade do sistema imunológico materno reconhecer como estranho componentes fetais na placenta como haloantígenos paternos (Davis et al., 2004). Esta deficiência na alo-reatividade entre vaca e bezerro pode prejudicar a ativação do sistema imunológico materno e, portanto, atrasar o reconhecimento de alo antígenos presentes na placenta fetal resultando em RP. Quanto a resposta imune inata, foi demonstrado que vacas que desenvolveram RP tiveram menor atividade quimiotática de leucócitos em relação ao sobrenadante de cotilédone, menor concentração de quimiotractante (IL-8) no sangue, diminuindo assim a migração de neutrófilos para os locais de fixação da placenta e diminuição da fagocitose de neutrófilos no período entre 2 semanas antes e 2 semanas após o parto (Kimura et al., 2002).

A duração da gestação também é um fator de risco para RP. Vieira-Neto et al. (2017) mostraram que vacas com curto período de gestação, entre 256 e 269 dias, tiveram maior incidência de RP comparadas com seus pares que tiveram duração de gestação entre 270 e 282 dias (primíparas: 18,4 vs. 5,2%; multíparas: 35,5 vs. 5,1%). Vacas com gestação curta podem não ser capazes de completar o processo de maturação da placenta e as mudanças associadas na expressão de complexo de histocompatibilidade maior (MHC) nos tecidos fetais, permitindo assim o reconhecimento materno destes tecidos como estranho (Davis et al., 2004). A distocia é outro fator de risco importante para RP. A involução uterina tardia e danos causados pela tração mecânica do bezerro durante um parto distócico impede a liberação normal das membranas fetais. Além disso, hipocalcemia também está associada com RP (Melendez et al., 2004a), que pode ser explicado por uma menor fagocitose e função neutrofílica (Martinez et al., 2012; Martinez et al., 2014) dificultando a degradação da matriz extracelular para separação das membranas.

2.4 Metrite

Metrite é considerada quando uma vaca apresenta descarga vaginal de origem uterina com consistência aquosa e de cor avermelhada ou amarronzada com odor fétido e acompanhada de aumento uterino com flacidez e falta de tônus muscular à palpação transretal, muitas vezes acompanhada de sintomatologia sistêmica como febre, anorexia, entre outros sintomas (Chenault et al., 2004; Sheldon et al., 2006). Entretanto, a definição de metrite varia entre estudos porque difere no tempo e características da doença. Kelton et al. (1998) considerou uma vaca com metrite se ela teve uma condição pós-parto caracterizada por uma descarga cervical anormal, corrimento vaginal, ou ambos, ou conteúdo uterino. Enquanto Sheldon et al. (2006) propôs que vacas com um útero aumentado, com conteúdo aquoso fétido vermelho-acastanhado e aumento de temperatura recebe a definição de metrite puerperal, enquanto o termo, metrite deve ser usado para vacas com involução uterina retardada e secreção fétida na ausência de aumento de temperatura. De maneira geral, hoje existe um consenso de que é definida como descrita no início deste parágrafo, de acordo com a descrição inicial de Chenault et al. (2004) e de Sheldon et al. (2006). Vários pesquisadores usam uma escala de secreção uterina de 5 pontos que foi derivada inicialmente de Chenault et al. (2004), indo de 0 a 4, e adaptada por Williams et al. (2005), indo de 1 a 5, sendo: 1, não fétido, conteúdo viscoso, claro, vermelho ou marrom; 2, secreção mucoide turva com manchas de pus; 3, secreção mucopurulenta não fétida com <50% de pus; 4, secreção mucopurulenta não fétida com ≥50% de pus; 5, conteúdo fétido vermelho-acastanhado, secreção aquosa. Vacas com pontuação ≤4 são classificadas como não tendo metrite, sendo que as com escore 5 são classificadas como com metrite. Se acompanhada de sintomatologia sistêmica, como febre (temperatura ≥39,5 ° C), estão são classificadas como tendo metrite aguda ou puerperal. Mesmo assim, foi demonstrado que as vacas com febre e sem febre têm comunidades bacterianas semelhantes e não associadas a carga bacteriana total ou bactérias específicas (Jeon et al., 2016), apesar que a taxa de cura difere entre elas sendo inferior para vacas com sintomatologia sistêmica junto ao caso de metrite (Lima et al., 2014). Portanto, metrite é diagnosticada nas primeiras 2 a 3 semanas pós-parto quando uma vaca apresenta descarga vaginal com consistência aquosa e de cor avermelhada ou amarronzada com odor fétido e acompanhada de aumento uterino com flacidez e falta de tônus muscular à palpação transretal, acompanhada ou não de sintomatologia sistêmica como febre e anorexia. A incidência de metrite varia de 20 a 40% (Curtis et al., 1985; Markusfeld, 1987;) e ocorre no início da lactação, com ~ 90% dos casos diagnosticados nos primeiros 14 DEL e com pico em torno de

5 a 7 dias pós-parto (Galvão, 2011). Metrite está associada a menor produção de leite, diminuição da reprodução e aumento no risco de descarte ou por morte ou vendas. Vacas que desenvolveram metrite produziram 5 kg/d de leite a menos que vacas que não desenvolveram metrite (Huzzey et al., 2007; Daetz et al., 2016). Vacas com metrite apresentam redução na prenhez por inseminação artificial (IA) e têm menor probabilidade de estarem cíclicas no final do período de espera voluntário quando comparadas com vacas sem metrite. Metrite está associada com um aumento do intervalo entre parto e concepção em ~ 36,5 dias (Giuliodori et al., 2013; Ribeiro et al., 2013; Goshen e Shpigel, 2006).

Até recentemente, acreditava-se que o útero gravídico era estéril e que a metrite era causada pela colonização do lúmen uterino logo ao parto por *Escherichia coli* (*E. coli*), sendo este agente o ponto de partida para a entrada de outros patógenos envolvidos no desenvolvimento da metrite (Sheldon et al., 2009). No entanto, vacas que não desenvolvem metrite e as que eventualmente são diagnosticadas com metrite compartilham a maioria dos gêneros de bactérias de maneira similar logo no dia do parto, assim como no dia 6 pós-parto (Jeon et al., 2015). Ou seja, a microbiota uterina presente que antecede os casos de metrite não difere entre vacas saudáveis e àquelas que eventualmente apresentam doença uterina. Essa microbiota uterina é composta principalmente por *Bacteroidetes, Fusobacteria, Proteobacteria, Tenericutes* e *Firmicutes*, independentemente de infecção (Jeon et al., 2015) e a mudança no crescimento de bactérias de *Bacteroides, Porphyromonas e Fusobacterium* do dia 0 ao dia 3 está associado com o desenvolvimento de metrite (Jeon et al., 2015). No caso de *E. coli*, a abundância relativa que foi encontrada no útero de vacas com metrite foi baixa (> 1%) (Jeon et al., 2015; Jeon et al., 2016), e sua presença muitas vezes é associada ao microbioma de vacas saudáveis (Jeon et al., 2016). Acreditava-se que a via de contaminação uterina era apenas pela vagina devido à contaminação por fezes (Sheldon et al., 2009). No entanto, a microbiota uterina é associada a bactérias que estão presentes na vagina, fezes e sangue (Jeon et al., 2017), portanto, sugerindo que as bactérias também podem chegar ao útero pela via hematógena, talvez originárias do trato gastrointestinal durante períodos de bacteremia durante a gestação. De qualquer maneira, após o parto, o ambiente uterino é alterado devido a abertura da cérvix, expulsão do feto, presença de remanescente de tecidos e sangue, lesões de mucosa, todos os quais favorecem a colonização e o crescimento bacteriano, principalmente de anaeróbios. Uma involução uterina tardia, interrupção do fluxo sanguíneo através da placenta, baixa tensão de oxigênio, necrose do epitélio caruncular e o acúmulo de sangue e fluido alantóico

são condições em que as bactérias anaeróbias podem prosperar, alterando assim o lóquio uterino para uma secreção purulenta e fétida.

Entre os fatores de risco de metrite, pode-se citar distocia, natimorto, gêmeos, RP, estação ao parto, duração da gestação e paridade. As chances de ter metrite em vacas com distocia, natimortos e gêmeos foram 4,32, 6,26 e 6,57 vezes maiores em comparação com vacas que não tiveram essas condições (Hossein-Zadeh, 2011). Vacas com parto anormal, ou seja, aquelas com distocia, natimortos e/ou gêmeos têm atraso na involução uterina em comparação com vacas com partos considerados normais (Fonseca et al., 1983). Após a integridade do epitélio do tecido uterino estar comprometida, as barreiras mecânicas, como o muco, têm a produção reduzida, e as bactérias têm fácil acesso ao tecido e podem causar infecção. A chance de ter metrite foi 27,74 vezes maior em vacas que desenvolvem RP em comparação com vacas que não desenvolveram RP (Hossein-Zadeh, 2011). O parto na estação fria também está associado à metrite. Hossein-Zadeh (2011) mostrou que vacas que pariram no inverno teve 2,45 maior chance de ter metrite quando comparado com as que pariram na primavera. Vacas com gestação curta tiveram maior incidência de metrite em comparação com vacas com uma gestação média (Vieira-Neto et al., 2017). A paridade é outro fator de risco para metrite. Vacas primíparas têm 1,68 maior chance de ter metrite do que vacas multíparas (Hossein-Zadeh, 2011).

Além destes, a hipocalcemia subclínica está associada à metrite. Vacas com hipocalcemia subclínica apresentaram risco 3,24 vezes maior de desenvolver metrite, e as mesmas apresentaram 11,5 vezes maior risco de desenvolver metrite puerperal (Martinez et al., 2012). Baixas concentrações de cálcio no sangue estão associadas ao comprometimento imunológico (Martinez et al., 2012; 2014), e causam baixa contratilidade (Al-Eknah e Noakes. 1989) do útero, portanto interrompe o processo de eliminação das bactérias. Além disso, Hammon et al. (2006) e Martinez et al. (2012) observaram que vacas com atenuação na imunidade inata, como neutrófilos com fagocitose prejudicada, tiveram maior risco de desenvolver metrite e outras doenças uterinas, portanto sugerindo que um sistema imunológico prejudicado aumenta o risco de metrite.

2.5 Cetose

Cetose ou hipercetonemia é uma condição caracterizada por concentrações elevadas de AGNE, BHB e corpos cetônicos, como ácido acetoacético e acetona nos tecidos e fluidos corporais. Mensuração de BHB no sangue ou soro é o método padrão para o diagnóstico da

hipercetonemia já que BHB é o componente originário de cetogênese mais predominante no sangue entre os corpos cetônicos. Estudos observacionais estabeleceram limites de AGNE e BHB para identificar vacas com alto risco de doenças ou de redução em produção de leite, portanto, estabelecendo os pontos de corte para hipercetonemia (Ospina et al. 2010a; Ospina et al. 2010b). Hipercetonemia ou a cetose pode ser clínica ou subclínica. A cetose subclínica é definida como uma vaca tendo uma concentração sanguínea de BHB ≥1,2 mmol/L, mas sem sinais clínicos, enquanto vacas com cetose apresentam sinais clínicos e estão associadas a concentrações de BHB maiores, normalmente acima de 3,0 mmol/L (Oetzel, 2004).

A incidência de cetose varia devido a diferentes definições entre os estudos (McArt et al., 2013b). No entanto, usando um limite de BHB ≥1,2 mmol/L McArt et al. (2012) relataram uma incidência de hipercetonemia de 44% com um pico de incidência no 5º dia pós-parto. Foi relatado que a hipercetonemia está associada com menor produção de leite e aumento do descarte. A associação entre produção de leite e hipercetonemia varia entre os estudos, sendo que alguns pesquisadores mostraram que vacas com hipercetonemia produziram mais leite, enquanto outros relataram menor produção de leite nas primeiras semanas pós-parto (McArt et al., 2013b). Chapinal et al. (2012) mostraram que vacas com de BHB ≥0,60 mmol/L durante a última semana pré-parto produziram menos 1,7 kg/d de leite em comparação com vacas com menores concentrações de BHB, enquanto vacas multíparas que tiveram AGNE ≥0,5 mmol/L produziram menos 1,6 kg/d de leite em comparação com vacas multíparas com menor concentrações de AGNE no sangue. Além disso, Chapinal et al. (2012) mostraram que hipercetonemia durante as primeiras 2 semanas pós-parto também foi associada a menor produção de leite. Mcart et al. (2012) relataram que para cada aumento de 0,1 mmol/L nas concentrações de BHB, no primeiro teste positivo, a produção de leite diminui cerca de 0,5 kg/dia. Outros estudos relataram uma associação entre hipercetonemia e maior produção de leite e outros menor produção de leite apenas em vacas multíparas e maior produção de leite em vacas primíparas (Ospina et al 2010a; McArt et al., 2013b).

O aumento da pontuação de escore de condição corporal (ECC) durante o período seco e paridade são os principais preditores de cetose. McArt et al. (2013a) mostrou que vacas com alto ECC durante o período seco tiveram maiores concentrações de BHB do dia 3 ao 6 pós-parto e tiveram um risco 1,2 maior de serem diagnosticadas com BHB ≥1,2 mmol/L em comparação com vacas que tiveram baixo ECC durante o período seco.

2.6 Mastite

A mastite é definida como uma inflamação da glândula mamária que normalmente é causada por agentes bacterianos. Mastite clínica é diagnosticada quando a vaca apresenta alterações visuais no leite como presença de coágulos, flocos, ou um leite aquoso. Essas alterações podem estar presentes em um ou mais quartos que podem ou não ser acompanhados por sinais de inflamação do tecido do úbere como calor, inchaço ou descoloração da pele (Kelton et al., 1998). Normalmente, vacas com tecido mamário saudável, sem presença de patógenos no leite, tem contagem de células somáticas (CCS) inferior a 200.000 células/mL. Portanto, o uso de CCS é padrão para identificar vacas com alteração no leite, sendo que a mastite subclínica está associada a concentrações de células somáticas acima de 200.000/mL e os casos clínicos com CCS em milhões por mL de leite (Schepers et al., 1997).

A mastite clínica tem um grande impacto econômico no rebanho leiteiro. Santos et al. (2004) mostraram que o momento da ocorrência do primeiro diagnóstico clínico de mastite teve impactos diferentes na produção de leite. Eles mostraram que vacas que desenvolveram mastite clínica antes da primeira inseminação artificial (IA) pós-parto tiveram uma redução de 2,2 kg/d de leite em comparação com vacas que não desenvolveram mastite, enquanto vacas diagnosticadas entre a primeira IA pós-parto e o diagnóstico de prenhez tiveram redução de 1,4 kg/d de leite na lactação. No entanto, se o primeiro caso clínico de mastite foi diagnosticado depois que a vaca estava prenhe a produção de leite não apresentou diferença estatística quando comparada com vacas que não desenvolveram mastite. A razão destas diferenças são basicamente o momento na lactação quando o primeiro caso foi diagnosticado. Quanto mais cedo ele ocorrer, maior o impacto em produção, em parte devido a afetar o tecido mamário nos períodos de maior produção, mas também por haver mais tempo para que ocorra recidivas na mesma lactação.

A mastite também prejudica a reprodução. Santos et al. (2004) mostrou que a mastite diagnosticada antes da primeira IA teve menor porcentagem de concepção na primeira IA, menor taxa de prenhez, maior incidência de aborto e maior número de dias em aberto em comparação com vacas que não desenvolveram mastite.

A mastite pode ser contagiosa ou ambiental. Na mastite contagiosa, a transmissão ocorre do quarto infectado para outro quarto saudável na mesma vaca ou em vacas diferentes por meio de um fômite, como equipamento de ordenha ou mãos contaminadas. Na mastite ambiental, os

microrganismos, presentes no meio ambiente, tem acesso à glândula mamária normalmente logo antes ou após a ordenha, sendo que a transmissão pode ocorrer a partir de fezes, material da cama, ou fômites (Smith, 2002). *Staphylococcus aureus, E. coli, Enterobacter spp. Streptococcus dysgalactiae, Trueperella pyogenes* e *Pseudomonas spp.* são identificados como os patógenos com maior incidência nos casos de mastite clínica (Levison et al., 2016).

Paridade, estação ao parto e conformação do teto estão entre os fatores de risco para mastite clínica. À medida que as vacas envelhecem, o úbere torna-se mais penduloso e há alteração na conformação do teto, presença de hiperqueratose do epitélio, o que dificulta a higienização, mudanças que os colocam em maior risco de ter mastite. Estação de ano pode influenciar o risco de mastite, principalmente devido a concentração de chuvas e a mudanças de temperatura. Onde chove nos meses de inverno, o risco de mastite aumenta. Em locais onde há aumento e umidade associados com os meses quentes, então este período se torna de maior risco. Vacas com ligamento acessório de úbere solto e baixa altura de úbere aumentaram as chances de diagnóstico de mastite clínica em 3,7 e 2,8, respectivamente, em comparação com vacas que tinham forte ligamento do úbere e um ponto de altura de úbere intermediário (Miles et al., 2019). Desequilíbrios metabólicos, como cetose subclínica também aumentam a chance de desenvolver mastite. Raboisson et al. (2014) mostrou que vacas com cetose subclínica tiveram 1,64 vezes mais chance de desenvolver quadro clínico de mastite em comparação com vacas sem cetose subclínica. Hammon et al. (2006) mostrou que vacas com função imunológica prejudicada por concentrações alteradas de AGNE, pode colocar as vacas em risco de doenças infecciosas, não sendo capazes de eliminar o patógeno adequadamente.

