UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS DE BOTUCATU

FATORES QUE AFETAM A EXPRESSÃO DE ESTRO DETECTADO POR MONITORES DE ATIVIDADE E RISCOS DE CONCEPÇÃO EM VACAS LEITEIRAS LACTANTES

AUGUSTO MESQUITA LACERDA MADUREIRA

Trabalho de apresentado ao programa de Pós-Graduação em Zootecnia como parte das exigências para obtenção do título de Mestre

BOTUCATU - SP

Fevereiro – 2016

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“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê. ” (Arthur Schopenhauer)

“A menos que modifiquemos à nossa maneira de pensar, não seremos capazes de resolver os problemas causados pela forma como nos acostumamos a ver o mundo”. (Albert Einstein)

“A persistência é o menor caminho do êxito”. (Charles Chaplin)

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LISTA DE ABREVIATURAS

AAM – Sistemas automatizados de monitoramento de atividade

AGNE – Ácidos graxos não esterificados

BEN – Balanço energético negativo

BO – Boumatic Heat-Seeker®

CL – Corpo lúteo

DEL – Dias em lactação

DU – Duração da atividade física

DP – Desvio padrão

ECC – Escore de condição corporal

E2 – Estradiol

FSH – Hormônio folículo estimulante

GnRH - Hormônio liberador de gonadotrofina

HT - Heatime®

IA – Inseminação artificial

IATF – Inseminação artificial em tempo fixo

LH – Hormônio luteinizante

P4 - Progesterona

PA – Pico de atividade física

P/IA – Prenhez por inseminação artificial

PGF2α – Prostaglandina F2α

ROC – Receiver operating characteristic

VPP – Valor preditivo positivo

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CAPITULO 1

CONSIDERÇÕES INICIAIS

1. Introdução

A detecção de estro, é um fator fundamental para se obter sucesso em programas reprodutivos (STEVENSON, 2001), pois, a ovulação em bovinos leiteiros ocorre por volta de 30 horas após o início do comportamento de estro (ROELOFS et al., 2005). A aceitação de monta é o principal evento de estro (ROELOFS et al., 2010), e a frequência desse comportamento tem diminuído com a alta produção de leite (LOPEZ et al., 2004; RIVERA et al., 2010).

A produção de leite tem sido negativamente correlacionada com o desempenho reprodutivo, embora, uma análise mais cuidadosa sugere que alta produção é compatível com boa fertilidade (SANTOS et al., 2008). O sistema de produção (manejo, nutrição e seleção genética) que é necessário para a alta produção de leite impôs diversas circunstâncias para os animais que podem afetar sua capacidade de emprenhar e manter a gestação, independentemente do nível de produção de leite. Isto foi devidamente questionado por LeBlanc (2010): fertilidade de fato está em declínio, ou as práticas atuais não são adequadas para os animais modernos?

Animais de alta produção têm fluxo sanguíneo hepático aumentado e, consequentemente, alto metabolismo de hormônios esteroides (SANGSRITAVONG et al., 2002; VASCONCELOS et al., 2003). Esses animais apresentam menores concentrações circulantes de estradiol, o que pode ser uma possível causa para menor expressão e intensidade de estro (LOPEZ et al., 2004; WILTBANK et al., 2006; ROELOFS et al., 2010).

O período de transição em vacas leiteiras também tem um papel importante no sucesso reprodutivo e pode afetar a intensidade da expressão do comportamento de estro. Animais após o parto passam por um balanço energético negativo (BEN) acentuado, o qual tem um efeito negativo na fertilidade, afetando os perfis metabólicos e endócrinos no fígado, ovário e funcionalidade do útero (WATHES et al., 2007b). Animais que apresentam um intenso BEN, apresentam menor concentrações de glicose, insulina e os fator de crescimento semelhante a insulina (IGF-I), como consequência uma menor frequência de pulsos de LH, que diminuirá a síntese de estradiol do folículo preovulatório (BUTLER, 2003) e consequentemente o comportamento de estro. IGF-I é um fator de crescimento essencial para a síntese de estradiol e de crescimento folicular (GARNSWORTHY et al., 2008) e esta correlacionado com interações funcionais no ciclo

estral e comportamento sexual (CARDONA-GÓMEZ et al., 2002; WOELDERS et al., 2014).

A inquietação do animal é comumente citada como uma característica secundária de estro em bovinos leiteiros (VAN EERDENBURG et al, 1996; ROELOFS et al, 2010) e contribui para o aumento da atividade física (JONSSON et al., 2011). A necessidade de tecnologias mais eficientes e confiáveis para a detecção de estro tem levado ao desenvolvimento de monitores de atividade que baseiam se no aumento relativo na atividade física do animal (KIDDY, 1977; ROELOFS et al, 2010) em vez da atividade de monta e outros sinais secundários (JÓNSSON et al., 2011). Estudos recentes utilizando monitores de atividade mostram taxas de detecção de estro entre 74,6 % e 90% (LOVENDAHL e CHAGUNDA, 2010; AUNGIER et al, 2015, respectivamente), demonstrando o benefício desta tecnologia.

Os efeitos da condição metabólica do animal e a dinâmica ovariana na P/IA tem sido extensivamente estudada em protocolos de inseminação artificial em tempo fixo (IATF), mas há dados limitados disponíveis de tais efeitos sobre a intensidade de estro, assim como também da intensidade de estro na fertilidade das vacas inseminadas. Informações quantitativas sobre a intensidade do estro, somente possíveis quando utilizados os dados dos monitores de atividade, poderiam ser usadas para melhorar a qualidade da detecção de cio, prever os resultados na prenhez, e especialmente ser usada como ferramenta estratégica para melhorar a tomada de decisões em programas reprodutivos (ex: utilização de sêmen ou embrião de melhor qualidade, re-sincronização, avaliação historica do animal, etc).

2. Revisão de Literatura

2.1.Fisiologia do Ciclo Estral

O ciclo estral, é um período cíclico de atividade ovariana, fase em que o animal esta receptível sexualmente (comportamento de estro) começando desde a puberdade (FORDE et al., 2011). Bovinos são poliestrais, tem duração do ciclo estral de 18 a 24 dias e demonstram comportamento de estro em média a cada 21 dias. O ciclo estral possui uma fase lútea de 14 a 18 dias de duração quando ocorre a formação do corpo lúteo (CL),

seguido por uma fase folicular de 4 a 6 dias na qual ocorre a maturação final e ovulação do folículo dominante. O estro ocorre devido a uma influência específica de hormônios esteroides ovarianos em centros comportamentais no cérebro de mamíferos. Os hormônios reguladores do ciclo estral são sintetizados em quatro locais: o hipotálamo (hormônio liberador de gonadotrofina; GnRH), na hipófise [hormônio folículo estimulante (FSH) e hormônio luteinizante (LH)], nos ovários [progesterona (P4) e estradiol (E2)] e no útero [prostaglandina F2α (PGF2α)] e funcionam por uma série de mecanismos de regulação.. O aumento do FSH, que promove o crescimento e proliferação das células foliculares, inicia duas ou três ondas de crescimento folicular por ciclo estral (ADAMS et al., 1992; SUNDERLAND et al., 1994) e cada onda é composta por: emergência de um grupo de folículos, seleção, dominância, atresia ou ovulação do folículo dominante (FORDE et al., 2011).

Somente alguns folículos continuam a crescer, enquanto que os demais sofrem atresia. Geralmente, quando o maior folículo em crescimento atinge um diâmetro de 8,3 a 9,0 mm (GINTHER et al. 1996; SARTORI et al. 2001), ocorre o desvio folicular, caracterizando a seleção do folículo dominante. O folículo dominante continua em desenvolvimento, o FSH induz a ativação de outros fatores de crescimento, aumentando a concentração de IGF-1 disponível para o crescimento celular e síntese de estradiol (RIVERA e FORTUNE, 2003; LUCY, 2007). O estradiol é sintetizado após o LH se ligar ao seu receptor na membrana da célula da teca, onde os andrógenos serão sintetizados nas células da teca a partir do colesterol e aromatizados (enzima aromatase P450arom, CYP19) em estradiol nas células da granulosa (Forde et al., 2011). A concentração de estradiol no fluido folicular do folículo dominante é duas vezes maior quando comparado com um folículo subordinado do mesmo diâmetro (Mihm et al., 2000).

A elevada concentração de estradiol que causa o comportamento de estro também é responsável pela supressão do FSH, o qual a onda original do folículo depende para o crescimento (SUNDERLAND et al., 1994; GINTHER et al., 2000a; b). Ao contrário dos folículos menores, que regridem, o folículo dominante pode funcionar independentemente de FSH pela presença de receptores de LH desenvolvidos nas células da granulosa, se tornando responsivo também ao LH (XU et al., 1995). A ovulação ocorre por volta de 30 horas (ROELOFS et al.,2005a) após o início do comportamento de estro, quando E2 promove feedback positivo no hipotálamo e na pituitária anterior, estimulando a liberação de GnRH e LH, e na maturação e ovulação do folículo dominante (ROCHE,

1996; WALKER et al., 1996). Após a ovulação, o LH intercede na formação do corpo lúteo (CL) a partir das células da teca e da granulosa do folículo dominante ovulado (FORDE et al., 2011). O CL produz alta concentração de P4 que é fundamental para a manutenção da gestação (SENGER, 2005).

A alta concentração de P4 durante a fase lútea impede a ocorrência de pulsos frequentes de LH que resultariam na ovulação de um folículo dominante (SENGER, 2005). Folículos dominantes que se desenvolvem durante esse período entram em atresia (FORDE et al., 2011). O reconhecimento embrionário é sinalizado pela produção de intereron tau (IFNT) pelo concepto, o IFNT age de forma parácrina inibindo a expressão de receptores de estrógeno e ocitocina evitando assim a liberação de pulsos de PGF2α e consequentemente evitando a luteólise (ANTONIAZZI, 2011). Se o reconhecimento materno não for sinalizado, o que ocorre por volta do dia 16 do ciclo, ocorre a luteólise do CL via troca em contracorrente de PGF2α na veia uterina para a artéria ovariana (SENGER, 2005; FORDE et al., 2011). Com a regressão do CL, ocorre uma drástica diminuição na concentração de P4, que resulta no aumento de GnRH permitindo assim, que o animal retome a fase folicular do ciclo estral (SENGER, 2005; FORDE et al., 2011).

