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

João Ricardo Malheiros de Souza

FERTILIDADE EQUINA: A INFLUÊNCIA DA IDADE MATERNA

Santa Maria, RS
2021

João Ricardo Malheiros de Souza

FERTILIDADE EQUINA: A INFLUÊNCIA DA IDADE MATERNA

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Sanidade e Reprodução Animal, da Universidade Federal de Santa Maria (UFSM-RS), como requisito parcial para obtenção do grau de **Doutor em Medicina Veterinária.**

Orientador: Prof. Paulo Bayard Dias Gonçalves
Coorientadora: Dra. Kalyne Bertolin

Santa Maria, RS
2021

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

Souza, João Ricardo Malheiros de
Fertilidade equina: a influência da idade materna / João Ricardo Malheiros de Souza.- 2021.
87 p.; 30 cm

Orientador: Paulo Bayard Dias Gonçalves
Coorientadora: Kalyne Bertolin
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Medicina Veterinária, RS, 2021

1. Cio do Potro 2. Mortalidade Embrionária 3. Transferência de Embriões 4. Progesterona 5. Embrião Equino I. Gonçalves, Paulo Bayard Dias II. Bertolin, Kalyne III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, JOÃO RICARDO MALHEIROS DE SOUZA, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Tese) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

João Ricardo Malheiros de Souza

FERTILIDADE EQUINA: A INFLUÊNCIA DA IDADE MATERNA

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Sanidade e Reprodução Animal, da Universidade Federal de Santa Maria (UFSM-RS), como requisito parcial para obtenção do grau de **Doutor em Medicina Veterinária**.

Aprovado em 10 de março de 2021:

Paulo Bayard Dias Gonçalves, Dr. (Unipampa)
(Presidente/Orientador)

Juliana Germano Ferst, Dra. (Unipampa)

Vitor Braga Rissi, Dr. (UFSC)

Claudio Alves Pimentel, Dr. (UFPel)

Anelise Maria Hammes Pimentel, Dra. (UFPel)

Santa Maria, RS
2021.

DEDICATÓRIA

A Deus, pelos sonhos e pela coragem concedida para realizá-los.

Aos meus pais, João Lima e Elenir, por terem me ensinado o valor do conhecimento e do esforço em busca dos nossos objetivos.

As minhas irmãs, Jane Elizabeth e Maria Carolina, pela amizade e companhia.

A Vó Maria *(in memorian)*, pela amizade, carinho e exemplo de vida.

Aos meus padrinhos, pelo apoio e compreensão nos momentos difíceis.

Aos meus orientadores, Prof. Dr. Paulo Bayard Dias Gonçalves e Dra. Kalyne Bertolin, pela oportunidade, confiança e incentivo. Além de excelentes orientadores, grandes amigos.

Aos Professores, Dr. Vilceu Bordignon, Dr. Alfredo Quites Antoniazzi, Dr. Fabio Vasconcellos Comim e Dr. Valerio Valdetar Marques Portela Junior pelos ensinamentos, apoio científico e conselhos sempre que precisei.

Aos Professores, Dr. Charles Ferreira Martins, Dr. Bernardo Gasperin, Dra. Monique Tomazele Rovani, Dr. Vitor Braga Rissi, Dr. Rudi Weiblen, Dr. Felipe Libardoni e Dra. Sandra Fiala Rechsteiner, por terem sido pessoas de referência durante minha vida acadêmica.

Ao Dr. Claudio Alves Pimentel e Dra. Anelise Maria Hammes Pimentel pela amizade, orientação e incentivo que oportunizaram minha formação profissional e um imenso crescimento pessoal.

Aos Drs. Reno Roldi de Araujo, Rogério Dereti, Werner Giehl Glanzner, Karina Gutierrez, Juliana Germano Ferst e Gustavo Freitas Ilha, grandes profissionais que tive o prazer de acompanhar durante a minha vida acadêmica.

A equipe BioRep pelo auxílio, companheirismo, aprendizado e convivência diária que oportunizaram a realização deste trabalho.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), à Financiadora de Estudos e Projetos (FINEP), à Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) e à Universidade Federal de Santa Maria (UFSM) pelo apoio financeiro e possibilidade de estudo.

Ao Programa de Pós-Graduação em Medicina Veterinária (PPGMV) e a secretária do PPGMV, Maria Moro da Rosa, pelo apoio administrativo.

RESUMO

FERTILIDADE EQUINA: A INFLUÊNCIA DA IDADE MATERNA

AUTOR: João Ricardo Malheiros De Souza
ORIENTADOR: Paulo Bayard Dias Gonçalves

A redução dos índices de fertilidade relacionados à idade é frequentemente relatada em humanos e equinos. Embora a caracterização da função seja mais evidente aos profissionais durante as avaliações ginecológicas, principalmente após o parto, a sua interpretação em conjunto com a idade da égua e a qualidade embrionária também devem ser consideradas para favorecer a eficiência reprodutiva da espécie equina. Nesse contexto, o primeiro objetivo desta tese foi investigar a influência da idade materna sobre a fertilidade de éguas Puro Sangue Inglês durante o pós-parto. Os resultados obtidos nesse primeiro estudo nos permitiram identificar que a idade influencia na fertilidade pós-parto da égua, sendo que a idade de dez anos é um ponto de corte para redução da fertilidade. Além disso, identificamos que o intervalo entre o parto e a cobertura pode influenciar na perda embrionária. Também foi possível identificar que a fertilidade do segundo estro após o parto é superior quando comparada com o primeiro estro (cio do potro). O segundo objetivo desta tese foi investigar o efeito da idade materna sobre a recuperação e qualidade de embriões recuperados de éguas Mangalarga Marchador em programas de transferência de embriões. Além disso, verificar a influência da suplementação de progesterona em éguas receptoras após a transferência de embriões com diferentes qualidades. Os resultados obtidos nesse segundo estudo nos permitiram identificar que a idade da égua doadora influencia na probabilidade de recuperação e na qualidade embrionária. Também foi possível identificar que a qualidade embrionária e o grupo de receptoras influenciaram na probabilidade de gestação aos 15 e 60 dias após a transferência. Enquanto que a suplementação de progesterona nas receptoras aumentou a probabilidade de gestação aos 15 e 60 dias em embriões de diferentes qualidades. No entanto, a perda embrionária foi dependente somente do grupo de receptoras. Em conjunto, os dados evidenciam que a idade materna influencia a fertilidade de éguas tanto no pós-parto quanto em programas de transferência de embriões.

Palavras chave: Cio do Potro. Mortalidade Embrionária. Transferência de Embriões. Progesterona. Embrião Equino.

ABSTRACT

EQUINE FERTILITY: THE INFLUENCE OF MATERNAL AGE

AUTHOR: João Ricardo Malheiros De Souza
ADVISOR: Paulo Bayard Dias Gonçalves

The reduction in age-related fertility rates is reported in humans and horses. Although the characterization of the function is more evident to professionals during gynecological evaluations, especially after foaling, its interpretation in conjunction with the mare's age and embryonic quality must also be considered to favor the reproductive efficiency of the equine species. In this context, the first objective of this thesis was to investigate the influence of maternal age on the fertility of Thoroughbred mares during the postpartum period. The results obtained in this first study allowed us to identify that age influences the mare's postpartum fertility, with the age of ten being a cut-off point for reducing fertility. In addition, we identified that the interval between delivery and coverage can influence embryonic loss. It was also possible to identify that the fertility of the second estrus after delivery is higher when compared to the first estrus (foal heat). The second objective of this thesis was to investigate the effect of maternal age on the recovery and quality of embryos recovered from Mangalarga Marchador mares in embryo transfer programs. In addition, to verify the influence of progesterone supplementation in recipient mares after the transfer of embryos with different qualities. The results obtained in this second study allowed us to identify that the age of the donor mare influences the probability of recovery and the embryonic quality. It was also possible to identify that the embryonic quality and the group of recipients influenced the probability of pregnancy at 15 and 60 days after the transfer. While supplementation of progesterone in the recipients increased the probability of gestation at 15 and 60 days in embryos of different qualities. However, embryonic loss was dependent only on the recipient group. Taken together, the data show that maternal age influences the fertility of mares both in the postpartum period and in embryo transfer programs.

Keywords: Heat Cycle. Embryo Loss. Embryo Transfer. Progesterone. Equine Embryo.

LISTA DE FIGURAS

ARTIGO I

ARTIGO II

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

A equinocultura é uma das principais atividades agropecuárias do Brasil. Com um rebanho superior a 5 milhões de animais, é responsável por movimentar aproximadamente 16 bilhões de reais anualmente. Estima-se que aproximadamente 3 milhões de pessoas estejam envolvidas em todo o complexo do agronegócio do cavalo, distribuídas entre diversos setores da economia relacionada aos animais, sejam eles utilizados para trabalho, esporte ou lazer (LIMA; CINTRA, 2015).

Uma crescente movimentação financeira tem ocorrido nos últimos anos devido ao aumento da criação voltada para o público urbano, tanto para o esporte quanto para o lazer (LIMA; CINTRA, 2015). Esses animais, normalmente necessitam maior atenção e cuidados, movimentando com maior intensidade a indústria de serviços. Paralelo ao mercado consumidor, cresce também o número e o tamanho dos eventos que envolvem a atividade, fortalecendo a seleção zootécnica de animais.

De modo geral, a seleção de animais na espécie equina é realizada pela sua aptidão esportiva ou ainda pelas suas características fenotípicas (ALVARENGA et al., 2017). E ao contrário do que ocorre na bovinocultura e ovinocultura, por exemplo, a fertilidade não é considerada no momento do planejamento dos acasalamentos entre animais de performance ou ainda no descarte de animais. Culminando com permanência de alterações de fertilidade através da descendência.

Embora possa variar entre raças, o início da vida atlética dos equinos inicia entre 12 a 24 meses, período em que também ocorre a puberdade (GINTHER, 1992; STEINER; UMPHENOUR, 2009). Nos machos de elevado valor zootécnico, a estação reprodutiva muitas vezes é intercalada com os períodos de treinamento e competição, ao contrário das fêmeas. As quais, são mantidas em competição enquanto permanecerem vitoriosas, postergando sua vida reprodutiva, ou são submetidas, intensamente ao uso de tecnologias reprodutivas, em raças que permitem sua utilização.

A redução dos índices de fertilidade relacionados à idade é frequentemente relatada em humanos (ASRM, 2014) e equinos (CARNEVALE; GINTHER, 1995; HENDRIKS et al., 2015). Embora a caracterização da função uterina (CARNVALE; GINTHER, 1992; MARINONE et al., 2017) seja mais evidente aos profissionais durante as avaliações ginecológicas, principalmente após o parto, a interpretação sobre as características oocitárias (CARNEVALE et al., 1999) também devem ser consideradas. Afinal, serão fundamentais para o início do desenvolvimento embrionário, seguida da manutenção gestacional.

Após a ovulação do gameta feminino e posterior fecundação, o embrião equino chega ao útero em 144 horas (BATTUT et al., 1997), iniciando o período de reconhecimento materno da gestação. Sob a influência da progesterona, uma série de situações fisiológicas ocorre no ambiente uterino a fim de receber a sinalização embrionária até sua fixação em um dos cornos uterinos (GINTHER, 1983; KASTELIC et al., 1987). Sabe-se que as etapas desse processo podem ser afetadas pela idade materna (CARNEVALE, 1995; MORRIS; ALLEN, 2002; CARNEVALE, 2008; RIZZO et al., 2019), modulando a qualidade embrionária (RAMBAGS et al., 2014; HENDRIKS et al., 2015; RIZZO et al., 2019).

A caracterização da fertilidade pós-parto equina em relação a idade ainda apresenta lacunas a serem preenchidas pelo conhecimento científico. Expandir o conhecimento a cerca das interações entre a idade materna e a qualidade de embriões equinos também é necessário. Portanto o objetivo desta tese foi estudar a influência da idade materna sobre a fertilidade de éguas.

2 REVISÃO BIBLIOGRÁFICA

2.1 CICLO ESTRAL

A espécie equina é caracterizada pela sua estacionalidade, onde o aumento da atividade reprodutiva ocorre durante a primavera e o verão (GINTHER, 1992), coincidindo com o aumento da exposição diária à luz (SHARP et al., 1975). Para as fêmeas, esse processo estimula a ocorrência de fases foliculares que culminam em ovulação (HANSEN, 1985). O intervalo entre ovulações é de 22 dias em média, embora esse período possa variar entre 16 e 25 dias (GINTHER; PIERSON, 1989; GINTHER, 2017a).

Considerando uma distribuição anual, o ciclo equino pode ser dividido em quatro períodos: 1) Folicular ou estro: período de receptividade ao acasalamento concomitante à crescente dinâmica folicular, culminando com a ovulação; 2) Luteal ou diestro: período de ausência da receptividade e com presença de corpo lúteo; 3) Anestro: período onde não há atividade ovariana, nem a ocorrência de ovulação; 4) Transicional: intervalo entre o anestro e a estação reprodutiva (GINTHER, 1992). Esse último, caracterizado pelo retorno a ciclicidade fisiológica da égua, que pode apresentar comportamento irregular de estro, visto que voltam a ocorrer os aumentos na secreção de gonadotrofinas e dos hormônios esteroides (DONADEU; WATSON, 2007). O tempo de duração desse período varia entre indivíduos, principalmente considerando que além dos ovários, o endométrio também necessita de um período para adaptação (KENNEY; DOIG, 1986), após os baixos níveis de hormônios esteroides do anestro.

2.2 DINÂMICA FOLICULAR

De modo geral, equinos são animais monovulatórios (GINTHER et al., 2003) e seu ciclo é composto por ondas foliculares. Essas ondas compreendem acontecimentos que obedecem à seguinte sequência: recrutamento, emergência, dominância e ovulação ou atresia. A caracterização de uma onda pode ser realizada a partir da população de folículos ovarianos, onde ondas maiores são compostas por folículo dominante e subordinados, enquanto ondas menores são compostas por folículos que não atingem o diâmetro de um dominante (GINTHER, 1992).

Ondas maiores ainda podem ser divididas como primárias: quando o folículo dominante se desenvolve e ovula durante o estro; ou secundárias: quando o folículo dominante se torna anovulatório ou ovula após a ovulação primária ou no diestro (GINTHER, 1993). Folículos ovulatórios, presentes em ondas primárias, emergem 6 ou 7 dias depois da última ovulação (aproximadamente 12 mm de diâmetro) e ovulam em torno de 15 dias mais tarde com aproximadamente 45 mm de diâmetro (GINTHER; BERGFELT, 1993)

As gonadotrofinas como o hormônio folículo estimulante (FSH) e o hormônio luteinizante (LH) além dos esteroides ovarianos são os principais reguladores endócrinos da função ovariana. A emergência de um *pool* de folículos ocorre em resposta à elevação dos níveis de FSH para iniciar a onda folicular (GINTHER, 1992). Durante a divergência folicular, ocorre a diminuição na produção de FSH e a diferença entre a taxa de crescimento entre os dois maiores folículos da onda (GINTHER, 2016), que apresentam em média 18,0-22,5mm (GINTHER et al., 2003). Esse período coincide com o aumento das concentrações de LH e estradiol.

O aumento da expressão de receptores de LH (LHR) na granulosa, teca, e células do cumulus tem sido correlacionada ao crescimento folicular (GOUDET et al., 1999). Recentemente, a utilização de biopsia intrafolicular facilitou a interpretação dos resultados sobre receptores de gonadotrofinas em éguas sem a necessidade de castração ou eutanásia. De modo geral, a maior expressão de LHR é detectada em camadas da parede de folículos de 30 mm em comparação com folículos de 10 mm (ISHAK et al., 2018).

A produção de estrógeno aumenta conforme o diâmetro folicular, e esse aumento induz a expressão de seus receptores (ERRα) (LIU et al., 2003). Assim como a inibina, o estradiol é um hormônio que atua na glândula pituitária inibindo gradualmente a secreção de FSH (GINTHER et al., 2002). E por isso, folículos com 30 e 20 mm apresentam maiores expressões de ERRα em comparação a folículos de 10 mm (BASHIR et al., 2016; ISHAK et al., 2018).

Alterações intrafoliculares, como o aumento da disponibilidade intrafolicular de IGF1 (fator de crescimento semelhante a insulina) livre também tem sido considerado um importante fator durante o processo de divergência folicular (BEG; GINTHER, 2006). A sua concentração pode aumentar no provável folículo dominante ainda antes do início desse processo, assegurando a dominância do selecionado (DONADEU; GINTHER, 2002; GINTHER, 2017b). Estudos demonstram que o IGF1 endógeno está envolvido na regulamentação da produção de inibina-A e ativina-A, mas não na produção de estradiol em um futuro folículo dominante (GINTHER et al., 2004a; GINTHER, 2017b).

A funcionalidade do IGF1 parece estar diretamente associada à ação de proteínas ligadoras do IGF (IGFBP) no fluido folicular, sendo IGFBP-2 e IGFBP-3, seguidas de suas proteases, as mais importantes em equinos (GÉRARD et al., 1999; GINTHER et al., 2004b). Essas podem ser degradadas a partir da sua ligação com a proteína plasmática-A associada à prenhez (PAPP-A), tornando IGF1 livre. Nesse contexto, sua concentração aumenta significativamente entre 16,0 a 19,0 mm no folículo dominante da égua (DONADEU; GINTHER, 2002), antes mesmo de apresentar diferenças morfológicas em relação aos seus subordinados (RIVERA; FORTUNE, 2003) e do período de divergência folicular (20,0 a 23,9 mm).

O estradiol inicia a elevação da sua concentração tanto no ambiente folicular do futuro folículo dominante (DONADEU; GINTHER, 2002; BASHIR et al., 2016; ISHAK et al., 2018), quanto no plasma sanguíneo, logo após o período de divergência folicular (GASTAL et al., 1999), independente da idade da égua (GINTHER et al., 2009). Embora esteja envolvido no processo de divergência como um supressor de FSH em bovinos (GINTHER, 2016), esse efeito não é verdadeiro para equinos (GINTHER, 2017a). O aumento da sua concentração está relacionada ao desenvolvimento de células da granulosa intrafoliculares expressando aromatase (BELIN et al., 2000) e da oscilação de LH durante a divergência (DONADEU; GINTHER, 2003; GINTHER, 2017a), e normalmente seu pico ocorre dois dias anteriores à ovulação em ondas espontâneas (GINTHER et al., 2006) ou induzidas (GINTHER et al., 2008).

A crescente concentração de inibina-A e ativina-A no futuro folículo dominante são desencadeadas pelo aumento de IGF1 livre no fluido folicular antes do desvio (DONADEU; GINTHER, 2002). Paralelo a esse evento, ocorre o declínio do FSH, indicando que a inibina é seu primeiro supressor após a emergência, até o aumento do estradiol local durante a divergência (GINTHER, 2017b). A partir de então esse binômio entre estradiol e inibina serão responsáveis pela ação na glândula pituitária inibindo gradualmente a secreção de FSH (BEG; GINTHER, 2006), contribuindo para o crescimento do folículo dominante.