2.7 Problemas digestivos

Os distúrbios digestivos podem incluir vacas com deslocamento de abomaso (DA) e indigestão. O DA é definido como o movimento do quarto compartimento do estômago para uma posição anormal do lado direito ou esquerdo do abdômen, detectado por ausculta de som de “pin” com percussão digital. A indigestão foi anteriormente definida como fezes escassas e falta de apetite com estase ruminal e intestinal (Stangaferro et al., 2016a). Casos como diarreia, inchaço, constipação, compactação e dilatação cecal também podem ser julgados como distúrbios digestivos que afetam o trato gastrintestinal (TGI)

A incidência de distúrbios digestivos, definidos como vacas com diarreia, diminuição da

motilidade ruminal, inchaço ou DA foi relatado como 9,4% (Vercouteren et al., 2015), enquanto DA foi relatado com uma incidência de 5% (Gröhn et al., 1998). Distúrbios digestivos afetam a produção de leite, reprodução e abate na fazenda leiteira. Vacas que desenvolvem acidose, gases, baixa ingestão de alimento ou inchaço tiveram menor produção de leite em comparação com vacas saudáveis, mas vacas com DA tiveram menor produção de leite em comparação com as saudáveis e vacas com outras indigestões (Edwards e Tozer. 2004). Além disso, Van Winden et al. (2003) descobriram que vacas com DA produziram menos 6,5 kg / d de leite em comparação com vacas saudáveis. Os distúrbios digestivos também prejudicam a reprodução. Vacas que desenvolveram diarreia, diminuição da motilidade ruminal, inchaço ou DA tiveram menor porcentagem de vacas cíclicas em 21 dias pós-parto (Vercouteren et al., 2015). Além disso, Ribeiro et al. (2013) mostraram que vacas que desenvolveram diarreia, inchaço ou DA tinham menos chances (OR = 0,19) de retomada da ciclicidade no dia 49 e redução da prenhez por IA no dia 60 após a primeira IA.

Há literatura limitada avaliando fatores de risco de distúrbios digestivos em vacas leiteiras. A condição que foi mais investigada foi DA. No entanto, foi demonstrado que o distúrbio digestivo está correlacionado com distúrbios metabólicos (Vercouteren et al., 2015). Da mesma forma, DA é associado a distúrbios metabólicos. Vacas com aumento no pré-parto de AGNE e BHB e baixo cálcio teve maior risco de DA (Ospina et al., 2010b; Chapinal et al., 2011; Rodriguez et al., 2017). Ospina et al. (2010b) mostraram que os limiares críticos de AGNE durante o pré-parto e pós-parto para prever DA foram 0,27 e 0,72 mEq / L, respectivamente, enquanto para BHB no pós-parto foi de 10 mg / dL. No entanto, o mecanismo de AGNE e BHB para causar DA não é bem compreendido. Um possível mecanismo pode ser porque as vacas estão usando principalmente AGNE como combustível, e há depressão do CMS em vacas com cetose (Goldhawk et al., 2009), assim o vazio do abomaso é preenchido com gás, levando ao seu deslocamento.

Outros fatores que estão associados a um risco aumentado de ter DA são elevados ECC, estação ao parto e paridade (Cameron et al. 1998). Contudo, o mecanismo da maioria desses fatores de risco para causar DA não são completamente compreendidos. Cameron et al (1998) sugeriu que inverno e verão são fatores de risco para DA por causa do estresse durante o verão que irá diminuir o CMS e maior demanda de energia durante o inverno, que pode aumentar AGNE e BHB. No caso do ECC, conforme mencionado anteriormente, vacas super condicionadas têm maior perda de peso durante o período de transição aumentando assim os metabólitos de AGNE e BHB. No caso de

paridade, o risco de DA foi maior em vacas multíparas em comparação com vacas primíparas, e isso pode estar relacionado ao fato de vacas multíparas serem mais propensas a ter cetose, portanto, aumentando AGNE e BHB no sangue.

2.8 Manqueira

Pontuações de locomoção geralmente são usadas para indicar a gravidade da claudicação e essas pontuações podem ser medidas manual ou automaticamente. A maioria das pontuações nos sistemas manuais utilizados pelos pesquisadores são baseados na marcha assimétrica, relutância em suportar peso e costas arqueadas, e geralmente é pontuado em uma escala de cinco níveis. Enquanto as medidas automáticas são baseadas na cinética de movimento e posturas, padrões de comportamento e variáveis de produção que podem indicar claudicação (Schlageter-Tello et al., 2014). No entanto, Bicalho et al., (2007a) avaliaram um sistema de pontuação visual com um sistema automatizado de pontuação de locomoção e mostrou que o sistema de pontuação visual feito por veterinários treinados teve melhor desempenho do que o sistema de pontuação automatizado.

A incidência de claudicação foi relatada como 23%, mas aumentando em paridade crescente (Bicalho et al., 2008). A claudicação está associada à perda de leite, piora no desempenho reprodutivo e abate. Depois de controlar a produção de leite durante as primeiras 3 semanas pós-parto Bicalho et al. (2008) mostraram que vacas que desenvolveram claudicação produziram menos 1,5 kg / d de leite em comparação com vacas sem claudicação. Por outro lado, Melendez et al. (2003) relataram que vacas com claudicação tiveram 25 pontos percentuais (pp) a menos para taxa de concepção no primeiro serviço, 7 pp menor taxa de prenhez e 14 pp maior para cistos ovarianos em comparação com vacas sem claudicação, e eles relataram que vacas com claudicação tinham um menor risco (OR = 0,43) de ficar gestante em comparação com vacas sem claudicação.

A claudicação pode ser infecciosa ou não infecciosa. Entre claudicação infecciosa, pode ser mencionadas dermatite digital, necrobacilose interdigital e dermatite interdigital e entre as não infecciosas estão as lesões como úlceras de sola e doença da linha branca. Os fatores de risco para claudicação não infecciosa são ECC e paridade. Baixo ECC tem maior risco de ter claudicação não infecciosa, isso pode ser devido à associação com a espessura do coxim digital (Machado et al., 2010). Como vacas perdem peso há uma perda na espessura da almofada digital levando a lesões. Por outro lado, vacas multíparas têm maior risco de apresentar claudicação não infecciosas

(Machado et al., 2010). Uma possível explicação é o enfraquecimento e diminuição da elasticidade do tecido conjuntivo. A acidose ruminal subaguda é outro fator de risco para claudicação não infecciosa. Bicalho et al. (2013) discutem a hipótese de que as toxinas liberadas do rúmen degradam as fibras de colágeno no casco, permitindo que a falange distal se mova livremente dentro da cápsula causando concussões do tecido mole e lesões. Os fatores de risco para dermatite digital incluem alojamento, estágio de lactação, conformação de casco, entre outros. Acesso a pasto, pisos limpos e texturizados estão entre as características de habitação que estão associadas a menor risco de dermatite digital.

Referências

Al-Eknah, M. M., and D. E. Noakes. 1989. **A preliminary study on the effect of induced hypocalcaemia and nifedipine on uterine activity in the parturient cow.** J. Vet Pharmacol. Ther. 12:237-239.

Atashi, H., A. Abdolmohammadi, M. Dadpasand, and A. Asaadi. 2012. **Prevalence, risk factors and consequent effect of dystocia in Holstein dairy cows in Iran.** Asian Australas. J. Anim. Sci. 25:447-451.

Attupuram, N. M., A. Kumaresan, K. Narayanan, and H. Kumar. 2016. **Cellular and molecular mechanisms involved in placental separation in the bovine: A review.** Mol. Reprod. Dev. 83:287-297.

Bicalho, R. C., S. H. Cheong, K. N. Galvão, L. D. Warnick, and C. L. Guard. 2007b. **Effect of twin birth calvings on milk production, reproductive performance, and survival of lactating cows.** J. Am. Vet. Med. Assoc. 231:1390-1397.

Bicalho, R. C., L. D. Warnick, and C. L. Guard. 2008. **Strategies to analyze milk losses caused by diseases with potential incidence throughout the lactation: A lameness example.** J. Dairy Sci. 91:2653-2661.

Bicalho, R. C. and G. Oikonomou. 2013. **Control and prevention of lameness associated with claw lesions in dairy cows.** Livest. Sci. 156:69-105.

Bicalho, R. C., S. H. Cheong, G. Cramer, and C. L. Guard. 2007a. **Association between a visual and an automated locomotion score in lactating Holstein cows.** J. Dairy Sci. 90:3294-3300.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. L. Carpenter. 2015. **Invited review: Inflammation during the transition to lactation: New adventures with an old flame.** J. Dairy Sci. 98:6631-6650.

Bromfield, J. J., J. E. P. Santos, J. Block, R. S. Williams, and I. M. Sheldon. 2015. **PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Uterine infection: Linking infection and innate immunity with infertility in the high-producing dairy cow.** J. Anim. Sci. 93:2021-2033.

Cameron, R. E., P. B. Dyk, T. H. Herdt, J. B. Kaneene, R. Miller, H. F. Bucholtz, J. S. Liesman, M. J. Vandehaar, and R. S. Emery. 1998. **Dry cow diet, management, and energy balance as risk factors for displaced abomasum in high producing dairy herds.** J. Dairy Sci. 81:132-139.

Carvalho, M. R., F. Peñagaricano, J. E. P. Santos, T. DeVries, B. McBride, and E. S. Ribeiro. 2019. **Long-term impact of clinical disease postpartum on milk production, reproduction, and culling of dairy cows.** J. Dairy Sci. 102:11701–11717.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. Santos, and S. J. LeBlanc. 2011. **The association of serum metabolites with clinical disease during the transition period.** J. Dairy Sci. 94:4897-4903.

Chapinal, N., S. J. Leblanc, M. E. Carson, K. E. Leslie, S. Godden, M. Capel, J. E. Santos, M. W. Overton, and T. F. Duffield. 2012. **Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance.** J. Dairy Sci. 95:5676-5682.

Chenault, J. R., J. F. McAllister, S. T. Chester Jr, K. J. Dame, F. M. Kausche, E. J. Robb. 2004. **Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows**. J. Am. Vet. Med. Assoc. 224:1634–1639.

Curtis, C.R., H.N. Erb, C.J. Sniffen, R.D. Smith, and D.S. Kronfeld. 1985. **Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows.** J. Dairy Sci. 68:2347-2360.

Daetz, R., F. Cunha, J. H. Bittar, C. A. Risco, F. Magalhaes, Y. Maeda, J. E. P. Santos, K. C. Jeong, R. F. Cooke, and K. N. Galvão. 2016. **Clinical response after chitosan microparticle administration and preliminary assessment of efficacy in preventing metritis in lactating dairy cows.** J. Dairy Sci. 99:8946-8955.

Davies, C. J., J. R. Hill, J. L. Edwards, F. N. Schrick, P. J. Fisher, J. A. Eldridge, and D. H. Schlafer. 2004. **Major histocompatibility antigen expression on the bovine placenta: its relationship to abnormal pregnancies and retained placenta.** Anim. Reprod. Sci. 83:267-280

Dematawewa, C. M. B. and P. J. Berger. 1997. **Effect of dystocia on yield, fertility, and cow losses and an economic evaluation of dystocia ccores for Holsteins**. J. Dairy Sci. 80:754-761.

do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. Bubolz, and G. E. Dahl. 2011. **Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows.** J. Dairy Sci. 94:86-96.

Drackley, J. K. **1999. Biology of dairy cows during the transition period: The final frontier?** J. Dairy Sci. 82:2259-2273.

Edwards, J. L., and P. R. Tozer. 2004. **Using activity and milk yield as predictors of fresh cow disorders.** J. Dairy Sci. 87:524-531.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. **Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open.** J. Dairy Sci. 66:1128-1147.

Fourichon, C., H. Seegers, and X. Malher. 2000. **Effect of disease on reproduction in the dairy cow: a meta-analysis.** Theriogenology 53:1729-1759.

French, P. D. 2006. **Dry matter intake and blood parameters of nonlactating Holstein and Jersey cows in late gestation.** J. Dairy Sci. 89:1057-1061.

Galvão, K. N., M. J. B. F. Flaminio, S. B. Brittin, R. Sper, M. Fraga, L. Caixeta, A. Ricci, C. L. Guard, W. R. Butler, and R. O. Gilbert. 2010. **Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows.** J. Dairy Sci. 93:2926-2937.

Galvão, K. N. 2011. **Identifying and treating uterine disease in dairy cows.** Proceedings 47th Florida Dairy Production Conference, Gainesville, March 30, 2011.

Giuliodori, M. J., R. P. Magnasco, D. Becu-Villalobos, I. M. Lacau-Mengido, C. A. Risco, and R. de la Sota. 2013. **Metritis in dairy cows: Risk factors and reproductive performance**. J. Dairy Sci. 96:3621-3631.

Goldhawk, C., N. Chapinal, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. **Prepartum feeding behavior is an early indicator of subclinical ketosis.** J. Dairy Sci. 92:4971-4977.

Goshen, T., and N.Y. Shpigel. 2006. **Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows.** Theriogenology 66:2210-2218.

Gröhn, Y. T., S. W. Eicker, V. Ducrocq, and J. A. Hertl. 1998. **Effect of diseases on the culling of Holstein dairy cows in New York State.** J. Dairy Sci. 81:966-978.

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. **Dry matter intake and energy balance in the transition period.** Vet. Clin. North Am. Food Anim. Pract. 20:447- 470.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. **Neutrophil function and energy status in Holstein cows with uterine health disorders.** Vet. Immunol. Immunopathol. 113: 21-29.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. **Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins.** J. Dairy Sci. 85:3430-3443.

Herath, S., D. P. Fischer, D. Werling, E. J. Williams, S. T. Lilly, H. Dobson, C. E. Bryant, I. M. Sheldon. 2006**. Expressão e função do receptor toll-like 4 nas células endometrial do útero.** Endocrinol. 147:562-570.

Hossein-Zadeh, N. G. and Ardalan, M. 2011. **Cow-specific risk factors for retained placenta, metritis and clinical mastitis in Holstein cows.** Vet. Research Com. 35:345-354.

Hostens, M., J. Ehrlich, B. Van Ranst, and G. Opsomer. 2012. **On-farm evaluation of the effect of metabolic diseases on the shape of the lactation curve in dairy cows through the MilkBot lactation model.** 95:2988-3007.

Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2007. **Prepartum behavior and dry matter intake identify dairy cows at risk for metritis.** J. Dairy Sci. 90:3220-3233.

Jeon, S. J., F. Cunha, X. Ma, N. Martinez, A. Vieira-Neto, R. Daetz, R. C. Bicalho, S. Lima, J. E. P. Santos, K. C. Jeong, and K. N. Galvão. 2016. **Uterine microbiota and immune parameters associated with fever in dairy cows with metritis.** PLoS One. 11:e0165740.

Jeon, S. J., F. Cunha, A. Vieira-Neto, R. C. Bicalho, S. Lima, M. L. Bicalho, and K. N. Galvão 2017. **Blood as a route of transmission of uterine pathogens from the gut to the uterus in cows.** Microbiome. 5:109.

Jeon, S. J., A. Vieira-Neto, M. Gobikrushanth, R. Daetz, R. D. Mingoti, A. C. Parize, S. L. de Freitas, A. N. da Costa, R. C. Bicalho, S. Lima, K. C. Jeong, and K. N. Galvão. 2015. **Uterine microbiota progression from calving until establishment of metritis in dairy cows.** Appl. Environ. Microbiol. 81:6324–6332.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. **Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle.** J. Dairy Sci. 81:2502-2509.

Kimura, K., J. P. Goff, M. E. J. Kehrli, and T. A. Reinhardt. 2002. **Decreased neutrophil function as a cause of retained placenta in dairy cattle.** J. Dairy Sci. 85:544-550.

Levison, L. J., E. K. Miller-Cushon, A. L. Tucker, R. Bergeron, K. E. Leslie, H. W. Barkema, and T. J. DeVries. 2016. **Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms.** J. Dairy Sci. 99:1341-1350.

Lima, F.S., A. Vieira-Neto, G.S.F.M. Vasconcellos, R.D. Mingoti, E. Karakaya, E. Solé, R.S. Bisinotto, N. Martinez, C.A. Risco, K.N. Galvão, and J.E.P. Santos. 2014. **Efficacy of ampicillin trihydrate or ceftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows**. J. Dairy Sci. 97: 5401-5414.

Machado, V. S., L. S. Caixeta, J. A. McArt, and R. C. Bicalho. 2010. **The effect of claw horn disruption lesions and body condition score at dry-off on survivability, reproductive performance, and milk production in the subsequent lactation.** J. Dairy Sci. 93:4071-4078.

Mahnani, A., A. Sadeghi-Sefidmazgi, and H. Keshavarzi. 2018. **Performance and financial consequences of stillbirth in Holstein dairy cattle.** Animal. 12:617-623

Markusfeld, O. 1987. **Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits**. J. Dairy Sci. 70:158- 166.

Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. N. Galvão, and J. E. P. Santos. 2012. **Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease.** J. Dairy Sci. 95:7158-7172.