2.2.Comportamento de Estro

Em mamíferos, estro é um sintoma e uma estratégia visual para garantir que a fêmea esteja receptiva à monta no momento próximo a ovulação (ROELOFS et al. 2010). A detecção de estro em bovinos leiteiros não é um problema recente e é um dos maiores problemas na indústria leiteira Norte Americana (HELMER e BRITT, 1985). Senger, (1994) relatou uma perda anual de US$ 300 milhões na indústria leiteira pela falha na detecção de estro, que tem sido correlacionada a perdas econômicas devido a intervalos de parto prolongados, perdas na produção de leite, custos veterinários, entre outros (NEBEL e JOBST, 1998). Portanto, a detecção de estro é fundamental em programas reprodutivos, pois a ovulação do folículo dominante ocorre por volta de 30 horas após a detecção deste comportamento (ROELOFS et al., 2005a).

Diversos autores descreveram o comportamento expresso por bovinos leiteiros durante o estro (WILLIAMSON et al., 1972; HURNIK et al., 1975; WALTON e KING,

1986; VAN VLIET e VAN EERDENBURG, 1996; LYIMO et al., 2000; SENGER, 2005). Williamson et al. (1972) realizaram um estudo em um rebanho leiteiro comercial para descrever os sinais de estro e o principal comportamento observado, foi a aceitação de monta, observada em 79% dos animais. Outros autores também descreveram o sinal de monta como sinal primário de estro (ALBRIGHT e ARAVE, 1997; ROELOFS et al., 2010).

Hurnik et al. (1975) mostraram que 90% dos animais observados demonstraram aceitação de monta. Por outro lado, Lyimo et al. (2000) observaram que apenas 53% dos animais tiveram aceitação de monta por detecção visual de estro, e o mesmo foi encontrado por LeBlanc et al. (1998), utilizando animais sincronizados. Van Vliet e Van Eerdenburg (1996) detectaram esse comportamento em somente 37% dos episódios de estro. Apesar de em todos os estudos anteriores os animais estarem alocados em um mesmo sistema (free-stall), houveram variações nas taxas de detecção de estro. A utilização de diferentes métodos para a definição do estro pode ser um fator relacionado a essas variações de resultado.

Reimers et al. (1985) mostraram a relação existente entre sinais de estro e a taxa de concepção na primeira inseminação, animais que aceitaram monta tiveram maior taxa de concepção quando comparados com aqueles que não expressaram esse comportamento. Gwasdauskas et al. (1986) relataram que inseminação artificial sem a aceitação de monta resultou em uma menor prenhez por inseminação (P/IA), aproximadamente 20% menor do que quando a aceitação de monta foi detectada.

O número de montas tem sido observado em diversos estudos. Britt et al. (1986) utilizando o método da observação visual estimaram que o número de montas foi de 3,7 ± 0,3 quando as vacas estavam alocadas em piquetes e 2,5 ± 0,2 quando as vacas estavam no concreto. Walker et al. (1996), usando o sistema HeatWatch, relataram que o número de eventos de monta por estro foi 10,1 ± 9,9 com 6 ± 7,2 montas ≥ 2 segundos de duração e uma duração total de monta de 24,1 ± 29,4 segundos. O total da duração de estro, estimado em somente 24,1 segundos, faz com que sinais secundários de estro sejam igualmente importantes.

O aumento da frequência de sinais secundários de estro, foi observado durante 6 horas antes da aceitação de monta (SVEBERG et al., 2011). As observações de sinais secundários de estro podem ser importantes para a melhora na detecção de estro (VAN

VLIET e VAN EERDENBURG, 1996) especialmente onde a aceitação de monta não é frequentemente observada.

Em 1927, John Hammond relatou que a duração do estro de vacas leiteiras era de 19,3 h, e um estudo realizado por Pennington et al. (1985), na década de 80 relataram duração semelhante, no entanto, experimentos mais recentes relatam um declínio acentuado na duração do estro (AT-TARAS e SPAHRS, 2001; LOPEZ et al., 2004; ROELOFS et al., 2005; SVEBERG et al., 2011).

Lopez et al. (2004) viram que a intensidade do estro era menor em vacas de alta produção. Vacas holandesas foram divididas em dois grupos com base na produção de leite e foi observado que a duração média do estro nas vacas de alta produção (46,4 kg leite/dia, n=146) era de apenas 6,2 h, 4,7 h menos do que a duração do estro no grupo de vacas com produção mais baixa (33,5 kg/dia, n=177) (LOPEZ et al.,2004). Vacas leiteiras de alta-produção têm maior fluxo sanguíneo hepático e, consequentemente, alto metabolismo de hormônios esteroides (SANGSRITAVONG et al., 2002; VASCONCELOS et al., 2003). Como resultado, esses animais podem apresentar uma diminuição nas concentrações circulantes de estradiol, o que poderia gerar menor expressão e menor intensidade de estro (LOPEZ et al., 2004; RIVERA et al., 2010; ROELOFS et al., 2010). Entretanto, como relatado por Van Eerdenburg, (2008), não houve uma correlação entre expressão de estro e produção de leite, mostrando que produção de leite pode ser relacionado com alta fertilidade em bovinos leiteiros lactantes (LÓPEZ-GATIUS et al. 2006).

2.3. Desenvolvimento de Sistemas de Monitoramento de Atividade

O aumento de atividade associado com a expressão de estro foi primeiro observado em ratos em 1923 (WANG, 1923), em bovinos leiteiros foi primeiramente descrito por Farris (1954). Kiddy (1977) avaliou a média de variação na atividade física em vacas de leite utilizando pedômetros para humanos e relatou que durante o estro o aumento de passos foi quatro vezes maior quando comparado com ao o período de não estro. Esses dois estudos foram a base para o desenvolvimento dos sistemas de monitoramento de atividade para a detecção de estro.

Existem dois tipos de monitores de atividade atualmente disponíveis: 1) pedômetros, que são usualmente utilizados na perna do animal e 2) acelerômetros, que tem sido utilizado no pescoço, perna ou orelha (SAINT-DIZIER E CHASTANT-MAILLARD, 2012). Pedômetros medem o número de passos dados e acelerômetros medem um movimento tridimensional, estimando a atividade geral do animal (FRICKE et al., 2014b).

O Heatime® (SCR Engineers, Natanya, Israel) é um sistema de monitoramento de atividade física. É um acelerômetro que calcula a atividade física conforme o movimento do animal. Calcula a média da atividade física de um período de sete dias, que é usada como o período de referência e é comparada com o mesmo intervalo de tempo do dia atual. Em outras palavras, o padrão de atividade para o dia 8 é comparado com a atividade média dos dias 1 a 7, que é dividida em intervalos de tempo (BAR, 2010). Afimilk ®, outro sistema de monitoramento de atividade, usa o valor médio de 10 dias anteriores (GALON, 2010). Este período de referência é incluído no cálculo para a detecção do alarme de estro de cada modelo comercial. Para definir o aumento de atividade física associado com estro, é preciso um período basal de referência, e então usá-lo como padrão para detectar o desvio padrão e o aumento relativo da atividade física do animal. Outros exemplos de monitores de atividade são: ALPRO (DeLaval; Sweden), SmartDairy Activity (Boumatic, USA), IceTag (IceRobotics,UK).

Alguns sistemas de atividade são baseados em uma tecnologia de "interruptor" de mercúrio. Um contador está ligado a este sistema, e conta o número de passos dependendo do movimento do animal (FIRK et al., 2002). Este sistema é provavelmente atrasado, quando comparado com as tecnologias mais recentes que utilizam acelerômetros que são comumente usadas em dispositivos eletrônicos como iPods, smartphones, GPS, etc. e baseiam-se em forças gravitacionais. Além do período de referência e metodologia para coleta de dados de atividade, a unidade de processamento de dados também incorpora um algoritmo usado na tradução dos dados obtidos. Os sistemas comerciais empregados atualmente comparam o nível atual de atividade com algum padrão médio ou desvio padrão da atividade basal de cada animal (ERADUS et al., 1992).

Estudos mostram a eficiência dos monitores de atividade em detectar estro. A porcentagem detectada pelos monitores de atividade varia entre 51 e 90% tanto em sistema de confinamento quanto em sistema à pasto (LEWIS e NEWMAN, 1984; REDDEN et al., 1993; ROELOFS et al., 2005a; HOCKEY et al., 2009; KAMPHUIS et

al., 2012; VALENZA et al., 2012; AUNGIER et al., 2015). Fricke et al., (2014b) relataram que os monitores podem detectar precisamente 70% dos animais em estro.

A sensibilidade dos monitores em detectar estro tem sido questionada, pois, podem existir falsos positivos (animais que são detectados, mas não estão em estro) e negativos (animais que não são detectados, mas estão em estro). Aungier et al., (2012) relataram grande número de falsos positivos, enquanto outros autores indicaram que uma maior sensibilidade dos monitores pode ser um grande problema (KAMPHUIS et al., 2012; VALENZA et al., 2012). Eventos falsos positivos aparentemente tem menor duração e intensidade, quando comparado com eventos verdadeiros de estro. Eventos verdadeiros tem uma relação positiva com pico de atividade e duração (AUNGIER et al., 2012). O uso de mais de uma medida, obtida pelos monitores de atividade, pode ajudar a reduzir falsos positivos (FIRK et al., 2012). Falsos negativos não serão detectados até o próximo ciclo estral, pois aquele estro não detectado só será detectado aproximadamente 21 dias depois desse episódio, quando o animal apresentar comportamento de estro novamente. Por isso, falso negativo representa um problema maior em relação aos eventos falso positivos.

2.4. Fatores que afetam a Expressão de Estro

Poucos estudos têm avaliado como o fator animal e o manejo do rebanho podem afetar a detecção de estro baseado na atividade física do animal. Arney et al. (1994) avaliaram os efeitos da produção de leite, paridade, estágio de lactação e nutrição na atividade de física. Não houve nenhuma associação entre a produção de leite, idade de vaca ou número de partos com a atividade física durante o estro, mas atividade diminuiu conforme o estágio da lactação avançou.