Efeitos como a angiogênese, a permeabilidade vascular e a estimulação de células endoteliais no folículo são funções do fator de crescimento endotelial vascular (VEGF) (REYNOLDS; REDMER, 1998). No folículo dominante, o VEGF aumenta 24 horas antes mesmo do início da divergência, sendo considerado um provável candidato à estimulação da vascularização folicular em função da dominância. No entanto, sua expressão diminui previamente à ovulação (BASHIR et al., 2016), provavelmente acompanhando o decréscimo da inibina-A, da IGF1 e da vascularização local (GINTHER et al., 2007a).

2.3 LUTEINIZAÇÃO

Após o pico ovulatório do LH e a ovulação, as células foliculares, teca e granulosa, iniciam o processo de luteinização, resultando em formação do corpo hemorrágico e, a seguir, do corpo lúteo (CL). De modo geral, a partir da ruptura da membrana basal existente entre a camada da teca e granulosa, ocorre uma série de alterações bioquímicas que resultam na secreção de progesterona (P4) (NISWENDER et al., 2000). Em ruminantes, há o crescimento de células endoteliais e fibroblastos além de uma rápida divisão mitótica das células esteroidogênicas pequenas (<20,0 µm, oriundas da teca) e grandes (20,0-30,0 µm, oriundas da granulosa) (ALILA; HANSEL, 1984). No entanto, em equinos, apenas a granulosa parece contribuir na formação celular do CL, em virtude da degeneração das células da teca (VAN NIEKERK et al., 1975; BROADLEY et al., 1994), ao contrário do que é observado em outras espécies domésticas, como: ovinos (FINCHER et al., 1986), bovinos (URSELY; LEYMARIE, 1979) e suínos (LEMON; MAULEON, 1982) em que ambas constituem as células luteais.

No processo ovulatório, o LH se liga a receptores acoplados à Proteína G com produção de adenosina monofosfato ciclíco (cAMP) e estimula a esteroidogênese. A partir do transporte de colesterol para interior celular e do meio intracelular para dentro da mitocôndria, ocorre a aceleração da conversão de colesterol em pregnenolona pela ação da enzima clivadora de cadeia lateral (P450scc), localizada na membrana interna da mitocôndria. Assim, a pregnenolona é convertida em progesterona pela enzima 3 beta-hidroxiesteróide desidrogenase (3Beta-HSD) que está presente no retículo endoplasmático liso (NISWENDER et al., 2000)

De um modo crescente, a progesterona atinge seu pico entre os dias 6 e 8 após a ovulação na égua (GINTHER et al., 2006; GINTHER et al., 2007b), simultaneamente ao aumento da perfusão endotelial local (GINTHER et al., 2007b) estimulado por fatores angiogênicos, como o VEGF produzido pelas células luteais (AL-ZI'ABI et al., 2003). Após esse período, ocorre uma redução em paralelo desses fatores até o início da luteólise, próximo ao dia 14 em não gestantes (GINTHER et al., 2007b), quando também é identificada a redução da expressão de P450scc mitocondrial (WATSON et al., 2005). Um papel regulador sobre a fase luteal tem sido sugerido também ao complexo de IGFs, pelo aumento de IGFBP-2 que diminui sua biodisponibilidade no CL (WATSON et al., 2005), enquanto que alterações no fluxo sanguíneo durante a luteólise não foram identificadas (GINTHER et al., 2007b).

A secreção endometrial de PGF2α durante o final da fase lútea é o sinal para o início da luteólise (MCCRACKEN et al., 1999). Em equinos, a liberação de PGF2α ocorre na circulação periférica, já que não existe sistema de contracorrente como em ruminantes (GINTHER, 1974). No entanto, acredita-se que o corpo lúteo equino tenha uma maior sensibilidade a PGF2α, provavelmente devido a uma maior afinidade de ligação. Assim como em outras espécies, a liberação pulsátil de PGF2α endometrial em equinos é estimulada pela ocitocina (OXT) ligada ao seu receptor (OXTR), que aumenta a atividade da fosfolipase C (PLC), elevando à produção de inositol trifosfato (IP3) e diacilglicerol (DAG) intracelulares. Esses mensageiros aumentam a expressão de fosfolipase A2 (PLA2), que influencia a disponibilidade de ácido araquidônico para ser metabolizado por prostaglandina endoperóxido sintetase 2 (PTGS2) ou cicloxigenase-2 (COX-2) em PGF2α (BAZER et al., 2012). Além disso, a expressão da COX-2 também aumenta próximo ao dia 15 após a ovulação em éguas não gestantes, e é inibida com a presença do concepto, sugerindo ser um evento chave na indução da luteólise ou no reconhecimento materno da gestação (BOERBOOM et al., 2004; ABABNEH et al., 2011).

2.4 GESTAÇÃO

Para que ocorra uma gestação, o concepto equino deve sinalizar sua presença ao útero materno, prolongando assim a permanência do corpo lúteo primário (LEITH; GINTHER, 1984; KASTELIC et al., 1987). Após a ovulação e posterior fecundação, o embrião chega ao útero em 144 horas (BATTUT et al., 1997), período que produz maiores quantidades de prostaglandina E2 (PGE2), responsável pelo relaxamento e auxílio da movimentação em musculatura lisa (WEBER et al., 1991; ROBINSON et al., 2000).

Utilizando o mesmo princípio para o mecanismo de sinalização, o embrião se movimenta pelos cornos uterinos e corpo do útero, apresentando pico da mobilidade entre os dias 11 e 14 (LEITH; GINTHER, 1984; STOUT; ALLEN, 2001) após a ovulação. Quando essa movimentação é limitada, uma redução no reconhecimento materno da gestação é verificada (MCDOWELL et al., 1988; OKADA et al., 2019), já que a superfície endometrial não foi sinalizada por inteiro (KLEIN, 2016) para inibir a ocorrência da luteólise (GINTHER, 1983). Intervenções medicamentosas durante esse período são realizadas com o intuito de inibir a liberação de PGF2α periférica após a transferência de embriões (TE) (KOBLISCHKE et al., 2008), ou ainda em animais com histórico de infertilidade. Estudos recentes sugerem que o uso indiscriminado de anti-inflamatórios não esteroides (AINEs) pode prejudicar a mobilidade embrionária (STOUT; ALLEN, 2001; OKADA et al., 2018), enquanto outros avaliam diferentes efeitos entre fármacos (OKADA et al., 2019) e necessitam ser melhor compreendidos. Após esse período crítico para o reconhecimento materno da gestação, a migração embrionária cessa devido ao aumento do tônus uterino e do tamanho do embrião, ocorrendo a sua fixação em um dos cornos uterinos entre os dias 16 e 17 (GINTHER, 1983; KASTELIC et al., 1987).

A progesterona é essencial para diversos fatores fisiológicos dinâmicos entre a vesícula embrionária e o útero, auxiliando na mobilidade, fixação e sobrevivência do concepto (KASTELIC et al., 1987). O corpo lúteo primário é o principal responsável pela secreção de progesterona, que mantém a gestação até aproximadamente 40 dias (GINTHER, 1992). A formação de corpos lúteos acessórios estimulados por FSH e Gonadotrofina Coriônica Equina (eCG), produzida pelos cálices endometriais uterinos (ALLEN, 2000), contribui com a gestação até aproximadamente 100 dias, quando a produção de progesterona passa a ser suportada pela placenta (HOLTAN et al., 1979).

Sob a influência da progesterona, uma série de situações fisiológicas ocorrem no ambiente uterino a fim de receber o concepto. Nesse contexto, torna-se um desafio estabelecer ferramentas que auxiliem a sincronia entre esses fatores, principalmente quando tecnologias reprodutivas, como a TE, são utilizadas. De forma geral, costuma-se sincronizar éguas receptoras cíclicas com a doadora através da aplicação intramuscular de PGF2α ou seus análogos quando o status reprodutivo estiver entre os dias 7 e 14 do diestro. Seguido da monitoração do crescimento folicular individual pelo exame ginecológico e ultrasonográfico, é possível identificar e optar pela maior sincronia entre doadora e receptora. Além disso, uma janela de 2 a 3 dias entre ovulações pode ser considerada (MCKINNON; SQUIRES, 2007).

A sincronização de receptoras acíclicas em programas de TE é uma das opções estabelecidas após a década de 80 (HINRICHS et al., 1986) e, logo em seguida, a eliminação da necessidade de intervenção cirúrgica (LAGNEAUX; PALMER, 1993). Embora diversos progestágenos, como: medroxiprogesterona, hidroxiprogesterona, norgestomet e megestrol tenham sido testados, somente o altrenogest foi capaz de manter a gestação, mesmo após a indução da luteólise com PGF2α (MCKINNON et al., 2000). Além disso, o altrenogest fornece condições semelhantes para o desenvolvimento do embrião independente da receptora ser cíclica ou não (GRECO et al., 2012; SILVA et al., 2015). Diversos protocolos hormonais utilizando estradiol (E2) associado à P4 de longa ação têm sido utilizados na sincronização de éguas receptoras de embrião acíclicas ou em diestro (GRECO et al., 2012; GRECO et al., 2016). Quando E2 e a P4 estão ligados aos seus receptores, modulam a expressão gênica e a síntese proteica de acordo com a fase do ciclo estral (HARTT et al., 2005; SILVA et al., 2014), e mesmo exógenos, simulam sinais clínicos semelhantes ao de éguas cíclicas (SILVA et al., 2017).

O efeito da manipulação do sistema endócrino sobre o sistema de defesa endometrial em éguas ainda é desconhecido e necessita ser compreendido. Em humanos, citocinas inflamatórias estão envolvidas durante o processo de implantação embrionária e, quando estão aumentadas tornam o prognóstico de manutenção gestacional desfavorável (VAN MOURIK et al., 2009). Enquanto que em bovinos (MAJEWSKA et al., 2010) e suínos (FRANCZAK et al., 2012) as interleucinas (IL) 1 e IL6 modulam a síntese de prostaglandinas, além de serem reconhecidas como marcadores de resposta inflamatória durante a fase aguda, envolvendo efeitos sistêmicos e locais. Ao longo do ciclo estral equino, diversas células específicas ao processo inflamatório possuem variação em sua expressão. Durante o estro, várias interleucinas são detectadas no endométrio (SZÓSTEK et al., 2013), principalmente em resposta a possibilidade de contaminação devido à abertura cervical e à cobertura (TROEDSSON et al., 2001). Após a ovulação, ocorre sua diminuição, tornando o diestro um período de suscetibilidade ao desenvolvimento de infecções persistentes, principalmente quando expostos durante o estro (EVANS et al., 1986; SZÓSTEK et al., 2013).

2.5 PARTO, PUERPÉRIO E INVOLUÇÃO UTERINA

Em muitas espécies, sabe-se que o feto determina o momento do nascimento, sendo esse controle mediado pelo aumento na produção de cortisol fetal resultante direto da maturação do eixo hipotálamo-hipófise-adrenal (PASHEN, 1984). Após um período de aproximadamente 330 dias de gestação, a égua apresenta um parto rápido em comparação a outras espécies domésticas, geralmente cerca de 45 minutos (ROSSDALE; RICKETTS, 1980). Muitas mudanças físicas na égua indicam a proximidade ao nascimento do potro, como a presença de colostro no úbere, o qual pode ser liberado em forma de gotejamento ou ainda pode ressecar formando uma cera no orifício do teto, redução na viscosidade do muco cervical, aumento do relaxamento da cérvix, inquietação e sudorese entre outros eventos importantes, os quais indicam a preparação da égua para parto (WESSEL, 2005).

O puerpério é denominado como o intervalo entre o retorno das atividades reprodutivas de uma fêmea e o parto. Caracterizado pela involução uterina e o reestabelecimento da atividade ovariana. Esse período dura em média 10 dias em equinos, mas pode variar de 35 a 50 dias em bovinos, bem como outras espécies domésticas de interesse zootécnico (GINTHER, 1992).

De modo a reparar tecidos adjacentes, reduzir o tamanho uterino e a auxiliar na eliminação de fluidos tissulares, a involução uterina é indispensável para aumentar a possibilidade de uma nova concepção (HAFEZ; HAFEZ, 2004). Considerando que 51 a 62% das éguas utilizadas em programas de reprodução são lactantes (GINTHER, 1992), torna-se extremamente importante gerar conhecimento fisiológico sobre esse período, principalmente em programas de reprodução que buscam produzir potros em intervalos inferiores a 12 meses.

2.6 PRIMEIRO ESTRO PÓS-PARTO - CIO DO POTRO

Há décadas esse assunto gera amplas discussões na indústria equina (STEVENSON, 1945). Comumente chamado de "cio do potro", é o primeiro estro ovulatório e fértil após o parto em equinos (GINTHER, 1992). Normalmente, os sinais característicos de estro ocorrem entre 5 e 12 dias após o parto. No entanto, pode haver ausência dos sinais clássicos de cio em 10% das éguas (GINTHER, 1992). Embora alguns autores ratifiquem que as suas taxas de fertilidade sejam comparadas as dos ciclos subsequentes (LOY, 1982; WOODS et al., 1987; SHARMA et al., 2010), outros salientam a diminuição da fertilidade (MERKT; GÜNZEL, 1979) e o aumento da perda embrionária (MEYERS et al., 1991).

3 ARTIGO I – AGE-DEPENDENT EFFECT OF FOAL HEAT BREEDING ON PREGNANCY AND EMBRYO MORTALITY RATES IN THOROUGHBRED MARES

ARTIGO PUBLICADO

Age-dependent effect of foal heat breeding on pregnancy and embryo mortality rates in Thoroughbred mares

João Ricardo Malheiros de Souza; Paulo Bayard Dias Gonçalves; Kalyne Bertolin; Rogério Ferreira; Ana Sílvia Sardinha Ribeiro; Djacy Barbosa Ribeiro; Sandra Mara da Encarnação Fiala Rechsteiner; Reno Roldi de Araújo; Cláudio Alves Pimentel;
Anelise Maria Hammes Pimentel

Journal of Equine Veterinary Science
2020 Jul;90:102982. doi: 10.1016/j.jevs.2020.102982.

Age-dependent effect of foal heat breeding on pregnancy and embryo mortality rates in Thoroughbred mares

João Ricardo Malheiros de Souza[1]; Paulo Bayard Dias Gonçalves[1,2]; Kalyne Bertolin[1]; Rogério Ferreira[3]; Ana Sílvia Sardinha Ribeiro[4]; Djacy Barbosa Ribeiro[5]; Sandra Mara da Encarnação Fiala Rechsteiner[6]; Reno Roldi de Araújo[7]; Cláudio Alves Pimentel[8]; Anelise Maria Hammes Pimentel[9]*

[1]Laboratório de Biotecnologia e Reprodução Animal (BioRep), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil. [2]Universidade Federal do Pampa (Unipampa), Uruguaiana, RS, Brasil. [3]Departamento de Zootecnia, Universidade do Estado de Santa Catarina, Chapecó, SC, Brasil. [4]Instituto Sócio Ambiental e dos Recursos Hídricos, Universidade Federal Rural da Amazônia (UFRA), Belém, PA, Brasil. [5]Instituto da Saúde e Produção Animal, Universidade Federal Rural da Amazônia (UFRA) Belém, PA, Brasil. [6]Historep, Instituto de Biologia, Universidade Federal de Pelotas (UFPEL), Campus da Saúde, Pelotas, RS, Brasil. [7]Brasil Reprodução Animal e Universidade Iguaçu – UNIG, Iguaçu, RJ, Brasil. [8]Departamento de Patologia Animal, Faculdade de Medicina Veterinária, Universidade Federal de Pelotas (UFPEL), Capão do Leão, RS, Brasil. [9]Departamento de Zootecnia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPEL), Capão do Leão, RS, Brasil.

*Dra. Anelise Maria Hammes Pimentel, Departamento de Zootecnia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPEL), Campus Capão do Leão, Capão do Leão, RS, Brasil.

Phone: +55-53-3275-7596

Email: anehammespimentel@gmail.com

Abstract

The aim of the present study was to evaluate the correlation of age and heat cycle to determine reproductive efficiency in young and aged Thoroughbred mares bred on foal heat (FH) or on second heat (SH) after foaling. Embryo mortality (EmbM) was determined every time a mare was found open after a positive pregnancy diagnosis. Parturition to breeding interval (PBI), pregnancy rate (PregR) and EmbM rate were the dependent variables and the treatments were breeding on the FH or on SH. The cutoff age to obtain above-average probability for the EmbM was 10 years old. PregR in mares bred on FH was lower compared to SH (P < 0.01), however it was neither affected by age mares (P > 0.05) nor by the age group of mares (P > 0.05). Regarding FH and SH, there was a difference in PregR in young mares (P < 0.01), unlike in aged mares (P > 0.05). EmbM rate was not different between mares bred on FH or SH (P > 0.05) although it was affected by age of mares (P < 0.01). EmbM was higher in oldest than young mares (P < 0.01). Aged mares bred on FH had a significantly higher EmbM rate compared to the young group also bred on FH (P < 0.01). In conclusion, the reproductive efficiency of Thoroughbred mares bred on FH is dependent of the age. Aged mares (≥10 years old) should be bred at their SH to reduce EmbM and improve reproductive performance.

Keywords: heat cycle, reproductive performance, embryo loss, equine

Introduction

Equine is a peculiar species as the mare shows a fertile estrus within two weeks after parturition. However, the decision to breed mares during the first postpartum estrus (foal heat [FH]) is very controversial [1-6]. It is not recommended to breed mares on FH when they have problems at parturition (e.g., dystocia, retained placenta, delayed uterine involution, fever). In these situations, mares should be properly treated and re-evaluated before breeding [3, 7].

Often, practitioners decide to breed mares on FH to make them pregnant early in the breeding season. In such situations fertility must be improved and several management protocols have been adopted, such as postponing breeding up to the second heat (SH) [8], delaying ovulation with progesterone [9, 10], anticipating the second ovulation with prostaglandins [11, 12], and post-breeding uterine lavage [13].

Several studies reported lower conception rates [4, 5, 8, 14-17] and higher embryo mortality (EmbM) rates [5, 18-22] in mares bred at the first postpartum estrus, even without any pathological situation. Previously, some authors suggested that mares should not be bred on FH to avoid embryo or fetal loss, regardless of periparturient disorders, due to uterine involution, which usually occurs after the second or third week from parturition in mares [8]. In some studies, pregnancy rates (PregRs) and embryonic losses have been similar in mares bred either on FH or on later postpartum period [1, 2, 23].

