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Original Research

Effect of the Flunixin Meglumine on Pregnancy Rates in an Equine Embryo Transfer Program

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ABSTRACT

During the equine embryo transfer (ET), manipulation of the recipients cervix can stimulate the release of prostaglandin F2α by the uterine environment. Nonsteroidal anti-inflammatory drugs such as flunixin meglumine (FM) are frequently used in order to prevent a potential luteolysis. However, despite the reduction of inflammatory reaction and release of prostaglandins, the benefits of FM in pregnancy rates (PRs) of mares submitted to ET are not conclusive, and there is no information about the early pregnancy loss (EPL) rate after FM injection. The objective of this study was to evaluate the effect of FM in the PR and EPL in embryo-recipient mares. The data from 409 ET from a commercial breeding center were used, which 179 mares formed the control group (CG) and 230 recipients received the treatment of FM 1.1 mg/kg immediately after ET. There was no difference ($P > .05$) in PRs at 15 days (70.95% in the CG and 75.22% in treated mares) and 60 days (65.92% in CG and 65.22% in FM treated mares). However, there was a trend in the increase of early the pregnancy loss rate in mares that received FM ($P = .0852$). From the results of the present experiment, FM does not improve the PR in embryo-recipient mares.

1. Introduction

In equine embryo transfer (ET), excessive manipulation of the recipient mares stimulates the prostaglandin F2α (PGF2α) release, which can be sufficient to initiate the luteolytic process [1,2].

In addition, ET can induce noninfectious acute subclinical inflammatory reaction in the endometrium and the increase of PGF2α 2–4 days after the procedure [2]. There are contradictions about prostaglandin production after the manipulation of the mares reproductive tract and whether the quantity released in the circulatory system can be sufficient to induce luteolysis [1,3,4].

Prostaglandins are produced by the enzyme prostaglandin G/H synthase, also called prostaglandin-endoperoxide synthase or cyclooxygenase (COX) [5–7]. The two isoforms most investigated of COX are COX-1, endogenous or constitutive, and COX-2, considered inductive [8]. The expression of endometrial COX-2 has been observed in nonpregnant mares, producing prostaglandins responsible for luteolysis induction. In pregnant mares, the COX-2 is downregulated by the embryo [9,10], whereas COX-1 expression is upregulated in early gestation [11].

Animal welfare/ethical statement: The study "Effect of the flunixin meglumine on pregnancy rates in an equine embryo transfer program" was approved by the Ethics and Animal use Committee, with protocol number 127/2016, from the São Paulo State University (UNESP), Botucatu campus, São Paulo, Brazil.

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Wilde et al [4] demonstrated the application of a nonsteroidal anti-inflammatory drug (NSAID) flunixin meglumine (FM) after manipulation of the cervix, which reduced the release of PGFM in mares. In an attempt to control endometrium inflammatory reaction after ET, Koblischke et al [2] used two NSAIDs: FM and meclofenamic acid. This resulted in the decrease of polymorphonuclear cells in the uterus and serum levels of PGFM, comparing treated mares to the control group.

According to Vernon et al [12], PGF2α in the uterine lumen of pregnant mares may be involved in maternal recognition of pregnancy instead of inducing luteolysis. The embryos produce PGF2α and PGE2 to stimulate myometrial contractions and promote embryo mobility, distributing uniformly the maternal recognition factors of gestation into the uterus [13].

There is discordance between the authors about the efficiency of FM application in equine ET. Although it prevents the uterine inflammatory reaction, some studies performed in Brazil showed reduction on fertility rates when FM was used [14,15], also interfering with embryo mobility [16]. Based on this information, the objective of this study was to evaluate the effect of the administration of FM immediately after ET on the pregnancy rate (PR) of recipient mares and in the early pregnancy loss (EPL) rate.

2. Material and Methods

2.1. Local and Animals

The study was conducted in Minas Gerais/Brazil (latitude: 21°07′12″S and longitude: 42°56′34″W) in the 2014/2015 breeding season. Animals that presented a history of anatomic or reproductive abnormalities that compromised of the ET procedure were not included in the study.