Recentemente, López-Gatius et al. (2005) relataram que produção de leite e número de partos estão significativamente associados com a atividade física. No estudo relataram que um aumento de 1 Kg na produção de leite e com um parto adicional, a atividade física no momento do estro foi reduzida em 1,6% e 21,4%, respectivamente. Ao contrário, Walker et al. (1996) descreveram que a duração do estro foi quase 50% mais curta para primíparas do que para vacas multíparas em lactação. Outras pesquisas mostraram que primíparas têm uma duração significativamente mais longa de estro (13,6

vs 10,8 h) e um estro mais intenso quando comparado com multíparas (ROELOFS et al., 2005b).

Sartori et al., (2004) compararam novilhas e multíparas e relataram que animais em lactação tiveram uma menor concentração de estradiol e progesterona e relataram que novilhas tiveram menor tamanho de folículos pré-ovulatórios e menor tecido luteal quando comparado com animais em lactação. Mesmo as estruturas ovarianas sendo maiores as concentrações de estradiol (7,9 vs 11,3 pg/ml) e progesterona (5,6 vs 14,9 ng/ml) foram menores em multíparas. A baixa concentração desses hormônios está diretamente relacionada com a alta taxa metabólica de animais com alta produção de leite e/ou alta taxa de ingestão (SANGSRITAVONG et al., 2002; VASCONCELOS et al., 2003).

Müller e Schrader (2005) investigaram a consistência da atividade física em duas lactações seguidas de 35 vacas Holandesas. Os resultados mostraram consistência no comportamento de atividade entre lactações, o que não foi influenciado por semana de lactação, ordem da lactação ou condições ambientais (temperatura ambiente e luz). No entanto, atividade só foi medida a partir de 105 dias em lactação, perdendo possíveis diferenças na atividade física no começo da lactação, quando o *status* metabólico podem diferir significativamente.

A alta produção de leite e consequente demandas adicionais de energia no início da lactação não podem ser atendidas através de dieta por si só, essencialmente a vaca deve obter os nutrientes para atingir os níveis de energia satisfatórios a partir de suas próprias reservas corporais, levando aos animais um estado temporário de balanço energético negativo (BEN; WATHES et al., 2007). Um balanço energético negativo severo, leva potencialmente ao atraso do retorno do ciclo estral em bovinos leiteiros e por consequência reduz o comportamento de estro, que é dependente do estrógeno (WATHES et al., 2007 e CHAGAS et al., 2007). Aungier et al. (2012), relataram que um aumento de 0,25 no escore de condição corporal (ECC) foi correlacionado significativamente com um aumento na atividade física antes da ovulação. Vacas que perderam menos de 100kg do peso vivo entre duas semanas pré-parto até cinco semanas pós-parto tiveram maior atividade nos dois primeiros eventos de estro pós-parto (BURNETT et al., 2015).

Arney et al. (1994) avaliaram o efeito do consumo de matéria seca e atividade física no momento do estro em 18 vacas holandesas divididas em grupos de alta e baixa energia. O nível de atividade durante o estro não foi diferente entre os dois grupos.

Løvendahl e Chagunda (2010) relataram que vacas com baixo escore de condição corporal tiveram maior intervalo entre o parto e a primeira atividade de estro, e a correlação entre o escore de condição corporal e a atividade física entre 90 a 120 dias em lactação (DEL) foi positiva.

Outro fator importante para expressão de estro é o número de animais em estro no mesmo período, Hurnik et al. (1975) demonstraram que quando mais de um animal está em estro no mesmo período, o número de montas aumenta consideravelmente de 11, 2 para 52, 6 montas por estro quando três animais estavam em estro simultaneamente. Roelofs et al. (2005a) demonstraram que o número de animais em estro resultou em um aumento de passos dados durante o estro. Embora este aumento não tenha sido significativo, a taxa de detecção de estro por pedômetros foi significativamente maior, quando mais de um animal estava em estro no mesmo período (67% quando apenas um animal estava em estro, contra 85% e 95%, quando dois ou mais de dois animais estavam em estro simultaneamente, respectivamente). Interações sociais provavelmente ocorreriam mais quanto mais vacas estivessem em estro ao mesmo tempo, aumentando sua atividade e, portanto, a probabilidade de detecção de estro.

A produção de leite é comumente relatada por influenciar diversos aspectos de desempenho reprodutivo. Lactação induz alterações metabólicas, tais como o BEN, que podem afetar os tecidos ovarianos, útero e outros tecidos que podem contribuir para o declínio histórico da performance reprodutiva (LUCY, 2001; WILTBANK et al., 2006). Vacas que produzem mais leite podem ter um BEN acentuado, o que aumentará o nível de ácidos graxos não esterificados (AGNE), que está correlacionado com baixa atividade física (VAN VLIET e VAN EERDENBURG, 1996). Embora não houve nenhuma correlação entre produção de leite e expressão do estro (VAN EERDENBURG, 2008), o *clearance* metabólico dos hormônios esteroides relacionados com alta produção de leite (SANGSRITAVONG et al., 2002) provavelmente reduz a manifestação comportamental do estro. Em contradição com Van Eerdenburg, (2008) um estudo realizado por Lopez et al. (2004a) relataram que animais que produzem mais leite (> 39,5 kg/dia) tiveram uma menor concentração de estradiol no dia do estro, e duração do estro foi reduzida em comparação com animais de baixa produção (< 39,5 kg/dia). Yaniz et al. (2006) também relataram redução na atividade física em animais com maiores produções de leite, ordem da lactação e índice de temperatura e umidade.

Holman et al. (2011) relataram que alta produção de leite e baixa condição corporal pode afetar negativamente a sensibilidade dos monitores de atividade, e acrescentando que problemas de claudicação também pode afetar a leitura dos monitores. Entretanto, ordem da lactação, idade do animal, produção de leite, dias em lactação e temperatura ambiente, em outros estudos relataram não haver efeito na atividade física detectada pelos monitores de atividade (LYMIO et al., 2000; AT-TARAS e SPAHR, 2001; VAN EERDENBURG et al., 2002). Muitos aspectos da produção leiteira sofreram mudanças ao longo dos anos, portanto deve-se ter cuidado ao concluir relação de causa e efeito entre alta produção e redução no desempenho reprodutivo (LE BLANC, 2010), pois, animais que tem baixa produção de leite podem estar doentes, afetando o trato reprodutivo, enquanto animais de alta produção, normalmente são os mais saudáveis (SANTOS et al., 2009).

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CAPITULO 2

FACTORS AFFECTING EXPRESSION OF ESTRUS MEASURED BY ACTIVITY MONITORS AND CONCEPTION RISK OF LACTATING DAIRY COWS

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1. Interpretive Summary

Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. *By Madureira et al.* The effects of reproductive, productive and other cow-related factors on estrus expression and conception rate were analyzed. Factors such as pre-ovulatory follicle diameter at estrus were not correlated with estrus intensity and duration, whereas parity, body condition and secondary signs of estrus significantly affected estrus measurements. Milk production was weakly correlated with estrus intensity and duration, but according to milk production categorized into quartiles, higher producing cows had lower intensity estrus. High estrus intensity yielded greater conception rates compared with estrus of low intensity. Quantitative data from estrus expression might be used to survey and predict fertility measures in dairy cows.

Running head: ESTRUS EXPRESSION AND CONCEPTION RATE

Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cow s

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2. Abstract

The objective of this study was to determine risk-factors affecting increase in physical activity during estrus and pregnancy per AI (**P/AI**) in lactating dairy cows. Cows were monitored continuously by two automated activity monitors (a collar-mounted accelerometer [Heatime, SCR Engineers; **HT**] and a leg-mounted pedometer [Boumatic Heat-seeker-TX; **BO**]). When an increase in activity was detected, body condition score (BCS) and blood samples were collected, ovaries were scanned by ultrasonography and, if the cow was eligible for breeding, AI was performed. Milk production and health-related data were recorded throughout the experimental period. Pregnancy diagnosis was performed at 42 ± 7 d of gestation. Data was analyzed using Pearson's correlation, ANOVA and logistic regression with SAS. A total of 1,099 true events of estrus from 318 lactating Holstein cows were recorded, averaging 3.46 ± 1.1 events per cow. Positive predictive value (PPV) for estrus episodes detected by the HT and BO systems were 89.6% and 85.5%, respectively. Mean peak activity at estrus (**PA**) recorded by the HT system was 71.6 ± 20.7 index-value, and 334.3 ± 155.7% relative increase by the BO system. Compared to primiparous, multiparous cows expressed estrus with lower PA (69.3 ± 0.8 vs. 75.9 ± 1.1 [HT]; 323.9 ± 6.0 vs. 354.8 ± 8.48 [BO]) and shorter duration (**DU**; 10.7 ± 0.2 vs. 12.0 ± 0.3); DU was measured by HT only. Lower BCS was associated with decreased PA measured by both systems, estrus DU and P/AI. Peak activity was weakly correlated with milk production on the day of AI (r = -0.20), however, when categorized into quartiles, the highest yield cows had lower PA and DU. Follicle diameter was not correlated with PA or DU, but cows with greater concentrations of estradiol had higher PA. Cows with greater PA in both systems had greater P/AI than those with lower PA (36.5 vs. 24.6% [HT], 33.5 vs. 21.4% [BO]). In conclusion, measurements of estrus events captured by AAM are correlated with BCS, parity and secondary behavior signs related to estrus. Surprisingly, estrus intensity and duration were only weakly correlated with milk production, pre-ovulatory follicle diameter and concentrations of estradiol at estrus. Cows that had measurements of high intensity estrus were significantly more fertile than low intensity estrus.

Key Words: automated activity monitor, pregnancy per AI, milk production, estrus expression

3. Introduction

Studies have indicated the negative effect of milk production on standing to be mounted and total time standing (Lopez et al., 2004, Rivera et al., 2010), while others do not report such an effect (Lymio et al., 2000; Van Eerdenburg et al., 2002). The use of automated activity monitor (**AAM**) technology to assist estrus detection may be used to retrieve useful data regarding estrous behavior parameters. The effects of the animal's metabolic status and ovarian dynamics on pregnancy per AI (**P/AI**) has been extensively studied when using timed AI programs, but limited data is available of such effects on estrus behavior as measured by current AAM technologies and consequent fertility from cows inseminated upon natural or induced estrus.