The histologic changes during postpartum uterine involution in the mare have been described [24]. Within 7 days postpartum, microcaruncles disappear by cellular shrinkage, lysis, and phagocytosis, by which time luminal epithelium and endometrial glands return to normal. The diameters of the gravid and non-gravid horn are similar and microscopic uterine involution is nearly complete after the first postpartum estrus. So, considering the overall uterine involution, there are no reasons for higher early embryonic death rates associated with the foal heat. Other factors such as parturition-to-breeding interval (PBI) [17], number of previous live foals or presence of uterine cysts [25], stallion fertility [26], stress [22] and age of the mare [27, 28] have been suggested as possible causes for the lower fertility observed when mares are bred on FH. However, there are no long-term studies with a significant number of animals to conclude about the effect of age on FH fertility in the mare.

It is well known that the reproductive performance of older mares can be lower than that of younger mares [29-31]. Changes related to uterine function [32, 33] and low oocyte competence to develop into embryos [34] could be responsible for embryo loss in old animals. Furthermore, information on fertility during FH is controversial [1, 4, 6, 31], and this knowledge is essential to equine practitioners for breeding management. Then, we hypothesize that fertility of FH is age-dependent in the mare. The aim of this investigation was to examine whether the fertility of the first or second postpartum estrus would be affected by the age of the Thoroughbred mare conducted over a 11-year follow-up study.

Material and Methods

Mare selection

Over an 11-year period, the data were recorded from 898 postpartum cycles in 179 Thoroughbred mares at a breeding farm located in the Southern region of Brazil (32º S and 54º W). Mares were maintained and foaled (breeding season ranged from August 15 to November 30) on pasture and every morning fed additional from 3 and 4 kg oat. Stallions were maintained on separate pastures with its own stalls and every morning and evening were fed concentrate (1% of bodyweight; 14% protein) and 3 kg alfafa hay. Animals had ad libitum access to water and mineral supplement.

Experimental design and breeding management

Mares were analyzed by age (average was 9.48±3.2 years; range 4-22 years old) and divided into two classes according to the age: young or aged. After, mares were randomly bred at the foal heat (FH) in 424 cycles or bred at the second postpartum heat (SH) in 474 cycles. Therefore, they were teased every other day starting at the fifth day after foaling, and then palpated and ultrasound scanned daily per rectum for follicular monitoring. When a 35 mm follicle was detected, mares were bred with stallions of known fertility every other day until ovulation. Parturition to breeding interval was also evaluated and mares were allotted in two groups: bred until day 10 of parturition or after. Mares included in the SH group were treated with luteolytic agent (Cloprostenol, 250 µg/ml, Ciosin, MSD, Brazil) at day 7 after the first ovulation and teased every other day until the subsequent estrus of FH, and then breeding was performed as in FH group. When ovulation did not occur within 48 h after breeding, mares were re-bred. In cases of abnormality at parturition (dystocia, retained placenta, metritis or systemic diseases) animals were not included in this study.

Pregnancy diagnosis was performed by rectal palpation and ultrasonography at day 14 and repeated once a week until 45 days post-ovulation. Embryo mortality (EmbM) was characterized when a mare was diagnosed open after a previous positive pregnancy.

Data analysis

Receiver operator characteristic (ROC) curve analysis was performed to determine the optimal cutoff for mare maturity (young or aged) using EmbM as the classifier and mare age as the predictor. Optimal cutoff was determined as the value giving the greatest sum of sensitivity and specificity. Logistic regression analysis was performed to evaluate the probability of PregR and EmbM, using breeding heat (foal heat or second heat), age, PBI and its interaction as explanatory variables. Year and month of breeding were included in statistical model and removed when $P \geq 0.05$. Relationships between the EmbM (dependent variable) and the age of the mares (independent variable) were also statistically described by means of linear regression analysis. All statistical analysis was run in JMP software (SAS Institute Company) and differences were considered significant at $P \leq 0.05$.

Results

The overall PregR was 58.5% and EmbM was 9.1%. Regarding the age distribution of embryonic maintenance, the probability of maintaining gestation was 89.6% during FH and 92.0% during the SH. As a result of ROC analysis in this population, young mares were considered with less than 10 years old (n = 543; FH group = 265, SH group = 278), and aged mares were equal or older than 10 years old (n = 355; FH group = 159, SH group = 196). There was no detected effect of the year or month of breeding and these variables were removed from statistical models.

PregR was lower in mares bred on FH compared to SH (52.6% vs 63.7%; respectively; $P < 0.01$; Fig. 1A). Moreover, PregR was neither affected by age of mares ($P > 0.05$) nor by the age group of the mares (young: 59.1% vs aged: 57.5%; $P > 0.05$; Fig. 1B). EmbM rate was not different between mares bred on FH versus SH (10.4% vs 8.0%; $P > 0.05$; Fig. 1A); however, it was affected by age of mares ($P < 0.01$), being higher in aged (14.1%) compared to young mares (5.9%; $P < 0.01$; Fig. 1B).

The multivariable models indicated the interaction between the age of the mare and breeding heat to predict PregR and embryo maintenance. Therefore, when only considering young mares, the PregR was also lower in FH (51.3%) compared to SH (66.6%; $P < 0.01$; Fig. 2A), while the PregR did not differ in aged mares (FH, 54.7% vs SH, 59.7%; $P > 0.05$; Fig. 2A). On the other hand, aged mares bred on FH had a significantly higher EmbM rate compared with SH (20.1% vs 9,2%; $P < 0.01$; Fig. 2B) and with young mares bred on FH

(4.5%; $P < 0.01$; Fig. 2B). No difference was observed between young and aged mares on SH (7.2% vs 9.2%; $P > 0.05$; Fig. 2B). The odds of embryo maintenance using the age of the mares as a risk factor was significant for mares bred on foal heat ($P < 0.01$; Table 1), but not on second heat.

Regression analysis showed that mares bred on FH had a significant linear trend to increase EmbM as the age of the mare increased based on the following equations: EmbM (Foal Heat) rate = -0.1484 + 0.0277 × age ($P < 0.01$; $R^2 = 0.077$); overall EmbM rate = -0.0520 + 0.0154 × age ($P < 0.01$; $R^2 = 0.028$); EmbM (Second Heat) rate = 0.0232 + 0.0060 × age ($P > 0.05$; $R^2 = 0.05$; Fig. 3).

The average from parturition to breeding interval (PBI) was 21.6 days, ranging from 6 to 60 days in 898 cycles analyzed. The overall fertility was higher when mares were bred with a PBI >10 days compared with ≤10 days (62.6% vs 50.3%; $P < 0.01$). In FH and SH cycles, the PBI average was 10 ± 0.12 days (from 6 to 20 days), and 32 ± 0.32 days (from 21 to 60 days), respectively. The interaction between PBI and age of mares was detected, observing an increase in the EmbM when aged mares are bred few days after parturition ($P < 0.01$; Fig. 4).

During the 11-year period of data collection, our significant findings are: 1) total PregR was higher when mares were bred on SH than on FH; 2) PregR was not affected by the age of the mares when FH and SH were compared; and 3) EmbM rate was higher in aged than younger mares when bred on FH. Moreover, we determined the age of 10 years old as the cutoff to define an aged mare and recommend they should be bred at their SH to decrease embryo loss and increase reproductive performance.

Discussion

The outcomes of this study are consistent with previous reports of increasing PregR when mares are bred on SH than on FH [14, 15, 17, 35-38]. This higher PregR in SH (by about 11 percentage points) are explained by the time needed for the uterus to return for normal cyclic histological status, which generally occurs within 9 to 10 days [39] or 14 days [24] after parturition in mares. During the first 2 to 4 days postpartum, the vascular perfusion in endometrial and mesometrial tissues increase, following a progressive decrease until the second week postpartum [40]. Therefore, these data denote that the lower fertility during FH could be a consequence of the longer period for the endometrium to be repaired after foaling. Physiologically, uterine luminal epithelium and endometrial glands return from pregnant to non-pregnant condition within 7 days postpartum [24]. After the first postpartum estrus, the uterine horn diameters of the pregnant and non-pregnant did not differ and microscopic uterine involution was nearly complete [24]. In addition, the bacterial uterine contamination present in postpartum period did not affect fertility during FH [41].

Pregnancy rates in first or second heat have been a subject of disagreement among researchers and equine practitioners. In general, young mares have been bred on FH under normal puerperium conditions, due to the fact that 97% of Thoroughbred mares ovulate before day 20 postpartum [17]. However, the decrease on PregR in young mares submitted to breeding on FH in comparison to SH was observed in the present study, opposed to that reported by other researches [42-44] and practitioners.

The overall PregR was not different between aged and young mares. The possible effect of aging on fertility in mares is very controversial. Some studies found a decrease in PregR as mares get older [27, 31, 45-49]; however, others studies did not find any difference between young and aged mares as observed in this study [19, 50]. This discrepancy may be explained by the age in which the mares were classified as young or aged. Old mares have been considered those aged >10 [47], >13 years [4, 51, 52], >15 years [23, 32, 33, 49], or even >17 years [21]. In the present work, fewer mares were found aged >15 years, thus the threshold of 10 years was used to group mares as old or young. Nowadays, in the

Thoroughbred farms, there is a tendency to remove aged mares (>15 years) from breeding programs due to the greater probability of reproductive problems.

Age-related decline in fertility is an important factor observed in clinical assistance in humans [53] and horses [28, 54]. Women ≥35 years old present a reduction in fertility status [55]; however, they can decide the best time to have children, generally based on their own personal or professional life. Commonly, Thoroughbred mares have their sport performance achievements before two years old and they are maintained for as long as they remain victorious in the races. In this horse breed, the use of reproductive technologies is not allowed. Therefore, mares subjected to sports performances would have their reproductive achievements later in life.

In the present study, the lower reproductive performance observed in aged mares can be explained by the higher EmbM rate compared with younger mares when bred on FH. The EmbM rate has been reported to range from 4,6% to 30,1% [19-22, 38, 56]. When mares were not grouped according to age, EmbM rate did not differ between mares bred either on FH or SH. Embryonic mortality has been attributed as an important cause of subfertility in older mares [30], attributed mainly to: 1) abnormalities in oviductal epithelium [30] which would determine an unfavorable environment for the early embryonic cleavage [57]; 2) abnormal oocytes due to a longer follicular phase, associated with a slower rate of follicular growth [28]; 3) reduced uterine tone [33]; 4) previous live foals or presence of uterine cysts [25]; 5) persistent endometritis [58] and 6) differential expression of uterine progesterone receptors [32].

In order to avoid reduction in productivity and impact industry profitability, is advisable mating mares within approximately 25 days of foaling [59]. The average interval from foaling to ovulation has been reported to be around 10 days [17], which was similarly found in 424 mares bred on FH in the present study. PregR was not different from mares bred before or after 10 days postpartum. On the other hand, interaction between PBI and age of mares was observed, suggesting increase of EmbM in aged mares bred with lower PBI. From one standpoint, the difference in PregR in mares bred before or after day 10 postpartum has been attributed to beneficial effect in earlier ovulation, which may indicate a tendency toward a better regulated endocrine system as the breeding season advances and estrus become shorter. Nevertheless, we cannot reject the possibility of an age unbalance among study groups to be the reason of differences observed in PregR, since mares were predominantly young (age <10 years).

The hypothesis that fertility of FH is age-dependent in the mare was confirmed. Through regression analysis were performed estimate embryo mortality as a function of mare age resulted in a linear trend. Also, there is a chance for the EmbM rate to increase by 22% for each year older when mares are breeding on FH. In studies of reproduction performance, the pregnancy is a binary outcome (yes/no), and the precision of an observed average value depends, in part, on the number of observations [60].

Taken together, the results obtained in the present study indicate that older mares should be bred on second heat as a breeding strategy to decrease EmbM rate and optimize reproductive efficiency. These findings are supported by large number of observations, giving a practical response to the equine industry. The reproductive efficiency of Thoroughbred mares bred on FH is dependent on the mare age, with higher EmbM rates in mares aged 10 years old or over.

Acknowledgements

The authors are grateful to the National Council of Technological and Scientific Development (CNPq) for the financial support.

References

[1] Sharma S, Davies Morel MC, Dhaliwal GS. Factors affecting the incidence of postpartum oestrus, ovarian activity and reproductive performance in Thoroughbred mares bred at foal heat under Indian subtropical conditions. Theriogenology. 2010;74:90-9.

[2] Blanchard TL, Thompson JA, Brinsko SP, Stich KL, Wendt KM, Varner DD, et al. Mating mares on foal heat: a five-year retrospective study. Lexington: American Association of Equine Practitioners (AAEP); 2004. p. 525-30.

[3] Macpherson ML, Blanchard TL. Breeding mares on foal-heat. Equine Veterinary Education. 2005;17:44-52.

[4] Ginther OJ. Reproductive Biology of the Mare: Basic and Applied Aspects: Equiservices; 1992.

[5] Lane EA, Bijnen ML, Osborne M, More SJ, Henderson IS, Duffy P, et al. Key Factors Affecting Reproductive Success of Thoroughbred Mares and Stallions on a Commercial Stud Farm. Reproduction in Domestic Animals. 2016;51:181-7.

[6] Scoggin CF. Not just a number: effect of age on fertility, pregnancy and offspring vigour in thoroughbred brood-mares. Reproduction, fertility, and development. 2015.

[7] LeBlanc MM. Common Peripartum Problems in the Mare. Journal of Equine Veterinary Science. 2008;28:709-15.

[8] Merkt VH. Fohlenrosse und Fruchtresorption. Reproduction in Domestic Animals. 1966;1:102-8.

[9] Loy RG, Hughes JP, Richards WP, Swan SM. Effects of progesterone on reproductive function in mares after parturition. Journal of reproduction and fertility Supplement. 1975:291-5.

[10] Loy RG, Evans MJ, Pemstein R, Taylor TB. Effects of injected ovarian steroids on reproductive patterns and performance in post-partum mares. Journal of reproduction and fertility Supplement. 1982;32:199-204.

[11] Burns SJ, Irvine CH, Amoss MS. Fertility of prostaglandin-induced oestrus compared to normal post-partum oestrus. Journal of reproduction and fertility Supplement. 1979:245-50.

[12] McKinnon AO, Squires EL, Harrison LA, Blach EL, Shideler RK. Ultrasonographic studies on the reproductive tract of mares after parturition: effect of involution and uterine fluid on pregnancy rates in mares with normal and delayed first postpartum ovulatory cycles. Journal of the American Veterinary Medical Association. 1988;192:350-3.

[13] Lenz TR. One Practitioner's Approach to Foal Heat Breeding. Proceedings of the annual meeting / Society for Theriogenology 1986. p. 111-9.

[14] Caslick EA. The sexual cycle and its relation to ovulation with breeding records of the Thoroughbred mare. . Cornell Veterinarian. 1937; 27:187–206.

[15] Trum BF. The estrous cycle of the mare. Cornell Veterinarian. 1950;40:17-23.

[16] Lieux P. Computerized results of a breeding practice. American Association of Equine Practitioners (AAEP)1973. p. 55.

[17] Loy RG. Characteristics of postpartum reproduction in mares. Veterinary clinics of North America Large animal practice 1980;2:345-9.

[18] Bain AM. Foetal losses during pregnancy in the thoroughbred mare: a record of 2,562 pregnancies. New Zealand veterinary journal. 1969;17:155-8.

[19] Woods GL, Baker CB, Baldwin JL, Ball BA, Bilinski J, Cooper WL, et al. Early pregnancy loss in brood mares. Journal of reproduction and fertility Supplement. 1987;35:455-9.

[20] Ball BA. Embryonic loss in mares. Incidence, possible causes, and diagnostic considerations. The Veterinary clinics of North America Equine practice. 1988;4:263-90.

[21] Brinsko SP, Ball BA, Miller PG, Thomas PG, Ellington JE. In vitro development of day 2 embryos obtained from young, fertile mares and aged, subfertile mares. Journal of reproduction and fertility. 1994;102:371-8.

[22] Malschitzky E, Pimentel AM, Garbade P, Jobim M, Gregory RM, Mattos RC. Management Strategies Aiming to Improve Horse Welfare Reduce Embryonic Death Rates in Mares. Reproduction in Domestic Animals. 2015;50:632-6.

[23] Camillo F, Marmorini P, Romagnoli S, Vannozzi I, Bagliacca M. Fertility at the first post partum estrous compared with fertility at the following estrous cycles in foaling mares and with fertility in nonfoaling mares. Journal of Equine Veterinary Science. 1997;17:612-6.

[24] Gygax AP, Ganjam VK, Kenney RM. Clinical, microbiological and histological changes associated with uterine involution in the mare. Journal of reproduction and fertility Supplement. 1979:571-8.

[25] de Mestre AM, Rose BV, Chang YM, Wathes DC, Verheyen KLP. Multivariable analysis to determine risk factors associated with early pregnancy loss in thoroughbred broodmares. Theriogenology. 2019;124:18-23.

[26] Kenney RM, Kingston RS, Rajamannon AH, Ramberg CF. Stallion semen characteristics for predicting fertility. American Association of Equine Practitioners Proceedings of the Annual Meeting of the American Association of Equine Practitioners 1971. p. 53-67.

[27] Vanderwall DK, Peyrot LM, Weber JA, Woods GL. Reproductive efficiency of the aged mare. Proceedings Annual Meeting Society of Theriogenology. Coeur d' Alene1989. p. 153–6.

[28] Carnevale EM, Ginther OJ. Defective oocytes as a cause of subfertility in old mares. Biology of Reproduction Monograph. 1995;1:209–14.

[29] Ball BA, Little TV, Weber JA, Woods GL. Survival of Day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. Journal of Reproduction and Fertility. 1989;85:187-94.

[30] Ball BA, Little TV, Hillman RB, Woods GL. Pregnancy rates at Days 2 and 14 and estimated embryonic loss rates prior to day 14 in normal and subfertile mares. Theriogenology. 1986;26:611-9.

[31] Vanderwall DK. Early Embryonic Loss in the Mare. Journal of Equine Veterinary Science. 2008;28:691-702.

[32] Marinone AI, Mucci N, Kaiser G, Losinno L, Armendano J, Rodriguez E, et al. Reproductive Characteristics in Old and Young Subfertile Mares: Are They Really Different? Journal of Equine Veterinary Science. 2017;55:90-6.

[33] Carnevale EM, Ginther OJ. Relationships of age to uterine function and reproductive efficiency in mares. Theriogenology. 1992;37:1101-15.

[34] Carnevale EM, Uson M, Bozzola JJ, King SS, Schmitt SJ, Gates HD. Comparison of oocytes from young and old mares with light and electron microscopy. Theriogenology. 1999;51:299.

[35] Jennings WE. Some Common Problems in Horse Breeding. Cornell Veterinarian. 1941;31:197–215.

[36] Bell RJ, Bristol FM. Fertility and pregnancy loss following delay of foal estrus with progesterone and estradiol 17b. Internactional Symposium on Equine Reproduction. Calgary1986. p. 36.

[37] Lowis TC, Hyland JH. Analysis of post-partum fertility in mares on a thoroughbred stud in southern Victoria. Australian veterinary journal. 1991;68:304-6.