Initially, 620 ETs were performed alternating FM applications. However, considering the experiment was accomplished in a commercial stud farm, some animals were excluded from the study because they received progesterone and/or antibiotic treatment, prescribed according to the assessment of the veterinarian in charge. Likewise, the ET procedures performed outside the breeding season, and recipient mares that received embryos from aged donor mares (>18 years) were excluded. Therefore, 230 animals were assigned to the treated group and 179 to the control group.

A total of 409 mares, aged between 3 and 10 years, body score between 5 and 7 on a scale of 1–9 [17], multiparous, cyclic from the second heat after anestrus period, were selected as recipients. These mares were retained in similar managements and pastures, with mineral salt supplement and water ad libitum.

2.2. Embryo Transfer

The uterine lavages for embryo collection and the transfers to recipient mares were always performed by the same veterinarian with experience in the ET technique. Donor mares were examined by ultrasonography (Ibex Pro, E.I. Medical Imaging, CO) until detection of a 35-mm follicle and grade 3 uterine edema (a scale of 0–5 [18–20]). The ovulations were induced with 1-mg deslorelin acetate (Sincrorelin, Ouro Fino, SP, Brasil) for artificial insemination (AI) in the next day [21].

The donor mares' ovulations were confirmed 1 day after AI, considering day 0 of the donor; thus, a cyclic recipient mare with a follicle of 35 mm or greater and uterine edema grades 2–3 was selected and induced to ovulation with 1 mg of deslorelin acetate, which were confirmed 2 days later.

Embryo collections were performed 7–9 days after ovulation of the donor using the flushing method from a transcervical bullet catheter (Embryo Flushing Catheter Bullet tip, Pets-Inc, Canton, TX) attached to a "Y" junction with a filter collector and a bottle of lactate ringer. The uterine lavage was performed up to three times with 1 L each.

Embryos (categories 1 and 2 [22]) were washed with embryo maintenance medium (Botuembryo, Botupharma, Botucatu, Brazil) and placed in an insemination pipette (PROVAR, São Paulo, Brazil) for transfer into the recipients via transcervical. Alternately, the recipients received FM (Flunixin Injectable, Chemitec, São Paulo, Brazil) intravenously immediately after ET or were not treated.

2.3. Treatment

In the moment of ET, recipient mares were between 4 and 7 days postovulation (D4–D7). Immediately after the procedure, a single injection of FM was administered at a dose of 1.1 mg/kg IV. The mares were distributed into two groups (treated and control) so that randomization occurred alternating the applications as the transfers were occurring in the center routine.

The diagnosis of gestation was performed with ultrasound evaluation 15 days (D15) from donor ovulation. The mares were again evaluated for observation of embryonic loss at 60 days using the same ultrasonography technique.

2.4. Statistical Analysis

The data were evaluated by the software Bioestat 5.4 (Belém, Pará, Brazil) using the simple logistic regression model, in which the conception rate at moments 15 and 60 was considered as dependent variables and the treatment effect as a predictor variable. The EPL rate was calculated by subtracting the number of recipients pregnant at D15 by the number of recipients pregnant at D60 and divided by the initial number of pregnant animals in each group, using the same simple logistic regression model. Differences were considered significant when $P < .05$, and tendencies were considered when $.05 < P < .10$.

3. Results

In the present study, PR did not differ ($P > .05$) between groups and moments of pregnancy diagnosis; however, statistical trend ($P = .0852$) was observed to increase EPL rates in mares that received FM, as represented in Table 1.

The mean age of mares that donate embryos to FM group is 7.6 ± 3.2 years and to control group is 7.8 ± 3.2 years. The embryos were transferred to recipient mares

Table 1
Reproductive rates between groups FM and control, considering (n) the number of animals in each group, pregnancy rates (PRs) according to the ultrasonographic diagnosis on day 15 (D15) and day 60 (D60) and early pregnancy loss (EPL).

Pregnancy Rates and Early Pregnancy Loss According to Treatment and Day of Diagnosis	N	D15 (PR)	D60 (PR)	EPL
FM	230	173 (75.22%)[a]	150 (65.22%)[b]	23 (13.29%)[c]
Control	179	127 (70.95%)[a]	118 (65.92%)[b]	9 (7.08%)[c]

Abbreviation: FM, flunixin meglumine.
[a,b,c] Lowercase letters mean difference between columns, $P < .05$.

with 4–7 days after ovulation, with a mean of 4.8 ± 1.1 days for the treated group and 4.8 ± 1.4 days for the control group. The average difference in the day of ovulation of donor and recipient mares across groups is 2.7 ± 1.4 days in FM group and 2.8 ± 1.5 days in control.