High–yielding dairy cows have an elevated metabolic rate, which increases liver blood flow and metabolism of steroid hormones (Sangsritavong et al., 2002; Vasconcelos et al., 2003). As a result, these cows experience decreased circulating estradiol concentrations that could be a possible cause of shorter and less intense expression of estrus (Lopez et al., 2004; Rivera et al., 2010; Roelofs et al., 2010). Historically, mounting behavior and standing to be mounted have been considered the clearest indicator of an estrus event, but its expression tends to decrease with greater milk production (Lopez et al., 2004; Rivera et al., 2010). The need for more efficient and reliable technologies for estrus detection has led to the development of activity monitors that rely on the relative increase in physical activity of the animal (Jónsson et al., 2011) instead of mounting activity and other secondary signs. The technology currently used on dairy farms varies because different products measure different features of physical activity (e.g. accelerometers, pedometers, neck-mounted, leg mounted, ear tags). A recent study conducted in Denmark (Lovendahl and Chagunda, 2010) using AAM showed a 74.6% estrous detection rate and 1.3% daily error rate, demonstrating the advantages of this technology compared to visual detection. Lopez-Gatius et al. (2005) reported that the two main factors affecting activity increases were lactation number and milk production and that there is a small but positive correlation between intensity of activity and P/AI. Moreover, Holman et al. (2011) reported that milk production affects the overall sensitivity of activity monitors, but others have reported no correlation between milk yield and expression of estrus behavior detected by the AAM (Lymio et al., 2000; Van Eerdenburg et al., 2002).

Few of the previous studies measured detailed reproductive physiological parameters associated with natural estrus behaviors, and the subsequent intensity and duration of activity detected by AAM. The effects of pre-ovulatory follicle diameter, circulating estradiol concentrations, and even a better understanding of the effect of estrus intensity and duration on P/AI are still unclear. In addition, the technology used has evolved, sensors have become more sensitive, and different algorithms are used to transform the raw data, which can interfere with interpretation and correlation with phenotypical measurements.

The objectives of this study were to identify risk factors associated with measurements of estrus expression and P/AI. The effects of parity, BCS, milk production, stage of lactation, secondary signs of estrus, pre-ovulatory follicle diameter and concentration of estradiol in plasma at the moment of increased activity on the intensity and duration of estrus detected by AAM systems were analyzed. In addition, risk factors associated with P/AI, particularly those related with estrus outcomes (intensity and duration) were assessed. Two AAM systems commonly used on dairy operations in North America were used to correlate and compare the estrus outcomes with production, reproduction, and other cow-related measurements.

4. Materials and Methods

This experiment was conducted between April 2012 and April 2013 at the University of British Columbia's Dairy Education and Research Centre in Agassiz, BC, Canada (latitude: 49°13'59" N; longitude: 121°46'01"W). The local Institutional Animal Care Committee following the requirements of the Canadian Animal Care Council (CACC) approved all experimental procedures.

4.1. Animals, Housing and Management

Lactating Holstein cows (n = 346) were enrolled onto the study during the first wk post-partum. The experimental herd had an average 305-d mature equivalent milk yield of 12,236 ± 2,219 kg/cow in 2013.

This experiment was an observational cohort study. A total of 1,161 estrus events were recorded, with 3.5 ± 1.1 events per animal. Cows were housed in sand-bedded free stalls (2.4 m long x 1.18 m wide x 0.40 m deep; at least 1 stall/cow) with concrete flooring. Milking was performed twice daily at 0500 h and 1500 h. Cows were fed a TMR twice daily at approximately

0700 h and 1600 h (pushed up 3 times/d). The TMR was formulated to meet or exceed the requirements of a 620 kg Holstein cow producing 40 kg/day of 3.5% fat corrected milk (NRC, 2001). Water and TMR were available for *ad libitum* intake.

4.2. Automated Activity Monitoring

Cows were monitored continuously by two AAM: 1) a neck-mounted accelerometer (**HT**; Heatime®, SCR Engineers, Israel) and, 2) a leg-mounted pedometer (**BO**; Boumatic Heat-seeker-TX®; Boumatic Dairy Equipment Co., Madison, WI). The HT was fitted to the upper left side at the cranial portion of the cow's neck at 7 ± 3 DIM and removed if a positive pregnancy diagnosis was detected at 47 ± 3 d post-AI. The BO sensor was attached to the right-front limb above the distal expansion of the metacarpal bone of each cow on the day of their first calving and remained with the animals throughout their entire time within the herd.

Activity data from HT was recorded in real time every 2 h by a wireless receiver box, which transmitted the data to a receiving unit connected to the farm manager's computer. The raw activity data from each cow was computed into an index value that is closely associated with the SD of the PA (calculated using the raw PA value and baseline values of the previous 14 d. Index values ranged from 0 to 100 and the threshold for an episode of high activity was set at 35. The time of the onset and end of high activity, peak of activity, and activity duration, were obtained from the consecutive timeframe the animal's activity was above the threshold. The peak of physical activity index (**PA**) was defined as the maximum index value at an estrus episode. The duration of each episode (**DU**) was considered as the interval from the start h of the first 2-h block above the activity threshold to the h after the last 2-h block above the activity threshold. An Excel macro (Excel; Microsoft Corporation, Redmond, WA) was used to identify estrus events from the exported HT backup files. The macro-edited file contained information of cow number, collar tag number, date and h (start h of the first 2-h block), DU of estrus and index value of PA for each estrus episode. Data from HT was also used to calculate the standard deviation of estrus activity from the average raw of the previous 14 d.

Data from BO system was used for comparison purposes; only PA was measured. The pedometer contains a mercury switch that registers and records movements when its position has changed. The data was recorded in 2-h increments and was retrieved twice daily by an electronic scanner attached to an archway at the milking parlor's entrance. An estrus event was recorded when activity exceeded 80% of the cow's baseline activity. The PA for the BO system was described as the relative percent increase in activity from the cow's baseline activity levels.

4.3. Blood Sampling, Body Condition Score and Analysis of Estradiol

Blood samples (7 mL) were harvested from the median coccygeal vein or artery utilizing Vacutainer tubes (Becton & Dickinson Vacutainer systems, Rutherford, NJ) with K_2EDTA. Blood samples were collected immediately following an estrus alert (increase in physical activity that exceeded the threshold) from the HT system. The HT system updated the information in real-time every 2 h, which allowed for blood sample collection at the earliest possible time after initiation of estrus. All samples were placed immediately on ice, transported to the laboratory and centrifuged at 2,000 x g for 15 min for separation of plasma. Plasma samples were then stored at -80 °C to be analyzed for estradiol concentration. Plasma concentrations of estradiol were determined using a double-antibody I125-based assay developed as described by Burke et al., (2003) with modifications. Average intra-assay CV was 4.2%, and inter-assay CV (18 assays) for pooled plasma samples containing 2.7, 5.8, and 12.3 pg/mL of estradiol were 6.3%, 5.2% and 4.1%, respectively. The average sensitivity of the estradiol assay was 1.1 pg/mL. All cows had BCS (Edmonson et al., 1989) recorded each time an estrus episode was detected by the AAM. Secondary estrus signs were documented at the time of increased activity and through examination of the reproductive tract (signs of repeated mounting activity, clear vaginal mucus and uterine tonus).

4.4. Ultrasonography, Artificial Insemination and Pregnancy Diagnosis

Cows had their ovaries examined by ultrasound (Aloka SSD-500, Aloka Co. Ltd, Wallingford, CT, USA) using a 7.5 MHz linear rectal transducer, immediately following an episode of high activity detected by the AAM. The presence and diameter of the largest follicles were measured and recorded. The following reproductive management protocol was used throughout the trial: cows were artificially inseminated upon estrus detected by the activity monitor using the a.m./p.m. rule. All cows were examined with an ultrasound at 47 ± 3 days post-AI for the detection of a viable embryo.

4.5. Statistical Analyses

This experiment was an observational cohort study. A total of 1,161 estrus 194 events were recorded, with 3.5 ± 1.1 events per animal. Means, SD, distributions, and normality tests were obtained using Univariate procedure in SAS software version 9.4 (SAS Institute Inc., Cary, NC). Normality was visually assessed and confirmed by the Kolmogorov-Smirnov method. Class variables used for analyses were described below. Parity was divided as cows in first lactation or

second lactation and higher (primiparous vs. multiparous). BCS classes were Low: BCS ≤ 2.5, Moderate: BCS ≥ 2.75 and BCS ≤ 3.0, and High: BCS ≥ 3.25. Lactation stage of the estrus episode was defined by DIM at the moment of the event: Fresh ≤ 45 DIM; Lactation: 45 to 120 DIM, and Late: 121 to 250 DIM. Milk production was classified into quartiles as follows: 1st quartile: ≤ 31.3 kg/d, 2nd quartile: 31.4 to 38.8 kg/d, 3rd quartile: 38.9 to 45.1 kg/d, and, 4th quartile: > 45.1 kg/d. Expression of secondary signs of estrus (signs of repeated mounting activity, clear vaginal mucus and uterine tonus) was classified as Weak (1 secondary sign), Normal (2 secondary signs), or Strong (all 3 secondary signs). The PA of estrus episodes from the HT system was divided as Low = 35 to 89 index, and High = 90 to 100 index, whereas for the BO system it was divided as Low = 80 to 321% relative increase, and High = 321 to 999% relative increase. Receiver operating characteristic (ROC) analysis using MedCalc version 11.1.0.0 (MedCalc software, Mariakerke, Belgium) was performed to determine the critical value of PA (index and relative increase) to optimize P/AI based on sensitivity and specificity. The ROC curve analysis plots the sensitivity against the false positive fraction (1 - specificity) to detect the best combination of sensitivity and specificity for P/AI.

The positive predictive value (PPV; true estrus events/total estrus events 216 detected by the AAM system) of each system was calculated using total estrus episodes 217 detected by both systems combined (true and false events; based on presence of a pre- 218 ovulatory follicle larger than 10 mm in diameter and absence of an active corpus luteum [more than 20 mm in diameter]).