Martinez, N., C.A. Risco, L.D.P. Sinedino, R.S. Bisinotto, E.S. Ribeiro, G.C. Gomes, F.S. Lima, L.F. Greco, D. Taylor-Rodriguez, J.P. Driver, W.W. Thatcher, and J.E.P. Santos. 2014. **Effect of induced subclinical hypocalcemia on physiological responses and function of immune cells in dairy cows.** J. Dairy Sci. 97:874-887.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012. **Epidemiology of subclinical ketosis in early lactation dairy cattle.** J. Dairy Sci. 95:5056-5066.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2013a. **Dry period and parturient predictors of early lactation hyperketonemia in dairy cattle.** J. Dairy Sci. 96:198- 209.

McArt, J. A., D. V. Nydam, G. R. Oetzel, T. R. Overton, and P. A. Ospina. 2013b. **Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance.** Vet. J. 198:560-570.

Mee, J. F. 2008. **Prevalence and risk factors for dystocia in dairy cattle: A review.** Vet J. 176:93-101.

Meijering, A. 1984. **Dystocia and stillbirth in cattle - A review of causes, relations and implications.** Livest. Prod. Sci. 11:143-177.

Melendez, P., J. Bartolome, L. F. Archbald, and A. Donovan. 2003. **The association between lameness, ovarian cysts and fertility in lactating dairy cows.** Theriogenology. 59:927-937.

Melendez, P., A. Donovan, C. Risco, and J. P. Goff. 2004a. **Plasma mineral and energy metabolite concentrations in dairy cows fed an anionic prepartum diet that did or did not have retained fetal membranes after parturition.** Am. J. Vet. Res., 65:1071-1076.

Miles, A. M., J. A. A. McArt, F. A. Leal Yepes, C. R. Stambuk, P. D. Virkler, and H. J. Huson. 2019. **Udder and teat conformational risk factors for elevated somatic cell count and clinical mastitis in New York Holsteins.** Prev. Vet. Med.163:7-13.

Moyes, K. M., J. K. Drackley, J. L. Salak-Johnson, D. E. Morin, J. C. Hope, and J. J. Loor. 2009. **Dietary-induced negative energy balance has minimal effects on innate immunity during a *Streptococcus uberis* mastitis challenge in dairy cows during midlactation.** J. Dairy Sci. 92:4301-4316.

Oetzel, G. R. 2004. **Monitoring and testing dairy herds for metabolic disease.** Veterinary Clinics of North America: Food Animal Practice. 20:651-674.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. **Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level.** J. Dairy Sci. 93:3595-3601.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. **Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: critical thresholds for prediction of clinical diseases.** J. Dairy Sci. 93:546-554.

Raboisson, D., M. Mounié, and E. Maigné. 2014. **Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review.** J. Dairy Sci. 97:7547-7563.

Ribeiro, E. S., W. R. Carvalho. 2017. **Impact and mechanisms of inflammatory diseases on embryonic development and fertility in cattle.** Anim. Reprod. 14: 589-600.

Ribeiro, E. S., G. Gomes, L. F. Greco, R. L. A. Cerri, A. Vieira-Neto, P. L. J. Monteiro Jr., F. S. Lima, R. S. Bisinotto, W. W. Thatcher, and J. E. P. Santos. 2016. **Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows.** J. Dairy Sci. 99:2201-2220.

Ribeiro, E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W. Thatcher, and J. E. P. Santos. 2013. **Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates.** J. Dairy Sci. 96:5682-5697.

Rodríguez, E.M., A. Arís, and A. Bach. 2017. **Associations between subclinical hypocalcemia and postparturient diseases in dairy cows.** J. Dairy Sci. 100:7427- 7434.

Santos, J. E. P., R. L. A. Cerri, M. A. Ballou, G. E. Higginbotham, and J. H. Kirk. 2004. **Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows.** Anim. Reprod. Sci. 80:31-45.

Santos, J. E. P., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thatcher. 2010. **Applying nutrition and physiology to improve reproduction in dairy cattle.** Soc. Reprod. Fertil. Suppl. 67:385–403.

Schepers, A.J., T.J. Lam, Y.H. Schukken, J.B. Wilmink, and W.J. Hanekamp. 1997. **Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters.** J. Dairy Sci.80:1833-1840.

Schlageter-Tello, A., E.A. Bokkers, P.W. Koerkamp, T. Van Hertem, S. Viazzi, C.E. Romanini, I. Halachmi, C. Bahr, D. Berckmans, and K. Lokhorst. 2014. **Manual and automatic locomotion scoring systems in dairy cows: a review.** Prev. Vet. Med. 116:12-25.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvão, and J. Workman. 2011. **Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows.** J. Dairy Sci. 94:5494-5501.

Sheldon, I.M., G.S. Lewis, S. LeBlanc, and R.O. Gilbert. 2006. **Defining postpartum uterine disease in cattle.** Theriogenology 65:1516-1530.

Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, H. Dobson. 2002. **Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle.** Reproduction 123:837-845.

Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio, and H. J. Schuberth. 2009. **Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle.** Biol. Reprod. 81:1025-1032.

Silva Del Río, N., S. Stewart, P. Rapnicki, Y. M. Chang, and P. M. Fricke. 2007. **An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle.** J. Dairy Sci. 90:1255-1264.

Smith, B. P. 2002. **Large Animal Internal Medicine.** Third ed. Mosby, Inc., St. Louis, Missouri.

Stangaferro, M. L., R. Wijma, L. S. Caixeta, M. A. Al-Abri, and J. O. Giordano. 2016a. **Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part I. Metabolic and digestive disorders.** J. Dairy Sci. 99:7395-7410.

Van Werven, T., Y. H. Schukken, J. Lloyd, A. Brand, H. Tj. Heeringa, M. Shea. 1992. **The effects of duration of retained placenta on reproduction, milk production, postpartum disease and culling rate.** Theriogenology 37:1191-1203.

Van Winden, S. C. L., R. Jorritsma, K. E. Muller, and J. P. T. M. Noordhuizen. 2003. **Feed intake, milk yield, and metabolic parameters prior to left displaced abomasum in dairy cows.** J. Dairy Sci.. 86:1465-1471.

Vercouteren, M. M., J. H. Bittar, P. J. Pinedo, C. A. Risco, J. E. P. Santos, A. Vieira-Neto, and K. N. Galvão. 2015. **Factors associated with early cyclicity in postpartum dairy cows.** J. Dairy Sci. 98:229-239.

Vergara, C. F., D. Dopfer, N. B. Cook, K. V. Nordlund, J. A. A. McArt, D. V. Nydam, and G. R. Oetzel. 2014. **Risk factors for postpartum problems in dairy cows: Explanatory and predictive modeling.** J. Dairy Sci. 97:4127-4140.

Vieira-Neto, A., K. N. Galvão, W. W. Thatcher, and J. E. P. Santos. 2017. **Association among gestation length and health, production, and reproduction in Holstein cows and implications for their offspring.** J. Dairy Sci. 100:3166-3181.

Williams, E. J., D. P. Fischer, G. C. W. England, H. Dobson, D. U. Pfeiffer, and I. M. Sheldon. 2005. **Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the inflammatory response to endometritis in cattle.** Theriogenology 63:102-117.

CAPÍTULO 2

Inflammatory diseases in dairy cows: risk factors and associations with pregnancy after embryo transfer

This article was published in the Journal of Dairy Science

ABSTRACT

The objectives of the present prospective cohort study were to identify risk factors for inflammatory diseases in Holstein-Gyr crossbred dairy cows and characterize the associations of those diseases with pregnancy per embryo transfer (ET). Diseases were diagnosed in the first 60 d postpartum in 252 primiparous and 481 multiparous cows. Uterine diseases (UTD) included retained placenta, metritis, clinical endometritis, and subclinical endometritis. Nonuterine diseases (NUTD) included mastitis, lameness, pneumonia, and displaced abomasum. Blood was sampled on d 0, 1, and 2 postpartum and analyzed for concentrations of haptoglobin, fatty acids, total Ca (tCa), P, and Mg, and again on d 8 postpartum and analyzed for concentration of β-hydroxybutyrate. The association between concentrations of metabolites in serum and inflammatory diseases was determined. Cows received a timed ET program starting 28 ± 3 d postpartum with first ET at 46 ± 3 d postpartum using fresh in vitro-produced embryos. Pregnancy was diagnosed on d 31 and 59 of presumptive gestation. Overall, 63.3% of the cows were diagnosed with UTD and 20.6% with NUTD. The risk factors for UTD included season of calving, parity group, calving problems, days with subclinical hypocalcemia, and serum concentrations of haptoglobin and Mg, whereas the risk factors for NUTD were parity group and serum Mg concentration. Cows that developed UTD had increased concentrations of haptoglobin on d 2 and fatty acids on d 1 and 2, and reduced concentrations of tCa on d 1 and 2 and of P and Mg on d 2 postpartum compared with cows without UTD. Cows that developed NUTD had increased concentrations of fatty acids on d 0 to 2 postpartum, and decreased concentrations of tCa and P on d 0 and 1, and of Mg on d 1 and 2 postpartum compared with cows without NUTD. Cows that developed NUTD had a 340-kg reduction in milk yield in the first 60 d postpartum. Inflammatory diseases were associated with lesser body condition score and increased loss of body condition in the first 70 d postpartum. Maintenance of pregnancy after ET was reduced in UTD cows following the first (41.7 vs. 25.4%) or all ET (46.4 vs. 36.2%), whereas maintenance of pregnancy was reduced in NUTD cows only at the second ET (39.0 vs 25.9%). The reduced pregnancy maintenance in UTD cows combined with a reduced 21-d service rate (61.9 vs. 54.8%) decreased the 21-d cycle pregnancy rate (28.6 vs. 19.9%) and the hazard of pregnancy to 300 d postpartum by 35%, resulting in an extra 32 d open. In conclusion, inflammatory diseases depressed fertility

in dairy cows receiving ET, with the greatest impact observed in UTD cows. This suggests that local inflammation of the uterus impairs maintenance of pregnancy in dairy cows following ET.

Key words: dairy cow, embryo transfer, inflammation, reproduction

1. INTRODUCTION

Calving and the onset of lactation are events that often result in inflammation in dairy cows (Bradford et al., 2015). Calving causes trauma, which increases the risk of uterine diseases (Vieira-Neto et al., 2016). Approximately 30 to 45% of postpartum cows develop some type of clinical disease in the first 30 to 60 d in lactation (Santos et al., 2010; Ribeiro et al., 2016; Carvalho et al., 2019), and most postpartum diseases are associated with reduced fertility (Santos et al., 2010; Ribeiro et al., 2016; Carvalho et al., 2019). Uterine diseases (UTD) are accompanied by signs of local or systemic illness (Sheldon et al., 2009). Similarly, mastitis, a nonuterine disease (NUTD) often diagnosed in early lactation, can lead to systemic signs of illness (Wenz et al., 2001). These early lactation diseases are associated with events that take place before or immediately after calving, and to some extent, they have common links such as negative nutrient balance and alterations in measures of immune function (Banos et al., 2013).

A common aspect of UTD and NUTD is sickness behavior with reduced DMI and the concurrent inflammatory response (Sheldon et al., 2009; Pérez-Báez et al., 2019). Pathogens and the resulting inflammatory response cause release of molecular patterns that stimulate residing immune cells to release cytokines (Sheldon et al., 2009; Bromfield et al., 2015), which, in turn, upregulate hepatic secretion of positive acute phase proteins (Cray et al., 2009). Furthermore, reduced DMI increases lipomobilization and reduces nutrient balance. Thus, it is not surprising that altered concentrations of biomarkers in blood have been associated with the risk of postpartum diseases in dairy cows (Huzzey et al., 2009; Chapinal et al., 2012; Martinez et al., 2012).

Diseases and concurrent inflammation affect reproduction in part because of disrupted endocrine signaling and perturbations in follicle or oocyte development (Sheldon et al., 2009; Bromfield et al., 2015). Cows that develop diseases in early lactation are more likely to have an extended anovulatory period (Santos et al., 2010). Uterine inflammation transiently reduces oocyte competence to develop to the morula stage embryo (Dickson et al., 2020). Also, inflammation creates a hostile uterine environment that prevents proper conceptus development (Ribeiro et al., 2016), thereby resulting in increased pregnancy loss (Santos et al., 2010). It is interesting that conceptuses from cows that had inflammatory diseases express molecular signatures compatible with inflammation (Ribeiro et al., 2016), suggesting shifts in the transcriptome that might

compromise conceptus survival. These molecular changes in reproductive tissues are observed even months after induced uterine inflammation (Horlock et al., 2020).

One method to circumvent the impacts of disease on fertility is the use of embryo transfer (ET). Also, synchronizing ovulation should minimize the impact of diseases mediated by anovulation. Therefore, timed ET allows for the evaluation of the association between diseases and maintenance of pregnancy, independent of early events needed to establish pregnancy in dairy cows. Inflammatory diseases affect pregnancy in dairy cattle receiving AI, although the underlying mechanisms are complex and multifaceted. Less is known about the impact of inflammatory diseases on maintenance of pregnancy following ET (Ribeiro et al., 2016; Barbosa et al., 2018; Estrada-Cortés et al., 2019). Segregating diseases into those that affect the uterus or other tissues may provide insight on potential underlying mechanisms of inflammation-associated subfertility that are directly linked to the reproductive tract or with systemic effects of inflammation.

It was hypothesized that periparturient events and plasma metabolites are risk factors for inflammatory diseases and that UTD and NUTD affect maintenance of pregnancy in an additive manner in crossbred dairy cows. Therefore, the objectives of the present study were to identify risk factors for inflammatory diseases in Holstein-Gyr crossbred dairy cows and characterize the association of those diseases with pregnancy per ET (P/ET) in the first 300 d postpartum.

2. MATERIALS AND METHODS

All procedures involving cows in this experiment were approved by the São Paulo State University regulatory animal research system committee (protocol number: CEUA-0060/2019) and followed the recommendations of the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (accessed at https://www.adsa.org/Portals/_default/SiteContent/ docs/AgGuide3rd/Chapter07.pdf).

2.1 Cows, Housing, and Management

The study was conducted on a commercial dairy farm milking approximately 1,600 cows in the state of Minas Gerais, Brazil, at a latitude of 20.72° South and longitude of 46.61° West. The farm was visited daily and weekly cohorts of cows were enrolled from August 2018 to January 2019. A total of 733 crossbred Holstein-Gyr dairy cows, 252 primiparous and 481 multiparous,

were enrolled in the study on the day of calving. Cows calving in the months of August and September were considered to have calved in the cool season (daily mean temperature 25°C).

All cows were either 50% Holstein and 50% Gyr (76 primiparous and 163 multiparous) or 75% Holstein and 25% Gyr (176 primiparous and 318 multiparous) derived from in vitro-produced embryos using in vivo-collected oocytes from purebred Gyr or 50% Holstein and 50% Gyr donors and fertilized with Holstein X-sorted semen from North American genetics.

Cows were housed in a compost barn during the last 3 wk of gestation and in another compost barn during the first 74.8 ± 13.1 d postpartum (ranged from 48 to 171 d, median 72 d). For the remainder of lactation, cows were on irrigated pastures of perennial Bermudagrass "Tifton 85" (*Cynodon spp.*). Prepartum and early postpartum cows housed indoors received diets as TMR (Table 1), whereas once on pasture, cows received the same grain mix used in the TMR that was fed at a rate of 1 kg per 2.7 kg of milk produced during each milking. The amount of grain mix fed to cows on pasture was adjusted twice monthly and pastures were managed in a rotational grazing system to have at least 15 kg of DM forage available per cow daily.

2.2 Characterization and Diagnosis of Health Problems and Survival

The hour of calving was recorded, and cows were observed for shedding of the placenta twice daily, at 12-h intervals. Cows that had not shed the placenta by 24 h after calving were considered to have retained fetal membranes. Cows that required assistance at calving were considered to have dystocia. The number of calves born and whether the calf was born alive or died immediately after birth was recorded. Cows with dystocia, stillbirth, or twins were considered to have calving problems. The hour of calving was categorized into 4 periods of 6 h each, from 0001 to 0600 h, 0601 to 1200 h, 1201 to 1800 h, and 1801 to 0000 h to characterize the distribution of calving's throughout the day.

Cows were evaluated for metritis on d 8 ± 3 postpartum by scoring the vaginal mucus using a vaginal mucus collection device (Metricheck, Simcro, New Zealand) concurrent with evaluation of rectal temperature and transrectal palpation. Metritis was characterized by fetid watery red-brown vaginal discharge (Sheldon et al., 2009), and cows with metritis and fever, based on rectal temperature >39.5°C, were considered to have puerperal metritis. Clinical endometritis was evaluated on d 21 ± 3 and 28 ± 3 postpartum by transrectal ultrasonography of the uterus to identify the presence of uterine content. Cows with visible uterine content had vaginal mucus scored using

the Metricheck device. Cows with echogenic uterine content or those with vaginal discharge score >2, on a 0 to 5 scale, were considered to have clinical endometritis (McDougall et al., 2007). The cut-point of vaginal discharge score to classify cows as having clinical endometritis was based on the depression in pregnancy rate observed by McDougall et al. (2007). Subclinical endometritis was evaluated on d 28 ± 3 postpartum by endometrial cytology using the cytobrush technique (Lima et al., 2013). A total of 200 cells were counted on the cytological slide and the proportion of PMN was determined. Subclinical endometritis was characterized when PMN constituted >9% of cells on the cytological slide (Galvão et al., 2009).

