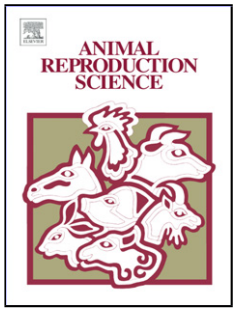

Oral misoprostol does not hasten oviductal transport of day-5 horse embryos

Celina M. Checura (Conceptualization) (Methodology) (Formal analysis) (Investigation) (Writing - original draft) (Visualization) (Project administration) (Funding acquisition), Harry W. Momont (Writing - review and editing), Catalina Castañeira (Investigation), Ana Flores-Bragulat (Investigation), Luis Losinno (Methodology) (Investigation) (Resources) (Writing - review and editing) (Project administration) (Funding acquisition)

PII: S0378-4320(20)30498-X

DOI: https://doi.org/10.1016/j.anireprosci.2020.106626

Reference: ANIREP 106626

To appear in: *Animal Reproduction Science*

Received Date: 12 June 2020

Revised Date: 7 October 2020

Accepted Date: 8 October 2020

Please cite this article as: Checura CM, Momont HW, Castañeira C, Flores-Bragulat A, Losinno L, Oral misoprostol does not hasten oviductal transport of day-5 horse embryos, *Animal Reproduction Science* (2020), doi: https://doi.org/10.1016/j.anireprosci.2020.106626

Oral misoprostol does not hasten oviductal transport of day-5 horse embryos

Celina M. Checura[a]*, Harry W. Momont[b], Catalina Castañeira[c]; Ana Flores-Bragulat[c], Luis Losinno[c]

[a]Piedmont Research and Education Center, Clemson University, Clemson, SC, USA; [b] School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA; [c] Laboratorio de Producción Equina, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

*Corresponding author. Piedmont Research and Education Center, Clemson University, 121 Poole and Ag Center, Clemson, SC, 29634, USA. *E-mail address*: cchecur@clemson.edu

Highlights

- Oral misoprostol did not hasten oviductal transport of equine embryos
- Oral misoprostol did not affect serum progesterone concentration
- Oral misoprostol did not alter corpus luteum echogenic characteristics

ABSTRACT

In horses, prostaglandin E_2 (PGE_2) is produced by embryos around Day 5 post-ovulation; PGE_2 functions directly at the oviduct promoting embryo transport into the uterus. Non-surgical collection of horse embryos for cryopreservation is recommended at Day 6.5 to

7 post-ovulation. It was proposed that misoprostol administered orally will hasten oviductal transport of horse embryos. In Experiment 1 (*n* = 15) there was comparison of time of embryo recovery (Day 6 and 6.5 post-ovulation) from mares administered misoprostol (Day 5 and 5.5) orally to that of untreated mares. On Day 6, embryo collections were attempted; if no embryo was collected, there was a second attempt on Day 6.5. In Experiment 2, (*n* = 16) misoprostol treatment was initiated on Day 4.5; there was the first embryo collection attempt on Day 5.5, followed by Day 6 and 6.5 if no embryo was collected. Blood samples were collected at 12 h intervals on Day 4.5 or 5, to Day 6.5. In Experiment 1, on days 6 and 6.5, respectively, there was collection of seven and one of a total of eight embryos detected at the time of collection per group (*P* = 1). In Experiment 2, 12 embryos were collected during 15 cycles with there being a total of three, two, and one collected from mares of both groups on Day 5.5, 6, and 6.5 post-ovulation, respectively (*P* = 1). Serum progesterone concentrations were not different (*P* ≥ 0.05). In conclusion, misoprostol, when administered orally, does not hasten oviductal transport of horse embryos.

Keywords: Equine; PGE_2; Misoprostol; Embryo; Oviduct

1. Introduction

In most mammals, embryos and oocytes are transported to the uterus. In mares, embryos, but not unfertilized oocytes, are selectively transported to the uterus (Betteridge and Mitchell, 1974). This selective mechanism has important implications for the embryo transfer industry.

The non-surgical collection of small embryos (<300 microns) requires that the uterine lavage occurs at 6 to 7 days post-ovulation. On Day 7 and later, the mean diameter of the embryo is >300 microns (Vanderwall, 2000; McCue et al., 2010). Reported pregnancy rates when there is transfer of small embryos into recipient females after cryopreservation can be as great as 70%. When there is transfer of expanded blastocysts (>300 microns) into recipient mares, there is only 10% to 20% pregnancy rate if there has been cryopreservation of these embryos using traditional methods (reviewed in Allen, 2001; McKinnon and Squires, 2009). There is need for use of more advanced techniques for blastocoel collapse before vitrification if pregnancy rates resulting from embryo transfer are going to be similar to those when there is transfer of small embryos (Checura and Parrish, 2006; Seidel Jr. et al., 2010; Troedsson et al., 2010; Squires and McCue, 2016).