Correlations between PA and DU within and between systems were determined by Pearson's correlation using the Corr procedure of SAS. Correlations were also calculated similarly for the relationship between physiological measurements (BCS, diameter of pre-ovulatory follicle, and plasma estradiol concentration) and automated measurements (PA from HT and BO and DU from HT). Continuous data was analyzed by ANOVA using the GLIMMIX procedure of SAS, with cow nested within estrus event as the random error. Only independent variables significantly different at the univariable level were included in the final model. Dichotomous outcome, such as P/AI, were evaluated by logistic regression using the Logistic procedure and frequency statistics were calculated using the Freq procedure of SAS. The model used in the logistical regression included the effects of parity, BCS, stage of lactation, PA and DU category and interactions. A backward stepwise regression model was used (Allison, 1999). Variables were continuously removed from the model by the Wald statistic criterion if the significance was > 0.15.

Differences with $P < 0.05$ were considered significant and those between $0.05 > P < 0.10$ were designated as tendency. Different letters in the table indicate difference between variables within columns ($P < 0.05$)

5. Results

5.1. Animals and Number of Events

There were 1,161 events detected. HT system detected 1,041 episodes, whereas BO system detected 993 estrus events, where 948 events were detected by both systems. Analysis of PPV included all the detected events. The HT system had a PPV of 89.6% (933/1,041), whereas the PPV of BO system was 85.5% (849/993).

Estrus events were considered true events after confirmation of a presence of a pre-ovulatory follicle larger than 10 mm in diameter and absence of an active corpus luteum less than 20 mm in diameter measured by ultrasonography. Only true estrus events were used for the analyses regarding risk factors affecting PA and DU of estrus events. Among all detected events, 1,099 were true estrus events. From these 1,099 estrus events, 629 were followed by AI with subsequent pregnancy diagnosis. The average milk production was 38.6 ± 9.0 kg/cow/d. Cows had a mean lactation number of 2.6 ± 1.8 and were 99.4 ± 60.2 DIM at the time of data collection.

5.2. Description of Estrus Events

The mean PA recorded by HT system was 71.6 ± 20.7 (index value). HT index has no measurement unit as it translates the raw data from the system into a scale from 35 to 100. The index is closely associated with the SD of the PA (calculated using the raw PA value and baseline values of the previous 14 d) with an r = 0.54 ($P < 0.01$; Figure 1A). The distribution of PA measured by HT was skewed to the left. The PA from BO system was 334.3 ± 155.7% relative increase with a normal distribution pattern. The correlation between PA data of the two systems was r = 0.55 ($P < 0.01$; Figure 1B). Mean estrus DU as measured by HT system was 11.2 ± 5.3 h. The PA and DU measured by HT system were positively correlated (r = 0.82, P < 0.01; Figure 1C). For HT measurements, estrus of High PA had longer DU (16.4 ± 0.3 h vs 9.8 ± 0.1 h; P < 0.01) when compared with estrus of Low PA.

5.3. Factors Affecting Peak Activity and Duration of Estrus Events: Animal Data

Parity had a marked effect on estrus measurements as multiparous cows expressed estrus of lower PA ($P < 0.01$) and shorter duration ($P < 0.01$) than primiparous cows, as shown in Table 1. The BCS of the cow at time of estrus affected PA in both HT and BO systems and DU ($P < 0.01$; Table 1), as cows with BCS ≤ 2.5 showed less intense estrus measurements. Lactation stage did not influence PA and DU of estrus events ($P > 0.1$; Table 1). Cows that displayed secondary

signs of estrus had greater PA measured by both systems and longer DU ($P < 0.01$; Table 1). These measurements progressively increased as more secondary signs were displayed concomitantly. Information regarding parity, BCS, lactation stage, secondary signs of estrus and milk production effects on PA and DU is depicted in Table 1. Milk production was negatively correlated with PA (r = -0.20, $P < 0.01$; Figure 2). Greater milk production was associated with lower PA and shorter DU of estrus (Table 1).

5.4. Factors Affecting Peak Activity and Duration of Estrus Events: Ovarian Data

Pre-ovulatory follicle diameter (19.0 ± 5.7 mm) was weakly correlated with PA (HT: r = 0.07, $P = 0.03$; BO: r = 0.01, $P = 0.04$; Figure 3) or DU (r = 0.06, $P = 0.08$) of estrus events. Similarly, the diameter of the follicle did not differ ($P = 0.72$) between estrus events classified as High or Low PA from both AAM. The concentration of estradiol in plasma was only weakly correlated with HT and BO PA of estrus events (r = 0.02, $P < 0.01$), although greater estradiol concentration was found in cows with High PA, compared to Low PA (9.5 ± 0.3 vs. 8.2 ± 0.2 ng/mL; 8.9 ± 0.2 vs. 8.1 ± 0.2 ng/mL, for HT and BO, respectively; $P < 0.02$). Follicle diameter and concentration of estradiol in plasma were affected by parity ($P < 0.01$) and BCS ($P < 0.05$), but not by stage of lactation ($P > 0.42$). Milk production was not correlated with both follicle diameter (r = 0.02; $P = 0.15$) and estradiol concentration (r = 0.02; $P = 0.10$). When milk production was divided in quartiles, follicle diameter increased with milk production ($P < 0.01$), but no difference in circulating concentrations of estradiol was found (P > 0.10). Data regarding pre-ovulatory follicle and estradiol concentrations is shown in Table 2.

5.5. Risk Factors for Pregnancy per AI

The P/AI was not influenced by parity ($P = 0.91$), stage of lactation ($P = 0.31$) or secondary signs of estrus ($P = 0.54$). Milk production also had no effect on P/AI ($P = 0.87$) with only marginal numerical difference among quartiles. In contrast, cows with greater BCS had greater P/AI than cows with lower BCS ($P < 0.01$; Table 1). The P/AI was not affected by pre-ovulatory follicle diameter ($P = 0.15$), or by estradiol concentrations ($P = 0.40$).

Cows that expressed High PA had greater P/AI compared with Low PA as measured by HT (37.4 % vs 23.9 %; $P = 0.004$, Figure 4A), as well as by BO (33.5 % vs 21.4 %; $P = 0.001$, Figure 4B). The distribution of P/AI percentages according to increased PA at estrus is illustrated in Figure 5.

6. Discussion

In the current study, some major risk factors related with PA and DU of estrus events were assessed. Even tough new technologies capture physical activity using sensors and algorithms for data processing that are significantly different than those used in recent past, it was interesting to observe a lack of correlation between measurements of estrus expression and milk production and pre-ovulatory follicle diameter. Although there was a greater concentration of estradiol in plasma from events classified as high intensity, the correlation with PA and DU was weak and was subjected to great variation. One interesting finding from this study was the substantial increase in P/AI from events of High PA. The improvement in fertility was somewhat expected from cows with greater PA; however, the common knowledge was that high intensity estruses were associated with differences in BCS, parity or even lactation stage and health, which in turn would affect fertility. In fact, we observed greater PA and DU as BCS increased as well as in primiparous cows, but greater P/AI still occurred in spite of those and other risk factors known to affect conception rates. The practical implications could be the intensive use of PA and DU measurements to predict fertility and improve decision-making in reproductive programs using activity monitors, and potentially use it as a tool to select animals of superior estrus expression.

Body condition score was the major factor that affected physical activity at estrus. This study supported conclusions by Løvendahl and Chagunda (2010), who stated that in the first 5 mo after calving, low BCS had a negative correlation with estrus activity found early postpartum. Further support is provided by Aungier et al. (2012), who reported that a 0.25 increase in BCS was significantly correlated with an increase in physical activity prior to ovulation. Cows that lost less than 100 kg of BW from 2 wk pre-calving to 5 wk post-calving had greater PA in the first 2 estrus episodes post-partum (Burnett et al., 2015). The specific mechanism by which a temporary state of negative energy balance reduces estrogen-dependent estrus behavior is unclear.

In our study, multiparous cows expressed lower PA and DU than primiparous. López-Gatius et al. (2005) found that parity was significantly associated with walking activity and reported that for each additional parity number, walking activity at an estrus episode was reduced by 21.4%. On the contrary, Walker et al. (1996) described that duration of estrus was nearly 50% shorter for primiparous than for multiparous lactating dairy cows. Our study does not support findings from recent studies that reported no association between parity and physical activity at estrus (Arney et al., 1994; Løvendahl and Chagunda 2010; VeerKamp et al., 2000). Methodological differences may explain variation among different studies on the association between parity and physical activity. This study used AAM that would record activity levels every 2 h. The AAM used by Løvendahl and Chagunda (2010) would transmit signals every h,

potentially increasing the rate of false-positives. Additionally, this study employed only Holstein cattle as test subjects, while Løvendahl and Chagunda (2010) used 3 breeds: Jersey, Holstein and Red Dane. Research conducted by Orihuela et al. (2000) highlights the importance of cattle breed on the level of physical activity expressed at estrus, which can potentially be extrapolated to include parity. Two different AAM were used in the current study and the correlation of PA of estrus episodes was only moderate between the systems. It seems that different AAM will have different calibrations on how to measure physical movement, consequently influencing measurements of baseline levels and relative increases in activity during estrus.

Greater milk production has been negatively correlated with standing to be mounted behavior at estrus (Lopez et al., 2004; Rivera et al., 2010). The decrease in concentrations of estradiol possibly caused by increased hepatic blood flow and steroid clearance (Sangsritavong et al., 2002, Vasconcelos et al., 2003) is a possible cause for the decreased estrus-related behavior, most notably standing to be mounted. The apparent contradiction between the results of the current study and others (Lopez et al., 2004; Rivera et al., 2010) may lie on the fact that AAM measure overall physical activity and report intensity as a relative increase, instead of absolute numbers. Recent studies from our group (Silper et al., 2015; Madureira et al., 2015) found that heifers and cows with lower baseline levels tend to have greater relative increase in PA, but not necessarily greater absolute increases in step counts during estrus. Moreover, PA during estrus was weakly associated with milk production, emphasizing the influence of other factors, such as BCS and parity, as well as group size, health status, and lameness (Van Vliet and Van Eerdenburg, 1996; López-Gatius et al., 2005; Morris et al., 2009). The role of genetic predisposition should be further investigated due to the observation of great individual variation related to PA and DU of estrus episodes in spite of the influence of other risk factors.