[38] Kurtz Filho M, Alda JLD, Deprá NM, Brass KÉ, Corte FDDL, Silva JHSD, et al. Fertilidade pós-parto em éguas Puro-Sangue de Corrida. Brazilian Journal of Veterinary Research and Animal Science. 1998;35.

[39] Gomez-Cuetara C, Flores JM, Sanchez J, Rodriguez A, Sanchez MA. Histological changes in the uterus during postpartum in the mare. Anat Histol Embryol. 1995;24:19-23.

[40] Lemes KM, Silva LA, Alonso MA, Celeghini ECC, Pugliesi G, Carvalho HF, et al. Uterine Vascular Perfusion and Involution During the Postpartum Period in Mares. Journal of Equine Veterinary Science. 2017;51:61-9.

[41] Glatzel PS, Belz JP. Fertility in mares after disturbed or undisturbed puerperium; evidence from clinical, microbiologic and hormone analysis. Berliner und Munchener tierarztliche Wochenschrift. 1995;108:367-72.

[42] Stevenson WG. Comparative Fertility of Mares. Canadian journal of comparative medicine and veterinary science. 1945;9:126-30.

[43] Bain AM. Estrus and infertility of the thoroughbred mare in Australia. Journal of the American Veterinary Medical Association. 1957;131:179-85.

[44] Tolksdorff E, Jöchle W, Lamond DR, Klug E, Merkt H. Induction of ovulation during the postpartum period in the thoroughbred mare with a prostaglandin analogue, Synchrocept™. Theriogenology. 1976;6:403-12.

[45] Hutton CA, Meacham TN. Reproductive efficiency on fourteen horse farms. Journal of Animal Science. 1968;27:434-8.

[46] Laing JA, Leech FB. The frequency of infertility in thoroughbred mares. Journal of reproduction and fertility Supplement. 1975:307-10.

[47] Rocha ALA. Características puerperais. taxas de concepção e morte embrionária em éguas Puro Sangue de Corrida cobertas no cio do potro: Universidade Federal do Rio Grande do Sul; 1994.

[48] Blanchard TL, Thompson JA, Love CC, Brinsko SP, Ramsey J, O'Meara A, et al. Influence of day of postpartum breeding on pregnancy rate, pregnancy loss rate, and foaling rate in Thoroughbred mares. Theriogenology. 2012;77:1290-6.

[49] Chevalier-Clément F. Pregnancy loss in the mare. Animal Reproduction Science. 1989;20:231-44.

[50] Rossi R, Silva Filho JM, Palhares MS, Martins RA, Anjos FR, Silva MM. Efeito da idade sobre a fertilidade e perdas embrionárias de éguas inseminadas com sêmen asinino

diluído e resfriado a 5ºC por 12 horas de armazenamento. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2014;66:1442-8.

[51] Ferreira JC, Canesin HS, Ignacio FS, Rocha NS, Pinto CR, Meira C. Effect of age and endometrial degenerative changes on uterine blood flow during early gestation in mares. Theriogenology. 2015;84:1123-30.

[52] Allen WR, Brown L, Wright M, Wilsher S. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. Equine Veterinary Journal. 2007;39:438-45.

[53] ASRM. Female age-related fertility decline. Fertility and Sterility. The American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and The Practice Committee of the American Society for Reproductive Medicine: Elsevier; 2014. p. 633-4.

[54] Rambags BP, van Boxtel DC, Tharasanit T, Lenstra JA, Colenbrander B, Stout TA. Advancing maternal age predisposes to mitochondrial damage and loss during maturation of equine oocytes in vitro. Theriogenology. 2014;81:959-65.

[55] Menken J, Trussell J, Larsen U. Age and infertility. Science. 1986;233:1389-94.

[56] Bosh KA, Powell D, Shelton B, Zent W. Reproductive performance measures among Thoroughbred mares in central Kentucky, during the 2004 mating season. Equine Veterinary Journal. 2009;41:883-8.

[57] Brinsko SP, Ignotz GG, Ball BA, Thomas PG, Currie WB, Ellington JE. Characterization of polypeptides synthesized and secreted by oviductal epithelial cell explants obtained from young, fertile and aged, subfertile mares. American journal of veterinary research. 1996;57:1346-53.

[58] Christoffersen M, Troedsson M. Inflammation and fertility in the mare. Reproduction in Domestic Animals. 2017;52 Suppl 3:14-20.

[59] Bosh KA, Powell D, Neibergs JS, Shelton B, Zent W. Impact of reproductive efficiency over time and mare financial value on economic returns among Thoroughbred mares in central Kentucky. Equine Veterinary Journal. 2009;41:889-94.

[60] Amann RP. Weaknesses in reports of “fertility” for horses and other species. Theriogenology. 2005;63:698-715.

Table 1. Odds for embryo maintenance per unit change in regressor (mare age) stratified by mares bred on foal heat (FH) or second heat (SH).

Category	Odds Ratio	Confidence Interval (95% CI)	P-value
FH	0.78	0.71 – 0.85	0.0001
SH	0.92	0.84 – 1.02	0.1328

Figure Legends

Figure 1. A) Pregnancy rate (PregR) and embryo mortality rate (EmbM) in 179 Thoroughbred mares evaluated during 11-y period bred on foal heat (n=424 breedings; black bars) or on second heat (n=474 breedings; white bars). B) Effect of age on PregR and EmbM rate comparing young (<10-y; n = 543 breedings; black bars) and aged (age ≥10-y; n = 355 breedings; white bars) Thoroughbred mares. Data are shown in percentage; (*) indicates statistical significance.

Figure 2. Effect of age on (A) pregnancy rate (PregR) and (B) embryo mortality rate (EmbM) in Thoroughbred mares bred on foal heat (young, black bars, n = 265; aged, white bars, n = 159) or second heat (young, black bars, n = 278; aged, white bars, n = 196). Data are shown in percentage; (*) indicates statistical significance.

Figure 3. Probability of embryo mortality as a function of age for Thoroughbred mares bred on foal heat ($P < 0.01$; $R^2 = 0.077$), second heat ($P > 0.05$; $R^2 = 0.05$) or overall ($P < 0.01$; $R^2 = 0.028$).

Figure 4. Probability of pregnancy loss as function of parturition to breeding interval and mare age (P < 0.01).

Figure 1

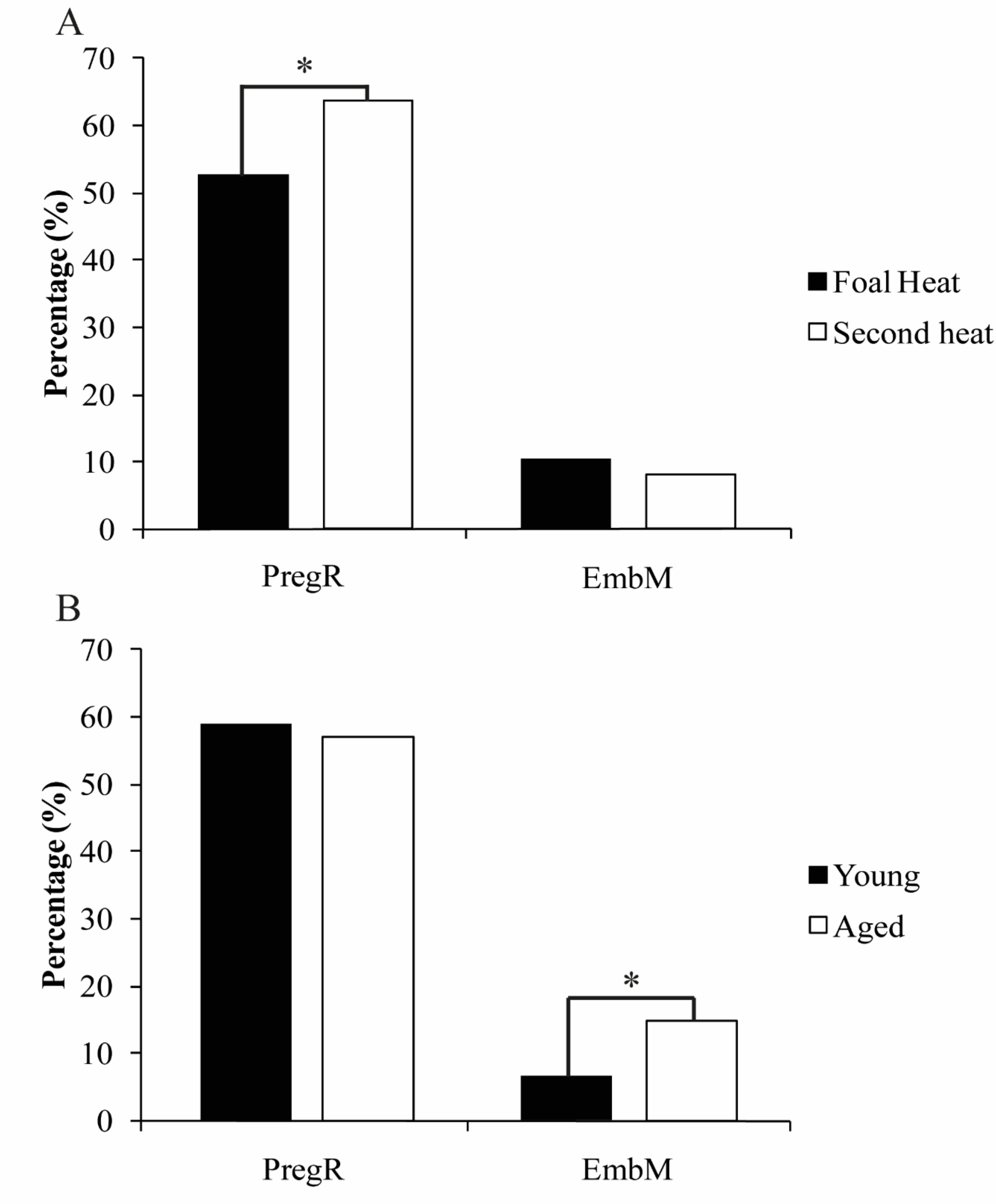

A
70
60
50
40
30
20
10
0
Percentage (%)
*
PregR
EmbM
Foal Heat
Second heat
B
70
60
50
40
30
20
10
0
Percentage (%)
*
PregR
EmbM
Young
Aged

Figure 2

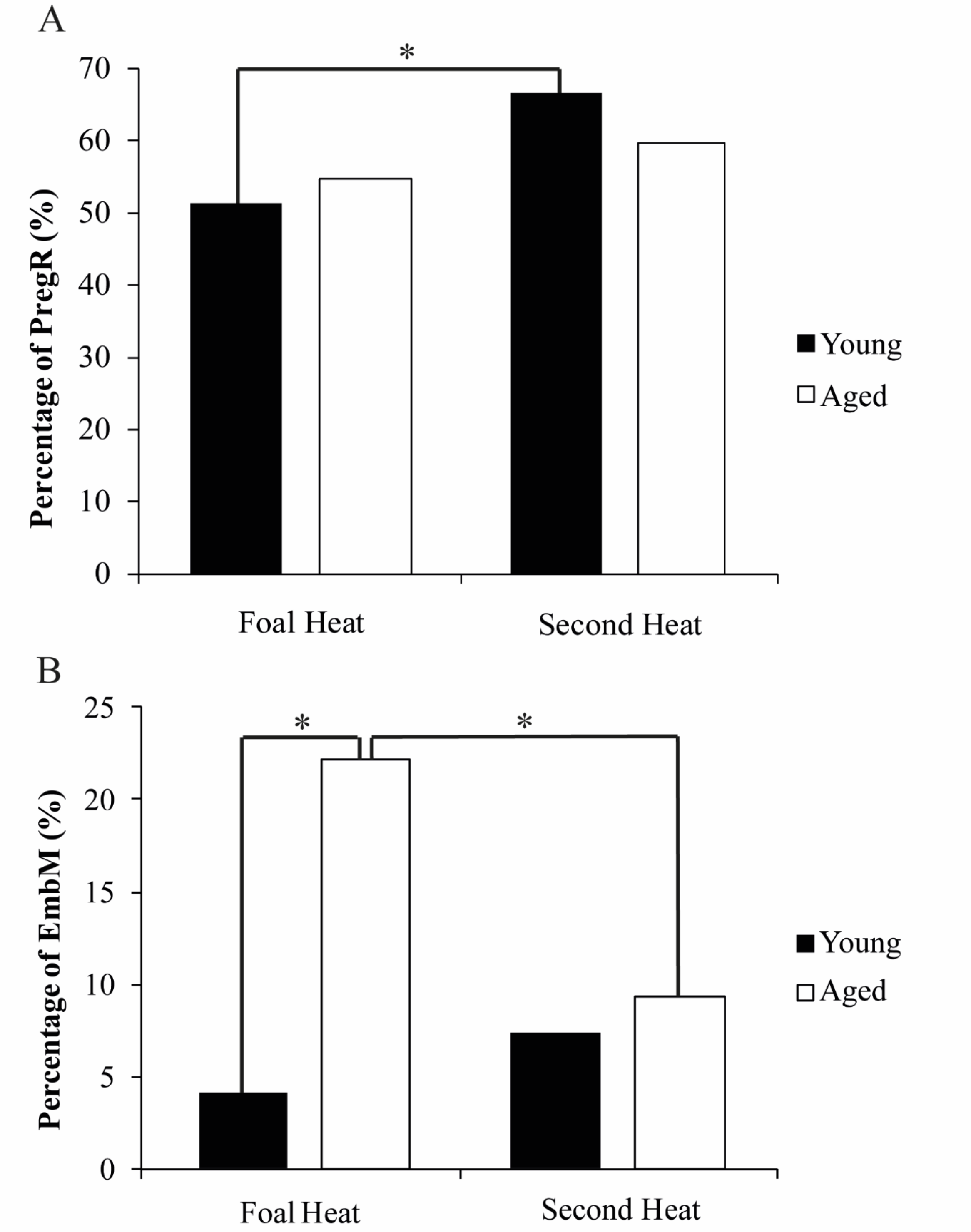
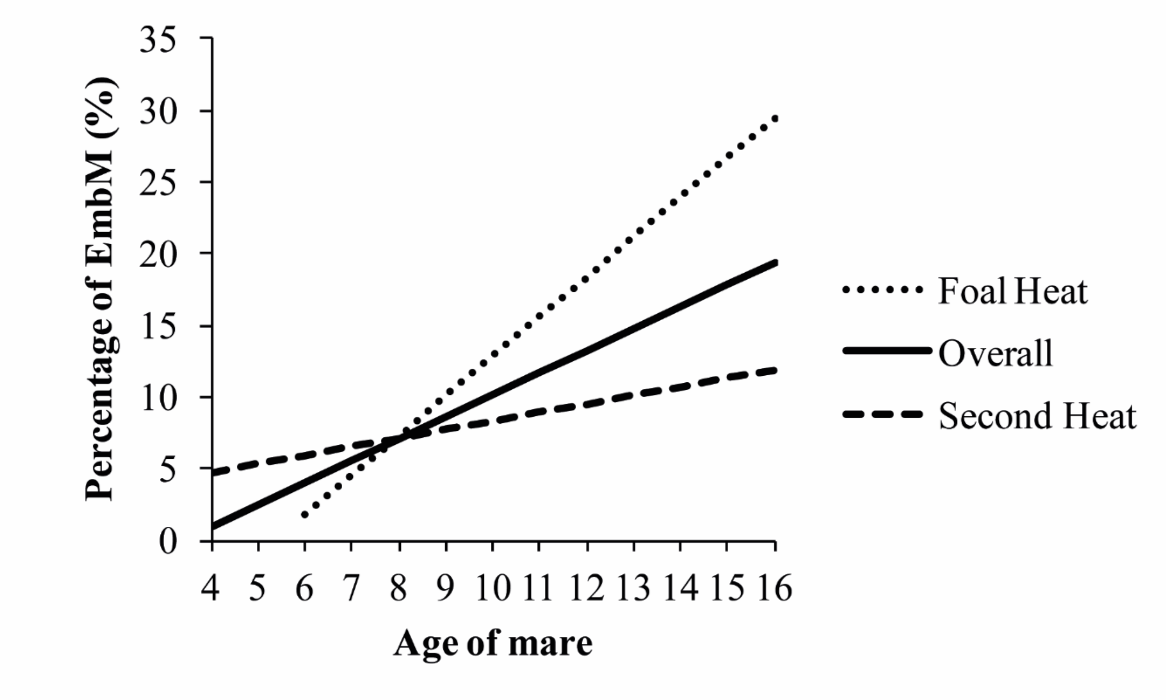
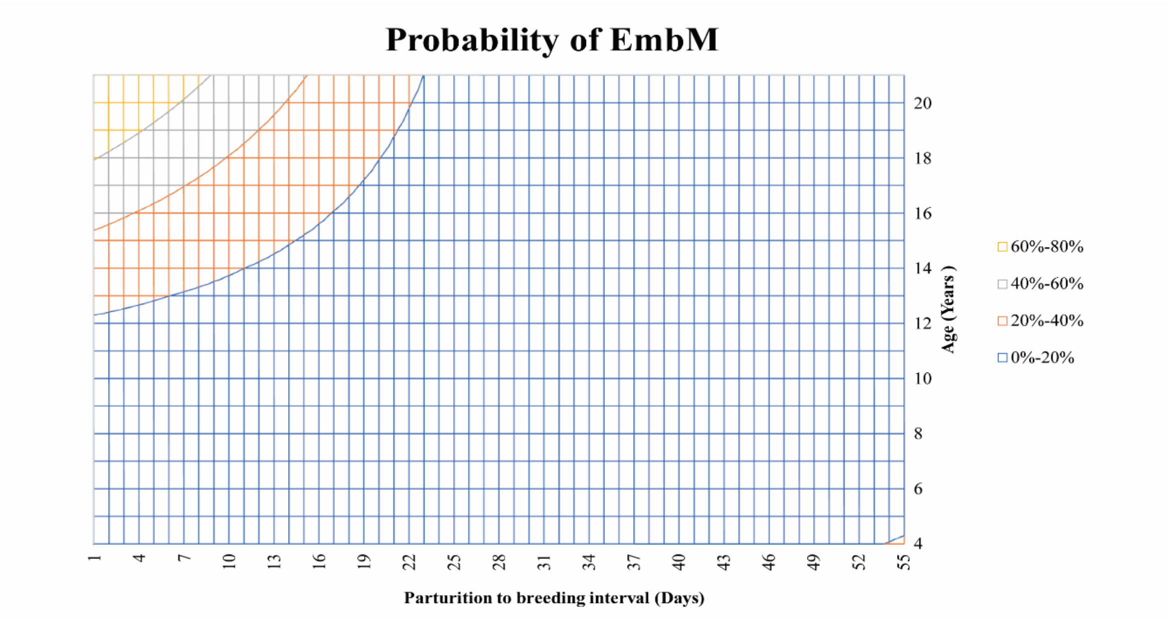

A
*
70
60
50
40
30
20
10
0
Percentage of PregR (%)
Foal Heat
Second Heat
Young
Aged
B
*
*
25
20
15
10
5
0
Percentage of EmbM (%)
Foal Heat
Second Heat
Young
Aged

Figure 3



35
30
25
20
15
10
5
0
Percentage of EmbM (%)
4 5 6 7 8 9 10 11 12 13 14 15 16
Age of mare
Foal Heat
Overall
Second Heat

Figure 4


Probability of EmbM
20
18
16
14
12
10
8
6
4
Age (Years)
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
Parturition to breeding interval (Days)
60%-80%
40%-60%
20%-40%
0%-20%

Highlights

- Aged mares bred on foal heat had significantly higher embryo loss.
- Embryo mortality was not different in mares when age was not taken into account.
- Pregnancy rate was higher when mares were bred after day 10 of parturition.
- Pregnancy rate was higher in mares bred on second heat compared with foal heat.

