

Evaluation of embryo collection and transfer days on pregnancy rate of Mangalarga Marchador mares during the breeding season

Evaluación de los días de recolección y transferencia de embriones sobre las tasas de preñez en yeguas Mangalarga Marchador durante la estación de monta
Avaliação do dia da colheita e da transferência de embriões equino da raça Mangalarga Marchador durante a estação de monta

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Abstract

Background: Proper timing for embryo collection and transfer in horses -which is critical for the success of this biotechnology- is still debated. Additionally, there is little information on this technology under tropical conditions.

Objective: To determine the best day for collection and transfer of embryos in Mangalarga Marchador mares under Brazilian northeast's conditions.

Methods: Donors (n= 30) and recipients (n= 76) in diestrus phase were selected based on both clinical and gynecology examinations. Estrus was induced on both donor and recipient mares by intramuscular injection of 5 mg Dinoprost, aiming to obtain an ovulation interval of -1 to +3 between recipient and donor. Ovulation was induced with buserelin acetate when the largest follicle reached at least 35 mm in diameter. At this time, mares were subjected to artificial insemination at 48-hour intervals until ovulation. The embryos were collected on days 7, 8, and 9 after ovulation.

Results: The embryo collection on day 8 was more efficient ($p < 0.05$) than on day 7, but it was not more effective ($p > 0.05$) than day 9, which presented the same efficiency ($p > 0.05$) as day 7. From a total of 76 embryos transferred to the recipients, that were between days 4 and 9 after ovulation, there was no influence ($p > 0.05$) of the day of transfer on pregnancy rate.

Conclusions: The embryo collection must be performed on day 8 after ovulation, and transfer can be performed on any day of that interval (4-9) without affecting the pregnancy rate.

Keywords: *assisted reproduction; embryo; embryo collection; embryo recipients; embryo transfer; Equus caballus; estrous synchronization; Mangalarga Marchador; mare.*

Resumen

Antecedentes: El momento mas apropiado para la recolección y transferencia de embriones en equinos - que es fundamental para el éxito de esta biotecnología- continua siendo sujeto de estudio. Además, es escasa la información sobre esta tecnología en condiciones tropicales.

Objetivo: Determinar el momento mas adecuado para la recolecta y transferencia de embriones en yeguas Mangalarga Marchador, en las condiciones del nordeste Brasileño.

Métodos: Donadoras (n= 30) y receptoras (n= 76) en la fase de diestro se seleccionaron con base en los exámenes clínicos y ginecológicos. El estro de las yeguas donadoras y receptoras fue inducido con 5 mg de Dinoprost, vía intramuscular, intentando obtener un intervalo de ovulación de -1 a +3 entre la receptora y la donadora. La ovulación fue inducida con acetato de buserelina cuando el folículo mayor alcanzó 35 mm de diámetro. En ese momento, las yeguas fueron sometidas a inseminación artificial en intervalos de 48 horas hasta la ovulación. Los embriones fueron recolectados en los días 7, 8 y 9 después de la ovulación.

Resultados: La recolecta de embriones en el día 8 fue más eficiente ($p < 0,05$) que en el día 7, pero no fue más efectivo ($p > 0,05$) que en el día 9, el cuál presentó la misma eficiencia ($p > 0,05$) que en el día 7. De un total de 76 embriones transferidos a las receptoras, que se encontraban entre el día 4 y 9 después de la ovulación, no se registró influencia ($p > 0,05$) del día de la transferencia en la tasa de preñez.

Conclusiones: La recolecta embrionaria debe ser realizada el día 8 después de la ovulación, y la transferencia puede ser realizada en cualquier día de este intervalo (4 a 9) sin que se afecte la tasa de preñez.

Palabras clave: *colección de embriones; embrión; Equus caballus; Mangalarga Marchador; receptora de embriones; reproducción asistida; sincronización del estro; transferencia de embriones; yegua.*

Resumo

Antecedentes: A importância do momento da colheita e da transferência do embrião equino para o sucesso dessa biotécnica em equino continua sem ser completamente entendida. Adicionalmente, existe pouca informação sobre essa tecnologia em condições tropicais.

Objetivo: Determinar o melhor dia para colheita e para transferência de embriões em éguas manga larga marchador nas condições do nordeste brasileiro.