In this study, we found that the HT system had a PPV of 89.6% whereas the BO system had 85.5%. The true episodes were verified from ovarian structures and are in agreement with recent studies reporting 72% (Aungier et al., 2012) and 90% (Aungier et al., 2015) PPV of estrous detection using the same technology. At-Taras and Spahr (2001) found a similar PPV for a pedometer system. Collectively, the current AAM systems detect a low percentage of false positives, but accurate calculation of sensitivity is still a field for future studies.

The correlation between the pre-ovulatory follicle diameter and plasma estradiol was weak ($r = 0.17$) and is in agreement with values reported elsewhere (Glencross et al., 1981; Cook et al., 1986; Sartori et al., 2004; Walker et al., 2008). Although reports have found that a larger follicle is associated with greater concentrations of estradiol in plasma (Cerri et al., 2004), it is clear from the current experiment that parity, BCS and milk production are the factors with the greatest impact on circulating concentrations of estradiol. Cows classified as High activity had similar

pre-ovulatory follicle diameter, but greater concentrations of estradiol in plasma than cows classified as Low. In spite of the differences in estradiol concentrations found when cows were divided in categories by estrus activity, the PA measured by both AAM systems was only weakly correlated with concentration of estradiol in plasma. A recent study by Aungier et al. (2015) observed no correlation between activity clusters measured by AAM and FSH, LH and estradiol profiles. However, a greater peak concentration of estradiol in plasma was in fact associated with standing and estrus-related behaviors. The expression of estrus behavior is triggered by high concentrations of estradiol-17β produced by the pre-ovulatory follicle (Allrich, 1994; Forde et al., 2011). It is reasonable to assume based on this and other studies (Walker et al., 2008; Aungier et al., 2015) that there is some level of dependence between PA at estrus and estradiol concentrations in plasma, but the influence of other risk-factors is probably greater than previously thought. For example, we observed that cows with lower milk production had greater concentrations of estradiol at estrus, and also greater PA and DU of estrus. One factor that has not been addressed in this or previous studies is the effect that sampling at random times of the day may have on estradiol concentrations, considering the acute changes in ovarian steroid concentrations observed in post-prandial periods (Vasconcelos et al., 2003). In addition, in spite of the efforts to draw a blood sample early in the estrus, it was impossible to determine when the sample was collected in relation to the LH surge and a possible negative effect on estradiol concentrations in plasma. The degree of variation found in the current study related to ovarian dynamics, PA and DU of naturally occurring estrus and further responses in P/AI is surprising nonetheless. Interestingly, Cummins et al. (2012) found that cows classified with a greater genetic merit for fertility had pronounced effects on corpus luteum development, progesterone concentration, preovulatory follicle diameter, and behavioral estrus. More detailed studies should be conducted to approach possible management, physiological or perhaps genetic explanations for the observed endocrine and behavioral variation and at times paradoxical correlations.

The PA during estrus was highly correlated with DU and is probably an acceptable measure of the intensity of the estrus episode. It was interesting to observe such an effect of PA (High vs Low) from both systems on P/AI. Among all risk factors included in the model of the analysis of P/AI, only BCS and PA at estrus were significant and remained in the final statistical model. Cows with High PA had approximately 12 percentage units greater P/AI than cows with Low PA, which represents 35% improvement in fertility. Previously, Lopez-Gatius et al. (2005) reported an improvement of 1.001-fold for every unit of relative increase in walking activity. In another recent study (Madureira et al., 2015) using a different pedometer system and only first AI responses, an even greater difference was observed (42.9 vs 28.9%) between cows displaying High and Low PA at estrus. It was previously mentioned that pre-ovulatory follicle diameter was not different between PA categories. The fact that follicle diameter was not different does not

imply that proestrus or dominance length was similar as there was no control of follicular emergence in this study. Therefore, proestrus and dominance length (Bleach et al., 2004; Cerri et al., 2009) cannot be ruled out as possible causes related to the reduced fertility observed. Increased length of proestrus (Ribeiro et al., 2012) and display of estrus at AI (Pereira et al., 2013) is associated with a reduction in pregnancy losses. Furthermore, Pereira et al. (2014) reported that animals that display estrus at AI had decreased pregnancy losses regardless of the diameter of the pre-ovulatory follicle. Peak concentration of estradiol at estrus was different between High and Low PA estrus episodes (approximately 1 pg/mL) to a similar degree found between the low vs high milk production groups, high vs low BCS groups and primiparous vs multiparous groups and probably sufficient to at least partly explain such change in fertility. When estradiol cypionate was used to induce estrus and ovulation, therefore bringing circulating concentrations of estradiol to high concentrations, the PA measured by a pedometer system still significantly affected P/AI results (Madureira et al., 2015).

The ovulation of pre-ovulatory follicles with similar diameter would suggest little change in concentrations of progesterone after AI. This information was not collected in the present study, but data from Madureira et al. (2015) suggests that concentrations of progesterone 10 d after AI was greater in cows displaying high intensity estrus at AI. The faster increase in progesterone early in the cycle could result in increased early embryonic development (Mann and Lamming, 2001; Bisinotto et al., 2010a) possibly due to changes in the endometrium receptor profile (Lonergan, 2011) and therefore could be a reason for increased P/AI found in animals with High PA in this study.

Another possible factor influencing P/AI is the ovulation rate from cows with different PA at estrus. Madureira et al. (2015) observed a greater failure of ovulation rate of cows that displayed estrus with a relative increase in PA from 80 to 100%. While this observation is certainly important to explain our observations, it is limited to cows expressing very low PA during estrus as the threshold dividing High and Low PA categories was around 400% relative increase in the current study. A possible explanation to correlate PA and P/AI, that has not been extensively studied, is that cows could have greater than expected individual variations in its ability to express estrogen receptors in the endometrium and perhaps more importantly in the hypothalamus. This would in turn generate cows that are more likely to translate circulating concentrations of estradiol into estrus-related behaviors, and later into a more adequate uterine environment for embryo development.

7. Conclusion

In conclusion, measurements of estrus events captured by AAM are correlated with BCS, parity and secondary behavior signs related to estrus. Estrus intensity and duration were only weakly correlated with milk production, pre-ovulatory follicle diameter and concentration of estradiol at estrus. Cows that had estrus of High intensity were significantly more fertile than those with Low intensity estrus. Both HT and BO automated activity monitor had high PPV for the detection of estrus events. More importantly, quantitative information from AAM systems could be intensively used to improve estrous detection quality, predict P/AI outcomes, improve decision-making in reproductive programs using AAM, and potentially be used as a tool to select animals of superior estrous expression.

8. Acknowlegments

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10. Figures and Tables

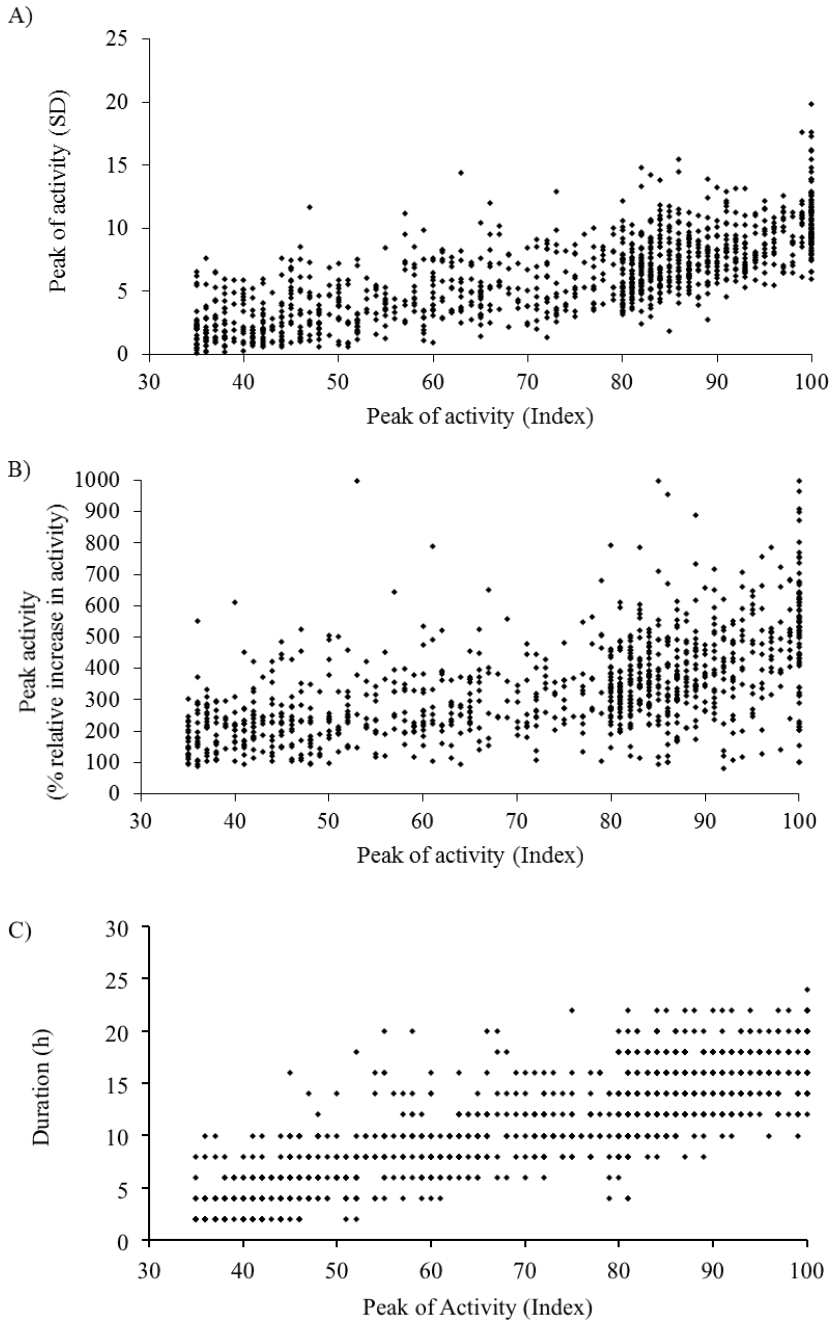


Figure 1. Correlations between peak of activity (index value; measured by a neck-mounted accelerometer; HT) and A) estrus activity in standard deviations (SD; calculated using the raw PA value and baseline values of the previous 14 d from HT data; r = 0.54, $P < 0.01$); B) percent relative increase in activity at estrus measured by a leg-mounted pedometer (BO; r = 0.55, $P < 0.01$); C) duration (h) of activity above the threshold (35 index-value) measured by HT (r = 0.82, $P < 0.01$).