From parturition until 60 DIM, mastitis, lameness, digestive, and respiratory problems were evaluated for all cows. All cows were examined for signs of clinical mastitis by the herd personnel immediately before each milking by stripping each quarter. Clinical mastitis was characterized by the presence of abnormal milk or by signs of inflammation in one or more quarters. Upon diagnosis of mastitis, a milk sample was collected aseptically from the affected quarter into a vial, which was identified and stored under refrigeration. Once daily, milk samples were cultured using a combination of media (blood agar, Edwards medium, and MacConkey) followed by Gram staining and biochemical assays for identification of the bacterial agent. Digestive problems included diarrhea, bloat, or displacement of abomasum. Respiratory problems were characterized as increased respiratory frequency associated with fever and detection of lung sounds such as wheezing, rhonchi, or crackling sounds at auscultation. Cows were scored twice for lameness (Sprecher et al., 1997), once on the day of calving and again at 28 ± 3 DIM, and those that stood and walked with an arched back and had short strides in one or more legs (lameness score >2) were classified as clinically lame. Except for clinical mastitis and calving problems, all other diseases were diagnosed by veterinarians in the research team during daily visits to the farm for data collection.

Cows were considered to have had inflammatory diseases if they were diagnosed with those that affected the uterus (UTD), which included retained fetal membranes, metritis, clinical endometritis, or subclinical endometritis; or those that affected other tissues defined as nonuterine diseases (NUTD), which included mastitis, displaced abomasum, respiratory disease, and lameness. Because cows could develop multiple diseases, many of those that had UTD also developed NUTD and vice versa. The DIM when a cow had the diagnosis of the first and the last

disease event in the first 60 postpartum were recorded. Cows that left the herd because they died or were culled were recorded for the first 300 DIM.

2.3 Blood Sampling and Analyses

Blood was sampled from all cows immediately after calving and again on d 1 and 2 postpartum. A third sample was collected once weekly on d 8 ± 3 postpartum. On d 0, 1, and 2, blood was collected by puncture of the coccygeal vessels into evacuated tubes with no additives (Vacutainer systems, Becton Dickinson, Franklin Lakes, NJ). Blood tubes were maintained at ambient temperature for 30 min for clotting and then placed in ice and transported to the laboratory within 3 h of collection. Tubes were centrifuged at 2,000 × g for 15 min for serum separation. Serum samples were frozen at −20°C and later analyzed for concentrations of haptoglobin, fatty acids, total Ca (tCa), Mg, and P. Blood collected on d 8 ± 3 was sampled from the coccygeal vessels and analyzed for concentrations of BHB in whole blood using a handheld meter for whole blood (KetoVet, ECO Diagnóstica, Nova Lima, MG, Brazil).

Concentrations of haptoglobin were assayed in serum by the colorimetric method based on peroxidase activity (Cooke and Arthington, 2013) and serum concentrations of fatty acids using a commercial kit (NEFA-C kit, Wako Diagnostics Inc., Richmond, VA) following the modifications by Johnson and Peters (1993). Concentrations of tCa and Mg were determined by atomic absorption spectrophotometer (AAnalyst 200, PerkinElmer Inc., Waltham, MA) according to Martinez et al. (2012). Concentrations of P were quantified using the molybdenum blue method (Quinlan and DeSesa, 1955). Control serum samples with predetermined large and small concentrations of the analytes were used to evaluate intra- and interassay coefficients of variation. The average intra- and interassay coefficients of variation were, respectively, 3.2 and 8.1% for haptoglobin, 5.5 and 4.0% for fatty acids, 1.2 and 1.4% for tCa, 1.0 and 2.3% for Mg, and 3.7 and 4.3% for P. Cows were considered to have subclinical hypocalcemia when serum tCa <2.00 Mm (Reinhardt et al., 2011).

2.4 Body Condition Score and Milk Yield

Cows were scored for body condition using a 1 to 5 scale with increments of 0.25 units as depicted in the Elanco BCS chart (Elanco Animal Health, 2009) at calving, and at 28 ± 3 and 70 ± 3 d postpartum by the same person. Cows were milked twice daily, at 12-h intervals, and daily

milk yield for the first 60 d postpartum was recorded for individual cows using milk meters (Flow indicator FI7, DeLaval Ltda., São Paulo, Brazil).

2.5 Reproductive Management

All breedings in the first 300 DIM were performed using timed ET. Embryos were produced in a commercial laboratory (In Vitro Brazil, Uberaba, MG, Brazil) with oocytes from purebred Gyr or crossbred 50% Gyr and 50% Holstein donor cows and X-sorted Holstein semen. Grade 1 morulae, early blastocysts, blastocysts, and expanded blastocysts were shipped fresh to the farm once a week and transferred on the same day.

The voluntary waiting period was 46 ± 3 DIM. Starting at 28 ± 3 DIM, cows had their estrous cycle and ovulation synchronized with a protocol as follows: d 0, i.m. injection of 2.0 mg of estradiol benzoate (Gonadiol; 1.0 mg/mL of estradiol benzoate; Zoetis, São Paulo, Brazil) and placement of an intravaginal progesterone insert (controlled internal drug release, CIDR-B, 1.9 g of progesterone, Zoetis); d 7, i.m. injections of 25 mg of PGF2α (Lutalyse; 5.0 mg/mL of dinoprost as tromethamine salt; Zoetis) and 300 IU of equine chorionic gonadotropin (Novormon; 200 IU/mL of equine chorionic gonadotropin; Zoetis); d 9, removal of the progesterone insert and i.m. injections of 12.5 mg of PGF2α and 1.0 mg of estradiol cypionate (2.0 mg/mL of estradiol cypionate; Zoetis). Two days later, on d 11 of the estrous synchronization protocol, it was anticipated to be d 0 of a new estrous cycle. All treatments were performed in the morning. On d 7 after presumptive estrus (i.e., d 18 of after starting the synchronization protocol), the ovaries of cows were scanned by ultrasonography using a 7.5-MHz linear array (Aloka SSD-500, Aloka, Tokyo, Japan) and cows with a visible corpus luteum were eligible to receive ET. Cows eligible to receive ET received an injection of 0.1 mg of GnRH (Fertagyl, 0.1 mg/mL of gonadorelin; MSD Animal Health, São Paulo, Brazil) concurrent with ET performed by a single experienced veterinarian.

2.6 Pregnancy Diagnosis and Resynchronization for Embryo Transfer

All cows were evaluated for pregnancy on d 31 of presumptive gestation (i.e., 24 d after ET). Diagnosis was performed using transrectal ultrasonography of the uterus and its contents and characterized by visualization of a live embryo with a heartbeat. Cows diagnosed as pregnant on d 31 were reexamined by transrectal ultrasonography 4 wk later, on d 59 of presumptive

pregnancy. Pregnancy per ET was calculated as the number of pregnant cows on d 31 or 59 of gestation divided by the total number of cows that received ET. Pregnancy loss was calculated as the number of cows that lost a pregnancy between gestation d 31 and 59 divided by the number of pregnant cows on d 31. Cows diagnosed as nonpregnant on d 31 or 59 had their estrous cycle resynchronized for timed ET using exactly the same timed protocol described for the first ET.

Pregnancy per ET on d 31 and 59, and pregnancy loss were calculated for the first 2 postpartum ET for each cow. Pregnancy per ET for all ET performed in the first 300 DIM was calculated only based on diagnosis performed on d 59 of presumptive pregnancy.

2.7 Calculations of 21-Cycle Service Rate, 21-d Cycle Pregnancy Rate, and Days Open

Service rate was calculated assuming that every eligible cow (those past the voluntary waiting period of 43 DIM and nonpregnant) should receive a breeding every 21 d. Cows became noneligible for subsequent breeding if they were diagnosed pregnant on d 59 of gestation, became "do not breed," left the herd, or completed 300 DIM, whichever happened first.

Days open was calculated for every cow as the interval from calving to pregnancy based on the diagnosis performed on d 59 of gestation. Cows that became "do not breed," left the herd without a pregnancy diagnosis, or remained nonpregnant by 300 DIM were censored using the date of whichever happened first.

2.8 Study Design and Statistical Analyses

The study design was a prospective cohort study. A sample size was calculated using the POWER procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC) based on the assumption that 50% of the cows would develop at least one inflammatory disease postpartum (Ribeiro et al., 2013, 2016) and that cows with inflammatory diseases would have at least an 11 percentage point decrease in P/ET. The mean P/ET of the farm in the preceding year was 40%. Therefore, it was assumed that cows without inflammatory diseases would have a P/ET of 45% and those with inflammatory diseases the P/ET would be 34%. The sample size, calculated using a likelihood ratio chi-squared test for 2 proportions with $\alpha = 0.05$ and $\beta = 0.20$, resulted in a total of 620 cows (310 per group). Because of potential attrition caused by diseases, a minimum sample size of 700 cows was deemed necessary for the study.

Binary data were analyzed by logistic regression using generalized linear mixed models with the GLIMMIX procedure of SAS (SAS/STAT, SAS Institute Inc.) fitting a binary distribution. Continuous data were analyzed by linear mixed models using the MIXED procedure of SAS (SAS/STAT, SAS Institute Inc.). Time to an event was analyzed by the Cox's hazard regression method using the PHREG procedure of SAS (SAS/ STAT, SAS Institute Inc.).

Risk factors for UTD and NUTD were investigated using univariable analyses. Factors considered were season of calving (cool vs. hot), lactation group (1 vs. 2 vs. >2 lactations), breed (50/50 Holstein-Gyr vs. 75/25 Holstein-Gyr), calving problems (no vs. yes), sex of the calf (female vs. male), BCS at calving, the greatest concentration of haptoglobin in serum on d 0 to 2 postpartum, the greatest concentration of fatty acids in serum on d 0 to 2 postpartum, blood BHB concentration on d 8 postpartum, days with subclinical hypocalcemia (0 vs. 1 vs. >1), the smallest concentration of Mg in serum on d 0 to 2 postpartum, and the smallest concentration of P in serum on d 0 to 2 postpartum. Variables associated with the response based on $P \leq 0.10$ were considered for inclusion in the multivariable models. The multivariable models included all explanatory variables as fixed effects that met the significance at $P \leq 0.10$ and a backward stepwise elimination procedure was applied, and at each step, the explanatory variable with largest P-value was removed if $P > 0.10$. At each step, model fit was assessed using the Akaike's information criterion. Therefore, the final multivariable models included only fixed effects with $P \leq 0.10$. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the F-tests in the multivariable statistical models.

To evaluate the association between inflammatory diseases and fertility of cows receiving ET, data were analyzed with multivariable logistic regression models with diseases classified as UTD or NUTD. For responses with a single measurement per cow, the models included the fixed effects of UTD, NUTD, and the interaction between UTD and NUTD. For responses with more than one measurement per cow such as 21-d cycle service rate, 21-d cycle pregnancy rate, and P/ ET for all ET, the models also included the fixed effects of replicate (21-d interval or ET number), the interaction between UTD and replicate, and the interaction between NUTD and replicate, and the random effect of cow nested within UTD and NUTD group. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the F-tests in the statistical models.

Milk yield, BCS, and concentrations of analytes in blood were analyzed with mixed models with the fixed effects of UTD, NUTD, the interaction between UTD and NUTD, time (day or week), the interactions between UTD and time, NUTD and time, and UTD and NUTD and time, and the random effect of cow nested within UTD and NUTD group. Time, day or week, was the factor in the REPEATED statement and the covariance structure that resulted in the best fit assessed based on the smallest Akaike's information criterion was selected. The first-order autoregressive structure was the most common covariance structure applied. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the F-tests in the statistical models. In all models for continuous data, the distribution of residuals and homogeneity of variance were evaluated after fitting the statistical models. Data that violated the assumptions of normality were subjected to transformation selected by a macro in SAS (SAS/STAT; SAS Institute Inc.) using the Box-Cox power transformation before statistical analyses (Piepho, 2009). The least squares means and standard error of the means of transformed data were back-transformed for data presentation according to Jørgensen and Pedersen (1998).

Time to event such as days open or days to leaving the herd were analyzed with the Cox's hazard regression method with models that included the fixed effects of UTD, NUTD, and the interaction between UTD and NUTD. If the interaction between UTD and NUTD resulted in $P > 0.10$, it was then removed from the final model. Proportionality of the hazards was assessed using the ASSESS PH and RESAMPLE functions in the PHREG procedure of SAS to perform graphical and numerical evaluations of the Martingale residuals and compute the P-value of the Kolmogorov-type supremum test (Lin et al., 1993). Differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered tendencies.

3. RESULTS

3.1 Risk Factors and Prevalence of Clinical and Subclinical Diseases

Uterine diseases affected 63.3% and NUTD affected 20.6% of the cows in the study. The prevalence of individual UTD and NUTD according to parity group is depicted in Table 2. The most prevalent UTD was subclinical endometritis, followed by clinical endometritis, then metritis and retained placenta. Because subclinical endometritis was included as a UTD, the proportion of endometrial PMN in cows without UTD was smaller ($P < 0.001$) than in those with UTD (3.0 ±

1.7 vs. 25.9 ± 1.0%). No difference (P = 0.80) in the proportion of endometrial PMN was observed between cows without or with NUTD (14.7 ± 0.8 vs. 14.2 ± 1.8%). Of the 733 cows, 26.2% (n = 192) were diagnosed with multiple UTD. The diagnosis of the first and last disease event in cows that developed UTD had, respectively, a mean (±SD) of 16.0 ± 11.0 and 28.6 ± 10.7 and a median of 19 and 27 d postpartum. Approximately 35% of primiparous and multiparous cows calved between 0001 and 0600 h and only 10% of cows calved between 1801 and 0000 h (Table 2).

The diagnosis of the first and last disease event in cows that developed NUTD had, respectively, a mean (±SD) of 15.7 ± 14.4 and 35.4 ± 16.1 and a median of 16.0 and 32.0 d postpartum. The most prevalent NUTD was mastitis with only a small proportion of cows diagnosed with other NUTD (Table 2). Of the 733 cows, only 1.9% (n = 14) were diagnosed with multiple NUTD in the first 60 d postpartum. Of the 118 cows diagnosed with mastitis, 80 had a single case, 30 had 2 cases, 6 had 3 cases, and 2 cows had 4 cases, resulting in a total of 166 cases of clinical mastitis. Of the 166 cases of mastitis, bacterial cultures included 33 cases of Streptococcus agalactiae, 13 cases of S. uberis, 7 cases of S. dysgalactiae, 2 cases of Staphylococcus coagulase negative, 2 cases of Corynebacterium sp., 1 cases of Bacillus sp., 9 cases of other gram-positive bacteria, 15 cases of coliform bacteria, 1 case of yeast, 2 contaminated samples with colonies of multiple microorganisms, and 81 samples with no growth.

Numerous risk factors were identified for UTD in the univariable analyses (Table 3), including season of calving, parity >2, breed, calving problems, days with subclinical hypocalcemia >1, blood concentration of BHB, and serum concentrations of haptoglobin, Mg, and P. After the multivariable analyses (Table 3), the risk factors that remained significant for UTD were season of calving, parity group >2, calving problems, days with subclinical hypocalcemia >1, and serum concentrations of haptoglobin and Mg.

Risk factors were identified for NUTD in the univariable analysis (Table 4), including parity group, days of subclinical hypocalcemia, and serum concentrations of fatty acids, Mg, and P; however, following multivariable analyses (Table 4) only parity group and serum concentration of Mg remained significant risk factors for NUTD.

3.2 Impacts of Inflammatory Diseases on Reproduction

Of the 733 cows enrolled, 675 cows (92.1%) received at least 1 ET (Table 5). A greater (P = 0.02) proportion of cows with UTD received ET than cows without UTD. Conversely, a smaller

($P < 0.001$) proportion of cows with NUTD received ET than those without NUTD. Furthermore, of the 733 cows enrolled, 686 (93.6%) were enrolled in the ovulation synchronization protocol at least once. For the first synchronization postpartum, 73.8% of the cows had a synchronized ovulation based on the presence of a corpus luteum on the day of ET. Both UTD ($P = 0.02$) and NUTD ($P = 0.03$) reduced the proportion of cows with a synchronized ovulation for ET, and UTD extended the interval from calving to first ET by 6 d (Table 5).

Maintenance of pregnancy following ET on d 59 of gestation was reduced ($P \leq 0.01$) in UTD cows for the first (41.7 vs. 25.4%) or all ET (46.4 vs. 36.2%) compared with cows without UTD. Maintenance of pregnancy on d 59 was reduced ($P = 0.02$) in NUTD at the second ET (39.0 vs. 25.9%; Table 5) compared with cows without NUTD. The reduced P/ET observed for cows with UTD combined with the reduced ($P = 0.007$) 21-d service rate (61.9 vs. 54.8%) resulted in smaller ($P < 0.001$) 21-d cycle pregnancy rate (28.6 vs. 19.9%) and a 35% decrease in the hazard of pregnancy, culminating in an extra 32 d open (Table 6, Figure 1A). Nonuterine diseases did not affect the rate of pregnancy or days open (Tables 5 and 6, Figure 1B).