Métodos: Doadoras (n = 30) e receptoras (n = 76) na fase de diestro foram selecionadas com base nos exames clínico e ginecológicos. O estro das éguas doadoras e receptoras foi induzido com 5 mg de Dinoprost administrado por via intramuscular, buscando obter um intervalo de ovulação de -1 a +3 entre a receptora e a doadora. A ovulação foi induzida com acetato de buserelina quando o folículo maior alcançou o tamanho de 35 mm de diâmetro. Nesse momento, as éguas foram submetidas a inseminação artificial em intervalos de 48 horas até a ovulação. Os embriões foram colhidos nos dias 7, 8 e 9 depois da ovulação.

Resultados: A colheita de embriões no dia 8 foi mais eficiente ($p < 0,05$) do que no dia 7, porém não foi mais efetivo ($p > 0,05$) do que o dia 9, o qual apresentou a mesma eficiência ($p > 0,05$) que o dia 7. De um total de 76 embriões transferidos para as receptoras que se encontravam entre os dias 4 e 9 depois da ovulação, não se registrou influência ($p > 0,05$) do dia da transferência sobre a taxa de prenhez.

Conclusões: A colheita embrionária deve ser realizada no dia 8 depois da ovulação, e a transferência pode ser realizada em qualquer dia desse intervalo (4-9) sem que a taxa de prenhez seja afetada.

Palavras-chave: colheita de embriões; égua; embriões; *Equus caballus*; Mangalarga Marchador; receptoras de embriões; reprodução assistida; sincronização do estro; transferência de embriões.

Introduction

Embryo transfer (ET) biotechnology in horses is a useful tool for allowing rapid genetic dissemination of valuable animals (Arruda *et al.*, 2001; Azevedo *et al.*, 2015). The ET makes it possible to obtain foals from aged mares, mares with problems to maintain gestation, and mares under training or competition. This biotechnology also allows obtaining foals of young mares (1 to 2 years) without impairing its development and shortens the generation interval of mares with a promising pedigree (Sieme *et al.*, 2018).

The ET technology is less complex than other reproductive biotechnologies, such as *in vitro* embryo production (Lira *et al.*, 2009; Claes *et al.*, 2016; Sanchez *et al.*, 2016; Rua *et al.*, 2016). Its efficiency depends on proper selection and management of mares (Squires *et al.*, 1999; Vanderwall, 2008), and estrus and ovulation synchrony (Iuliano *et al.*, 1985; Taveiros *et al.*, 2003/2008; Rabelo *et al.*, 2009; Hinrichs, 2012; Azevedo *et al.*, 2014).

Equine embryos are transported from the oviduct to the uterus from the compact morulae to the blastocyst stages, between the 5th and 6th days after ovulation (Oguri and Tsutsumi, 1972). Embryos obtained by post-ovulation artificial insemination (AI) reach the uterus later compared to their counterparts obtained by pre-ovulation AI, possibly due to delayed fertilization (Bertani *et al.*, 1997). Therefore, embryo collections should be performed around 12 hours after day 7 (Day 7.5) and should not exceed day 8 (Cuervo- Arango *et al.*, 2009).

The most appropriate day for embryo collection remains controversial. On one hand, for collections between days 6 and 9 after fertilization, collections made between days 7 and 8 retrieved greater embryo numbers (Cuervo-Arango *et al.*, 2009; Sieme *et al.*, 2018). On the other hand, similar embryo recovery rates could be achieved from days 5 to 8 post- ovulation (Vogelsang *et al.*, 1985). Also, according to Squires and Seidel (1995), day 6 is the most advantageous for embryo cryopreservation, and day 9 -although less used for ET- shows lower results in comparison to days 7 and 8.

The ET into previously synchronized mares can be performed by either surgical or transcervical methods (Fleury *et al.*, 2001). Despite ongoing controversy, the most frequently used period for ET has been from days 5 to 9 after donor ovulation (Iuliano *et al.*, 1985; Vogelsang *et al.*, 1985; Fleury *et al.*, 2001; Hajčić *et al.*, 2008; Sieme *et al.*, 2018).

Given that day for collection and ET in horses plays a crucial role in its overall success, a limited number of reports on the subject under the Brazilian northeast condition are available. The northeast of Brazil is a semi-arid region that in spite of stable luminosity throughout the year, faces substantial variation in forage supply due to low and uneven distribution of rainfall. The breeding season spans

from September to March, which is during the dry season. Therefore, the objective of this study was to define the most appropriate days for collection and transfer of embryos in Mangalarga Marchador mares under Brazilian northeast's conditions.