Non-surgical embryo recovery rates for Days 7, 8, and 9 following ovulation are similar, while the recovery rate on Day 6 is less. This lesser embryo recovery rate may be attributed to failure of 1) some embryos to be transported into the uterus by Day 6, 2) to collect embryos when the procedure for collection is conducted, or 3) accurate determination of time of ovulation. In some studies, mares from which there was failure to collect an embryo on Day 6.5 were detected to be pregnant later without being rebred

(reviewed in Vanderwall, 2000; Allen, 2001; McKinnon and Squires, 2009; Squires and McCue, 2016). Based on results form a study from Freeman et al. (1991), some embryos are transported into the uterus after Day 6, and therefore, embryo recovery rates on Day 6.5 should be greater than when there are attempts to collect embryos on Day 5.9 or earlier subsequent to ovulation.

There is a clear function of prostaglandin E_2 (PGE_2) on the oviductal transport of horse embryos. There are relatively greater concentrations of PGE_2 by Day-5 and -6 subsequent to ovulation and this secretion is temporally associated with PGE_2-specific binding to the oviductal wall of mares and with the oviductal transport of the embryo (Weber et al., 1991b; Weber et al., 1992). In addition, the administration of a continuous intra-oviductal infusion of PGE_2 resulted in a markedly accelerated transport of embryos in more mares compared to when the administration with a continuous intra-oviductal infusion of vehicle or no treatment administration. Oviductal transport, however, was apparently not hastened after intramuscular, intrauterine, or intraperitoneal PGE_2 administration to inseminated mares (Weber et al., 1991a). Laparoscopic administration of PGE_2 directly on the oviductal surface was effective leading to an increased embryo collection rate on Day 5 (reviewed in Allen, 2001). Surgical administration of PGE_2, however, is expensive and impractical for the sole purpose of recovering small embryos for cryopreservation.

Misoprostol is a synthetic prostaglandin E_1 analog that interacts with E-prostanol (EP) receptors EP2, EP3, and EP4 (Blikslager et al., 2001). In horses, misoprostol is indicated for the treatment of right dorsal ulcerative colitis associated with non-steroidal anti-inflammatory drug (NSAID)–induced administration (Jones, 2006) as well as other

gastroenteropathies (Lopp et al., 2019; Martin et al., 2019). In addition to the gastrointestinal protective applications of misoprostol; it is administered to women as a cervical-softening agent, for abortion in early pregnancy, induction of labor, and augmentation of uterine contractions when there is need for treatment of serious postpartum hemorrhage (Zhang et al., 2015; Bilgin and Komurcu, 2019). Conversely, when misoprostol was administered orally, there were no adverse effects when there was treatment of pregnant mares during early (Linton and McDonnell, 2014) or mid-gestation (Jacobson et al., 2013). Furthermore, the intra-cervical administration of PGE_2 for induction of cervical relaxation in mares (Nie and Barnes, 2003; LeBlanc, 2006) is controversial (McNaughten et al., 2014). There is intra-uterine administration of misoprostol directly onto the oviductal papilla for the treatment of presumptive blockage of the oviducts (Alvarenga and Segabinazzi, 2018). Misoprostol, therefore, has the potential to stimulate PGE_2 oviductal receptors and hasten oviductal transport of early-developing embryos into the uterus.

Prostaglandin E_2 has an important function in the ovulatory cascade and luteinization of follicular cells (reviewed in Checura, 2015). The effects of PGE_2 in progesterone production of the corpus luteum are complex. Treatment with PGE_2 resulted in increased secretions of both progesterone and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from cultured luteal cells of cattle (Del Vecchio et al., 1995) but treatment with PGE_2 also induces a reduction in receptors for both PGE_2 and $PGF_{2\alpha}$ (Weems et al., 2012). Systemic administration of misoprostol, therefore, may affect progesterone production patterns in early diestrus.

Timing recoveries of embryos at Day 6.5 following ovulation entails assessing the mares twice a day for estimating the time of ovulation. These procedures are easy to accomplish in referral centers with a mare on site, but there are problems with logistic, management of mares, and increased expenses for small scale breeders and ambulatory veterinary practitioners. To collect small embryos when mares are assessed once a day for estimating the time of ovulation, the recovery of the embryo should occur 6 days after ovulation is detected. Increasing the recovery rate of small embryos at the time of uterine flushing on Day 6 with use of a simple and relatively inexpensive process will make cryopreservation more efficient and widely used in mares (Squires and McCue, 2016). It was hypothesized that oral administration of misoprostol could be an alternative to PGE_2 administrations directly on the oviduct to hasten oviductal transport of horse embryos at the Day-5 developmental stage.