3.3 Impacts of Inflammatory Diseases on Milk Yield, Body Condition, and Survival

Inflammatory diseases were associated with altered milk yield. Cows diagnosed with UTD produced 94.5 kg more ($P = 0.03$) milk in the first 60 DIM than those without UTD (Figure 2A), an increase of 7.3%, which was observed in wk 6 and 7 postpartum (Figure 2B). Conversely, cows that developed NUTD had decreased ($P < 0.001$) cumulative milk yield by 60 d postpartum, a reduction of 340 kg or 22.4% observed throughout the 60 DIM (Figures 2A and 2C). No interaction ($P > 0.30$) was observed between UTD and NUTD for milk yield.

Inflammatory disease was associated with reduced BCS in the first 70 d postpartum (Figure 3). Cows that developed UTD had reduced BCS ($P = 0.03$) compared with those that did not develop UTD (Figure 3A), although the change in body condition from calving to 70 d postpartum did not differ ($P = 0.12$) between the 2 groups (without UTD = -0.25 ± 0.2 vs. with UTD -0.21 ± 0.01). An interaction ($P = 0.006$) between NUTD and day was observed for BCS and cows that developed NUTD had reduced BCS compared with those without NUTD but only at and after 28 d postpartum (Figure 3B). Cows with NUTD had increased ($P = 0.003$) loss of BCS in the first 70 d postpartum (-0.19 ± 0.01 vs. -0.27 ± 0.03 units). No interaction ($P > 0.50$) was observed between UTD and NUTD for BCS or change in BCS in the first 70 DIM.

Of the 733 cows enrolled in the study, 645 (88%) remained in the herd until 300 DIM. Cows that developed NUTD had a greater (P = 0.005) hazard (adjusted hazard ratio = 1.92; 95% CI = 1.22 to 3.01) of leaving the herd than those that did not develop NUTD [no NUTD = 10.3 (60/582) vs. UTD = 18.5% (28/151)]. No association (P = 0.37) with UTD and no interaction (P = 0.92) between UTD and NUTD was associated with the hazard of removal from the herd.

3.4 Associations Between Inflammatory Diseases and Concentrations of Analytes in Blood

Cows with UTD had increased haptoglobin (P = 0.03), decreased tCa (P = 0.0001), decreased P (P = 0.03), and tended to have increased BHB (P = 0.09) compared with cows without UTD (Figures 4 and 5). Cows with NUTD had increased fatty acids (P = 0.0008), increased BHB, decreased P (P = 0.01), and decreased Mg (P = 0.03) compared with cows without NUTD (Figures 4 and 5). No interactions were observed between UTD and NUTD for concentrations of haptoglobin, fatty acids, tCa, P, Mg, or BHB. Interactions (P < 0.01) between UTD and day postpartum were observed for haptoglobin, fatty acids, tCa, P, and Mg as differences between cows with UTD and without UTD were only observed after the day of calving (Figures 4 and 5). An interaction (P = 0.004) between NUTD and day was observed for tCa because cows with NUTD had smaller concentrations of tCa on d 0 and 1 postpartum than cows without NUTD (Figure 5B). Furthermore, when the largest serum concentrations of haptoglobin and fatty acids, and the smallest serum concentrations of tCa, P, and Mg from d 0 to 2 postpartum from each cow were analyzed, the same pattern of response to UTD and NUTD were observed (Supplemental Figure S1; https://doi.org/10.3168/jds .2020-19070). Cows with UTD had the greatest concentration of haptoglobin, whereas those with NUTD had the greatest concentration of fatty acids. Cows with both UTD and NUTD had the smallest concentrations of tCa, P, and Mg in serum.

4. DISCUSSION

Inflammatory diseases are known to be prevalent in the early postpartum period in dairy cows and the present prospective cohort study identified risk factors for UTD and NUTD and the association of those diseases with reproduction of cows receiving ET. The significant risk factors for UTD were multifactorial, including season of calving, parity, calving problems, subclinical

hypocalcemia, and serum concentrations of haptoglobin and Mg. Risk factors identified for NUTD included parity group and serum concentration of Mg. In the present study, inflammatory diseases were associated with a marked depression in reproduction in crossbred cows, corroborating previous reports of either high-producing cows in confinement systems or in grazing cows receiving AI (Santos et al., 2010; Ribeiro et al., 2013); however, the impact was associated mostly with UTD and only minor impacts were observed from NUTD. It is important to note that cows in the present study were bred only by timed ET, which was expected to bypass the effects that diseases might have on estrous expression, the developing follicle, and oocyte, fertilization, and embryo development for the first 7 d. Embryo recipients diagnosed with metritis in the early postpartum period have reduced P/ET, suggesting that uterine inflammation is a major contributor to pregnancy failure (Estrada-Cortés et al., 2019). Results herein corroborate those of Ribeiro et al. (2016) who showed that cows that had inflammatory diseases, particularly those with UTD diseases, have an impaired ability to maintain pregnancy after AI and ET.

Almost half of the cows in this study had uterine inflammation based on endometrial cytology on 28 d postpartum, and therefore, it is possible that endometrial function might have been altered by inflammatory molecules present in the uterus at the first ET. Another possibility is that cows with UTD had altered luteal lifespan that might interfere with maintenance of pregnancy. Furthermore, it is possible that UTD might influence expression of estrus, the endocrine milieu critical for endometrial receptivity of the conceptus, and affect conceptus and endometrial prostaglandin secretion needed for conceptus elongation and placentation (Brooks et al., 2014). Expression of estrus was not assessed in the present study, but it is well documented that cows that express estrus following an ovulation synchronization protocol that resembles that used in the present study have increased P/ET (Pereira et al., 2016). Finally, factors that predispose cows to develop uterine diseases, including those identified in the present study, might also influence maintenance of pregnancy in dairy cows. Pregnancy in cattle receiving ET depends on a transcriptome and metabolome that conveys a receptive endometrium for further conceptus development and maintenance of pregnancy. An elegant experiment by Mazzoni et al. (2020) investigated the endometrial transcriptome of dairy cows immediately before receiving an in vitro-produced embryo between estrous cycle d 6 and 8. Cows were subsequently slaughtered and pregnancy determined. As anticipated, cows that maintained pregnancy up to slaughter had an endometrial transcriptome that differed from those that failed to maintain pregnancy. Among the

differentially expressed genes and subsequent pathways that differed in the d 6 to 8 endometrium in cows that maintained pregnancy included transcripts involved in inflammation and in the balance between pro- and antiinflammatory responses that lead to tolerance for the conceptus (Mazzoni et al., 2020). It is conceivable that UTD results in endometrial inflammation that not only affects the transcriptome (Horlock et al., 2020), but the microbiota present in the uterus of cows with UTD might also differ and be less conducive to estrous cyclicity and pregnancy (Moore et al., 2019).

Inflammation in early lactation has been shown to disrupt early embryo development during the preimplantation period with reduced morula quality, conceptus elongation, and survival in dairy cows (Ribeiro et al., 2016). Products of induced endometrial inflammation are known to compromise early embryo development in vitro (Hill and Gilbert, 2008). It is unquestionable that bacterial products and mediators of the inflammatory cascade, including some proinflammatory cytokines, impair fertilization and reduce meiotic competence and development of in vitro-produced embryos to the blastocyst stage (Soto et al., 2003; Hansen et al., 2004), and alter granulosa cell and oocyte mRNA expression (Piersanti et al., 2019). These effects of inflammation on the follicle, oocyte, and early embryo help explain the reduction in fertilization, conceptus development, and pregnancy per AI observed in dairy cows that develop UTD (Ribeiro et al., 2016). In addition, when virgin dairy heifers were subjected to endometrial inflammation by utero-pathogenic bacteria, the transcriptome was altered 3 mo later in the endometrium, oviduct, and granulosa cells compared with control heifers (Horlock et al., 2020), suggesting a long-term effect of utero-pathogenic bacteria on reproductive tissues that may mediate the impacts of UTD on reproduction observed in cows receiving ET in the present study.

During the inflammatory process of cows that develop diseases in early lactation, pathogen-associated molecular patterns, localized or systemic host responses, subsequent release of inflammatory mediators, and concurrent tissue injury may contribute to the reduced maintenance of pregnancy observed in cows following ET. Chapwanya et al. (2009) described elevated expression of genes encoding toll-like receptors, inflammatory mediators, and effector molecules such as amyloid precursor protein and antimicrobial peptides in the uterus of cows 2 wk postpartum during uterine involution. Toll-like receptors appeared to be functional as endometrial epithelial cells secreted prostaglandin E2 in response to detection of pathogen-associated molecular patterns, leading to increased expression of antimicrobial peptides and amyloid

precursor proteins, providing nonspecific defenses against microbes on mucosal surfaces (Davies et al., 2008). Nonetheless, when the inflammatory response is exacerbated, disease develops, and excessive oxidative stress is often generated that damages tissue and causes dysfunction. Indeed, endometrial markers of oxidative stress have been associated with pregnancy success following ET in humans (Rahiminejad et al. 2016). Cows in the present study were synchronized to receive their first ET at 46 ± 3 DIM, a period in which uterine inflammation may still have been present, based on the findings of others (Lima et al., 2013). Lima et al. (2013) performed consecutive cytological examinations of the endometrium and demonstrated that cows that had not resolved inflammation by 46 DIM had reduced pregnancy per AI and increased pregnancy loss compared with those that resolved postpartum uterine inflammation. It is possible that the local cellular changes taking place in the endometrium of cows that developed UTD make it less capable of establishing tolerance to conceptus alloantigens and favoring rejection by the maternal immune system, or that the altered endometrial molecular signatures that persist for a long period after inflammation impair the ability of the uterus to support a pregnancy (Horlock et al., 2020).

The mechanisms of how NUTD reduce pregnancy in dairy cows receiving ET are not clear. Diagnosis of mastitis either before or after AI is associated with reduced pregnancy per AI, whereas mastitis after pregnancy had been diagnosed is associated with increased pregnancy loss (Santos et al., 2004). Isolation of major pathogens from milk of cows in the week of ET is associated with a marked reduction in P/ET, whereas cows with SCC above 4 × 105 /mL have reduced P/ET (Barbosa et al., 2018). This suggests that active infection and inflammation of the mammary gland when ET is performed is detrimental to maintenance of pregnancy. Indeed, oocyte competence to develop to the blastocyst stage is reduced in cows with spontaneous or induced mastitis (Roth et al., 2013; Asaf et al., 2014). Here, cows with NUTD had reduced milk yield, which probably reflects the effect of those diseases on DMI and nutrient balance. Also, bacterial products and cytokines result in hyperthermia, a well-characterized phenomenon that compromises reproduction in cattle by interrupting embryonic development, interfering with preimplantation events, and altering uterine function (Edwards and Hansen, 1997; Sakatani et al., 2004). In the current study, cows received ET bypassing events related to oocyte quality, fertilization, and early embryo development. In cows diagnosed with NUTD, the diagnosis of the last disease event was at 35.4 d postpartum and the first ET occurred at 59.9 d postpartum, thus an interval of 24.5 d. Unless the inflammation in the NUTD cows became chronic, it is unlikely that

inflammatory mediators or hyperthermia would still be present after 3 wk to affect maintenance of pregnancy after ET (Edwards and Hansen, 1997; Hansen et al., 2004). In fact, half of the cases of mastitis, the predominant NUTD diagnosed, had no bacterial isolate identified presumably because the agent had been cleared from milk. Also, 24 (14.5%) cases of mastitis the isolate agent was a gram-negative bacterium, which can cause an acute inflammatory response, but often of short duration. Therefore, it is more likely that inflammation caused by NUTD in early lactation might interfere with reproduction by compromising aspects related to follicle development or oocyte competence which can affect pregnancy many weeks following the insult (Ribeiro et al., 2016). On the other hand, if cows present infection and inflammation of the mammary gland in the week of ET, then the ongoing inflammatory process is associated with reduced maintenance of pregnancy following ET in dairy cows (Barbosa et al., 2018).

It is important to note that both UTD and NUTD reduced synchronization of ovulation for the first ET, and UTD delayed the first ET postpartum. Also, service rate for the subsequent cycles was reduced in cows that had UTD likely because of a combination of reduced synchrony of ovulation and reduced P/ET. Diseases and concurrent inflammatory response cause release of pathogen-associated and damage-associated molecular patterns that stimulate residing immune cells to release cytokines and, collectively, they can disrupt endocrine signaling and affect the developing follicle (Sheldon et al., 2009; Bromfield et al., 2015). This might explain the altered ability of cows to respond to the hormonal treatments and synchronize ovulation, which eventually affected the rate of pregnancy. In particular, the synchronization protocol used estradiol to induce ovulation, and pathogen-associated molecular patterns can disrupt the spontaneous LH surge in dairy cows (Lavon et al., 2008).

Cows with NUTD had a marked reduction in milk yield, which was not surprising given that most cases of NUTD involved mastitis that is known to depress productive performance. On the other hand, an unexpected increase in milk yield in the first 60 DIM was observed for cows with UTD, and the increase was less in cows that did not have NUTD (51 kg or 3.4%) than in those that had NUTD (138 kg or 12.4%). Metritis is known to be associated with reduced milk yield (Sheldon et al., 2009), but of the 464 cows with UTD, only 101 or 21.8% had metritis and the remainder had either clinical or subclinical endometritis, which typically are not associated with systemic signs of disease or depression in milk yield. Also, because the increase in total milk yield observed for cows with UTD originated mostly within those with NUTD, it is possible that

severity of NUTD, in particular mastitis, was less in those cows that also had UTD than those without UTD.

The risk of UTD or NUTD was dependent on several environmental and cow-level factors, many of which were associated with peripartum metabolism and management of transition cows. It is interesting that cows calving during the cool season had greater risk of developing UTD than those calving in the hot season. It is clear that heat stress during the entire dry period suppresses lactation performance and immune function (Dahl et al., 2017), but less is known about the effects of dry period heat stress on disease incidence. Preliminary data suggest that cooling prepartum cows during the summer months to alleviate heat stress increases the risk of metritis (Santos et al., 2014). One of the risk factors for uterine disease is trauma caused during calving (Vieira-Neto et al., 2016), and larger calves are more likely to cause dystocia and trauma (Johanson and Berger, 2003); here calving problems was one of the risk factors identified for UTD. Heat stress and the resulting hyperthermia reduces gestation length and the BW of the newborn calf (Dahl et al., 2017), which might ease delivery and reduce the risk of dystocia. Also, as lactation number increased, the risk of UTD and that of NUTD also increased. Older cows are more likely to have retained placenta and mastitis (Markusfeld, 1987; Vieira-Neto et al., 2016; Taponen et al., 2017), which was observed in the present study. Nevertheless, NUTD reduced milk yield likely because mastitis directly affects the mammary gland resulting in a direct impairment or death of milk secreting cells.

The number of days with subclinical hypocalcemia and serum concentrations of haptoglobin and Mg were risk factors for UTD. The increase in haptoglobin on d 2 postpartum in cows that developed UTD is likely related to the activation of the inflammatory process with release of cytokines from residing immune cells, which in turn stimulate hepatic secretion of positive acute phase proteins (Cray et al., 2009). Others have shown that haptoglobin increases in the first few days after calving and the increment is greater with increased severity of uterine disease (Huzzey et al., 2009). Also, as cows developed more persistent subclinical hypocalcemia, the risk of UTD increased. It has been shown that hypocalcemia increases the risk of metritis, and one of the possible underlying mechanisms is the reduced innate immune function observed in cows with subclinical hypocalcemia (Martinez et al., 2012). Calcium is the most abundant mineral in milk and Mg is critical in the diet of dairy cows because little is stored in the body. The prepartum diet fed in the present study was not designed to prevent hypocalcemia with a DCAD

of +160 mEq/kg. Also, although the dietary content of K pre- and postpartum was not excessive, dietary Mg was not high, which might have influenced the ability of cows to maintain adequate concentrations of tCa and Mg in serum. The incidence of cows with at least 1 d with subclinical hypocalcemia was high in both primiparous (52%) and multiparous (73.8%) cows, values greater than reported in Holstein cows in confinement herds in the United States (Reinhardt et al., 2011). Because alkalogenic diets and those that result in limited intake of Mg favor hypocalcemia and hypomagnesemia (Goff, 2008), and those were risk factors for UTD, it is desirable that improvements in metabolic health by dietary means be implemented to reduce the risk of diseases that might interfere with fertility.

5. CONCLUSIONS

Inflammatory diseases are prevalent in the early postpartum period and have long-lasting impacts on maintenance of pregnancy in dairy cattle. Results from this prospective cohort study in a population of crossbred cows showed that 63.3% were affected by clinical or subclinical uterine diseases (or both) and 20.6% were affected by nonuterine diseases. Cows affected by uterine inflammation had a marked reduction in reproductive performance, likely because of localized effects that reduced service rate and maintenance of pregnancy following ET. Subsequently, the rate of pregnancy decreased and days open increased in cows with uterine inflammation. Nonuterine diseases, however, compromised milk yield in the first 60 d in lactation with minor impacts on reproduction. The fact that reproduction was affected to a greater degree in cows with uterine diseases than those with nonuterine diseases after ET suggests that uterine inflammation compromises its ability to support pregnancy, whereas nonuterine inflammation might affect the developing follicle, oocyte, fertilization, very early embryo development, or a combination of these. Risk factors for inflammatory diseases included blood markers of early postpartum inflammation, energy, and mineral metabolism. Collectively, these results suggest that improving the maintenance of pregnancy in dairy cows requires prevention of early postpartum diseases, in particular those affecting the uterus, by minimizing calving problems and disturbances of mineral and energy metabolism at the onset of lactation.