Material and Methods

Ethical considerations

The study was approved by the Ethics Committee for Animal Research at Universidade Federal Rural de Pernambuco (License: 011/2013).

Location and animals

The experiment was conducted in Limoeiro (Pernambuco state, Brazil). The farm is located at the following geographic coordinates, 7° 52' 29'' S latitude and 35° 27' 01'' W longitude. Mean annual temperature is 24.6 °C and mean total rainfall is 1,007 mm³.

Mangalarga Machador donor and recipient mares were pluriparous, non-lactating, aged between 5 and 15 years, and body weight ranged from 450 to 550 kg. All mares were raised under semi-extensive conditions, mostly grazing on cultivated pastures (*Digitaria decumbes*) with access to water and mineralized salt (Suprafós 73, Supranor®, Recife, PE, Brazil) *ad libitum*. Mares were also supplemented with hay (*Cynodon spp*), alfalfa (*Medicago sativa*), and 4 kg/day of a pelletized commercial horse ration containing 16% crude protein, 12.0% fiber, 3,080 kcal/kg fat, 1.3% calcium, 0.75% phosphorus, and 6.0% ether extract (Corcel Reprodução, Presence, São Lourenço da Mata, PE, Brazil).

The body condition score of mares was between 5 and 7, in a 1 to 9 scale, as described by Henneke *et al.* (1983). Other factors considered for selection were: reproductive performance, clinical condition, and gynecological examination by ultrasonography for identification of uterine fluid and endometrial alterations that could compromise fertility, as suggested by Taveiros *et al.* (2008). For this examination, an Aquila Pro, Esaote, equipped with a linear multi-frequency transducer (6 and 8 MHz) was used.

Estrus was induced in donors (n= 30) and recipients (n= 76) with IM injection of 5 mg Dinoprost (Lutalyse®, Pfizer, São Paulo, SP, Brazil), and a single estrous cycle was used per mare. This strategy was carried out to obtain an -1 to +3 ovulation synchronies between the recipient and donor ovulations, as proposed by Squires (1993). Ovulation was induced with buserelin acetate (Conceptal®, MSD Saúde Animal, São Paulo, SP, Brazil) after the largest follicle had reached at least 35 mm diameter. At this time, mares were inseminated with fresh semen from a single stallion of proven fertility, using 500 x 10⁶ viable sperm concentration in 48-hour intervals until ovulation occurred, as recommended by Rabelo *et al.* (2009).

Embryo collections were performed on days 7 (D7), 8 (D8), and 9 (D9) post- ovulation (single embryo collection per mare). The procedure was carried out by the open method, which requires the introduction of a catheter (Bivona, St. Paul, Minnesota, USA) into the uterus body to fixate the balloon to the uterine walls. A total of 3 L of Ringer-Lactate solution was used, divided into three fractions (Fleury and Alvarenga, 1999). After the last uterine flushing, the balloon was emptied, and the catheter was removed. The filtered content was deposited in Petri dishes for embryo recovery using a stereomicroscope (80X), as suggested by Taveiros *et al.* (2008). Immediately after collection, donor mares received an additional Dinoprost injection to avoid an unwanted pregnancy.

After recovery, embryos were evaluated for the stage of development and graded based on their morphology, as described by McKinnon *et al.* (1993). Only grades I and II embryos (n= 76) were transferred immediately to recipients at various post-ovulation stages (D4, D5, D6, D7, D8, and D9). Pregnancy diagnosis was performed by ultrasonography on day 25 after ET. All ultrasonographic exams were carried out by the same technician.

Statistical analysis

The statistical analysis of binomial data was performed using a chi-square test with Yates correction (Preacher, 2001). The significance level of 5% was used.

Results

Table 1 shows results relative to embryo collection at different days post-ovulation. Day 8 was more efficient than day 7 for embryo recovery ($p < 0.05$), but it did not differ with day 9 ($p > 0.05$). Moreover, day 9 was not more efficient than day 7 ($p > 0.05$), although showing a trend towards such a difference ($p = 0.061$). Regarding transferable embryos, there was no difference among the days of collection ($p < 0.05$).

Table 1. Influence of the day of the collection on embryo recovery (Positive) and morphological evaluation (Transferable) in Manga Larga Marchador donor mares.