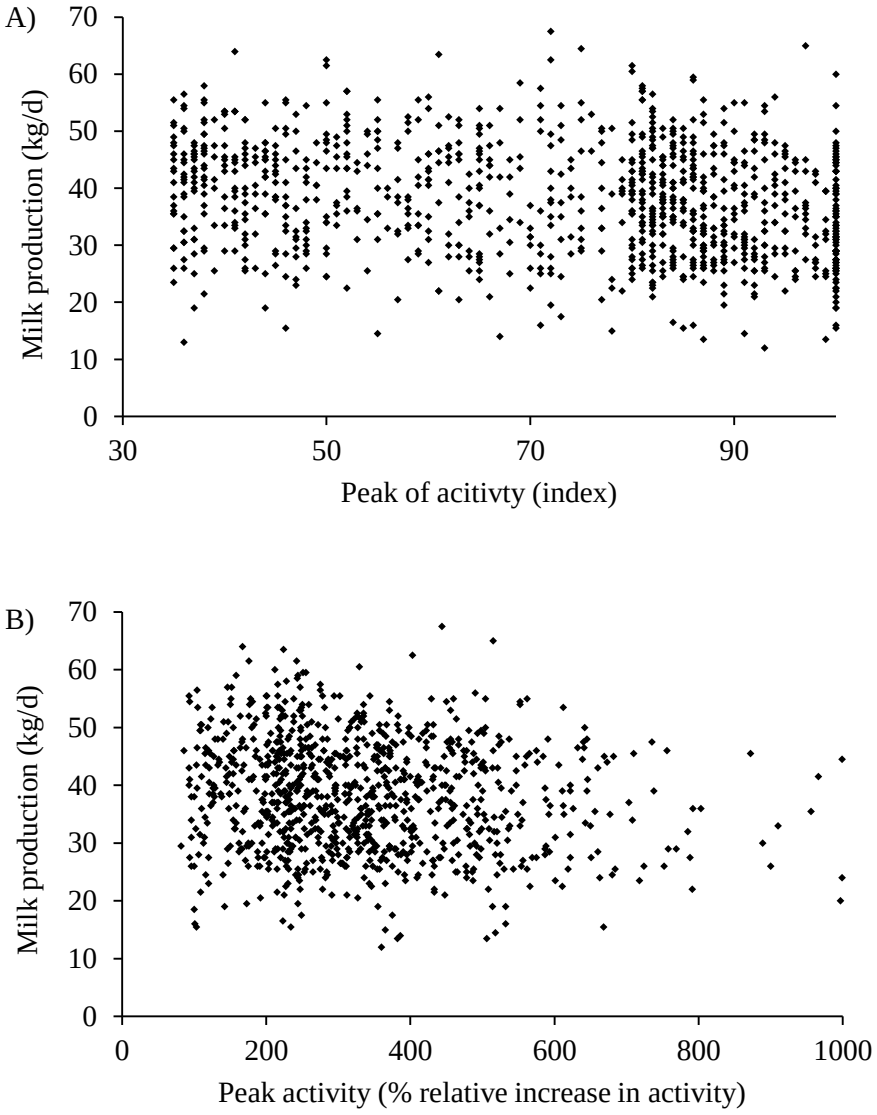


Figure 2. Correlations between milk production at the day of AI and, A) peak of activity (index value) measured by HT (r = 0.20, $P < 0.01$), and B) percent relative increase in activity measured by BO (r = 0.05, $P < 0.01$).

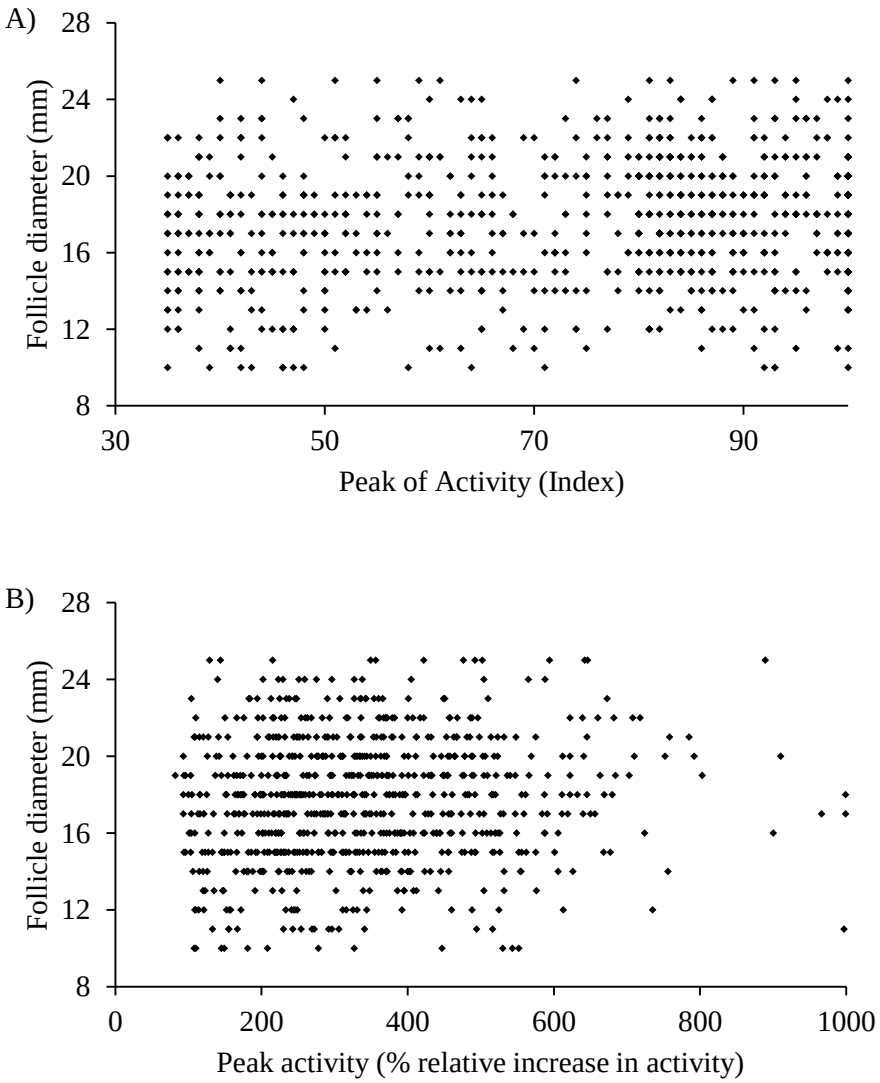


Figure 3. Correlations between diameter of the pre-ovulatory follicle and A) peak of activity (index value) measured by HT (r = 0.07, *P* = 0.03) and B) percent relative increase in activity measured by BO (r = 0.01, *P* = 0.02).

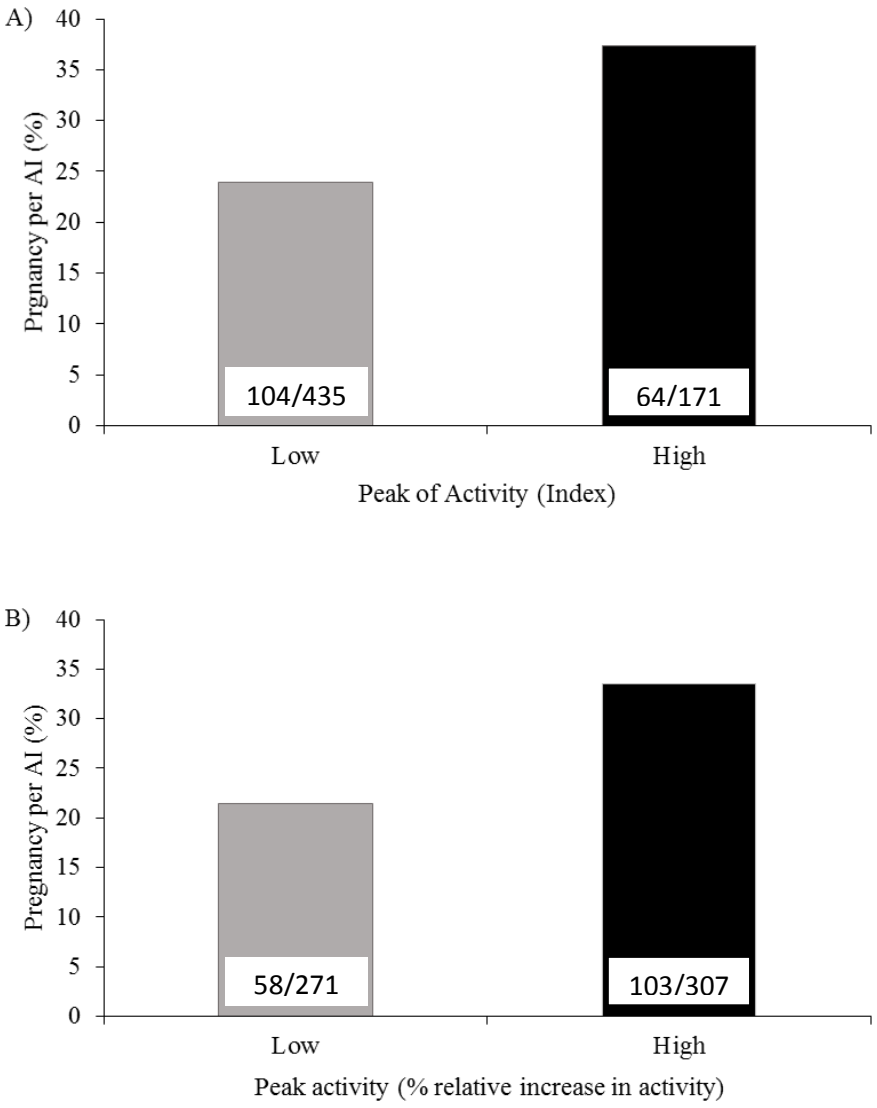


Figure 4. Pregnancy per AI (%) according to categories of peak activity during estrus: A) Low (35 to 89 index) or High (90 to 100 index) peak activity (index) at estrus episodes detected by HT (*P* = 0.004); and B) Low (80 to 321%) or High (> 321%) relative increase in activity (%) at estrus episodes detected by BO (*P* = 0.001).