4. Discussion

Studies developed in the last decades have demonstrated that ET or manipulation of mares' reproductive tract can cause the release of PGF2α, the luteolysis precursor hormone. Betteridge et al [3] identified an increase in serum prostaglandin levels after manipulation of the genital tract with uterine lavage, but this increase was not observed during ET. A similar result was observed by Wilde et al [4], in which cervical manipulation did not stimulate the release of PGF2α. With divergent results, Kask et al [1] reported that 6 of the 9 mares used as embryo recipient showed high values of PGFM after ET, but there was no luteolysis induction.

The use of FM in ET is recommended by Koblischke et al [2], aiming to attenuate the endometrial inflammatory process caused by the procedure, in addition to decrease plasma PGFM. Resende [14] showed a decrease in PGFM levels after FM, applied 6 or 3 hours before or immediately after ET; however, it was inefficient to control the uterine inflammatory process because there was no positive results of FM application in mares with uterine inflammation, periglandular fibrosis, lymphatic dilatation, fibrosis, or atrophy. According to Wilde et al [4], excessive manipulation of the cervix did not increase PGFM, but the plasma concentrations were lower in mares that received NSAIDs.

The experiment was proposed in order to facilitate the accomplishment in a commercial reproduction center, with FM application immediately after the ET procedure. As reported by Resende [14], a similar decrease in PGFM was observed between FM treatments 6 and 3 hours before and immediately after ET. Furthermore, PGF2α is not released within the first hour posttransfer, but belatedly, as consequence of subclinical endometritis and not by cervix manipulation [2].

Resende [14] observed from 165 ETs, a significant decrease at 60 days PR in embryo-recipient mares that were treated with FM immediately after the procedure, resulting in 58.13% of pregnant mares in the treated group and 72.15% in the control group. Caiado et al [15] also observed higher PR at 28 days in the mares that did not receive a single application of FM 15 minutes before ET (control group 75%—15/20) than in the treated group (55%—11/20). In both studies, there were no data about EPL.

In the present study, the PR obtained at 15 days of gestation (75.22% in FM group and 70.95% in the control) and 60 days (65.22% in FM group and 65.92% in control), values that corroborate with other studies [23–28]. However, FM did not decrease the PR as found by the experiments by Resende [14] and Caiado et al [15].

Conforming to the literature, the EPL in an equine reproduction program can range between 10.4% and 15.4% at 40–60 days of gestations [29–33]. In our study, EPL at 60 days in recipient mares treated with FM was 13.29%, almost twice compared with mares that did not receive the medication (7.08%).

Released in small quantity, the embryonic prostaglandins have local action and are not able to induce luteolysis [34], activating the mobility mechanism that promotes the embryo displacement in the entire uterine lumen, essential to maternal recognition of gestation [35]. This adequately ensures the distribution of the antiluteolytic factor [36]. The PGE2 stimulates cell mitogenesis and endothelial proliferation to form new blood vessels. In addition, it is immunomodulatory and antiapoptotic [37,38].

The gene expression of COX-1 is upregulated in the endometrium due to embryonic estrogen at the beginning of gestation. Considering the downregulation of COX-2 at this stage, COX-1 is the principal enzyme in the prostaglandin production cascade [11]. Cell culture of endometrium in the presence of an embryo depressed PGF synthesis, but the concentrations of PGE and 6-keto-PGF-1α were still elevated [39]. The endometrium synthesizes the prostanoid PGE due to increased prostaglandin E synthase expression at the beginning of gestation, simultaneously with the embryo, stimulating uterine contraction [9]. Therefore, there is a consensus in the literature that local concentration of prostaglandins is essential for establishment of pregnancy in mares [16,34,35,40,41].

The FM impairs the stimulus for the uterine smooth muscle contraction and reduces the embryo mobility, as demonstrated by Stout and Allen [16], which observed a significant decrease in the number of embryonic movements after the application of FM in mares with 12–14 days of gestation. According to McDowell et al [36], the restriction to mobility by decreasing the contact surface between the endometrium and the embryo causes EPL.