ACKNOWLEDGMENTS

The authors thank the owner and staff of Santa Luzia dairy farm from the Cabo Verde Group (Passos, MG, Brazil) for use of their cows and facilities. Partial funding for this project was provided by Phibro Animal Health (Teaneck, NJ) and the National Council for Scientific and Technological Development (CNPq) from Brazil under protocol number 134068/2019-0. Ingrid N. F. Edelhoff was supported by the graduate student fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) from Brazil under the code 001 and by funding from the University of Florida. Marcos H. C. Pereira was supported by the graduate student fellowship from CAPES under protocol number 88882.317784/2019-01. The authors have not stated any conflicts of interest.

References

Asaf, S., G. Leitner, O. Furman, Y. Lavon, D. Kalo, D. Wolfenson, and Z. Roth. 2014. Effects of Escherichia coli- and Staphylococcus aureus-induced mastitis in lactating cows on oocyte developmental competence. Reproduction 147:33–43.

Banos, G., E. Wall, M. P. Coffey, A. Bagnall, S. Gillespie, G. C. Russell, and T. M. McNeilly. 2013. Identification of immune traits correlated with dairy cow health, reproduction and productivity. PLoS One 8:e65766.

Barbosa, L. F. S. P., W. V. C. Oliveira, M. H. C. Pereira, M. B. Moreira, C. G. C. Vasconcelos, B. F. Silper, R. L. A. Cerri, and J. L. M. Vasconcelos. 2018. Somatic cell count and type of intramammary infection impacts fertility from in vitro produced embryo transfer. Theriogenology 108:291–296.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. L. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650.

Bromfield, J. J., J. E. P. Santos, J. Block, R. S. Williams, and I. M. Sheldon. 2015. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Uterine infection: Linking infection and innate immunity with infertility in the high-producing dairy cow. J. Anim. Sci. 93:2021–2033.

Brooks, K., G. Burns, and T. Spencer. 2014. Conceptus elongation in ruminants: Roles of progesterone, prostaglandin, interferon tau and cortisol. J. Anim. Sci. Biotechnol. 5:53.

Carvalho, M. R., F. Peñagaricano, J. E. P. Santos, T. DeVries, B. McBride, and E. S. Ribeiro. 2019. Long-term impact of clinical disease postpartum on milk production, reproduction, and culling of dairy cows. J. Dairy Sci. 102:11701–11717.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. P. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301–1309.

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.

Cooke, R. F., and J. D. Arthington. 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. J. Anim. Physiol. Anim. Nutr. (Berl.) 97:531–536.

Cray, C., J. Zaias, and N. H. Altman. 2009. Acute phase response in animals: A review. Comp. Med. 59:517–526.

Dahl, G. E., S. Tao, and J. Laporta. 2017. Late gestation heat stress of dairy cattle programs dam and daughter milk production. J. Anim. Sci. 95:5701–5710.

Davies, D., K. G. Meade, S. Herath, P. D. Eckersall, D. Gonzalez, J. O. White, R. S. Conlan, C. O'Farrelly, and I. M. Sheldon. 2008. Tolllike receptors and antimicrobial peptide expression in the bovine endometrium. Reprod. Biol. Endocrinol. 6:53.

Dickson, M. J., R. L. Piersanti, R. Ramirez-Hernandez, E. B. Oliveira, J. V. Bishop, T. R. Hansen, Z. Ma, K. C. Jeong, J. E. P. Santos, I. M. Sheldon, J. Block, and J. J. Bromfield. 2020. Experimentally induced endometritis impairs the developmental capacity of bovine oocytes. Biol. Reprod. ioaa069.

Edwards, J., and P. Hansen. 1997. Differential responses of bovine oocytes and preimplantation embryos to heat shock. Mol. Reprod. Dev. 46:138–145.

Elanco Animal Health. 2009. The 5-point body condition scoring system. Bulletin AI 10752. Elanco Animal Health, Greenfield, IN.

Estrada-Cortés, E., W. G. Ortiz, R. C. Chebel, E. A. Jannaman, J. I. Moss, F. C. de Castro, A. M. Zolini, C. R. Staples, and P. J. Hansen. 2019. Embryo and cow factors affecting pregnancy per embryo transfer for multiple-service, lactating Holstein recipients. Transl. Anim. Sci. 3:60–65.

Galvão, K. N., M. Frajblat, S. B. Brittin, W. R. Butler, C. L. Guard, and R. O. Gilbert. 2009. Effect of prostaglandin F2α on subclinical endometritis. J. Dairy Sci. 92:4906–4913.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176:50–57.

Hansen, P. J., P. Soto, and R. P. Natzke. 2004. Mastitis and fertility in cattle – Possible involvement of inflammation or immune activation in embryonic mortality. Am. J. Reprod. Immunol. 51:294–301.

Hill, J., and R. Gilbert. 2008. Reduced quality of bovine embryos cultured in media conditioned by exposure to an inflamed endometrium. Aust. Vet. J. 86:312–316.

Horlock, A. D., R. L. Piersanti, R. Ramirez-Hernandez, F. Yu, Z. Ma, K. C. Jeong, M. J. D. Clift, J. Block, J. E. P. Santos, J. J. Bromfield, and M. Sheldon. 2020. Uterine infection alters the transcriptome of the bovine reproductive tract three months later. Reproduction 160:93–107.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. Von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92:621–625.

Johanson, J. M., and P. J. Berger. 2003. Birth weight as a predictor of calving ease and perinatal mortality in Holstein cattle. J. Dairy Sci. 86:3745–3755.

Johnson, M. M., and J. P. Peters. 1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim. Sci. 71:753–756.

Jørgensen, E., and A. R. Pedersen. 1998. How to obtain those nasty standard errors from transformed data-and why they should not be used. Biometry Research Unit-Internal report 7. Danish Institute of Agricultural Sciences, Aarhus, Denmark.

Lavon, Y., G. Leitner, T. Goshen, B. Braw-Tal, S. Jacoby, and D. Wolfenson. 2008. Exposure to endotoxin during estrus alters the timing of ovulation and hormonal concentrations in cows. Theriogenology 70:956–967.

Lima, F. S., R. S. Bisinotto, E. S. Ribeiro, L. F. Greco, H. Ayres, M. G. Favoreto, M. R. Carvalho, K. N. Galvão, and J. E. P. Santos. 2013. Effects of 1 or 2 treatments with prostaglandin F2α on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. J. Dairy Sci. 96:6480–6488.

Lin, D. Y., L. J. Wei, and Z. Ying. 1993. Checking the Cox model with cumulative sums of martingale-based residuals. Biometrika 80:557–572.

Markusfeld, O. 1987. Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. J. Dairy Sci. 70:158–166.

Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. Galvão, and J. E. P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158–7172.

Mazzoni, G., H. S. Pedersen, M. B. Rabaglino, P. Hyttel, H. Callesen, and H. N. Kadarmideen. 2020. Characterization of the endometrial transcriptome in early diestrus influencing pregnancy status in dairy cattle after transfer of in vitro-produced embryos. Physiol. Genomics 52:269–279.

McDougall, S., R. Macaulay, and C. Compton. 2007. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. Anim. Reprod. Sci. 99:9– 23.

Moore, S. G., A. C. Ericsson, S. K. Behura, W. R. Lamberson, T. J. Evans, M. S. McCabe, S. E. Poock, and M. C. Lucy. 2019. Concurrent and long-term associations between the endometrial microbiota and endometrial transcriptome in postpartum dairy cows. BMC Genomics 20:405.

Pereira, M. H. C., M. C. Wiltbank, and J. L. M. Vasconcelos. 2016. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. J. Dairy Sci. 99:2237–2247.

Pérez-Báez, J., C. A. Risco, R. C. Chebel, G. C. Gomes, L. F. Greco, S. Tao, I. M. Thompson, B. C. do Amaral, M. G. Zenobi, N. Martinez, C. R. Staples, G. E. Dahl, J. A. Hernández, J. E. P.

Santos, and K. N. Galvão. 2019. Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part I. Calving disorders and metritis. J. Dairy Sci. 102:9138–9150.

Piepho, H. P. 2009. Data transformation in statistical analysis of field trials with changing treatment variance. Agron. J. 101:865–869.

Piersanti, R. L., J. E. P. Santos, I. M. Sheldon, and J. J. Bromfield. 2019. Lipopolysaccharide and tumor necrosis factor-alpha alter gene expression of oocytes and cumulus cells during bovine in vitro maturation. Mol. Reprod. Dev. 86:1909–1920.

Quinlan, K. P., and M. A. DeSesa. 1955. Spectrophotometric determination of phosphorus as molybdovanadophosphoric acid. Anal. Chem. 27:1626–1629.

Rahiminejad, M. E., A. Moaddab, M. Ganji, N. Eskandari, M. Yepez, S. Rabiee, M. Wise, R. Ruano, and A. Ranjbar. 2016. Oxidative stress biomarkers in endometrial secretions: A comparison between successful and unsuccessful in vitro fertilization cycles. J. Reprod. Immunol. 116:70–75.

Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188:122–124.

Ribeiro, E. S., G. Gomes, L. F. Greco, R. L. A. Cerri, A. Vieira-Neto, P. L. J. Monteiro Jr., F. S. Lima, R. S. Bisinotto, W. W. Thatcher, and J. E. P. Santos. 2016. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J. Dairy Sci. 99:2201–2220.

Ribeiro, E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W. Thatcher, and J. E. P. Santos. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J. Dairy Sci. 96:5682–5697.

Roth, Z., A. Dvir, D. Kalo, Y. Lavon, O. Krifucks, D. Wolfenson, and G. Leitner. 2013. Naturally occurring mastitis disrupts developmental competence of bovine oocytes. J. Dairy Sci. 96:6499–6505.

Sakatani, M., S. Kobayashi, and M. Takahashi. 2004. Effects of heat shock on in vitro development and intracellular oxidative state of bovine preimplantation embryos. Mol. Reprod. Dev. 67:77–82.

Santos, J. E. P., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thatcher. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Soc. Reprod. Fertil. Suppl. 67:385–403.

Santos, J. E. P., R. L. A. Cerri, M. A. Ballou, G. E. Higginbotham, and J. H. Kirk. 2004. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim. Reprod. Sci. 80:31–45.

Santos, J. E. P., E. S. Ribeiro, E. Karakaya, K. N. Galvão, and F. S. Lima. 2014. Influences of heat stress and uterine diseases on reproduction of dairy cows. J. Dairy Sci. 97(Suppl. 1):202. (Abstr.)

Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio, and H. J. Schuberth. 2009. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol. Reprod. 81:1025–1032.

Soto, P., P. R. Natzke, and P. J. Hansen. 2003. Actions of tumor necrosis factor-α on oocyte maturation and embryonic development in cattle. Am. J. Reprod. Immunol. 50:380–388.

Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47:1179–1187.

Taponen, S., E. Liski, A.-M. Heikkilä, and S. Pyörälä. 2017. Factors associated with intramammary infection in dairy cows caused by coagulase-negative staphylococci, Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Corynebacterium bovis, or Escherichia coli. J. Dairy Sci. 100:493–503.

Vieira-Neto, A., F. S. Lima, J. E. P. Santos, R. D. Mingoti, G. S. Vasconcellos, C. A. Risco, and K. N. Galvão. 2016. Vulvovaginal laceration as a risk factor for uterine disease in postpartum dairy cows. J. Dairy Sci. 99:4629–4637.

Wenz, J. R., G. M. Barrington, F. B. Garry, R. P. Dinsmore, and R. J. Callan. 2001. Use of systemic disease signs to assess disease severity in dairy cows with acute coliform mastitis. J. Am. Vet. Med. Assoc. 218:567–572.

Table 1. Diets fed to cows prepartum and early postpartum[1]

Ingredient	Prepartum[2]	Early postpartum[3]
Corn silage	74.9	37.9
Elephant grass silage	4.9	2.5
Whole cottonseed	3.7	7.4
Cracked corn	1.5	0.6
Soybean meal	13.2	10.0
Citrus pulp	---	4.0
Grain mix[4]	---	34.9
Prepartum mineral and vitamin[5]	2.0	---
Postpartum mineral and vitamin[6]	---	2.7
Nutrient content, [7] DM basis		
OM, %	92.8 ± 1.5	93.4 ± 1.0
CP, %	15.6 ± 1.2	17.6 ± 1.1
NDF, % OM	34.9 ± 3.2	26.0 ± 1.0
Ether extract, %	3.2 ± 0.2	3.3 ± 0.6
NFC, %	39.1 ± 2.2	46.5 ± 2.4
Ca, %	0.72 ± 0.11	0.86 ± 0.13
P, %	0.34 ± 0.05	0.37 ± 0.03
K, %	1.43 ± 0.14	1.14 ± 0.18
Mg, %	0.27 ± 0.01	0.23 ± 0.02
Na, %	0.10 ± 0.01	0.22 ± 0.04
Cl, %	0.40 ± 0.05	0.50 ± 0.08
S, %	0.22 ± 0.03	0.17 ± 0.02
DCAD, mEq/kg	161 ± 25	136 ± 42

[1] Samples collected weekly and composited for chemical analyses. Means and SD from chemical analysis of 4 composite samples per diet.

[2] Diet fed in the last 3 wk of gestation.

[3] Diet fed in the first 74 DIM.

[4] Each kilogram contained (DM basis) 63.0% ground corn, 20.3% soybean hulls, 9.9% soybean meal, 4.6% dried citrus pulp, 0.9% urea, 0.6% sodium chloride, and 0.7% sodium bentonite. The

nutrient content (DM basis) was 16.4% CP, 21.2% NDF, 47.6% starch, 57.7% NFC, 3.6% fat, and 2.7% ash.

[5] Each kilogram contained (DM basis) 190 g of Ca, 17 g of P, 80 g of Mg, 30 g of Na, 40 g of S, 2,400 mg of Zn, 1,500 mg of Mn, 1,200 mg of Cu, 30 mg of I, 16 mg of Co, 12 mg of Se, 36 mg of Cr, 75 mg of biotin, 750,000 IU of vitamin A, 250,000 IU of vitamin D_3, 7,500 IU of vitamin E, and 750 mg of sodium monensin.

[6] Each kilogram contained (DM basis) 220 g of Ca, 40 g of P, 25 g of Mg, 65 g of Na, 10 g of S, 2,375 mg of Zn, 2,375 mg of Mn, 562 mg of Cu, 31 mg of I, 13 mg of Co, 15 mg of Se, 57 mg of biotin, 100,000 IU of vitamin A, 25,000 IU of vitamin D_3, 625 IU of vitamin E, and 750 mg of sodium monensin.

[7] Values are means ± SD.

Table 2. Descriptive statistics of data from the 733 cows enrolled in the study according to parity group

Item, mean ± SD or % (n/n)	All cows	Primiparous	Multiparous
Cows, n	733	34.4 (252/733)	65.6 (481/733)
Distribution of calving			
0001 to 0600 h	35.2 (258/733)	36.9 (93/252)	34.3 (165/481)
0601 to 1200 h	25.6 (188/733)	25.8 (65/252)	25.6 (123/481)
1201 to 1800 h	28.8 (211/733)	26.6 (67/252)	29.9 (144/481)
1801 to 0000 h	10.4 (76/733)	10.7 (27/252)	10.2 (49/481)
Season of calving			
Cool	25.2 (185/733)	25.8 (65/252)	25.0 (120/481)
Hot	74.8 (548/733)	74.2 (187/252)	75.0 (361/481)
BCS at calving, 1 to 5	3.28 ± 0.30	3.31 ± 0.25	3.27 ± 0.33
Calving problems[1]	8.7 (64/733)	7.1 (18/252)	9.6 (46/481)
Uterine diseases[2]	63.3 (464/733)	58.0 (146/252)	66.1 (318/481)
Retained placenta	11.4 (84/733)	8.7 (22/252)	12.9 (62/481)
Metritis	13.8 (101/733)	11.5 (29/252)	15.0 (72/481)
Clinical endometritis	34.5 (243/704)	24.8 (61/246)	39.7 (182/458)
Subclinical endometritis	48.5 (333/686)	46.1 (112/243)	49.9 (221/443)
Nonuterine diseases[3]	20.6 (151/733)	8.7 (22/252)	26.8 (129/481)
Mastitis	16.1 (118/733)	7.1 (18/252)	20.8 (100/481)
Lameness	4.1 (30/733)	1.6 (4/252)	5.4 (26/481)
Pneumonia	1.2 (9/733)	0	1.9 (9/481)
Displaced abomasum	0.7 (5/733)	0	1.0 (5/481)
Haptoglobin,[4] x 100 AU/mL			
Mean	3.93 ± 3.56	4.70 ± 4.04	3.52 ± 3.20
Largest value	6.42 ± 5.56	7.42 ± 5.94	5.89 ± 5.28
Fatty acids,[4] m*M*			
Mean	0.48 ± 0.24	0.45 ± 0.20	0.49 ± 0.25
Largest value	0.67 ± 0.33	0.64 ± 0.33	0.68 ± 0.33
BHB,[5] m*M*	0.78 ± 0.37	0.72 ± 0.31	0.81 ± 0.39

Days of SCH[6]			
0	33.7 (247/733)	48.0 (121/252)	26.2 (126/481)
1	21.0 (154/733)	18.3 (46/252)	22.5 (108/481)
>1	45.3 (332/733)	33.7 (85/252)	51.3 (247/481)
Total Ca,[4] m*M*			
Mean	2.01 ± 0.18	2.08 ± 0.15	1.98 ± 0.18
Smallest value	1.89 ± 0.22	1.98 ± 0.18	1.84 ± 0.23
Total Mg,[4] m*M*			
Mean	0.95 ± 0.12	0.97 ± 0.11	0.94 ± 0.12
Smallest value	0.87 ± 0.12	0.90 ± 0.13	0.86 ± 0.12
Total P,[4] m*M*			
Mean	1.37 ± 0.25	1.46 ± 0.22	1.33 ± 0.25
Smallest value	1.13 ± 0.25	1.20 ± 0.24	1.08 ± 0.24
Milk yield			
First 60 DIM, kg/d	25.3 ± 7.1	21.4 ± 5.8	27.3 ± 6.9
Cumulative 60 DIM, kg	1,498 ± 467	1,275 ± 374	1,615 ± 467

[1] Includes dystocia, stillbirths, or twin calving.