Collection (Day)	Uterine flushing		Transferable embryos n (%)
	Total (n)	Positive n (%)	
D7	21	9 (42.8) ^A	6 (66.6) ^A
D8	70	51 (72.8) ^B	39 (76.4) ^A
D9	59	39 (66.1) ^{AB}	31 (79.4) ^A
Total	157	97 (61.7)	76 (78.3)

Different superscript letters (^A, ^B) within the same column indicate significant difference ($p < 0.05$).

Table 2 contains the results of pregnancy and conceptus loss about the recipients' post-ovulatory stage (in days) at the time of ET. There was no effect of recipient's post-ovulation day at ET on pregnancy rates and conceptus loss ($p > 0.05$).

Table 2. Influence of the recipients' post-ovulatory stage (Day) on pregnancy rates in Manga Larga Marchador mares submitted to embryo transfer.

Embryo Transfer			
Day (post- ovulation)	Embryos (n)	Pregnancy n (%)	Conceptus loss n (%)
D4	6	2 (33.3)	4 (66.7)
D5	20	18 (90.0)	2 (10.0)
D6	17	13 (76.4)	4 (23.6)
D7	15	9 (60.0)	6 (40.0)
D8	11	7 (63.6)	4 (36.4)
D9	7	3 (42.8)	4 (57.2)
Total	76	52 (68.4)	24 (31.6)

Discussion

The equine embryo reaches the uterus between the fifth and sixth day after ovulation (Oguri and Tsutsumi, 1972; Webber *et al.*, 1991). That is why the day of collection is described as an essential factor on quantity (Montechiesi, 2015) and quality of retrieved embryos (Betteridge *et al.*, 2000; Allen, 2001). The studies mentioned above tested different days for embryo collection. While day 7 was the least preferable, these reports are in contrast to Vogelsang *et al.* (1985), Iuliano *et al.* (1985) and Fleury *et al.* (2001), who suggested day 7 as the most promising day for embryo collection.

The most plausible explanation for this discrepancy is that embryos from Mangalarga Marchador mares at day 7 post-ovulation are smaller in size than other breeds, thus making their recovery more challenging during uterine flushing. Despite the embryo potential for adapting to unsynchronized uterine environments, it is probable that under such conditions, it may have initially faced slower development at gene, cellular, and tissue levels (Gibson *et al.*, 2017).

The results described herein may further support that collection can be preferentially performed at day 8, but can also be carried out at day 9 without diminishing embryo recovery. In a previous report, Iuliano *et al.* (1985) assumed that collections could be performed at day 8, while Fleury *et al.* (2001) supported that day 8 is the most promising day for embryo collection. Albeit the possibility that embryos at day 7 post-ovulation can be smaller than their day 9 counterparts, these later embryos are free from the zona pellucida and are more likely to succumb to mechanical damage during uterine flushing (Stout *et al.*, 2005). Nevertheless, their morphological quality was similar, since the percentage of transferable embryos did not differ.

The ET in horses can be performed by surgical or transcervical methods into previously synchronized recipients (Fleury *et al.*, 2001). Despite the controversy among reports on the best day for ET, it has been mostly described during days 5 to 9 after donor ovulation (Iuliano *et al.*, 1985; Vogelsang *et al.*, 1985; Fleury *et al.*, 2001; Stout, 2006; Hajčić *et al.*, 2008; Azevedo *et al.*, 2015).

As described above, donor and recipient mare management, selection, ovulation synchrony, and particularly- embryo quality for ET could have positively affected the results. This reasoning is based on the fact that pregnancy rates from day four to nine could be similar is ruled out. Thus, the remaining data would be in agreement with those published by several researchers (Wilson *et al.*, 1987; McKinnon and Squires, 1988; Fleury *et al.*, 2001; Taveiros *et al.*, 2008).

Although the absence of statistical difference among pregnancy results, those obtained on days five and six can be outlined from the remaining. In spite of lack of scientific support for these days as the best time-points for ET, it is possible to account for this pregnancy data to the more extended period that embryos have to develop for maternal to fetal crosstalk in the uterus. This crosstalk, among other possibilities, can be in consequence of conceptus migration within uterine horns, thus inhibiting PGF2 α production and release (Conley, 2016).

Therefore, embryos transferred more precociously may not have acquired the necessary competence to promote the signaling above, since it is the exact

moment of embryonic activation (Stout, 2006). It is possible that this problem overlaps with fluctuating P4 concentration during this time, and P4 available may not be sufficient for establishing uterine conditions to support embryonic development (Irvine *et al.*, 2000).