2. Materials and methods

2.1. Animal use and care protocols

Animal use and care protocols were reviewed and approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee for Experiment 1, Animal Use Protocol number V005218; and by the Comité de Ética at the Universidad Nacional de Río Cuarto for Experiment 2, number 207/18-FAV-UNRC.

2.2. Experiment 1

The objectives for Experiment 1 were to compare the time at which the embryo was recovered (Day 6 and Day 6.5 following ovulation) from mares treated with two doses

of oral misoprostol compared with untreated control mares, and to determine effects of misoprostol when administered orally on serum progesterone concentrations at days 5 to 6.5 post-ovulation.

2.2.1. Animals and procedures

Fifteen estrous cycling mares of mixed light breeds, 3 to 17 years of age, were assigned for use in Experiment 1. All mares were examined by a board certified theriogenologist at the beginning of the experiment and were considered reproductively sound. Mares were located in Madison, WI, USA (43°04'29.5"N 89°25'09.2"W) and the data were collected during two breeding seasons. Following detection of endometrial edema and a preovulatory (>35 mm) follicle, ovarian functions were monitored every 12 h and mares were artificially inseminated with ≥ 0.5×10^9 motile spermatozoa every other day until ovulation (ovulation day = Day 0). Ovulation was detected by ultrasonography to have occurred when the preovulatory follicle present at the previous ultrasound examination was observed to have evacuated >90% of its follicular fluid (Ginther, 1995). Inseminated mares were randomly assigned to not be treated (untreated control group) or to be treated with misoprostol. Misoprostol (Gavis Pharmaceuticals, Somerset, NJ, USA) was administered orally at 0.009 mg/kg BW, on Days 5 and 5.5. On Day 6, standard non-surgical embryo recovery procedures were conducted (see section 2.3) utilizing two liters of embryo recovery media (Vigro™ Complete Flush Solution, Vetoquinol USA, Inc., Fort Worth, TX, USA) to flush the uterus in an attempt to collect the embryos. If an embryo was not collected on Day 6, there was a second attempt at collection on Day 6.5. The time at which an embryo was collected from each mare (paired samples) was the end-

point for the statistical analysis; therefore, data were only included in the statistical analyses if there were embryos collected from mares after the procedures for both the treatment and control experimental regimens had been imposed. After the initial randomized assignment, mares were assigned to alternate experimental groups until an embryo was recovered, then the mare was assigned to the other group until the second embryo was recovered. When there were unilateral double ovulations in a mare, the data were excluded from the analyses. Blood samples were collected from the jugular vein at 12 h intervals on Day 5 to 6.5. On Day 6.5, all mares were administered $PGF_{2\alpha}$ (10 mg dinoprost tromethamine – Lutalyse, Zoetis, Parsippany, NJ, USA – intramuscular).

2.3. Experiment 2

The objectives for Experiment 2 were to compare the time at which the embryo was recovered (Day 5.5 or Day 6 or 6.5 following ovulation) from mares treated twice with an oral dose of misoprostol compared with mares not administered misoprostol, and to determine the effect of oral misoprostol on serum progesterone concentrations and corpus luteum echogenic characteristics at days 4.5 to 6.5 following the time when ovulation was detected to have occurred.

2.3.2. Animals and procedures

Estrous cycling mares of mixed light breeds (*n* = 16), 2.5 to 15 years of age, were assigned for use in Experiment 2. All mares were examined by a board certified theriogenologist at the beginning of the experiment and were considered reproductively sound. Mares were located in Río Cuarto, Córdoba, Argentina (33°06'33.0"S

64°18'06.7"W) and the data were collected for a period of 30 days during the breeding season. Following detection of endometrial edema and a preovulatory (>35 mm) ovarian follicle, ovarian activity was monitored every 12 h using ultrasonic procedures and mares were artificially inseminated with 1 x 10^9 motile spermatozoa every other day until ovulation (ovulation day = Day 0). Ovulation was detected by ultrasonography to have occurred when the preovulatory follicle present at the previous ultrasound examination was observed to have evacuated >90% of its follicular fluid (Ginther, 1995). Inseminated mares were alternately assigned to a treatment group at the time when ovulation was detected to have occurred (untreated control or misoprostol treatment: 0.009 mg/kg, per os, two doses 12 h apart), and all treatments were administered starting on Day 4.5. On Day 5.5, standard non-surgical procedures were conducted using 2 liters of embryo recovery media (Lactate ringer solution – 624-L, Rivero laboratories, Ciudad Autónoma de Buenos Aires, Argentina – with 0.01% polyvinyl alcohol – P-8136 Sigma-Aldrich, Saint Louis, MO, USA) to flush the uterus in an attempt to collect embryos, and whether there was an embryo collected or not collected was recorded. If an embryo was not collected, there was a second attempt at embryo collection on Day 6, and again, if an embryo was not recovered on Day 6, a third attempt at embryo collection occurred on Day 6.5. After the initial randomized assignment, mares were assigned to the alternate treatment group for conducting the other experimental regimen. Each estrous cycle was considered as an independent variable for statistical purposes and the time at which the embryo was collected was the end-point value recorded for the variable when conducting the statistical analysis. Data, therefore, from an estrous cycle were only included for statistical analyses when there was an embryo collected. Data collected during an estrous cycle when there