[2] Includes retained placenta, metritis, clinical endometritis, and subclinical endometritis.

[3] Includes mastitis, displaced abomasum, pneumonia, and lameness.

[4] Serum samples analyzed on days 0, 1, and 2 postpartum. AU = arbitrary units.

[5] Whole blood sample analyzed on d 8 ± 3 postpartum.

[6] Subclinical hypocalcemia (SCH) based on serum total Ca < 2.0 m*M* on d 0, 1, or 2 postpartum.

Table 3. Risk factors for uterine diseases[1]

			Univariable		Multivariable[2]	
Item	Level or unit	% (n/n) or mean ± SD	OR[3] (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Season of calving	Hot	60.0 (329/548)	Referent		Referent	
	Cool	73.0 (135/185)	1.80 (1.25 – 2.60)	0.002	1.66 (1.12 – 2.44)	0.01
Parity group	1	58.0 (146/252)[b]	Referent		Referent	
	2	60.5 (164/271)[b]	1.11 (0.78 – 1.58)	0.55	1.20 (0.82 – 1.75)	0.36
	> 2	73.3 (154/210)[a]	2.00 (1.34 – 3.00)	< 0.001	1.79 (1.14 – 2.82)	0.01
Breed[4]	50% H	58.6 (140/239)	Referent		Referent	
	75% H	65.6 (324/494)	1.36 (0.99 – 1.89)	0.06	1.28 (0.88 – 1.79)	0.22
Calving problems	No	61.1 (409/669)	Referent		Referent	
	Yes	86.0 (55/64)	3.57 (1.75 – 7.14)	0.004	3.01 (1.41 – 6.25)	0.004
Sex of the calf	Female	63.9 (423/662)	Referent			
	Male	57.8 (41/71)	0.80 (0.49 – 1.32)	0.37	---	---
BCS at calving	1 to 5	3.28 ± 0.30	0.71 (0.43 – 1.17)	0.18	---	---

Days of SCH[5]	0	55.1 (136/247)[b]	Referent		Referent	
	1	55.2 (85/154)[b]	1.01 (0.67 – 1.51)	0.98	0.73 (0.47 – 1.13)	0.16
	> 1	73.2 (243/332)[a]	2.23 (1.57 – 3.16)	< 0.001	1.47 (1.00 – 2.18)	0.05
Haptoglobin[6]	x100 AU/mL	6.42 ± 5.56	1.07 (1.04 – 1.10)	< 0.001	1.07 (1.02 – 1.10)	< 0.001
Fatty acids[6]	m*M*	0.67 ± 0.33	1.49 (0.93 – 2.38)	0.10	0.88 (0.40 – 1.95)	0.63
Total Mg[7]	m*M*	0.85 ± 0.12	0.07 (0.02 – 0.26)	< 0.001	0.15 (0.03 – 0.62)	0.01
Total P[7]	m*M*	1.10 ± 0.25	0.40 (0.21 – 0.74)	0.003	0.78 (0.38 – 1.59)	0.49
BHB on d 8 ± 3	m*M*	0.78 ± 0.37	1.08 (0.67 – 1.74)	0.03	1.49 (0.92 – 2.43)	0.11

[a,b,c] Superscripts depicted for the categories of an explanatory variable differ in the multivariable model ($P < 0.05$).

[1] Uterine diseases include retained placenta, metritis, clinical endometritis, and subclinical endometritis.

[2] The multivariable models included only fixed effects from univariable models that resulted in $P \leq 0.10$.

[3] OR = odds ratio.

[4] Crossbred cows were either 50% Holstein (H) 50% Gyr or 75% H and 25% Gyr.

[5] SCH = subclinical hypocalcemia based on serum total Ca < 2.00 m*M*.

[6] Largest value measured on d 0, 1 and 2 postpartum. AU = arbitrary units.

[7] Smallest value measured on d 0, 1, and 2 postpartum.

Table 4. Risk factors for nonuterine diseases[1]

			Univariable		Multivariable[2]	
Item	Level or unit	% (n/n) or mean ± SD	OR[3] (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Season of calving	Cool	23.2 (43/185)	Referent			
	Hot	19.7 (108/548)	0.81 (0.54 – 1.22)	0.30	---	---
Parity group	1	8.7 (22/252)[c]	Referent		Reference	
	2	21.0 (57/271)[b]	2.79 (1.64 – 4.72)	< 0.001	2.54 (1.49 – 4.33)	< 0.001
	> 2	34.3 (72/210)[a]	5.45 (3.23 – 9.20)	< 0.001	4.82 (2.84 – 8.20)	< 0.001
Breed[4]	50% H	21.0 (50/239)	Referent			
	75% H	20.4 (101/494)	0.97 (0.66 – 1.42)	0.88	---	---
Calving problems	No	20.0 (134/669)	Referent			
	Yes	26.6 (17/64)	1.45 (0.81 – 2.63)	0.22	---	---
Sex of the calf	Female	20.4 (135/662)	Referent			
	Male	22.5 (16/71)	1.13 (0.63 – 2.04)	0.67	---	---
BCS at calving	1 to 5	3.28 ± 0.30	0.93 (0.52 – 1.70)	0.83	---	---

Days of SCH[5]	0	13.8 (34/247)	Referent		Referent	
	1	22.1 (34/154)	1.78 (1.05 – 3.00)	0.03	1.34 (0.77 – 2.33)	0.30
	>1	25.0 (83/332)	2.09 (1.35 – 3.24)	0.001	1.19 (0.73 – 1.95)	0.48
Haptoglobin[6]	x100 AU/mL	6.42 ± 5.56	1.01 (0.98 – 1.04)	0.64	---	---
Fatty acids[6]	m*M*	0.67 ± 0.33	1.94 (1.16 – 3.23)	0.01	1.43 (0.83 – 2.47)	0.19
Total Mg[7]	m*M*	0.85 ± 0.12	0.10 (0.02 – 0.45)	0.002	0.21 (0.04 – 0.98)	0.05
Total P[7]	m*M*	1.10 ± 0.25	0.30 (0.14 – 0.64)	0.002	1.99 (0.31 – 4.35)	0.48
BHB on d 8 ± 3	m*M*	0.78 ± 0.37	1.08 (0.66 – 1.74)	0.76	---	---

[a,b,c] Superscripts depicted for the categories of an explanatory variable differ in the multivariable model ($P < 0.05$).

[1] Nonuterine diseases include mastitis, displaced abomasum, pneumonia, and lameness.

[2] The multivariable models included only fixed effects from univariable models that resulted in $P \leq 0.10$.

[3] OR = odds ratio.

[4] Crossbred cows were either 50% Holstein (H) and 50% Gyr or 75% H and 25% Gyr.

[5] SCH = subclinical hypocalcemia based on serum total Ca < 2.00 m*M*.

[6] Largest value measured on days 0, 1 and 2 postpartum. AU = arbitrary units.

[7] Smallest value measured on days 0, 1, and 2 postpartum.

Table 5. Impact of inflammatory diseases on reproductive performance of dairy cows receiving embryo transfer (ET)

Item, % (n/n)	Group[1]				*P*-value[2]		
or LSM ± SEM	UTD-/ NUTD-	UTD+/ NUTD-	UTD-/ NUTD+	UTD+/ NUTD+	UTD	NUTD	UTD x NUTD
Cows, n	230	352	39	112	---	---	---
Received ET	91.7 (211/230)	95.2 (335/352)	76.9 (30/39)	88.4 (99/112)	0.02	< 0.001	0.67
First ET							
Synchronization[3]	79.8 (170/213)	75.7 (256/338)	76.7 (23/30)	54.3 (57/105)	0.02	0.03	0.13
Day postpartum	52.1 ± 1.5	55.8 ± 1.2	53.4 ± 4.0	61.7 ± 2.2	0.02	0.14	0.36
Pregnant day 31	54.8 (115/210)	37.9 (127/335)	43.3 (13/30)	30.3 (30/99)	0.007	0.09	0.80
Pregnant day 59	41.9 (88/210)	25.1 (84/335)	40.0 (12/30)	26.3 (26/99)	0.004	0.97	0.77
Pregnancy loss	23.5 (27/115)	33.9 (43/127)	7.7 (1/13)	13.3 (4/30)	0.07	0.02	0.93
Second ET							
Day postpartum	96.8 ± 2.5	104.7 ± 1.7	92.9 ± 6.4	105.7 ± 3.3	0.008	0.71	0.54
Pregnant day 31	51.8 (59/114)	45.6 (108/237)	23.5 (4/17)	35.9 (23/64)	0.60	0.01	0.21
Pregnant day 59	44.7 (51/114)	36.3 (86/237)	17.7 (3/17)	28.1 (18/64)	0.73	0.02	0.19
Pregnancy loss	13.6 (8/59)	20.4 (22/108)	25.0 (1/4)	21.7 (5/23)	0.82	0.53	0.62
All ET							
21-d service rate	61.6 (430/698)	55.7 (833/1,502)	64.1 (66/103)	52.3 (228/436)	0.007	0.87	0.31
Pregnancy per ET[*†]	46.7 (200/428)	35.9 (300/836)	43.9 (29/66)	37.6 (86/229)	0.01	0.17	0.56
21-d pregnancy rate	28.7 (200/698)	20.0 (300/1,502)	28.2 (29/103)	19.7 (86/436)	< 0.001	0.95	0.97

[1] Crossbred Holstein-Gyr cows were classified according to diagnosis of uterine diseases (UTD) or nonuterine diseases (NUTD) in the first 60 DIM into 4 groups: UTD-/NUTD- = UTD negative and NUTD negative; UTD+/NUTD- = UTD positive and NUTD negative; UTD-/NUTD+ = UTD negative and NUTD positive; and UTD+/NUTD+ = UTD positive and NUTD positive.

[2] UTD = effect of UTD; NUTD = effect of NUTD; UTD x NUTD = interaction between UTD and NUTD.

[3] Synchronization of ovulation based on the presence of a visible corpus luteum on the day of timed ET.

[*] Interaction between UTD and ET number ($P < 0.05$); † Interaction between NUTD and ET number ($P < 0.05$).

Table 6. Cox's proportional hazard regression for days open in dairy cows receiving embryo transfer[1]

Item	Pregnant,[2] % (n/n)	Days open		Adjusted HR[3] (95% CI)	*P*-value
		Mean ± SEM	Median (95% CI)		
UTD					
No	85.1 (229/269)	103.6 ± 3.9	87 (81 - 102)	Referent	
Yes	83.2 (386/464)	132.4 ± 3.5	119 (111 - 128)	0.65 (0.55 - 0.77)	< 0.001
NUTD					
No	85.9 (500/582)	121.2 ± 3.1	107 (97 - 115)	Referent	
Yes	76.2 (115/151)	126.0 ± 6.0	115 (102 - 131)	1.00 (0.81 - 1.23)	0.98

[1] Crossbred Holstein-Gyr cows were classified according to diagnosis of uterine diseases (UTD) or nonuterine diseases (NUTD) in the first 60 DIM. The interaction between UTD and NUTD was not significant (P = 0.86); therefore, results are presented for the main effects only.

[2] Pregnant = cows that became pregnant in the first 300 d postpartum based on the diagnosis performed on d 59 of the presumptive pregnancy.

[3] HR = hazard ratio.

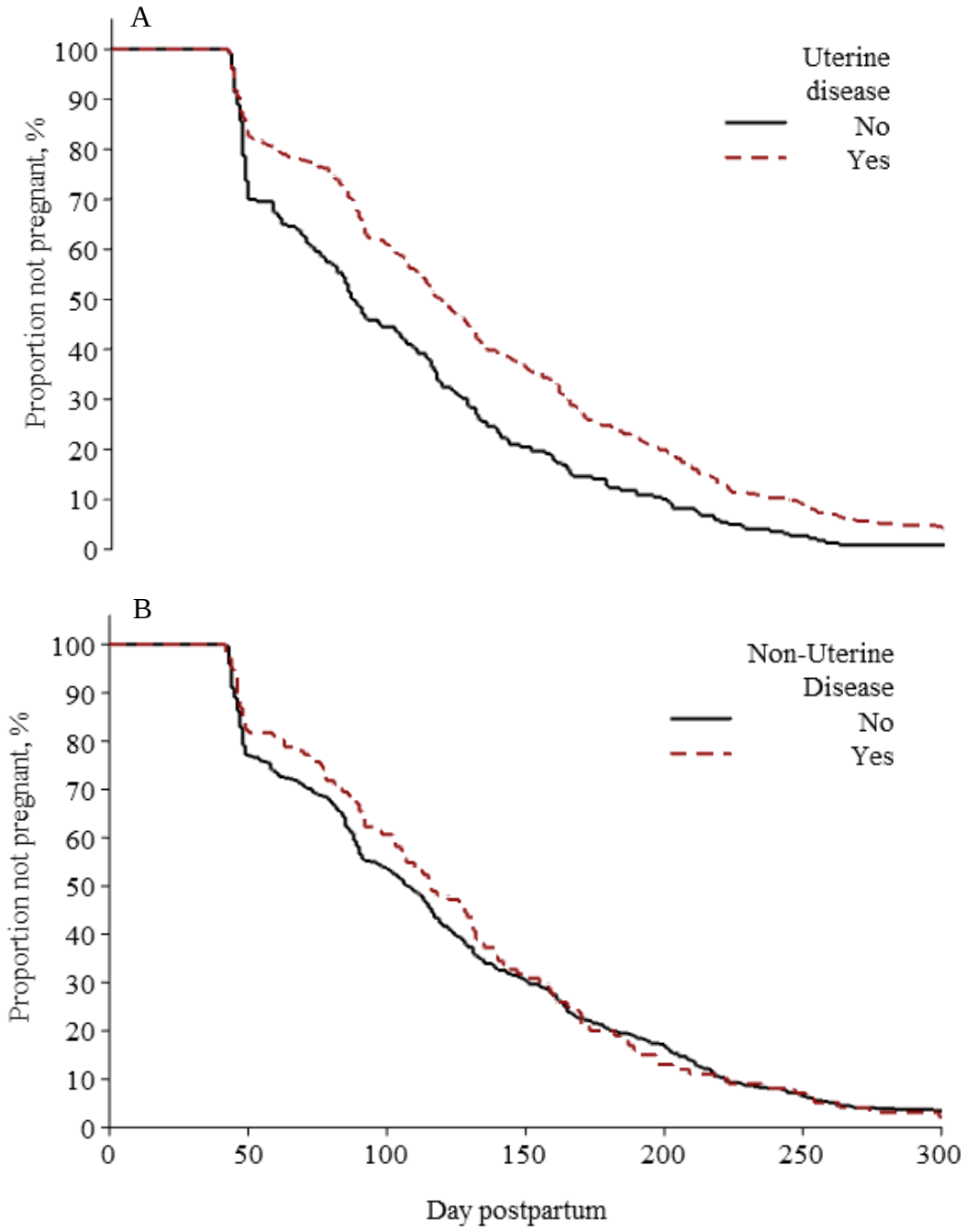

Figure 1. Survival curves for interval from calving to pregnancy in crossbred Holstein-Gyr cows receiving embryo transfer. Cows were classified according to diagnosis of uterine diseases (UTD) or nonuterine diseases (NUTD) in the first 60 DIM. (A) Effect of UTD (yes or no) on time to pregnancy ($P < 0.001$). (B) Effect of NUTD (yes or no) on time to pregnancy ($P = 0.98$). The interaction between UTD and NUTD was not significant ($P = 0.86$).