In conclusion, equine embryos can be collected eight days post-ovulation, and their transfer can be performed in a relatively broad period without any detrimental effect on pregnancy rates.

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Conflict of Interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

References

- Allen WR. Fetomaternal interactions and influences during equine pregnancy. *Reproduction* 2001; 121(4):513-527. <http://www.reproduction-online.org/content/121/4/513.full.pdf+html>
- Arruda RP, Visintin JA, Fleury JJ, Garcia AR, Madureira EH, Celeghini ECC, Neto JRN. Existem relações entre tamanho e morfoecogenicidade do corpo lúteo detectados pelo ultra-som e os teores de progesterona plasmática em receptoras de embriões equinos?. *Braz J Vet Res An Sci* 2001; 38(5):233-

239.[https:// www.revistas.usp.br/bjvras/article/view/5877/7408](https://www.revistas.usp.br/bjvras/article/view/5877/7408)

Azevedo MV, Souza NM, Ferreira-Silva JC, Batista IO, Moura MT, Oliveira MA, Oliveira MAL, Lima PF. Induction of multiple ovulations in mares using low doses of GnRH agonist Deslorelin Acetate at 48 hours after luteolysis. *Pferdeheilkunde* 2015; 31:160-164. www.hippiatrika.com/download.htm?id=20150208

Azevedo MV, Souza NM, Ferreira-Silva JC, Batista IO, Sales FABM, Alvarenga MA, Oliveira MAL, Lima PF. Indução de ovulações duplas utilizando baixa dose de deslorelina em éguas. *Ciênc Vet Tróp* 2014; 17 (1/2):42-47. <http://revistas.bvs-vet.org.br/cvt/article/view/28811>

Bertani GR, Scheid IR, Fialho FB, Rubin MIB, Wentz I, Goncalves PBD. Effect of the time of artificial insemination with frozen-thawed or fresh semen on embryo viability and early pregnancy rate in gilts. *Theriogenology* 1997; 48(6):933-945. <https://www.sciencedirect.com/science/article/pii/S0093691X9700321X>

Betteridge KJ. Comparative aspects of equine embryonic development. *Anim Reprod Sci* 2000; 60:691-702. https://ac.els-cdn.com/S03784320000000750/1-s2.0-S03784320000000750-main.pdf?_tid=b8214fdf142348549064cb20cc46e2cd&acdnat=1522156136_0d8c17138a5e1ac99fee76ae74fcb703

Claes A, Galli C, Colleoni S, Necchi D, Lazzari G, Deelen C, Beitsma M, Stout T. Factors influencing oocyte recovery and in-vitro production of equine embryos in a commercial OPU/ICSI program. *J Equine Vet Sci* 2016; 41:68-69. [http://www.j-evs.com/article/S0737-0806\(16\)30179-4/fulltext](http://www.j-evs.com/article/S0737-0806(16)30179-4/fulltext)

Conley AJ. Review of the reproductive endocrinology of the pregnant and parturient mare. *Theriogenology* 2016; 86(1):355-365. [http://www.theriojournal.com/article/S0093-691X\(16\)30088-7/pdf](http://www.theriojournal.com/article/S0093-691X(16)30088-7/pdf)

Cuervo-Arango J, Aguilar J, Newcombe JR. Effect of type of semen, time of insemination relative to ovulation and embryo transfer on early equine embryonic vesicle growth as determined by ultrasound. *Theriogenology* 2009; 71(8):1267-1275. [http://www.theriojournal.com/article/S0093-691X\(09\)00019-3/pdf](http://www.theriojournal.com/article/S0093-691X(09)00019-3/pdf)

Douglas RH. Some aspects of equine embryo transfer. *J Reprod Fertil* 1981; 32(S1): 405- 408. <http://europepmc.org/abstract/med/6962874>

Duchamp G, Bour B, Combarnous Y, Palmer E. Alternative solutions to hCG induction of ovulation in the mare. *J Reprod Fertil* 1986; 35(S1):221-228. <https://www.ncbi.nlm.nih.gov/pubmed/3479577>

Farias LD, Neves AP, Rechsteiner SMDEF, Tarouco AK. Indução da ovulação em éguas: uma revisão. *Rev Bras Reprod Anim* 2016; 40(1):17-21. [http://cbra.org.br/pages/publicacoes/rbra/v40/n1/p17-21%20\(RB611\).pdf](http://cbra.org.br/pages/publicacoes/rbra/v40/n1/p17-21%20(RB611).pdf)