Embryos of 10–32 days, when incubated in cell culture, produce significant quantity of PGE2 and PGF2α. However, when FM is added in the culture, there is an inhibition to the PGE2 release by the embryo. The PGF2α release is preserved because it is preformed and reserved [34].

The lower PR after FM treatment in previous studies [14,15] and the increase in EPL in our study could be explained as consequence of prostaglandins inhibition by FM. The prostaglandins E-2, F, and I-2 produced by equine embryos are important to signaling its presence to the uterus [39], which is essential to the embryo maternal communication.

In conclusion, flunixin meglumine does not improve PR in embryo-recipient mares. Flunixin meglumine treatment showed a tendency to increase the pregnancy loss rate; however, further studies are necessary to confirm these observations.

References

[1] Kask K, Odensvik K, Kindahl H. Prostaglandin F2α release associated with an embryo transfer procedure in the mare. Equine Vet J 1997; 29:286–9.
[2] Koblischke P, Kindahl H, Budik S, Aurich J, Palm F, Walter I, et al. Embryo transfer induces a subclinical endometritis in recipient mares which can be prevented by treatment with non-steroid anti-inflammatory drugs. Theriogenology 2008;70:1147–58.
[3] Betteridge KJ, Renard A, Goff AK. Uterine prostaglandin release relative to embryo collection, transfer procedures and maintenance of the corpus luteum. Equine Vet J Suppl 1985;17:25–33.
[4] Wilde MH, Dinger JE, Hoaglang TA, Woody RL, Graves-Hoagland CO. The effects of cervical dilation on plasma pgfm, progesterone and the duration of luteal function in diestrous mares. Theriogenology 1989;32:675–81.
[5] Hamberg M, Svensson J, Wakabayashi T, Samuelsson B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc Natl Acad Sci U S A 1974;71:345–9.
[6] Samuelsson B, Goldyne M, Granstrom E, Hamberg M, Hammarstrom S, Malmsten C. Prostaglandins and thromboxanes. Annu Rev Biochem 1978;47:997–1029.
[7] Needleman P, Truk J, Jakschik BA, Morrison AR, Lefkowith JB. Arachidonic acid metabolism. Annu Rev Biochem 1986;55:69–102.
[8] Habenicht AJR, Goerig M, Grulich J, Rothe D, Gronwald R, Loth U, et al. Human platelet-derived growth factor stimulates prostaglandin synthesis by activation and by rapid de novo synthesis of cyclooxygenase. J Clin Invest 1985;75:1381–7.
[9] Boerboom D. Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. Biol Reprod 2004;70:391–9.
[10] Ealy AD, Eroh ML, Sharp DC. Prostaglandin H synthase Type 2 is differentially expressed in endometrium based on pregnancy status in pony mares and responds to oxytocin and conceptus secretions in explant culture. Anim Reprod Sci 2010;117:99–105.
[11] Atli MO, Kurar E, Kayis SA, Aslan S, Semacan A, Celik S, et al. Evaluation of genes involved in prostaglandin action in equine endometrium during estrous cycle and early pregnancy. Anim Reprod Sci 2010;122:124–32.
[12] Vernon MW, Zavy MT, Asquith RL, Sharp DC. Prostaglandin F2alpha in the equine endometrium: steroid modulation and production capacities during the estrous cycle and early pregnancy. Biol Reprod 1981;25:581–9.
[13] Allen WR. The physiology of early pregnancy in the mare. AAEP Proc 2000;46:338–54.
[14] Resende ÁMDE. Efeito do tratamento anti- inflamatório na histologia endometrial, produção de prostaglandina e taxa de gestação após transferência de embriões e/ou manipulação cervical em éguas. Thesis. Lavras, MG, Brazil: Universidade Federal de Lavras (UFLA); 2009.
[15] Caiado JRC, Fonseca FA, Silva JFS, Caiado JCC, Silva Fontes R da. Aplicação do flunixin meglumine antes da transferência não-cirúrgica de embriões em éguas da raça Mangalarga Marchador. Rev Bras Ciência Vet 2005;12:11–5.
[16] Stout TAE, Allen WR. Role of prostaglandins in intrauterine migration of the equine conceptus. Reproduction 2001;121:771–5.
