Journal of Equine Veterinary Science 62 (2018) 40–43

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

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

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ARTICLE INFO

Article history:
Received 1 September 2017
Received in revised form 24 October 2017
Accepted 24 October 2017
Available online 3 November 2017

Keywords:
Embryo transfer
Flunixin meglumine
Pregnancy rate
Mare

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.

Conflict of interest statement: The authors declare no conflicts of interest.

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https://doi.org/10.1016/j.jevs.2017.10.023

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