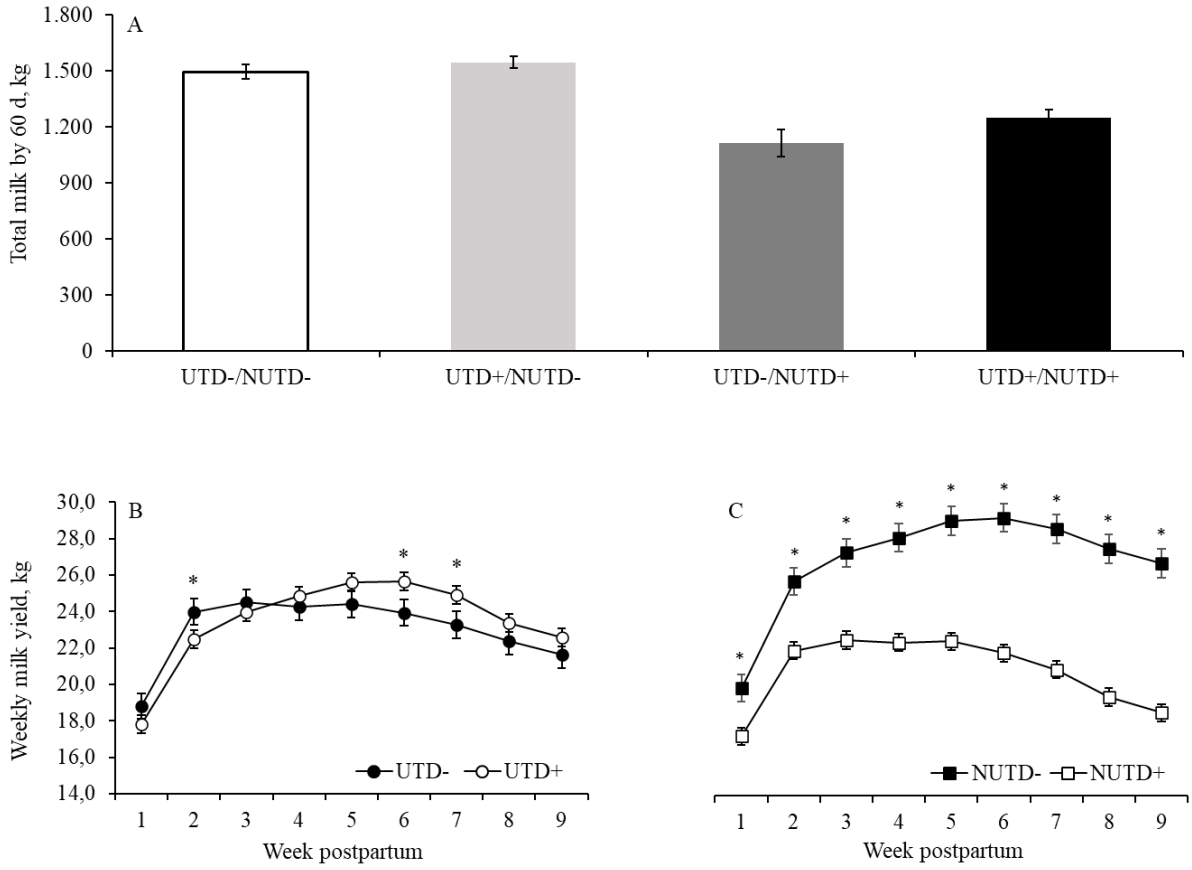

Figure 2. Cumulative milk yield (A) in the first 60 d postpartum according to uterine diseases (UTD) and nonuterine diseases (NUTD), and weekly milk yield in cows according to diagnosis of UTD (B) or NUTD (C). Crossbred Holstein-Gyr cows were classified according to diagnosis of UTD or NUTD in the first 60 d postpartum into 4 groups: UTD-/NUTD- = UTD negative and NUTD negative; UTD+/NUTD- = UTD positive and NUTD negative; UTD-/NUTD+ = UTD negative and NUTD positive; and UTD+/NUTD+ = UTD positive and NUTD positive. (A) Effects of UTD ($P = 0.03$), NUTD ($P < 0.001$) and interaction between UTD and NUTD ($P = 0.30$). (B) Effects of UTD ($P = 0.44$), week ($P < 0.0001$), and interaction between UTD and week ($P = 0.008$). (C) Effects of NUTD ($P < 0.001$), week ($P < 0.0001$), and interaction between NUTD and week ($P < 0.0001$). Within week, * denotes difference between groups ($P < 0.05$). Error bars represent SEM.

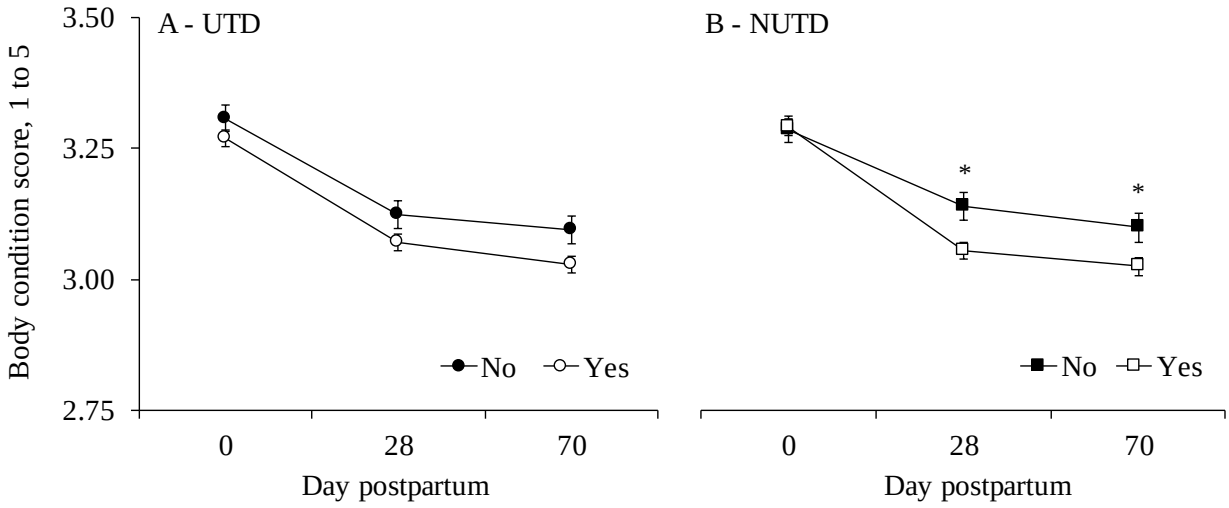

Figure 3. Body condition score of cows according to uterine diseases (UTD; A) or nonuterine diseases (NUTD; B) in the first 70 d postpartum. (A) Effect of UTD (P = 0.03) and interaction between UTD and day (P = 0.70). (B) Effect of NUTD (P = 0.04) and interaction between NUTD and day (P = 0.006). Within day, * denotes difference between groups ($P < 0.05$). Error bars represent SEM.

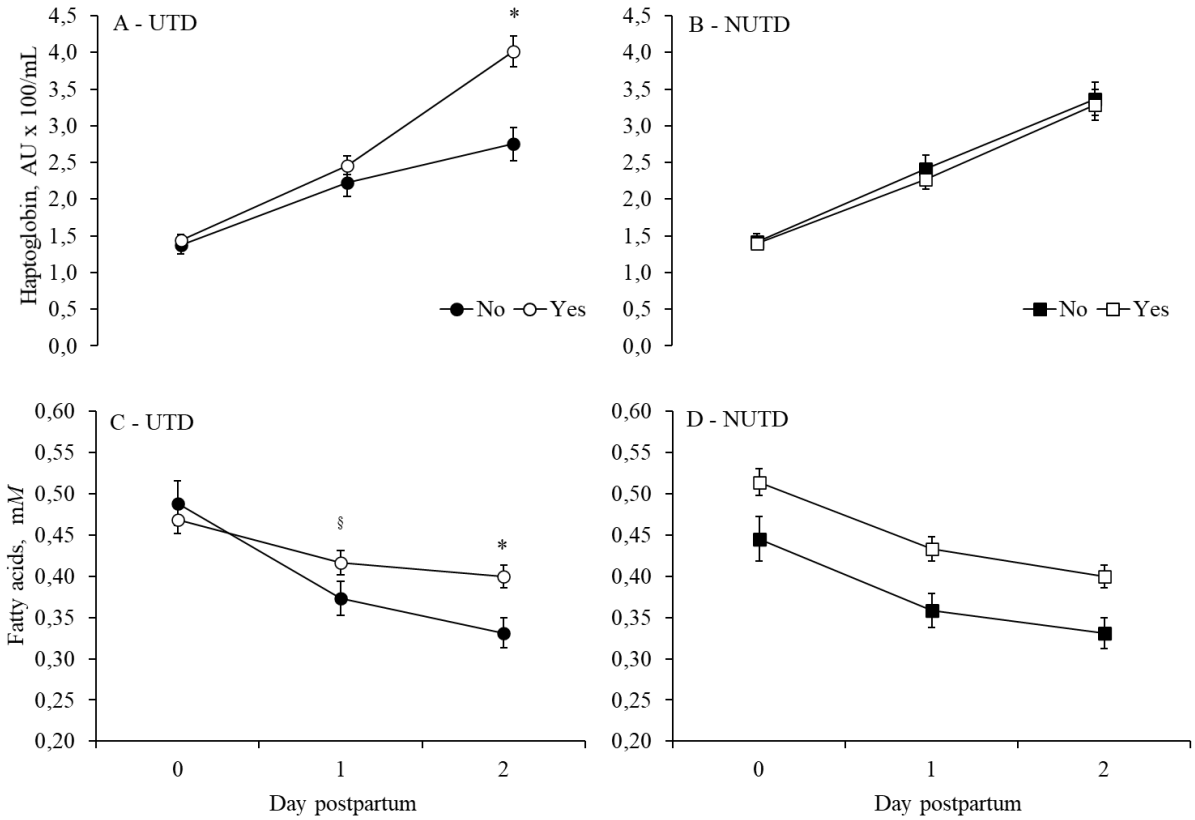

Figure 4. Serum concentrations of haptoglobin according to uterine diseases (UTD; A) or nonuterine diseases (NUTD; B), and serum concentration of fatty acids according to UTD (C) or NUTD (D). (A) Effect of UTD (P = 0.03) and interaction between UTD and day (P = 0.002). (B) Effect of NUTD (P = 0.66) and interaction between NUTD and day (P = 0.79). (C) Effect of UTD (P = 0.10) and interaction between UTD and day (P = 0.008). (D) Effect of NUTD (P = 0.0008) and interaction between NUTD and day (P = 0.76). Within day, * denotes difference between groups (P < 0.05); within day, § denotes a tendency for difference between groups (P = 0.09). Error bars represent SEM.

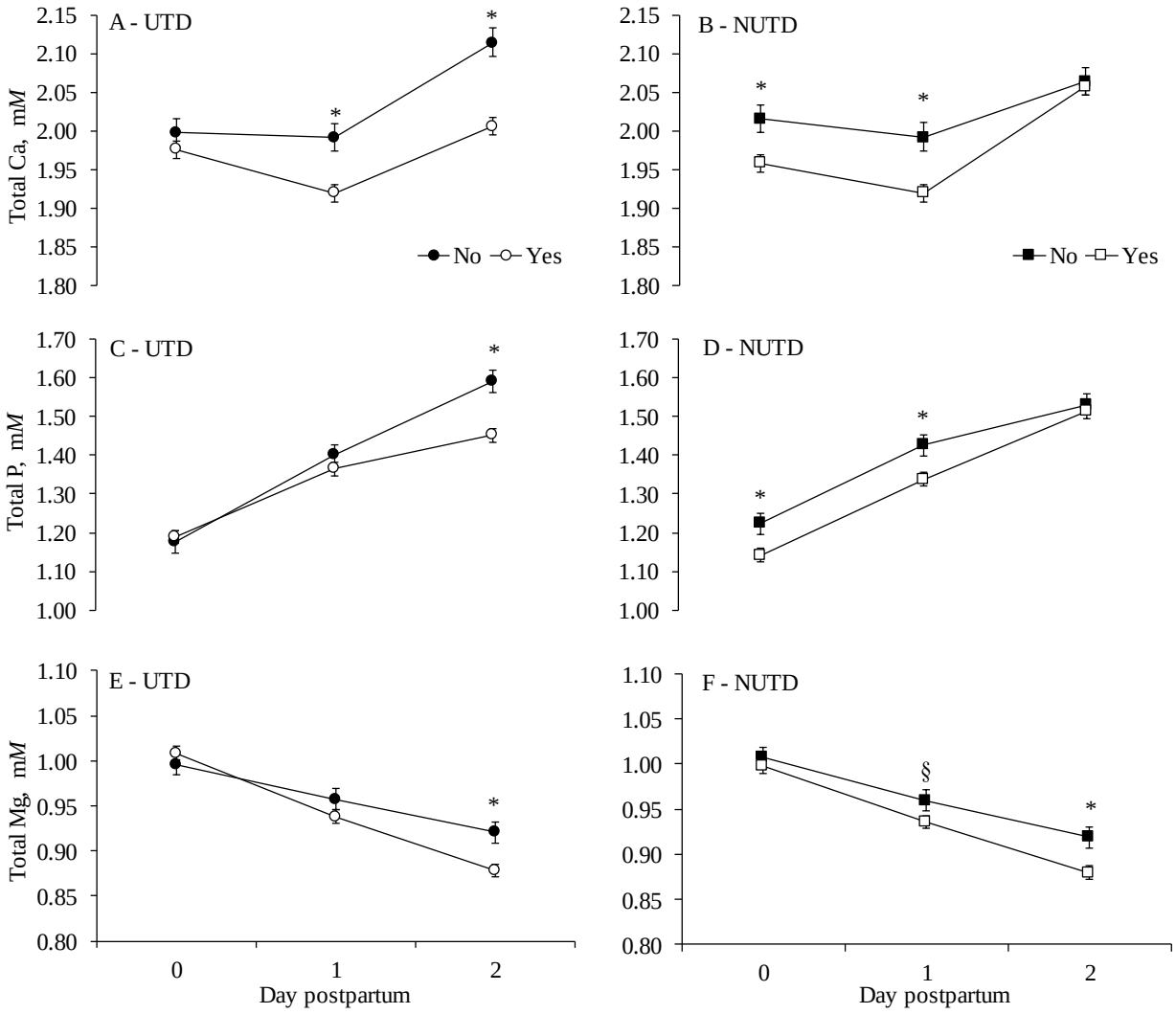

Figure 5. Serum concentrations of total Ca according to uterine diseases (UTD; A) or nonuterine diseases (NUTD; B), serum concentrations of total P according to UTD (C) or NUTD (D), and serum concentrations of total Mg according to UTD (E) or NUTD (F). (A) Effect of UTD ($P = 0.0001$) and interaction between UTD and day ($P = 0.003$). (B) Effect of NUTD ($P = 0.009$) and interaction between NUTD and day ($P = 0.004$). (C) Effect of UTD ($P = 0.03$) and interaction between UTD and day ($P = 0.0007$). (D) Effect of NUTD ($P = 0.01$) and interaction between NUTD and day ($P = 0.12$). (E) Effect of UTD ($P = 0.15$) and interaction between UTD and day ($P = 0.0008$). (F) Effect of NUTD ($P = 0.03$) and interaction between NUTD and day ($P = 0.12$). Within day, * denotes difference between groups ($P < 0.05$) and § denotes a tendency to differ ($P = 0.08$). Error bars represent the SEM.

IMPLICAÇÕES

Está bem estabelecido que doenças do periparto estão associadas com eventos reprodutivos e vacas que desenvolvem doença tem menor probabilidade de se tornarem gestantes ao recebem IA, embora os mecanismos subjacentes sejam complexos e múltiplos. Menos se sabe sobre o impacto das doenças inflamatórias na manutenção da prenhez após TE, que contorna as falhas nos primeiros estágios necessários para o estabelecimento da prenhez. Além disso, segregar doenças inflamatórias naquelas que afetam o útero ou afetam outros tecidos pode trazer informações sobre os potenciais mecanismos subjacentes que estão diretamente ligados ao trato reprodutivo ou aos efeitos sistêmicos da inflamação.

É importante observar que o manejo reprodutivo das vacas no presente estudo foi todo baseado em TE em tempo fixo, com ciclo estral e ovulação sincronizadas, que é esperado contornar os impactos que as doenças podem ter na expressão do estro, na competência e desenvolvimento folicular e oocitária, fertilização e desenvolvimento embrionário durante os primeiros 7 dias de vida. Portanto, o presente estudo nos permitiu a avaliação do impacto das doenças exclusivamente na manutenção da gestação após o dia 7 de prenhez, independente dos estágios iniciais necessários para estabelecer a prenhez em vacas leiteiras. Além disso, sincronizar o ciclo estral pela indução da ovulação deve minimizar o impacto que as doenças têm em aumentar a prevalência de vacas anovulares.

Como de praxe em estudos populacionais envolvendo doenças, o presente estudo é de corte prospectivo, o que nos permitiu estabelecer associações entre doenças durante o início da lactação com a prenhez por TE ou o intervalo entre o parto até a prenhez. Apesar de associativo, é hipotenizado que haja uma relação de causa e efeito entre doenças e a redução no desempenho reprodutivo aqui observado. Estudos futuros deverão ser feitos para investigar de forma mais especifica a biologia da subfertilidade atribuída as doenças inflamatórias, se o momento de ocorrência e a origem das doenças uterina ou não uterina afetam a fertilidade de maneira diferente.