4 ARTIGO II – DONOR AGE AND RECIPIENT PROGESTERONE TREATMENTS AFFECT EMBRYO QUALITY AND CONCEPTION RATE IN MARE EMBRYO TRANSFER PROGRAMS

ARTIGO SUBMETIDO PARA PUBLICAÇÃO

Donor age and recipient progesterone treatments affect embryo quality and conception rate in mare embryo transfer programs

João Ricardo Malheiros de Souza; Reno Roldi de Araújo; Rogério Ferreira; Kalyne Bertolin; Sandra Mara da Encarnação Fiala Rechsteiner; Paulo Bayard Dias Gonçalves

Theriogenology, 2021.

Abstract

The physiological process that characterizes horses as a long-day breeding has been managed by veterinarians to start the reproductive activity early in the breeding season and improve the efficiency of reproductive biotechnologies. The aim of this investigation was to evaluate: 1) the effect of donor age on embryo recovery (ER) rate; 2) the effect of donor age on EQ; and 3) the effect of progesterone supplementation in cyclic and acyclic recipient mares (recipient mare group; RmG) on pregnancy rates in mares. Mares were divided into the following categories: 2 to 6 years old (young), 7 to 12 years old (adult), and 13 to 28 years old (aged). Logistic regression analysis was performed on the data from three separate analyses. The first model compared donor age, breeding season, and month of breeding to the probability of ER. The ER and EQ rates from young and adult mares were higher than aged mares ($P \leq 0.05$). Among the explanatory variables analyzed, only EQ and RmG affected the probability of PregR15 and PregR60 ($P \leq 0.0001$). For grade 1 and 2 embryos, the highest PregR15 and PregR60 was observed in recipient mares that were cycling and received P4 (CL/P4). We also observed the effect of RmG on EmbM ($P \leq 0.0276$). Embryonic mortality between cyclic and acyclic mares did not differ. However, progesterone treatment of cyclic recipients (CL/P4) decreased embryonic mortality compared to acyclic recipients with progesterone (P4). These findings may be the basis for determining new strategies to increase reproductive efficiency in embryo transfer programs in horses.

Keywords: embryo transfer, embryo loss, equine, fertility, pregnancy outcome

Introduction

The reproductive seasonality is very pronounced in equine species, in which ovarian activity increases during spring and summer [1], coinciding with the increase in daylight [2]. However, these responses can start at different times in different individuals. Temperature, photoperiodic history, cycles during winter, and food availability also influence the breeding season onset [3, 4]. During early onset of reproductive activity (return to cyclicity), the mare may show irregular estrus behavior due to a cyclical increase in gonadotropin and steroid hormone secretions [5]. This period varies between mares, especially considering that in addition to the ovaries, the endometrium [6] and myometrium [7] also require time to adapt after steroid hormone level variation. The first spontaneous ovulation of the season depends on physiological factors, such as age [8], number of available follicles [9], rate of response to hCG [10], ovulation time [11], and number of services per cycle [12].

Ovulation is a complex mechanism that triggers the oocyte release from follicle [13]. In the periovulatory period, the oocyte that is arrested in prophase I (PI) of the first meiotic division undergoes several progressive modifications, chromatin condensation and nuclear membrane breakdown (germinal vesicle, GVBD), progressing to metaphase II (MII), completing nuclear, cytoplasmic and molecular maturation [1, 14]. These processes are influenced by mare's age [15-18] and are fundamental to future embryo quality (EQ) [18-20]. When oocytes are fertilized, the zygote is transported through the utero-tubal junction [21] and moves through the uterus until maternal recognition of pregnancy that is required for corpus luteum to produce progesterone (P4), and consequently, pregnancy/embryo maintenance [22, 23].

Progesterone production by the corpus luteum (CL) is required in cyclic mares during early pregnancy to initiate uterine changes and ensure embryo survival [1, 24-26]. In assisted reproduction biotechnologies, such as embryo transfer (ET), veterinarians manage this period to synchronize the donor and recipient [12, 27-29]. In general, this technique is used to increase the number of foals born from competing young mares [30, 31] or improve the performance of aged donors [32, 33].

There has been a worldwide increase in the horse embryo industry volume, with approximately 31,000 embryo recoveries (ER) recorded in the America continent per year [34]. The mare breeding season onset and maximization of embryo per donors have required more recipients by ET program [29, 35]. The major challenges to the ET success rate include the precise maintenance and management of the number of recipients per donor (2–3) and the high cost of an ET program [33].

The synchronization of acyclic recipients for ET was established after the 1980s [36] and later without surgical intervention in mares [37]. Several protocols using estradiol (E2) associated with long-acting P4 have been used to synchronize acyclic or diestrus recipient mares [24, 35, 38]. Endogenous E2 and P4 modulate gene expression and protein synthesis according to the estrous cycle stage [39, 40], and they simulate clinical signs similar to those of cyclical mares when they are exogenously administered [41]. However, there are no long-term studies with a significant number of ETs to determine the effect of hormonal intervention on the embryo maintenance rate with different EQ. Thus, our hypotheses were that donor age affects the recovery of quality embryos and P4 supplementation in cyclic recipient mares improve the embryo quality and pregnancy rate in mare embryo transfer programs. This investigation aimed to evaluate the effects of 1) donor age on ER success, 2) donor age on EQ, and 3) P4 supplementation in cyclic and acyclic recipient on pregnancy rate.

Material and Methods

Experimental animals and breeding management

The experiment was performed during four breeding seasons, ranged from August 15 to April 30. A total of 1712 uterine lavages were performed from 340 donors of Mangalarga Marchador breed at a four-breeding farm located in the southern part of Brazil (parallel 30o). Age of the donors ranged from 2 to 28 years (mean 9.47±0.11 years), and were divided into the following categories: 2 to 6 years old (young; 31.37%), 7 to 12 years old (adult; 45.68%), and 13 to 28 years old (aged; 22.95%). Mares weigh 380 to 520 kg, and were kept on pasture with ad libitum access to water and mineral supplement. They were also checked every other day for estrus behavior by clinical and ultrasonographic examination of the genital tract per rectum (Sonoscape A5, Shinzhen, China). Therefore, mares were classified as acyclic or cyclic recipient according to their reproductive status by only one experienced veterinarian.

The reproductive status of donor mares was checked every other day and ovulation was induced with 750μg of deslorelin acetate (i.m.; Sincrorelin®, Ouro Fino, Brazil) when preovulatory follicle reached ≥35 mm and uterine edema was detected. The insemination with fresh or cooled semen (doses containing 500 x 106) was processed 24 h hours after induction of ovulation. Mares were inseminated at 48 h intervals and checked every 24 h until ovulation was detected (D0; disappearance of large preovulatory follicle).

Embryo recovery and Embryo transfer

Embryo recovery was performed seven or eight days after ovulation, by non-surgical technique with transcervical catheter (32FR, Minitube, Brazil) connected to 0.75 μm embryo filter (WTA, Brazil). Uterine flushing was repeated three times with lactated Ringer solution prewarmed to 36ºC. The embryos were washed in holding media (TQC Holding Plus®, Vitrocell) and scored for quality (EQ: embryo classification; 1 to 4) [42] under a binocular microscope at 10x to 20x magnification. Embryos were transferred by only one veterinarian to recipients using non-surgical technique (ET).

Recipient-mares design

Recipient mares were classified according to the reproductive status described below:

CL Group: control group. The mares were cycling and ovulation (D0) was or was not induced with GnRH analogous (Deslorelin acetate, 250µg/ml, IM, Sincrorrelin, Brazil), without post-ovulation hormonal treatment. The mares that were used as embryo recipients had corpus luteum and were synchronized with 2 to 5 days from the donors.

CL/P4 Group: cyclic group with P4. The mares were cycling and ovulation (D0) was or was not induced with GnRH analogous (Deslorelin acetate, 250µg/ml, IM, Sincrorrelin, Brazil). The recipient mares received, intramuscularly, 1.5 g of long-acting progesterone (P4LA, IM, Botupharma®, Botucatu, SP, Brasil) on the day of embryo transfer (D5), which was repeated weekly until 120 days of gestation.

P4 Group: acyclic recipient group with P4. The mares received 10 mg of Estradiol Benzoate (EB, 1mg/ml, i.m., Estrogin, USA) and, after 48 hours, 1.5 g of long-acting progesterone, which was repeated weekly until 120 days of gestation.

Pregnancy diagnosis

Pregnancy diagnosis was performed by transrectal palpation and ultrasonography at 7 days after ET (PregR15). When pregnancy diagnosis was confirmed, the mare was examined once a week until 60 days after ET (PregR60). Pregnancy loss (embryo mortality – EmbM) was considered when a mare was diagnosed open after a previous positive pregnancy.

Data analysis

Analysis was performed by logistic or ordinal regression using three separate models. The first model was included the age of the donor mare, breeding seasons and month of breeding to probability of embryo recovery (ER; 0 = negative, 1 = positive). The second model used the same interaction to embryo quality, classified as previously established [42] (EQ; 1 to 4) in cases of success of ER. The third model evaluated PregR15, PregR60, EmbM and their interactions with the following variables: age of the donor mare, breeding seasons, month of breeding, EQ and recipient mare group (RmG; CL, CL/P4 or P4). Explanatory variables were included in statistical model and removed when $P>0.05$. Binary logistic regression with maximum likelihood estimation was also used to analyze the difference between variable with significant effect on PregR15, PregR60 and EmbM. All statistical analysis was run in JMP software (SAS Institute Company) and differences were considered significant at $P\leq0.05$.

Results

The effect of the donor age on the ER was evaluated in 1,712 uterine lavages from 340 donor mares divided into three categories of animals (young, adult and aged). The effect of the breeding season or month of breeding was not detected; therefore, these two variables were removed from the statistical models. A total of 1,085 embryos (63.37%) were obtained from these donor mares. The ER rate was affected by the donor age (P≤0.01; Tables 1 and 2); however, it did not differ between young and adult mares (65.85% and 64.61%; respectively; P = 0.64). As expected, the ER rates from these two categories of animals were higher than those obtained from aged mares (56.74%; P≤0.05; Tables 1 and 2).

A total of 1,085 embryos were recovered, from which 978 were classified as grade 1 (90.14%), 91 as grade 2 (8.39%) and 16 as grade 3 (1.47%; Table 3). EQ was also affected by age, being higher in young and adult mares than in aged mares (P≤0.01; Figure 1). The of embryo recovery of grades 1, 2 and 3 were, respectively, 318 (91.64%), 26 (7.49%) and 3 (0.87%) from young mares, which did not differ from the 471 (91.46%), 37 (7.18%) and 7 (1.36%) embryos obtained from adult mares. However, aged mares had lower percentage of embryo quality 1 (n= 189; 84.75%) and higher rate of embryo quality 2 (n= 28; 12.56%) and 3 (n= 6; 2.69%) than young and adult mares. The comparison among age groups has to be analyzed with caution because the number of grade 3 embryos was very low.

The effects of donor age, breeding season, month of breeding, EQ and RmG on pregnancy at 7 days after ET (PregR15) were analyzed. However, only EQ and RmG affected PregR15 (P≤0.0001, Table 3). The other variables had no effect and were excluded from the model. We transferred 1,072 embryos from 1,085 recovered (98,8%). For grade 1 embryos, the highest PregR15 was found for the CL/P4 (n=262; 96.60%) group, following the P4 (n=307; 87.60%) and CL (n=402; 77.60%; P≤0.01; Figure 2A) groups. When considered grade 2 embryos, the CL/P4 group presented 100.00% (n=22) of PregR15, significantly higher than the CL and P4 group (n=35; 62.90% and n=34; 76.50%, P≤0.01). The numbers of grade 3 embryos transferred in the CL (n=7; 28.60%), CL/P4 (n=1; 100.00%) and P4 (n=2; 0.00%) groups were too small for statistical analysis.

Pregnancy rates at 60 days after ET (PregR60) followed a similar trend to that observed in the pregnancy diagnosis at 15 days of embryonic life (PregR15). The likelihood analysis for PregR60 revealed that EQ and RmG affected the model ($P \leq 0.0001$, Table 4). PregR60 with grade 1 embryos were higher in the CL/P4 group (n=262; 96.56%; $P \leq 0.01$) than in the P4 (n=307; 85.89%) and CL groups (n=401; 77.61%; $P \leq 0.01$; Figure 2B). Likewise, grade 2 embryos had the highest PregR60 for CL/P4 (n=22; 100.00%; $P \leq 0.01$), followed by P4 (n=34; 62.86%) and CL groups (n=35; 76.47%; $P \leq 0.01$; Figure 2B). The numbers of grade 3 embryos transferred in the CL/P4 (n=1; 100.00%), CL (n=7; 14.29%) and P4 (n=2; 00.00%) groups were too small for statistical analysis.

The effects of donor age, breeding season, month of breeding, EQ and RmG were also analyzed in relation to embryonic mortality. However, only RmG affected EmbM ($P \leq 0.0276$; Table 5). The other variables were excluded from the model. The highest EmbM rate was observed in acyclic recipient treated with P4 (P4 group; n=46/343; 13.40%), which differed from CL/P4 (n=20/285; 7.00%; $P \leq 0.01$), but did not differ statistically from the CL group (n=43/444; 9.70%). EmbM in cyclic recipient with P4 (CL/P4) or without P4 (CL) did not differ ($P > 0.05$; Figure 3).

Discussion

The results presented here were from four reproductive seasons, 1,712 uterine lavages, 340 donors, 1085 embryos and 1,072 embryo transfers. The new and main findings are the positive effect on the pregnancy and embryo mortality rates in response to the long-acting progesterone administration to cyclic embryo recipient mares. In addition, we observed that the age of mare donor influenced embryo recovery and EQ. Also, the quality of the embryos transferred to the recipient affected the pregnancy rate in horses.

It is well established that age-related fertility decline has multifactorial etiological factors in women [43] and mares [18, 44]. In the present study, ER and EQ were negatively affected in aged mares (≥ 15 years), which is consistent with previous reports [16, 45-47]. There are evidences that oocytes from old mares show alterations in meiotic division, chromosome recombination and spindle formation [18]. Aneuploidies have been reported in oocytes from aged mares [18, 48-50], and particularly frequently in >14-year-old mares [16, 51, 52].

The equine embryo reaches the uterus 5.5 d post-ovulation [53], after embryonic genome activation, at the early blastocyst stage. These important developmental events occurring during its passage through the oviduct, which plays a specific role in EQ and early embryonic development in horses [54]. During this physiological process of embryo development, P4 plays an important role in preparing the oviduct for embryonic transport and initial development [55] and in preparing the uterus for embryo implantation and pregnancy maintenance [22, 56].

The pregnancy rates were affected by the EQ at 7 and 60 d after ET, consistent with previous studies in mares [47] and cows [57]. Although most embryos collected were grade 1, the presence of low-quality embryos was also observed in young mares, which may be associated with the selection of superior animals, which was also detected in high milk production cows [58]. The P4 administration increased the pregnancy rate in cyclic recipient mares after embryo transfer of either embryo grade 1 or 2. In addition to the well-known role of P4 produced by the primary corpus luteum at the beginning of pregnancy, followed by equine chorionic gonadotropin (eCG) secretion, leading to the formation of supplementary corpora lutea [24], recent studies have shown that P4 increases the secretion of prostaglandin in the equine myometrium and endometrium tissues during the middle luteal phase [7], which also occurs during early pregnancy in other species [59, 60]. Furthermore, P4 supplementation reduces the expression of its receptors, and this endometrial suppression is associated with uterine receptivity required for the embryo and fetus development, maternal recognition of pregnancy, and subsequent implantation [26, 61]. The endometrium in P4-treated mares shows a slight but significant increase in the number of polymorphonuclear neutrophils [61], and improved uterine immune function [62].

We did not observe the effect of embryo grade 1 or 2 on EmbM. It is important to note that grade 3 embryos were obtained in insufficient numbers for a reliable statistical analysis. The timing of the increase in P4 concentration after fertilization seems to be essential for the development of fair- and poor-quality embryos. Regardless of the embryo quality, embryonic survival is similar whether the embryos are transferred to synchronous cattle recipients [63]. P4 supplementation might have improved the synchrony between the recipient and embryos in the present study.

In conclusion, the donor age influenced the embryo recovery and embryo quality success rates. Moreover, P4 supplementation improved pregnancy rate after embryo transfer in cyclic recipient.

Acknowledgments

This study was supported by grants from Brazilian National Council for Scientific and Technological Development (CNPq; 409981/2018-4/302031/2019-7), Research Support Foundation of the State of Rio Grande do Sul (FAPERGS; Edital PRONEX 12/2014 - FAPERGS/CNPq, 16/2551-0000494-3) and Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES; Finance Code 001).

Conflict of interest

None of the authors have any conflict of interest to declare.

References

[1] Ginther OJ. Reproductive Biology of the Mare: Basic and Applied Aspects: Equiservices; 1992.

[2] Sharp DC, Kooistra L, Ginther OJ. Effects of artificial light on the oestrous cycle of the mare. J Reprod Fertil Suppl. 1975:241-6.

[3] Guillaume D, Duchamp G, Salazar-Ortiz J, Nagy P. Nutrition influences the winter ovarian inactivity in mares. Theriogenology. 2002;58:593-7.

[4] Nagy P, Guillaume D, Daels P. Seasonality in mares. Animal Reproduction Science. 2000;60-61:245-62.

[5] Donadeu FX, Watson ED. Seasonal changes in ovarian activity: lessons learnt from the horse. Anim Reprod Sci. 2007;100:225-42.

[6] Kenney RM, Doig PA. Equine endometrial biopsy. In: Morrow DA, editor. Current therapy in theriogenology. Philadelphia: W.B. Saunders Company; 1986. p. 1143.

[7] Piotrowska-Tomala KK, Jonczyk AW, Skarzynski DJ, Szóstek-Mioduchowska AZ. Luteinizing hormone and ovarian steroids affect in vitro prostaglandin production in the equine myometrium and endometrium. Theriogenology. 2020.

[8] Ginther OJ, Gastal EL, Gastal MO, Beg MA. Dynamics of the Equine Preovulatory Follicle and Periovulatory Hormones: What's New? Journal of Equine Veterinary Science. 2008;28:454-60.