Fleury JJ, Alvarenga MA. Effects of collection day on embryo recovery and pregnancy rates in a nonsurgical equine embryo transfer program. *Theriogenology* 1999; 51(1):261. [http://www.theriojournal.com/article/S0093-691X\(99\)91820-4/pdf](http://www.theriojournal.com/article/S0093-691X(99)91820-4/pdf)

Fleury JJ, Pinto AJ, Marques A, Lima CG, Arruda RP. Fatores que afetam a recuperação embrionária e os índices de prenhez após transferência transcervical em equinos da raça Mangalarga. *Braz J Vet Res An Sci* 2001; 38(1):29-33. <https://www.revistas.usp.br/bjvras/article/view/5915/7446>

Gibson C, Ruijter-Villani M, Stout TA. Negative uterine asynchrony retards early equine conceptus development and upregulation of placental imprinted genes. *Placenta* 2017; 57:175-182. [http://www.placentajournal.org/article/S0143-4004\(17\)30656-2/pdf](http://www.placentajournal.org/article/S0143-4004(17)30656-2/pdf)

Hajčić B, Samardžija M, Grizelj J, Dobranić T, Getz I, Prvanović N. Embryo transfer in mares. *Vet Stanica* 2008; 39(6):335-348. <http://bib.irb.hr/prikazi-rad?rad=380659>

Henneke DR; Potter GD; Kreider JL; Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* 1983; 15(4):371-372. <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.2042-3306.1983.tb01826.x>

Hinrichs K. Assisted reproduction techniques in the horse. *Reprod Fertil Dev* 2012; 25(1):80-93. <http://www.publish.csiro.au/rd/RD12263>

Imel KJ; Squires EL; Elsdén RP; Shideler RK. Collection and transfer of equine embryos. *J Am Vet Med Assoc* 1981; 179(10):987-991. <http://europepmc.org/abstract/med/7341563>

Irvine CH, Alexander SL, McKinnon AO. Reproductive hormone profiles in mares during the autumn transition as determined by collection of jugular blood at 6 h intervals throughout ovulatory and anovulatory cycles. *J Reprod Fertil* 2000; 118(1):101-109. <http://www.reproduction-online.org/content/118/1/101.full.pdf>

Iuliano MF, Squires EL, Cook VM. Effect of age of equine embryos and method of transfer on pregnancy rate. *J Anim Sci* 1985; 60(1):258-263. <https://pdfs.semanticscholar.org/4cfe/643c0ced98947fddb3aac548585f44ad6d9.pdf>

Lira RA, Peixoto GCX, Silva AR. Transferência de embrião em equinos: revisão. *Acta Vet Brasilica* 2009; 3(4):132-140. <https://periodicos.ufersa.edu.br/index.php/acta/article/view/1421/782>

McKinnon AO, Nobeliuś AM, Figueroa S, Skidmore J, Vasey JR, Trigg TE. Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. *Equine Vet J* 1993; 25(4):321-323. <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.2042-3306.1993.tb02972.x>

McKinnon AO, Squires EL. Equine embryo transfer. *Vet Clin North Am Equine Pract* 1988; 4(2):305-333. <https://www.sciencedirect.com/science/article/pii/S0749073917306430?via%3Dihub>

Montechiesi DF. Transferência de embriões em equinos e os fatores relacionados as taxas de prenhez. *Ciênc Anim* 2015; 25(1):187-194. http://www.uece.br/cienciaanimal/dmdocuments/palestra15_p187_194.pdf

Oguri N, Tsutsumi Y. Non-surgical recovery of equine eggs, and an attempt at non surgical egg transfer in horses. *J Reprod Infertil* 1972; 31(2):187-195. <http://www.reproduction-online.org/content/31/2/187.full.pdf+html>

Preacher KJ (2001). Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [software].