[17] Henneke DR, Polter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983;15:371–2.
[18] Samper JC. Ultrasonographic appearance and the pattern of uterine edema to time ovulation in mares. Proc Annu Conv AAEP 1997;43: 189–91.
[19] Samper JC. Uterine edema in the mare. In: Samper JC, editor. Equine breeding Management Artificial Insemination. 2nd edition. St. Louis, MO: Elsevier Inc; 2009. p. 133–8.
[20] Hayes KEN, Pierson RA, Scraba ST, Ginther OJ. Effects of estrous cycle and season on ultrasonic uterine anatomy in mares. Theriogenology 1985;24:465–77.
[21] Melo CM, Papa FO, Filho JNPP, Araújo GH, Dell'Aqua Jr JA, Alvarenga MA. Eficiência do acetato de deslorelina e do extrato de pituitária equina na indução da ovulação em éguas. Veterinária E Zootec 2012;19:392–8.
[22] McCue PM, Ferris RA. How to evaluate equine embryos. Las Vegas, NV, USA: AAEP Proceedings; 2009.
[23] Fleury JJ, Alvarenga MA. Effects of collection day on embryo recovery and pregnancy rates. Theriogenology 1999;51:261.
[24] Ball BA, Little TV, Hillman RB, Woods GL. Pregnancy rates at days 2 and 14 and estimated embryonic loss rates. Theriogenology 1986; 26:611–9.
[25] Camargo CE, Weiss RR, Kozicki LE, Duarte MP, Garcia Duarte MC, Lunelli D, et al. Some factors affecting the rate of pregnancy after embryo transfer derived from the brazilian jumper horse breed. J Equine Vet Sci 2013;33:924–9.
[26] Foss R, Wirth N, Schiltz P, Jones J. Nonsurgical embryo transfer in a private practice. AAEP Proc 1999;45:210–2.
[27] Riera FL, McDonough J. Commercial embryo transfer in polo ponies in Argentina. Equine Vet J 1993;25:116–8.
[28] Squires EL, McCue PM, Vanderwall D. The current status of equine embryo transfer. Theriogenology 1999;51:91–104.
[29] Carnevale EM, Ramirez RJ, Squires EL, Alvarenga MA, Vanderwall D, McCue PM. Factors affecting pregnancy rates and early embryonic death after equine embryo transfer. Theriogenology 2000;54: 965–79.
[30] Villahoz MD, Squires EL, Voss JL, Shindeler RK. Some observations on early embryonic death in mares. Theriogenology 1985;23: 915–24.
[31] Carney NJ, Squires EL, Cook VM, Seidel GE, Jasko DJ. Comparison of pregnancy rates from transfer of fresh versus cooled transported equine embryos. Theriogenology 1991;36:23–32.
[32] Ginther OJ. Embryonic loss in mares: incidence, time of occurrence, and hormoral involvement. Theriogenology 1985;23:77–89.
[33] Panzani D, Vannozzi I, Marmorini P, Rota A, Camillo F. Factors affecting recipients' pregnancy, pregnancy loss, and foaling rates in a commercial equine embryo transfer program. J Equine Vet Sci 2016;37:17–23.
[34] Stout TAE, Allen WR. Prostaglandin E2 and F2α production by equine conceptuses and concentrations in conceptus fluids and uterine flushings recovered from early pregnant and dioestrous mares. Reproduction 2002;123:261–8.
[35] Leith GS, Ginther OJ. Characterization of intrauterine mobility of the early equine conceptus. Theriogenology 1984;22:401–8.
[36] McDowell KJ, Sharp DC, Peck LS, Cheves LL. Short Report - effect of restricted conceptus mobility on maternal recognition of pregnancy in mares. Equine Vet J 1985;17:23–4.
[37] Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493–501.
[38] Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998;93:705–16.
[39] Watson ED, Sertich PL. Prostaglandin production by horse embryos and the effect of co-culture of embryos with endometrium from pregnant mares. J Reprod Fertil 1989;87:331–6.
[40] Stout TAE. Embryo – maternal communication during the fi rst 4 weeks of equine pregnancy. Theriogenology 2016;86:349–54.
[41] Weber JA, Woods GL, Freeman DA, Vanderwall DK. Prostagiandin E2 secretion by day-6 to day-9 equine embryos. Prostaglandins 1992;43.