[9] Wesson JA, Ginther OJ. Puberty in the female pony: reproductive behavior, ovulation, and plasma gonadotropin concentrations. Biology of Reproduction. 1981;24:977-86.

[10] Carnevale EM, Squires EL, McKinnon AO, Harrison LA. Effect of human chorionic gonadotropin on time to ovulation and luteal function in transitional mares. Journal of Equine Veterinary Science. 1989;9:27-9.

[11] Staempfli S, Clavier S, Thompson D, Burns P, Lyle S, McKinnon A. Effect of a Single Injection of Long-acting Progesterone on the First Ovulation in Early and Late Spring Transitional Mares. Journal of Equine Veterinary Science - J EQUINE VET SCI. 2011;31.

[12] Cuervo-Arango J, Clark A. The first ovulation of the breeding season in the mare: The effect of progesterone priming on pregnancy rate and breeding management (hCG response rate and number of services per cycle and mare). Animal Reproduction Science. 2010;118:265-9.

[13] Pincus G, Enzmann EV. The Comparative Behavior of Mammalian Eggs in Vivo and in Vitro : I. The Activation of Ovarian Eggs. The Journal of experimental medicine. 1935;62:665-75.

[14] Edwards RG. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. Nature. 1965;208:349-51.

[15] Carnevale EMG, O.J. Defective oocytes as a cause of subfertility in old mares. Biol Reprod. 1995;1:209-2014.

[16] Carnevale EM. The mare model for follicular maturation and reproductive aging in the woman. Theriogenology. 2008;69:23-30.

[17] Morris LH, Allen WR. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Equine Veterinary Journal. 2002;34:51-60.

[18] Rizzo M, Ducheyne KD, Deelen C, Beitsma M, Cristarella S, Quartuccio M, et al. Advanced mare age impairs the ability of in vitro-matured oocytes to correctly align chromosomes on the metaphase plate. Equine Veterinary Journal. 2019;51:252-7.

[19] Hendriks WK, Colleoni S, Galli C, Paris DB, Colenbrander B, Roelen BA, et al. Maternal age and in vitro culture affect mitochondrial number and function in equine oocytes and embryos. Reproduction, fertility, and development. 2015.

[20] Rambags BP, van Boxtel DC, Tharasanit T, Lenstra JA, Colenbrander B, Stout TA. Advancing maternal age predisposes to mitochondrial damage and loss during maturation of equine oocytes in vitro. Theriogenology. 2014;81:959-65.

[21] van Niekerk CH, Gerneke WH. Persistence and parthenogentic cleavage of tubal ova in the mare. Onderstepoort J Vet Res. 1966;33:195-232.

[22] Allen WR. Fetomaternal interactions and influences during equine pregnancy. Reproduction. 2001;121:513-27.

[23] Smits K, Gansemans Y, Tilleman L, Nieuwerburgh F, Van de Velde M, Gerits I, et al. Maternal Recognition of Pregnancy in the Horse: Are MicroRNAs the Secret Messengers? Int J Mol Sci. 2020;21:419.

[24] Pinto CRF. Impact of the corpus luteum on survival of the developing embryo and early pregnancy in mares. Theriogenology. 2020.

[25] Caballeros JE, Camacho C, Cazales N, Estradé MJ, Fiala-Rechsteiner S, Jobim MIM, et al. Ultrastructural and histological characteristics of the equine endometrium at day 5 post ovulation. Theriogenology. 2019;132:106-12.

[26] Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Frontiers in bioscience : a journal and virtual library. 2002;7:d1879-98.

[27] Cuervo-Arango J, Claes AN, Ruijter-Villani M, Stout TA. Likelihood of pregnancy after embryo transfer is reduced in recipient mares with a short preceding oestrus. Equine Veterinary Journal. 2018;50:386-90.

[28] Cuervo-Arango J, Martín-Peláez MS, Claes AN. A practical guide to estimate the age of the early CL by ultrasonography in mares examined for the first time to be used as recipients in a commercial embryo transfer program. Theriogenology. 2020;153:48-53.

[29] Oliveira Neto IV, Canisso IF, Segabinazzi LG, Dell'Aqua CPF, Alvarenga MA, Papa FO, et al. Synchronization of cyclic and acyclic embryo recipient mares with donor mares. Animal Reproduction Science. 2018;190:1-9.

[30] Pinto MR, Miragaya MH, Burns P, Douglas R, Neild DM. Strategies for Increasing Reproductive Efficiency in a Commercial Embryo Transfer Program With High Performance Donor Mares Under Training. Journal of Equine Veterinary Science. 2017;54:93-7.

[31] Mortensen CJ, Choi YH, Hinrichs K, Ing NH, Kraemer DC, Vogelsang SG, et al. Embryo recovery from exercised mares. Animal Reproduction Science. 2009;110:237-44.

[32] Squires EL, McCue PM, Vanderwall D. The current status of equine embryo transfer. Theriogenology. 1999;51:91-104.

[33] Squires E. Embryo transfer challenges and perspectives. Revista Brasileira de Reprodução Animal. 2013;37:105-7.

[34] Viana JHM. 2018 Statistics of embryo production and transfer in domestic farm animals. Embryo Technology Newsletter. 2019;36:26.

[35] Greco GM, Burlamaqui FLG, Pinna AE, Queiroz FJRd, Cunha MPdS, Brandão FZ. Use of long-acting progesterone to acyclic embryo recipient mares. Revista Brasileira de Zootecnia. 2012;41:607-11.

[36] Hinrichs K, Sertich PL, Kenney RM. Use of altrenogest to prepare ovariectomized mares as embryo transfer recipients. Theriogenology. 1986;26:455-60.

[37] LAGNEAUX D, PALMER E. Embryo transfer in anoestrous recipient mares: attempts to reduce altrenogest administration period by treatment with pituitary extract. Equine Veterinary Journal. 1993;25:107-10.

[38] Greco GM, Fioratti EG, Segabinazzi LG, Dell'Aqua JA, Jr., Crespilho AM, Castro-Chaves MMB, et al. Novel Long-Acting Progesterone Protocols Used to Successfully Synchronize Donor and Recipient Mares With Satisfactory Pregnancy and Pregnancy Loss Rates. Journal of Equine Veterinary Science. 2016;39:58-61.

[39] Hartt LS, Carling SJ, Joyce MM, Johnson GA, Vanderwall DK, Ott TL. Temporal and spatial associations of oestrogen receptor alpha and progesterone receptor in the endometrium of cyclic and early pregnant mares. Reproduction. 2005;130:241-50.

[40] Silva ESM, Scoggin KE, Canisso IF, Troedsson MHT, Squires EL, Ball BA. Expression of receptors for ovarian steroids and prostaglandin E2 in the endometrium and myometrium of mares during estrus, diestrus and early pregnancy. Animal Reproduction Science. 2014;151:169-81.

[41] Silva ESM, Ignácio FS, Fritsch SC, Zanoni DS, Pantoja JCF, Oliveira-Filho JP, et al. Administration of 2.5 mg of estradiol followed by 1,500 mg of progesterone to anovulatory mares promote similar uterine morphology, hormone concentrations and molecular dynamics to those observed in cyclic mares. Theriogenology. 2017;97:159-69.

[42] McKinnon AO, Squires EL. Morphologic assessment of the equine embryo. Journal of the American Veterinary Medical Association. 1988;192:401-6.

[43] ASRM. Female age-related fertility decline. Fertility and Sterility. The American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and The Practice Committee of the American Society for Reproductive Medicine: Elsevier; 2014. p. 633-4.

[44] Souza JRMd, Gonçalves PBD, Bertolin K, Ferreira R, Ribeiro ASS, Ribeiro DB, et al. Age-Dependent Effect of Foal Heat Breeding on Pregnancy and Embryo Mortality Rates in Thoroughbred Mares. Journal of Equine Veterinary Science. 2020;90:102982.

[45] Carnevale EM, Ramirez RJ, Squires EL, Alvarenga MA, Vanderwall DK, McCue PM. Factors affecting pregnancy rates and early embryonic death after equine embryo transfer. Theriogenology. 2000;54:965-79.

[46] Carnevale EM, Ginther OJ. Defective oocytes as a cause of subfertility in old mares. Biology of Reproduction Monograph. 1995;1:209–14.

[47] Panzani D, Rota A, Marmorini P, Vannozzi I, Camillo F. Retrospective study of factors affecting multiple ovulations, embryo recovery, quality, and diameter in a commercial equine embryo transfer program. Theriogenology. 2014;82:807-14.

[48] Shilton CA, Kahler A, Davis BW, Crabtree JR, Crowhurst J, McGladdery AJ, et al. Whole genome analysis reveals aneuploidies in early pregnancy loss in the horse. Sci Rep-Uk. 2020;10:13314.

[49] de Mestre AM, Rose BV, Chang YM, Wathes DC, Verheyen KLP. Multivariable analysis to determine risk factors associated with early pregnancy loss in thoroughbred broodmares. Theriogenology. 2019;124:18-23.

[50] Rizzo M, du Preez N, Ducheyne KD, Deelen C, Beitsma MM, Stout TAE, et al. The horse as a natural model to study reproductive aging-induced aneuploidy and weakened centromeric cohesion in oocytes. Aging. 2020;12:22220-32.

[51] Lane EA, Bijnen ML, Osborne M, More SJ, Henderson IS, Duffy P, et al. Key Factors Affecting Reproductive Success of Thoroughbred Mares and Stallions on a Commercial Stud Farm. Reproduction in Domestic Animals. 2016;51:181-7.

[52] Scoggin CF. Not just a number: effect of age on fertility, pregnancy and offspring vigour in thoroughbred brood-mares. Reproduction, fertility, and development. 2015.

[53] Ginther OJ. Fixation and orientation of the early equine conceptus. Theriogenology. 1983;19:613-23.

[54] Lazzari G, Colleoni S, Lagutina I, Crotti G, Turini P, Tessaro I, et al. Short-term and long-term effects of embryo culture in the surrogate sheep oviduct versus in vitro culture for different domestic species. Theriogenology. 2010;73:748-57.

[55] Allen WR. The Physiology of Early Pregnancy in the Mare. American Association of Equine Practitioners (AAEP). 2000;46.

[56] Kastelic JP, Adams GP, Ginther OJ. Role of progesterone in mobility, fixation, orientation, and survival of the equine embryonic vesicle. Theriogenology. 1987;27:655-63.

[57] Erdem H, Karasahin T, Alkan H, Dursun S, Satilmis F, Guler M. Effect of embryo quality and developmental stages on pregnancy rate during fresh embryo transfer in beef heifers. Tropical Animal Health and Production. 2020;52:2541-7.

[58] Leroy JLMR, Opsomer G, De Vliegher S, Vanholder T, Goossens L, Geldhof A, et al. Comparison of embryo quality in high-yielding dairy cows, in dairy heifers and in beef cows. Theriogenology. 2005;64:2022-36.

[59] Franczak A, Kotwica G, Kurowicka B, Oponowicz A, Wocławek-Potocka I, Petroff BK. Expression of enzymes of cyclooxygenase pathway and secretion of prostaglandin E2 and F2alpha by porcine myometrium during luteolysis and early pregnancy. Theriogenology. 2006;66:1049-56.

[60] Franczak A, Zmijewska A, Kurowicka B, Wojciechowicz B, Petroff BK, Kotwica G. The effect of tumor necrosis factor alpha (TNFalpha), interleukin 1beta (IL1beta) and interleukin 6 (IL6) on endometrial PGF2alpha synthesis, metabolism and release in early-pregnant pigs. Theriogenology. 2012;77:155-65.

[61] Willmann C, Budik S, Walter I, Aurich C. Influences of treatment of early pregnant mares with the progestin altrenogest on embryonic development and gene expression in the endometrium and conceptus. Theriogenology. 2011;76:61-73.

[62] Peltier MR, Grant TR, Hansen PJ. Distinct physical and structural properties of the ovine uterine serpin. Biochimica et biophysica acta. 2000;1479:37-51.

[63] Hasler JF, McCauley AD, Lathrop WF, Foote RH. Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. Theriogenology. 1987;27:139-68.

Figure legends

Figure 1. Embryo quality distribution on mare age. Grade 1: 318 (Young), 471 (Adult) and 189 (Aged); Grade 2: 26 (Young), 37 (Adult) and 28 (Aged); Grade 3: 3 (Young), 7 (Adult) and 6 (Aged). Data are show in percentage. Differ letters indicate significance difference.

Figure 2. A) Pregnancy rate of recipient mares 15 days after embryo transfer (PregR15) on recipient treatments from differ embryo quality. B) Pregnancy rate of recipient mares 60 days after embryo transfer (PregR60) on recipient treatments from differ embryo quality. Grade 1: CL group, dark gray, n=401; P4/CL group, light gray, n=262; P4 group, gray, n=307. Grade 2: CL group, dark gray, n=35; P4/CL group, light gray, n=22; P4 group, gray, n=34. Data are show in percentage. Differ letters indicate significance difference.

Figure 3. Embryo mortality rate (EmbM) in recipient mares after embryo transfer. CL group, dark gray, n=43; P4/CL group, light gray, n=20; P4 group, gray, n=46. Data are show in percentage. Differ letters indicate significance difference.

Table legends

Table 1. Perform of embryo recovery in mares.

Table 2. Odds for embryo recovery per unit change in regressor (embryo grade) stratified by mare age.

Table 3. Analysis for each variable, statistical probability (p-value) and the 95% confidence interval (CI) for the variables associated with the PregR15 after embryo transfer by group of recipient mares.

Table 4. Analysis for each variable, statistical probability (p-value) and the 95% confidence interval (CI) for the variables associated with the PregR60 after embryo transfer by group of recipient mares.

Table 5. Analysis for each variable, statistical probability (p-value) and the 95% confidence interval (CI) for the variables associated with the EmbM after embryo transfer by group of recipient mares.

Highlights

- Age of mares did influence the probability of success on ER and EQ;
- EQ did influence on the probability of PregR15 and PregR60;
- The progesterone supplementation in cyclic recipient mares improve PregR15 and PregR60;
- The progesterone supplementation improve mare fertility.

Table 1. The effect of mare's age on embryo recovery rate.

Donor age	Uterine lavages n	Embryo recovery n	Embryo recovery %
Young	537	347	64.61a
Adult	782	515	65.85a
Aged	393	223	56.74b
Total	1712	1085	63.37

Columns with different letters are significantly different ($P \leq 0.05$).

Table 2. Odds ratio (OR) for embryo recovery per unit change in regression (embryo grade) stratified by age group.

Variable	OR	p-Value	95% CI (OR)
Age	1.03	0.001	1.01 - 1.06
Young-Adult*	1.05	0.642	0.83 – 1.32
Aged-Adult*	1.47	0.002	1.14 – 1.88
Aged-Young*	1.39	0.015	1.06 – 1.81

* Effect of age-group (overall p-Value = 0.007)

Table 3. Odds ratio (OR) for each variable, statistical probability (p-value) and the 95% confidence interval (CI) for the variables associated with pregnancy at 7 days after embryo transfer (PregR15) by group of recipient mares.

Variable	OR	p-Value	95% CI (OR)
Effect of RmG in PregR15 by EQ 1 (overall p-value = 0.001)			
CL-CL/P4	8.10	0.001	4.00-16.41
CL-P4	2.04	0.001	1.35-3.08
CL/P4-P4	0.25	0.001	0.11-0.53
Effect of RmG in PregR15 by EQ 2 (overall p-value = 0.001)			
CL-CL/P4	4.75	0.992	0-.
CL-P4	1.92	0.222	0.67-5.47
CL/P4-P4	0.00	0.992	0-.
Effect of RmG in PregR15 by EQ 3 (overall p-value = 0.146)			
CL-CL/P4	7.39	0.997	0-.
CL-P4	8.44	0.996	0-.
CL/P4-P4	0.00	0.995	0-.

Group CL: control group.
Group CL/P4: cyclic group with long-acting progesterone (P4).
Group P4: anestrus group with P4.
RmG = recipient mare group (CL, CL/P4, P4)
EQ= embryo quality

Table 4. Odds ratio (OR) for each variable, statistical probability (p-value) and the 95% confidence interval (CI) for the variables associated with pregnancy at 60 days after embryo transfer (PregR60) by group of recipient mares.

Variable	OR	p-Value	95% CI (OR)
Effect of RmG in PregR60 by EQ 1 (overall p-value = 0.001)			
CL-CL/P4	3.63	0.001	2.34-5.65
CL-P4	1.32	0.093	0.95-1.85
CL/P4-P4	0.36	0.001	0.22-0.58
Effect of RmG in PregR60 by EQ 2 (overall p-value = 0.001)			
CL-CL/P4	9.55	0.992	0-.
CL-P4	1.69	0.277	0.65-4.40
CL/P4-P4	0.00	0.992	0-.
Effect of RmG in PregR60 by EQ 3 (overall p-value = 0.118)			
CL-CL/P4	1.77	0.997	0-.
CL-P4	0.00	0.996	0-.
CL/P4-P4	0.00	0.995	0-.

Group CL: control group.
Group CL/P4: cyclic group with long-acting progesterone (P4).
Group P4: anestrus group with P4.
RmG = recipient mare group (CL, CL/P4, P4)
EQ= embryo quality

Table 5. Odds ratio (OR) for each variable, statistical probability (p-value) and the 95% confidence interval (CI) for the variables associated with embryo mortality (EmbM) after embryo transfer by group of recipient mares.

Variable	OR	p-Value	95% CI (OR)
CL	Referent		
CL+P4	0.7	0.2129	0.40 - 1.22
P4	1.44	0.103	0.93 - 2.25

Effect of Recipient-mares classification (overall p-value = 0.0276)

Figure 1.

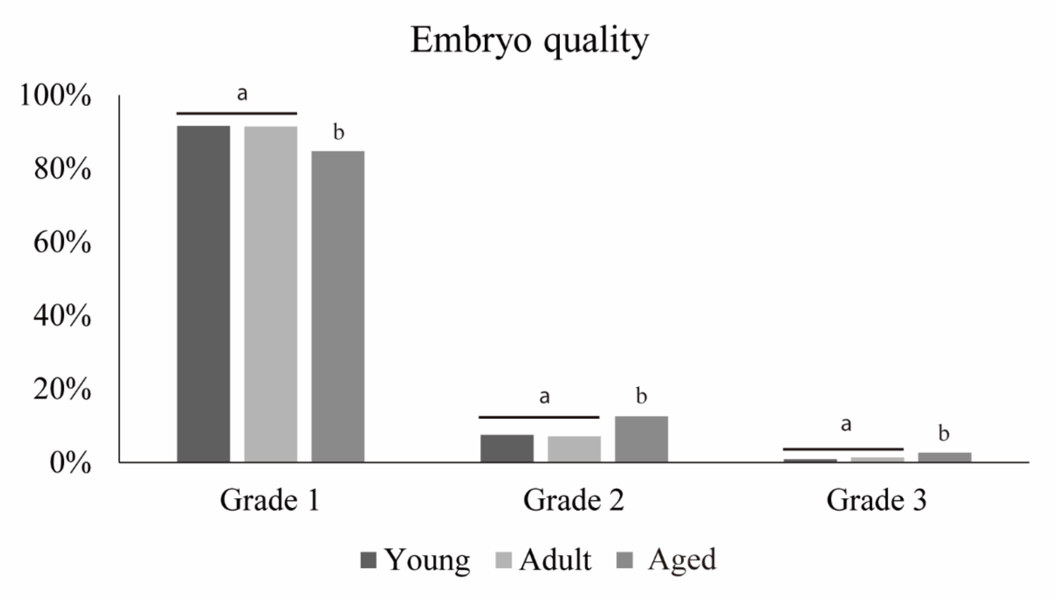

Embryo quality
100%
80%
60%
40%
20%
0%
a
b
a
b
a
b
Grade 1
Grade 2
Grade 3
Young
Adult
Aged

Figure 2.