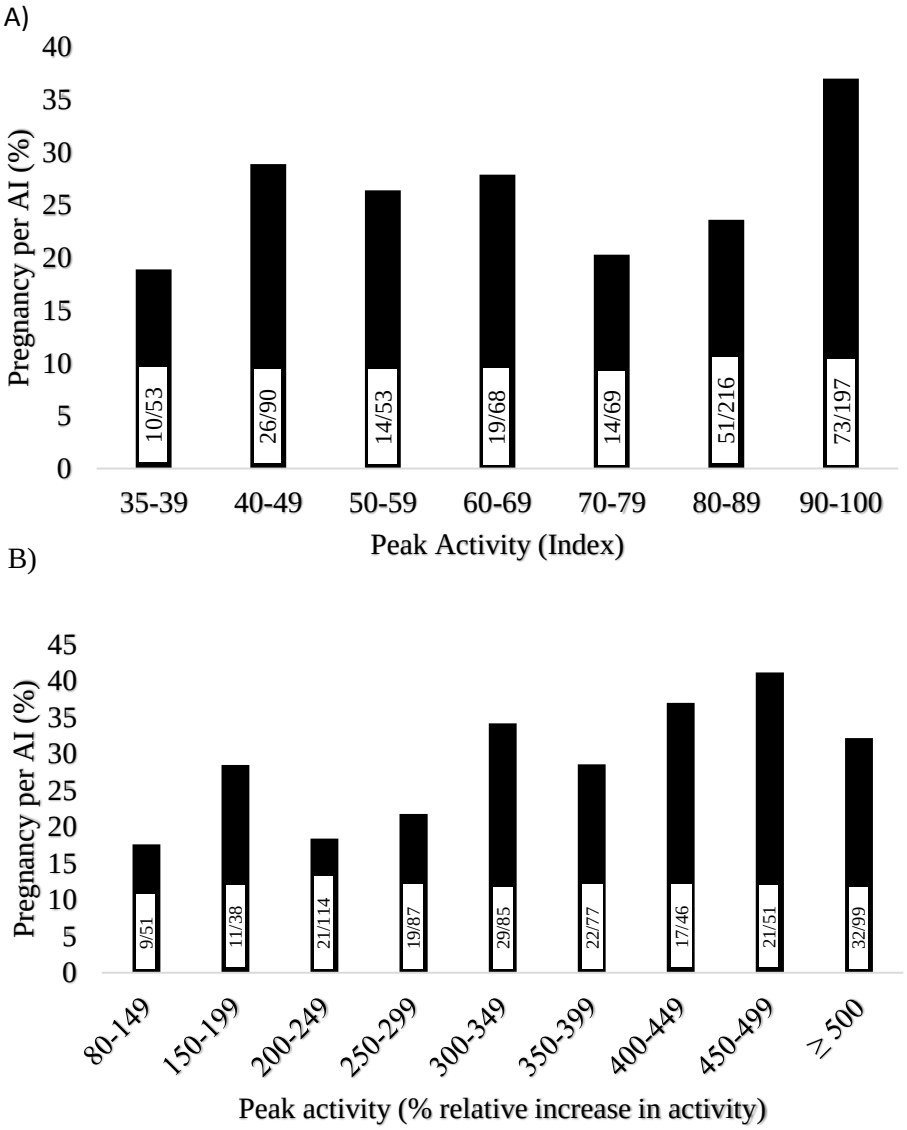


Figure 5. Distribution of pregnancy per AI (%) according to peak activity during estrus detected by A) HT and B) BO.

Table 1. Means ± SD for peak estrus activity (index and percent relative increase in activity), estrus duration, on index activity and pregnancy per artificial insemination according to body condition score, parity, milk production, lactation stage and secondary signs of estrus.

Variable	Peak activity		Duration (h)[3]	Pregnancy Per AI (%)[4]
	Index[1]	% Relative increase[2]		
BCS				
Low (≤ 2.5)	65.2 ± 1.5[a]	294.7 ± 11.3[a]	9.6 ± 0.4[a]	16.8[a] [7/29]
Medium (2.75 to 3.0)	73.8± 0.8[b]	345.7 ± 6.0[b]	11.4 ± 0.5[b]	28.6[b] [122/427]
High (≥ 3.25)	71.9± 1.9[b]	339.2 ± 14.9[b]	11.6 ± 0.2[b]	39.7[c] [17/101]
Parity				
Primiparous	75.9 ± 1.1[a]	354.8 ± 8.4[a]	12.0 ± 0.3[a]	27.3 [59/216]
Multiparous	69.3 ± 0.8[b]	323.9 ± 6.0[b]	10.2 ± 0.2[b]	27.7 [115/415]
Secondary signs of estrus[5]				
Weak	66.6 ± 1.0[a]	308.3 ± 7.7[a]	10.1 ± 0.3[b]	28.6 [47/191]
Normal	73.3 ± 1.1[b]	340.7 ± 9.0[b]	11.7 ± 0.3[a]	24.6 [63/216]
Strong	76.8 ± 1.2[c]	362.9 ± 8.9[c]	12.2 ± 0.3[a]	29.2 [64/224]
Milk production (kg/d)				
≤ 31.3	74.6 ± 1.4[a]	350.3 ± 11.1[a]	12.1 ± 0.3[a]	26.9 [42/164]
31.4 – 38.8	71.6 ± 1.4[b]	322.4 ± 11.2[b]	11.6 ± 0.3[a]	28.9 [51/176]
38.9 – 45.1	68.5 ± 1.5[b]	312.8 ± 11.3[b]	10.3 ± 0.3[b]	25.3 [40/158]
> 45.0	64.4 ± 1.4[b]	307.2 ± 11.2[b]	9.6 ± 0.3[b]	28.6 [39/139]
Lactation stage				
Fresh (≤ 45 DIM)	67.9 ± 1.7[a]	308.8 ± 12.8[a]	9.8 ± 0.4[a]	-
Lactating (45 to 120 DIM)	71.6 ± 1.0[b]	334.8 ± 8.2[b]	11.4 ± 0.2[b]	29.6 [111/374]
Late (121 to 250 DIM)	69.8 ± 1.3[ab]	325.9 ± 10.2[b]	11.6 ± 0.3[b]	29.1 [63/256]

Different letters indicate difference between variables within columns ($P < 0.05$)

[1] Index represents standard deviations from each cow's own basal activity (HT); [2] % Relative increase in activity was calculated as the percent increase in activity from each cow's own basal activity (BO); [3] Duration is the total time above the threshold (35 index value; from HT); [4] Percentage (number of cows pregnant at 32 d/number of cows inseminated); [5] Secondary signs (signs of repeated mounting activity, clear vaginal mucus and uterine tonus) were classified as Weak (at least 1 sign), Normal (at least 2 signs), or Strong (3 secondary signs

Table 2. Means ± SD for pre-ovulatory follicle diameter and estradiol concentration according to milk production, lactation stage, peak of activity, parity and body condition score

Variable	Follicle Diameter (mm)	Estradiol Concentration (pg/mL)
Milk production (kg/d)		
≤ 31.3	18.0 ± 0.4	9.2 ± 0.3
31.4 – 38.8	18.4 ± 0.4	8.3 ± 0.3
38.9 – 45.0	19.3 ± 0.4	8.2 ± 0.3
> 45.0	20.1 ± 0.4	8.2 ± 0.3
Lactation stage		
Fresh (≤ 45 DIM)	19.1 ± 0.5	8.1 ± 0.3
Lactating (45 to 120 DIM)	18.9 ± 0.3	8.5 ± 0.2
Late lactation (121 to 250 DIM)	19.1 ± 0.3	8.0 ± 0.2
Peak of activity[1]		
High	18.9 ± 0.2	9.4 ± 0.3[a]
Low	19.1 ± 0.4	8.2 ± 0.1[b]
Parity		
Primiparous	18.1 ± 0.3[a]	9.1 ± 0.2[a]
Multiparous	19.5 ± 0.2[b]	8.2 ± 0.1[b]
BCS		
Low (≤ 2.5)	18.4 ± 0.4[a]	7.6 ± 0.3[a]
Medium (2.75 – 3.0)	18.9 ± 0.2[a]	8.7 ± 0.1[b]
High (≥ 3.25)	20.4 ± 0.6[b]	8.7 ± 0.4[c]

[1] Peak of activity measured by HT: Low = 35 to 89 index, High = 90 to 100 index

Within the same column and subheading, different letters indicate difference at $P < 0.05$

CAPÍTULO 3

CONSIDERAÇÕES GERAIS E IMPLICAÇÕES

1. Considerações Gerais e Implicações

Os sistemas automatizados de monitoramento de atividade utilizado para a detecção de estro é uma ferramenta viável para melhorar as taxas de serviço e possivelmente auxiliar as tomadas de decisões em manejos reprodutivos em propriedades leiteiras. No presente estudo fatores tais como a produção de leite, diâmetro do folículo pré-ovulatório e a concentração plasmática de estradiol em estro não foram correlacionados com a duração e intensidade do estro. Animais que tiveram eventos de maior intensidade durante o estro, no entanto, tiveram maior taxa de concepção quando comparados com animais que tiveram eventos de menor intensidade. Informações quantitativas dos sistemas automatizados de monitoramento de atividade podem ser usadas como:

Ferramenta para mensurar a qualidade de estro;

Associar essas informações com taxa de ovulação e prenhez por IA;

Melhorar a tomada de decisão para inseminação em programas reprodutivos:

Inseminação por estro espontâneo ou por protocolos de sincronização (IATF)

Transferência de embriões

Existem diversos sistemas automatizados de monitoramento de atividade física disponíveis para produtores de leite, porém, ainda há a necessidade de mais estudos em relação aos monitores. Alguns destes sistemas possuem recursos que podem ser adaptáveis dependendo da fazenda ou do grupo de animais, e pode ser feito ajustes de acordo com a raça, ordem da lactação, condição corporal dos animais e estação do ano. Podendo este ser personalizado para cada rebanho