Rabelo MC, Taveiros AW, Melo PRM, Chaves RM, Santos Junior ER, Aguiar Filho CR, Bezerra FQG, Almeida-Irmão JM, Freitas Neto LM, Lima PF, Oliveira MAL. Taxas de prenhez e de perda embrionária em éguas da raça Mangalarga Marchador em diferentes status reprodutivos utilizadas como receptoras em programas de transferência de embriões. *Med Vet (UFRPE)* 2009; 3(4):13-19. <http://www.ead.codai.ufrpe.br/index.php/medicinaveterinaria/article/view/656/535>

Rua MAS, Quirino CR, Pacheco A, Júnior AB, Vega HO, Ribeiro MDS. Follicular aspiration, in vitro maturation and intracytoplasmic injection in mares. *PUBVET* 2016; 10(3):248-256. <https://www.cabdirect.org/cabdirect/abstract/20163103862>

Sanchez R, Herrera C, Blanco M, Rosati I, Lazzari G, Colleoni S, Sieme H, Galli C. In Vitro Production of Equine Embryos by Ovum Pick Up and Intracytoplasmic Sperm Injection in Young Mares. *J Equine Vet Sci* 2016; 41:77. [http://www.j-evs.com/article/S0737-0806\(16\)30198-8/fulltext](http://www.j-evs.com/article/S0737-0806(16)30198-8/fulltext)

Sieme H, Sieme, H, Rau J, Tiedemann D, Oldenhof H, Barros L, Sanchez R, Blanco M, Martinsson

G, Herrera C, Burger D. Equine Embryo Transfer. In: Animal Biotechnology 1. Springer 2018;179-192. https://doi.org/10.1007/978-3-319-92327-7_8

Squires EL, McCue PM, Vanderwall DK. The current status of equine embryo transfer. *Theriogenology* 1999; 51:91-104. [http://www.theriojournal.com/article/S0093-691X\(98\)00234-9/pdf](http://www.theriojournal.com/article/S0093-691X(98)00234-9/pdf)

Squires EL. Use of progestins in open and pregnant mares. *Anim Reprod Sci* 1993; 33(14):183-193. <https://www.sciencedirect.com/science/article/pii/0378432093901147>

Stout TAE. Equine embryo transfer: review of developing potential. *Equine Vet J* 2006; 38(5):467-478. <https://onlinelibrary.wiley.com/doi/abs/10.2746/042516406778400529>

Stout TAE, Meadows S, Allen WR. Stage-specific formation of the equine blastocyst capsule is instrumental to hatching and to embryonic survival in vivo. *Anim Reprod Sci* 2005; 87(3-4):269-281. https://ac.els-cdn.com/S0378432004002532/1-s2.0-S0378432004002532-main.pdf?_tid=220d09a5-db36-4d79-af3180b00c09cd5a&acdnat=1522159334_0761d5d3a73277ec2ba1ef226f01f157

Taveiros AW, Melo PRM, Freitas Neto LM, Aguiar Filho CR, Silva ACJ, Lima PF, Oliveira MAL. Produção de embriões de éguas Mangalarga Marchador utilizadas nas Regiões Nordeste e Sudeste do Brasil. *Med Vet (UFRPE)* 2008; 2(3):19-24. <http://www.journals.ufrpe.br/index.php/medicinaveterinaria/article/view/687/566>

Taveiros AW, Oliveira MA, Lima PF, Tenório FF, Bartolomeu CC, Santos MHB, Oliveira LR, Iunes-Souza TC, Freitas JC. Ultrasonographic monitoring of 103 recipient mares of different reproductive status during the first 30 days after embryo transfers. *Vet Rec* 2003; 153(18):558-560. <https://www.ncbi.nlm.nih.gov/pubmed/14627236>

Vanderwall DK. Early embryonic loss in the mare. *J Equine Vet Sci* 2008; 28(11):691-702. [http://www.j-evs.com/article/S0737-0806\(08\)00331-6/abstract](http://www.j-evs.com/article/S0737-0806(08)00331-6/abstract)

Vogelsang SG, Bondioli KR, Massey JM. Commercial application of equine embryo transfer. *Equine Vet J* 1985; 17(S3):89-91. <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.2042-3306.1985.tb04603.x>

Weber JA, Freeman DA, Vanderwall DK, Woods GL. Prostaglandin E2 hastens oviductal transport of equine embryos. *Biol Reprod* 1991; 45(4):544-546. <https://academic.oup.com/biolreprod/article/45/4/544/2763008>

Wilson JM, Rowley MB, Rowley WK, Smith HA, Webb RL, Tolleson DR. Successful non-surgical transfer of equine embryos to post-partum lactating mares. *Theriogenology* 1987; 27(1):295. [http://www.theriojournal.com/article/0093-691X\(87\)90172-5/pdf](http://www.theriojournal.com/article/0093-691X(87)90172-5/pdf)