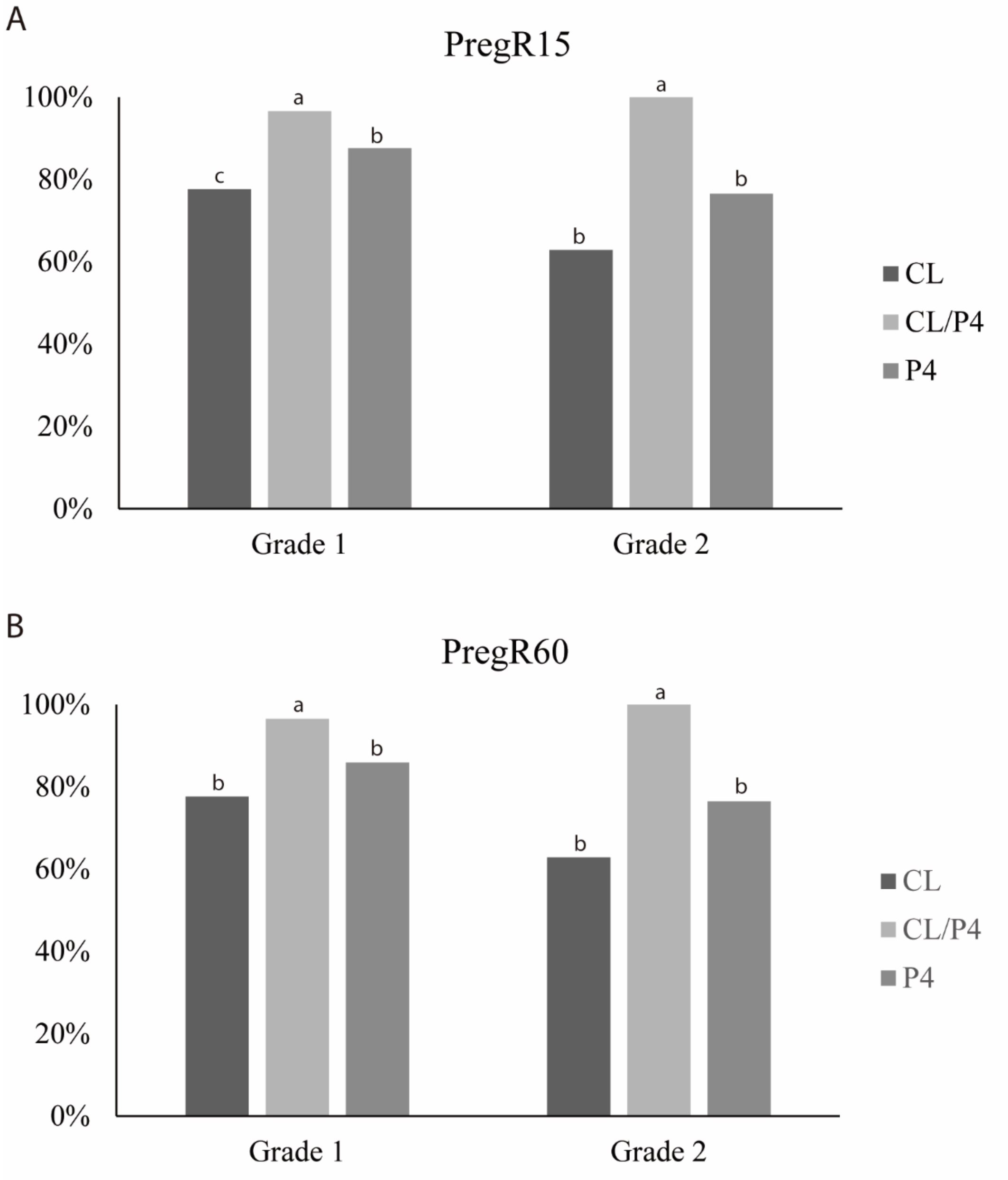
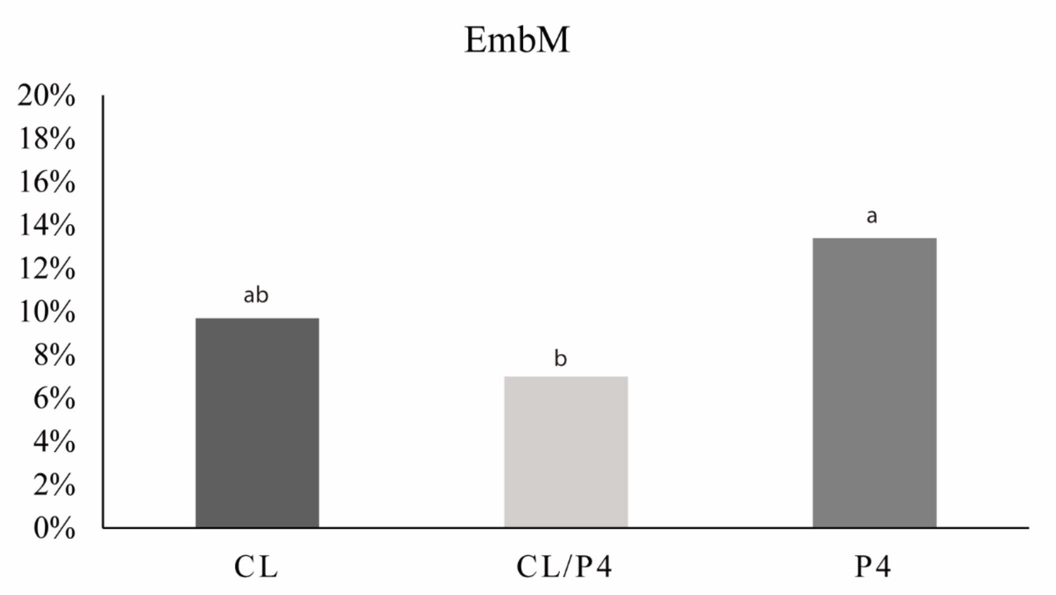

A
PregR15
100%
80%
60%
40%
20%
0%
c
a
b
b
a
b
Grade 1
Grade 2
CL
CL/P4
P4
B
PregR60
100%
80%
60%
40%
20%
0%
b
a
b
b
a
b
Grade 1
Grade 2
CL
CL/P4
P4

Figure 3.



5 DISCUSSÃO

A eficiência reprodutiva na espécie equina é considerada baixa, com taxa de natalidade entre 50 e 60% (GINTHER, 1992). Esse quadro está relacionado ao fato de que o modelo de seleção dos animais é atrelado ao desempenho e não por fertilidade (ALVARENGA et al., 2017). Falhas de manejo, problemas ligados ao garanhão e particularidades reprodutivas da égua, tais como: estacionalidade, imposição de uma estação reprodutiva arbitrária e manutenção de animais "problema", independente da idade ou dos problemas reprodutivos que possam apresentar, são as principais razões para esses resultados.

O avanço em pesquisas científicas e a melhoria no manejo dos animais tem buscado melhorar a eficiência ao longo das décadas (RICKETTS; YOUNG, 1990). No entanto, os resultados são, muitas vezes, confrontados com a redução da fertilidade paralela ao avanço da idade tanto em humanos (ASRM, 2014), quanto em equinos (CARNEVALE; GINTHER, 1995; HENDRIKS et al., 2015). De forma geral, as éguas iniciam sua estação reprodutiva mais tarde em comparação aos machos, e quando seus descendentes estão sendo comprovados pelo desempenho, elas apresentam escassas possibilidades de maximizar sua genética.

Durante muitos anos buscou-se reduzir o intervalo entre partos para maximizar a produção de animais e reduzir os custos de produção. Com isso, a cobertura das éguas durante o primeiro estro após o parto tornou-se um tema amplamente discutido, com resultados muitas vezes controversos e de difícil casualização e interpretação clínica (MERKT; GÜNZEL, 1979; LOY et al., 1982; SHARMA et al., 2010; MURE et al., 2021). Além disso, muitas éguas são submetidas a reprodução com idades avançadas, reduzindo a taxa de gestação por ciclo e aumentando a taxa de morte embrionária (RICKETTS; ALONSO, 1991; HENDRIKS et al., 2015).

Nesse contexto, os resultados obtidos no primeiro estudo desta tese nos permitiram identificar que a idade influencia na fertilidade pós-parto da égua, sendo que a idade de dez anos é um ponto de corte para redução da fertilidade. Dessa forma os profissionais podem considerar a idade da égua no momento da tomada de decisão sobre cobrir a égua ou realizar outra abordagem. Além disso, identificamos que éguas cobertas após 10 dias subsequentes ao parto apresentam maior probabilidade de gestação em relação as éguas cobertas em um intervalo inferior. Esse resultado pode ser explicado por uma melhor involução uterina das éguas após o parto. Também foi possível identificar que a fertilidade do segundo estro após o parto é superior quando comparada com o primeiro estro (cio do potro). Recentemente mais estudos contribuíram com nossos resultados, indicando que o encurtamento do diestro após o cio do potro, com a administração de prostaglandina 2α, favorece a fertilidade das éguas (MURE et al., 2021).

Em animais que são submetidos ao uso de biotecnologias reprodutivas, como a transferência de embriões, a idade também é um dos pontos a serem considerados. Acredita-se que atualmente no Brasil, 30 % das éguas doadoras de embriões sejam idosas (JACOB et al., 2019), as quais possuem dinâmica folicular mais lenta quando comparadas a éguas jovens (GINTHER et al., 2009) e aumento de alterações genômicas resultando em morte embrionária (SHILTON et al., 2020). Além disso, o efeito da idade da doadora sobre a taxa de recuperação embrionária e de gestação pós-transferência têm sido controversas (MARINONE et al., 2015), provavelmente pela dificuldade de detectar influência da qualidade dos embriões através dos modelos propostos.

Os resultados obtidos no segundo artigo desta tese nos permitiram identificar que a idade das éguas doadoras influencia na recuperação e qualidade embrionária. A partir disso, novas abordagens podem ser realizadas nos animais a fim de maximizar sua genética, anterior a redução dessas variáveis. Também foi possível verificar que a qualidade embrionária e égua receptora influenciam a probabilidade de gestação aos 15 e 60 dias após a transferência do embrião. Enquanto que somente a égua receptora influencia a probabilidade de mortalidade embrionária pós-transferência. Receptoras cíclicas suplementadas com progesterona apresentaram maior probabilidade de gestação aos 15 e 60 dias após a transferência de embriões. Esse resultado contribui com a eficiência reprodutiva da espécie, principalmente de animais submetidos aos programas de transferências de embriões, aumentando a taxa de natalidade e reduzindo a mortalidade embrionária.

Nesta tese diferentes aspectos relacionados a eficiência reprodutiva equina foram explorados e podem contribuir com estudos futuros. O primeiro trabalho identificou a influência da idade na fertilidade pós-parto da égua; o segundo trabalho verificou a influência da idade na recuperação e qualidade embrionária de éguas doadoras, bem como a influência da qualidade embrionária e da égua receptora sobre a probabilidade de gestação após a transferência de embrião. Éguas receptoras cíclicas suplementadas com progesterona apresentaram maior probabilidade de gestação aos 15 e 60 dias após a transferência de embriões. Além disso, somente a égua receptora influencia a probabilidade de morte embrionária pós-transferência.

6 CONCLUSÃO

Com a realização deste trabalho, conclui-se que éguas envelhecidas cobertas no primeiro cio após o parto apresentaram maior mortalidade embrionária em comparação as éguas jovens. A taxa de gestação foi maior quando as éguas foram cobertas após o décimo dia pós-parto. Bem como, a taxa de gestação foi maior em éguas cobertas no segundo cio em comparação com o cio do potro. Também é possível concluir que a idade da égua doadora de embriões influencia na taxa de recuperação e qualidade embrionária. E que a qualidade embrionária e a égua receptora influenciam na taxa de gestação pós-transferência de embrião, enquanto que somente a égua receptora influencia na mortalidade embrionária. Além disso, a suplementação de éguas receptoras cíclicas com progesterona favorece a probabilidade de gestação aos 15 e 60 dias após a transferência de embriões.

REFERÊNCIAS

ABABNEH, M. et al. Expression of Cytosolic Phospholipase A2 in Equine Endometrium during the Oestrous Cycle and Early Pregnancy. **Reproduction in Domestic Animals**, v. 46, n. 2, p. 268-274. 2011. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-0531.2010.01657.x>. Acesso em: 20 jul. 2016. doi: doi:10.1111/j.1439-0531.2010.01657.x.

ALILA, H. W.; HANSEL, W. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. **Biol Reprod**, v. 31, n. 5, p. 1015-25, 1984. Disponível em: 20 jul. 2016.

ALLEN, W. R. The physiology of later pregnancy in the mare. **Proceedings of the Annual Convention of the AAEP**: AAEP PROCEEDINGS, 2000. p. 338-354.

AL-ZI'ABI, M. O. et al. Angiogenesis and vascular endothelial growth factor expression in the equine corpus luteum. **Reproduction**, v. 125, n. 2, p. 259-70. 2003. Disponível em: 20 jul. 2016.

BASHIR, S. T. et al. Changes in intrafollicular concentrations of free IGF-1, activin A, inhibin A, VEGF, estradiol, and prolactin before ovulation in mares. **Theriogenology**, v. 85, n. 8, p. 1491-8. 2016. Disponível em: em: 20 jul. 2016. doi: 10.1016/j.theriogenology.2016.01.013.

BATTUT, I. et al. Success rates when attempting to nonsurgically collect equine embryos at 144, 156 or 168 hours after ovulation. **Equine Vet J Suppl**, n. 25, p. 60-2, 1997. Disponível em: 20 jul. 2016. doi.

BAZER, F. W. et al. Roles of Conceptus Secretory Proteins in Establishment and Maintenance of Pregnancy in Ruminants. **Asian-Australasian Journal of Animal Sciences**, v. 25, n. 1, p. 1-16. 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4092924/>. Acesso em: 20 jul. 2016. doi: 10.5713/ajas.2011.r.08.

BEG, M. A.; GINTHER, O. J. Follicle selection in cattle and horses: role of intrafollicular factors. **Reproduction**, v.132, n.3, p.365-77. 2006. Disponível em: 20 jul. 2016. doi: 10.1530/rep.1.01233.

BELIN, F. et al. Intrafollicular concentrations of steroids and steroidogenic enzymes in relation to follicular development in the mare. **Biol Reprod**, v. 62, n. 5, p. 1335-43, 2000. Disponível em: 20 jul. 2016.

BOERBOOM, D. et al. Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. **Biol Reprod**, v. 70, n. 2, p. 391-9, 2004. Disponível em: 20 jul. 2016. doi: 10.1095/biolreprod.103.020800.

BROADLEY, C. et al. Isolation of cell populations from the mare corpus luteum: comparison of mechanical and collagenase dissociation. **J Reprod Fertil**, v. 102, n. 1, p. 7-15, 1994. Disponível em: 20 jul. 2016.

DE RUIJTER-VILLANI, M. et al. Effect of pregnancy on endometrial expression of luteolytic pathway components in the mare. **Reproduction, Fertility and Development**, v. 27, n. 5, p. 834-845, 2015. Disponível em: <https://www.publish.csiro.au/paper/RD13381>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1071/RD13381.

DONADEU, F. X.; GINTHER, O. J. Changes in Concentrations of Follicular Fluid Factors During Follicle Selection in Mares1. **Biology of Reproduction**, v. 66, n. 4, p. 1111-1118, 2002. Disponível em: <http://dx.doi.org/10.1095/biolreprod66.4.1111>. Acesso em: 20 jul. 2016. doi: 10.1095/biolreprod66.4.1111.

DONADEU, F. X.; GINTHER, O. J. Suppression of circulating concentrations of FSH and LH by inhibin and estradiol during the initiation of follicle deviation in mares. **Theriogenology**, v. 60, n. 8, p. 1423-34, 2003. Disponível em: 20 jul. 2016.

DONADEU, F. X.; WATSON, E. D. Seasonal changes in ovarian activity: lessons learnt from the horse. **Anim Reprod Sci**, v. 100, n. 3-4, p. 225-42, 2007. Disponível em: 20 jul. 2016. doi: 10.1016/j.anireprosci.2006.12.001.

EVANS, M. J. et al. Clearance of bacteria and non-antigenic markers following intra-uterine inoculation into maiden mares: Effect of steroid hormone environment. **Theriogenology**, v. 26, n. 1, p. 37-50, 1986. Disponível em: <http://www.sciencedirect.com/science/article/pii/0093691X8690110X>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/0093-691X(86)90110-X.

FINCHER, K. B. et al. Proteins secreted by the sheep conceptus suppress induction of uterine prostaglandin F-2 alpha release by oestradiol and oxytocin. **J Reprod Fertil**, v. 76, n. 1, p. 425-33, 1986. Disponível em: 20 jul. 2016. doi.

FRANCZAK, A. et al. The effect of tumor necrosis factor alpha (TNFalpha), interleukin 1beta (IL1beta) and interleukin 6 (IL6) on endometrial PGF2alpha synthesis, metabolism and release in early-pregnant pigs. **Theriogenology**, v. 77, n. 1, p. 155-65, 2012. Disponível em: 20 jul. 2016. doi: 10.1016/j.theriogenology.2011.07.029.

GASTAL, E. L. et al. Follicle deviation and intrafollicular and systemic estradiol concentrations in mares. **Biol Reprod**, v. 61, n. 1, p. 31-9, 1999. Disponível em: 20 jul. 2016.

GÉRARD, N. et al. Relationships between follicular fluid composition and follicular/oocyte quality in the mare. **Livestock Production Science**, v. 60, n. 2, p. 243-253, 1999. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0301622699000974>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/S0301-6226(99)00097-4.

GINTHER, O. J. et al. Age-related dynamics of follicles and hormones during an induced ovulatory follicular wave in mares. **Theriogenology**, v. 71, n. 5, p. 780-8, 2009. Disponível em: 20 jul. 2016. doi: 10.1016/j.theriogenology.2008.09.051.

GINTHER, O. J. et al. Controlling interrelationships of progesterone/LH and estradiol/LH in mares. **Anim Reprod Sci**, v. 95, n. 1-2, p. 144-50, 2006. Disponível em: 20 jul. 2016. doi: 10.1016/j.anireprosci.2005.10.008.

GINTHER, O. J. et al. Critical role of insulin-like growth factor system in follicle selection and dominance in mares. **Biol Reprod**, v. 70, n. 5, p. 1374-9. 2004b. Disponível em: 20 jul. 2016. doi: 10.1095/biolreprod.103.026195.

GINTHER, O. J. et al. Follicle and systemic hormone interrelationships during spontaneous and ablation-induced ovulatory waves in mares. **Anim Reprod Sci**, v. 106, n. 1-2, p. 181-7, 2008. Disponível em: 20 jul. 2016. doi: 10.1016/j.anireprosci.2007.12.018.

GINTHER, O. J. et al. In Vivo Effects of an Intrafollicular Injection of Insulin-Like Growth Factor 1 on the Mechanism of Follicle Deviation in Heifers and Mares1. **Biology of Reproduction**, v. 70, n. 1, p. 99-105, 2004a. Disponível em: <http://dx.doi.org/10.1095/biolreprod.103.021949>. Acesso em: 20 jul. 2016. doi: 10.1095/biolreprod.103.021949.

GINTHER, O. J. et al. Luteal blood flow and progesterone production in mares. **Anim Reprod Sci**, v. 99, n. 1-2, p. 213-20, 2007b. Disponível em: 20 jul. 2016. doi: 10.1016/j.anireprosci.2006.05.018.

GINTHER, O. J. et al. Mechanism of follicle deviation in monovular farm species. **Anim Reprod Sci**, v. 78, n. 3-4, p. 239-57, 2003. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/12818647>. Acesso em: 20 jul. 2016.

GINTHER, O. J. et al. Relationships of follicle versus oocyte maturity to ultrasound morphology, blood flow, and hormone concentrations of the preovulatory follicle in mares. **Biol Reprod**, v. 77, n. 2, p. 202-8, 2007a. Disponível em: 20 jul. 2016. doi: 10.1095/biolreprod.107.061184.

GINTHER, O. J. et al. Role of low circulating FSH concentrations in controlling the interval to emergence of the subsequent follicular wave in cattle. **Reproduction**, v. 124, n. 4, p. 475-82, 2002. Disponível em: em: 20 jul. 2016.

GINTHER, O. J. Fixation and orientation of the early equine conceptus. **Theriogenology**, v. 19, n. 4, p. 613-23, 1983. Disponível em: em: 20 jul. 2016. doi.

GINTHER, O. J. Follicle Selection in Mares: 90 Years from Observation to Theory. **Journal of Equine Veterinary Science**, v. 54, p. 24-31, 2017a. Disponível em: <https://doi.org/10.1016/j.jevs.2017.01.017>. Acesso em: 20 jul. 2016. doi: 10.1016/j.jevs.2017.01.017.

GINTHER, O. J. Internal regulation of physiological processes through local venoarterial pathways: a review. **J Anim Sci**, v. 39, n. 3, p. 550-64, 1974. Disponível em: 20 jul. 2016.

GINTHER, O. J. Major and Minor Follicular waves During the Equine Estrous Cycle. **Journal of Equine Veterinary Science**, v. 13, n. 1, p. 18-25, 1993. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0737080607800128>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/S0737-0806(07)80012-8.

GINTHER, O. J. **Reproductive Biology of the Mare: Basic and Applied Aspects**: Equiservices. 1992.

GINTHER, O. J. Systemic and intrafollicular components of follicle selection in mares. **Domest Anim Endocrinol**, v. 59, p. 116-133, 2017b. Disponível em: 20 jul. 2016. doi: 10.1016/j.domaniend.2016.12.005.

GINTHER, O. J. The theory of follicle selection in cattle. **Domestic Animal Endocrinology**, v. 57, p. 85-99, 2016. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0739724016300753>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/j.domaniend.2016.06.002.

GINTHER, O. J.; BERGFELT, D. R. Growth of small follicles and concentrations of FSH during the equine oestrous cycle. **J Reprod Fertil**, v. 99, n. 1, p. 105-11, 1993. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/8283426>. Acesso em: 20 jul. 2016.

GINTHER, O. J.; PIERSON, R. A. Regular and irregular characteristics of ovulation and the interovulatory interval in mares. **Journal of Equine Veterinary Science**, v. 9, n. 1, p. 4-12, 1989. Disponível em: <http://www.sciencedirect.com/science/article/pii/S073708068980 1108>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/S0737-0806(89)80110-8.

GOUDET, G. et al. Intrafollicular content of luteinizing hormone receptor, alpha-inhibin, and aromatase in relation to follicular growth, estrous cycle stage, and oocyte competence for in vitro maturation in the mare. **Biol Reprod**, v. 60, n. 5, p. 1120-7, 1999. Disponível em: 20 jul. 2016.

GRECO, G. M. et al. Novel Long-Acting Progesterone Protocols Used to Successfully Synchronize Donor and Recipient Mares With Satisfactory Pregnancy and Pregnancy Loss Rates. **Journal of Equine Veterinary Science**, v. 39, p. 58-61, 2016. Disponível em: <https://doi.org/10.1016/j.jevs.2015.07.012>. Acesso em: 20 jul. 2016. doi: 10.1016/ j.jevs.2015.07.012.

GRECO, G. M. et al. Use of long-acting progesterone to acyclic embryo recipient mares. **Revista Brasileira de Zootecnia**, v. 41, p. 607-611, 2012. Disponível em: <http://www. scielo.br/scielo.php?script=sci_arttext&pid=S1516-35982012000300019&nrm=iso>. Acesso em: 20 jul. 2016.

HAFEZ, E. S. E.; HAFEZ, B. **Reprodução Animal**. 2004.

HANSEN, P. J. Photoperiodic regulation of reproduction in mammals breeding during long days versus mammals breeding during short days. **Animal Reproduction Science**, v. 9, n. 4, p. 301-315, 1985. Disponível em: <http://www.sciencedirect.com/science/article/pii/037843 2085900594>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/0378-4320(85)90059-4.

HARTT, L. S. et al. Temporal and spatial associations of oestrogen receptor alpha and progesterone receptor in the endometrium of cyclic and early pregnant mares. **Reproduction**, v. 130, n. 2, p. 241-50, 2005. Disponível em: 20 jul. 2016. doi: 10.1530/rep.1.00596.

HINRICHS, K. et al. Use of altrenogest to prepare ovariectomized mares as embryo transfer recipients. **Theriogenology**, v. 26, n. 4, p. 455-60, 1986. Disponível em: 20 jul. 2016.

HOLTAN, D. W. et al. Effect of ovariectomy on pregnancy in mares. **J Reprod Fertil Suppl**, n. 27, p. 457-63, 1979. Disponível em: 20 jul. 2016. doi.

ISHAK, G. M. et al. In vivo antral follicle wall biopsy: a new research technique to study ovarian function at the cellular and molecular levels. **Reproductive Biology and Endocrinology**, v. 16, n. 1, p. 71, 2018. Disponível em: <https://doi.org/10.1186/s12958-018-0380-8>. Acesso em: 20 jul. 2016. doi: 10.1186/s12958-018-0380-8.

KASTELIC, J. P. et al. Role of progesterone in mobility, fixation, orientation, and survival of the equine embryonic vesicle. **Theriogenology**, v. 27, n. 4, p. 655-63, 1987. Disponível em: 20 jul. 2016.

KENNEY, R. M.; DOIG, P. A. Equine endometrial biopsy. In: D. A. Morrow (Ed.). **Current therapy in theriogenology**. Philadelphia: W.B. Saunders Company, v. 2. ed., 1986. Equine endometrial biopsy, p. 1143.

KLEIN, C. Maternal Recognition of Pregnancy in the Context of Equine Embryo Transfer. **Journal of Equine Veterinary Science**, v. 41, p. 22-28, 2016. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0737080616300697>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/j.jevs.2016.04.001.

KOBLISCHKE, P. et al. Embryo transfer induces a subclinical endometritis in recipient mares which can be prevented by treatment with non-steroid anti-inflammatory drugs. **Theriogenology**, v. 70, n. 7, p. 1147-1158, 2008. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0093691X08004068>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/j.theriogenology.2008.06.037.

LAGNEAUX, D.; PALMER, E. Embryo transfer in anoestrous recipient mares: attempts to reduce altrenogest administration period by treatment with pituitary extract. **Equine Veterinary Journal**, v. 25, n. S15, p. 107-110, 1993. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.2042-3306.1993.tb04840.x>. Acesso em: 20 jul. 2016. doi: 10.1111/j.2042-3306.1993.tb04840.x.

LEITH, G. S.; GINTHER, O. J. Characterization of intrauterine mobility of the early equine conceptus. **Theriogenology**, v. 22, n. 4, p. 401-8, 1984. Disponível em: 20 jul. 2016.

LEMON, M.; MAULEON, P. Interaction between two luteal cell types from the corpus luteum of the sow in progesterone synthesis in vitro. **J Reprod Fertil**, v. 64, n. 2, p. 315-23, 1982. Disponível em: 20 jul. 2016.

LIU, D. et al. Estrogen stimulates estrogen-related receptor alpha gene expression through conserved hormone response elements. **Endocrinology**, v. 144, n. 11, p. 4894-904, 2003. Disponível em: 20 jul. 2016. doi: 10.1210/en.2003-0432.

LOY, R. G. Reproductive performance in post-partum mares. **Reproduction, Fertility and Development**, v. 32, n. Suppl., p. 642, 1982. Disponível em: 20 jul. 2016.

MAJEWSKA, M. et al. Is interleukin-1alpha a luteotrophic or luteolytic agent in cattle? **Reproduction**, v.139, n.3, p.665-72. 2010. Disponível em: 20 jul. 2016. doi: 10.1530/rep-09-0328.

MCCRACKEN, J. A. et al. Luteolysis: a neuroendocrine-mediated event. **Physiol Rev**, v. 79, n. 2, p. 263-323, 1999. Disponível em: 20 jul. 2016. doi: 10.1152/physrev.1999.79.2.263.

MCDOWELL, K. J. et al. Restricted Conceptus Mobility Results in Failure of Pregnancy Maintenance in Mares1. **Biology of Reproduction**, v. 39, n. 2, p. 340-348, 1988. Disponível em: <http://dx.doi.org/10.1095/biolreprod39.2.340>. Acesso em: 20 jul. 2016. doi: 10.1095/biolreprod39.2.340.

MCKINNON, A. O. et al. The inability of some synthetic progestagens to maintain pregnancy in the mare. **Equine Veterinary Journal**, v. 32, n. 1, p. 83-85, 2000. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.2746/042516400777612035>. Acesso em: 20 jul. 2016. doi: doi:10.2746/042516400777612035.

MCKINNON, A. O.; SQUIRES, E. L. Embryo transfer and related Technologies. **Current Therapy in Equine Reproduction**. J. C. Samper, J. F. Pycock, et al. Philadelphia: W.B. Sounders**:** 319-334 p. 2007.

MERKT, H.; GÜNZEL, A.-R.. A survey of Early Pregnancy Losses in West German Thoroughbred Mares. **Equine Veterinary Journal**, v. 11, n. 4, p. 256-258, 1979. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.2042-3306.1979.tb01359.x>. Acesso em: 20 jul. 2016. doi: 10.1111/j.2042-3306.1979.tb01359.x.

MEYERS, P. J. et al. Quantifying the occurrence of early embryonic mortality on three equine breeding farms. **Can Vet J**, v. 32, n. 11, p. 665-72, 1991. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/17423895https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1481100/pdf/canvetj00072-0027.pdf>. Acesso em: 20 jul. 2016.

NISWENDER, G. D. et al. Mechanisms controlling the function and life span of the corpus luteum. **Physiol Rev**, v. 80, n. 1, p. 1-29, 2000. Disponível em: 20 jul. 2016. doi: 10.1152/physrev.2000.80.1.1.

OKADA, C. T. C. et al. Effect of the Flunixin Meglumine on Pregnancy Rates in an Equine Embryo Transfer Program. **Journal of Equine Veterinary Science**, v. 62, p. 40-43, 2018. Disponível em: <https://doi.org/10.1016/j.jevs.2017.10.023>. Acesso em: 20 jul. 2016. doi: 10.1016/j.jevs.2017.10.023.

OKADA, C. T. C. et al. The effect of flunixin meglumine, firocoxib and meloxicam on the uterine mobility of equine embryos. **Theriogenology**, v. 123, p. 132-138, 2019. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0093691X18308501>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/j.theriogenology.2018.09.026.

PASHEN, R. L. Maternal and foetal endocrinology during late pregnancy and parturition in the mare. **Equine Veterinary Journal**, v. 16, n. 4, p. 233-238, 1984. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.2042-3306.1984.tb01918.x>. Acesso em: 20 jul. 2016. doi: 10.1111/j.2042-3306.1984.tb01918.x.

REYNOLDS, L. P.; REDMER, D. A.. Expression of the angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in the ovary. **J Anim Sci**, v. 76, n. 6, p. 1671-81. 1998. Disponível em: 20 jul. 2016. doi.

RIVERA, G. M.; FORTUNE, J. E. Selection of the dominant follicle and insulin-like growth factor (IGF)-binding proteins: evidence that pregnancy-associated plasma protein A contributes to proteolysis of IGF-binding protein 5 in bovine follicular fluid. **Endocrinology**, v. 144, n. 2, p. 437-46, 2003. Disponível em: 20 jul. 2016. doi: 10.1210/en.2002-220657.

ROBINSON, S. J. et al. Modulation of oviductal transport in mares by local application of prostaglandin E2. **J Reprod Fertil Suppl**, n. 56, p. 587-92, 2000. Disponível em: 20 jul. 2016.

ROSSDALE, P. D.; RICKETTS, S. W. **Equine stud farm medicine**. London: Bailliere Tindall, 1980. viii + 564 pp.

SHARMA, S. et al. Factors affecting the incidence of postpartum oestrus, ovarian activity and reproductive performance in Thoroughbred mares bred at foal heat under Indian subtropical conditions. **Theriogenology**, v. 74, n. 1, p. 90-9, 2010. Disponível em: <http://www.ncbi.nlm. nih.gov/pubmed/20207409>. Acesso em: 20 jul. 2016. doi: 10.1016/j.therio genology.2010.01.018.

SHARP, D. C. et al. Effects of artificial light on the oestrous cycle of the mare. **J Reprod Fertil Suppl**, n. 23, p. 241-6, 1975. Disponível em: 20 jul. 2016.

SILVA, E. S. A. M. D. et al. Ultrasonography of the conceptus development from days 15 to 60 of pregnancy in non-cyclic recipient mares. **Ciência Rural**, v. 45, p. 512-518, 2015. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782015000300512&nrm=iso>. Acesso em: 20 jul. 2016.

SILVA, E. S. M. et al. Administration of 2.5 mg of estradiol followed by 1,500 mg of progesterone to anovulatory mares promote similar uterine morphology, hormone concentrations and molecular dynamics to those observed in cyclic mares. **Theriogenology**, v. 97, p. 159-169, 2017. Disponível em: <http://www.sciencedirect.com/science/article/ pii/S0093691X17301917>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/ j.theriogenology.2017.04.031.

SILVA, E. S. M. et al. Expression of receptors for ovarian steroids and prostaglandin E2 in the endometrium and myometrium of mares during estrus, diestrus and early pregnancy. **Animal Reproduction Science**, v. 151, n. 3, p. 169-181, 2014. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0378432014003510>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/j.anireprosci.2014.11.001.

STEVENSON, W. G. Comparative Fertility of Mares. **Can J Comp Med Vet Sci**, v. 9, n. 5, p. 126-30, 1945. Disponível em: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1660941/ pdf/vetsci00306-0010.pdf>. Acesso em: 20 jul. 2016.

STOUT, T. A. E. Embryo–maternal communication during the first 4 weeks of equine pregnancy. **Theriogenology**, v. 86, n. 1, p. 349-354, 2016. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0093691X16300875>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/j.theriogenology.2016.04.048.

STOUT, T. A.; ALLEN, W. R. Role of prostaglandins in intrauterine migration of the equine conceptus. **Reproduction**, v. 121, n. 5, p. 771-5, 2001. Disponível em: 20 jul. 2016.

SZÓSTEK, A. Z. et al. Impairment of the Interleukin System in Equine Endometrium During the Course of Endometrosis1. **Biology of Reproduction**, v. 89, n. 4, p. 79, 1-13-79, 1-13, 2013. Disponível em: <http://dx.doi.org/10.1095/biolreprod.113.109447>. Acesso em: 20 jul. 2016. doi: 10.1095/biolreprod.113.109447.

TROEDSSON, M. H. T. et al. Interaction between equine semen and the endometrium: the inflammatory response to semen. **Animal Reproduction Science**, v. 68, n. 3, p. 273-278, 2001. Disponível em: <http://www.sciencedirect.com/science/article/pii/S037843200100 1646>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1016/S0378-4320(01)00164-6.

URSELY, J.; LEYMARIE, P. Varying response to luteinizing hormone of two luteal cell types isolated from bovine corpus luteum. **J Endocrinol**, v. 83, n. 3, p. 303-10, 1979. Disponível em: 20 jul. 2016.

VAN MOURIK, M. S. et al. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. **J Leukoc Biol**, v. 85, n. 1, p. 4-19, 2009. Disponível em: 20 jul. 2016. doi: 10.1189/jlb.0708395.

VAN NIEKERK, C. H. et al. Relationship between the morphology of and progesterone production by the corpus luteum of the mare. **J Reprod Fertil Suppl**, n. 23, p. 171-5, 1975. Disponível em: 20 jul. 2016.

WATSON, E. D. et al. Expression of mRNA encoding insulin-like growth factor binding protein-2 (IGFBP-2) during induced and natural regression of equine corpora lutea. **Theriogenology**, v. 64, n. 6, p. 1371-80, 2005. Disponível em: 20 jul. 2016. doi: 10.1016/j.theriogenology.2005.02.015.

WEBER, J. A. et al. Prostaglandin E2 secretion by oviductal transport-stage equine embryos. **Biol Reprod**, v. 45, n. 4, p. 540-3, 1991. Disponível em: 20 jul. 2016.

WESSEL, M. Staging and Prediction of Parturition in the Mare. **Clinical Techniques in Equine Practice**, v. 4, n. 3, p. 219-227, 2005. Disponível em: <http://www.sciencedirect.com/science/article/pii/S1534751605000673>. Acesso em: 20 jul. 2016. doi: https://doi.org/10.1053/j.ctep.2005.07.003.

WOODS, G. L. et al. Early pregnancy loss in brood mares. **J Reprod Fertil Suppl**, v. 35, p. 455-9, 1987. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/3479600>. Acesso em: 20 jul. 2016.