was a unilateral double ovulation that occurred were excluded from the statistical analyses. Blood samples were collected from the jugular vein at 12 h intervals from Days 4.5 to 6.5 subsequent to the time ovulation was detected to have occurred. The B-Mode ultrasonic images of the corpus luteum were collected at 12 h intervals from Days 4.5 to 6.5 after ovulation was detected to have occurred. On Day 6.5, all mares were administered $PGF_{2\alpha}$ (10 mg Dinoprost tromethamine – Lutalyse, Zoetis, Parsippany, NJ, USA –, intramuscular).

2.3.3. Ultrasonic images

Ultrasonic images were analyzed using ImageJ (National Institute of Health, Bethesda, MD, USA). To determine average pixel area and pixel intensity, the outer edge of the corpus luteum was delineated by an operator in each image and a pixel histogram analysis was conducted. Each image was delineated and analyzed three times and the averages for pixel area (number of pixels) and pixel intensity (scale 0 to 255, where 0 is black and 255 is white) were used for statistical analysis.

2.4. Embryo recovery procedure

For embryo recovery, the mare was placed in stocks, the tail wrapped, the perineal area cleaned and dried. A silicone balloon-tipped catheter (86 cm, 32 Fr, 75 cc balloon, Bioniche Animal Health, ON, Canada) was passed through the cervix into the uterine body. Sterile sleeve and sterile lubricant were used by the operator to manually insert the catheter into the uterine body. The balloon-cuff was inflated with approximately 30 to 60 cc of air and pulled caudally to a position where it was placed cranially next to the internal

cervical os. The uterus was filled and emptied ("flushed") three to four times with a total of 2 liters of embryo recovery solution (Experiment 1: Vigro™ Complete Flush Solution, Vetoquinol USA, Inc., Fort Worth, TX, USA; Experiment 2: Lactate ringer solution – 624-L, Rivero laboratories, Ciudad Autónoma de Buenos Aires, Argentina – with 0.01% polyvinyl alcohol – P-8136 Sigma-Aldrich, Saint Louis, MO, USA). Massage of the uterus per rectum and manipulation of the catheter was conducted to distribute the fluid in both uterine horns and to facilitate fluid recovery. The recovered fluid was filtered (Em-Con™ -0.75 µm- Immuno Systems, Spring Valley, WI, USA, or EmSafe -0.65 µm- filters Minitube USA, Verona, WI, USA) and there were observations for embryos in the fluid using a stereomicroscope (Vanderwall, 2000).

2.5. Serum progesterone samples

Blood samples were centrifuged immediately after collection and serum was stored at -20 ºC until hormone assays were conducted. All serum progesterone concentrations were determined using a radioimmunoassay with I125 at a commercial laboratory in the USA (BET Laboratories, Lexington, KY, USA): Progesterone CT kits (MP Biomedicals LLC, Irvine, CA, USA) were used; the standard curve was based on seven standards analyzed at the same time as the samples; four control samples 0.1, 1.0, 5.0, and 20.0 ng/ml used to ascertain hormone recovery values when the assays were conducted and CV's were less than 10%. All samples, including standards, were analyzed in duplicate.

2.6. Statistical analysis

Serum progesterone and pixel data were analyzed as repeated measures over time using the mixed procedure in the Statistical Analysis System software (SAS Institute, Cary, NC, USA). Time and treatment were considered to be fixed effects and mare-within-treatment was a random effect. The interaction of treatment by time was included in the model. For the repeated measures analysis, the first order autoregressive covariance structure (AR1) was used to fit a time series-type covariance structure in which the correlation decreases as a function of time. Normality of distribution for the data was tested using the univariate procedure in SAS, with emphasis on the residual plot, the Sharpiro-Wilk and the Kolmogorov-Smirnov tests. There were considered to be differences in mean values when there was a $P \leq 0.05$ and there was considered to be a tendency for differences when there was a $0.05 < P < 0.10$ value.

3. **Results**

3.1. Experiment 1

Of the 15 mares in this experiment, there was only detection of an embryo in the flushing solution of eight mares in both experimental groups. Reasons for exclusion of data were: detection of a double ovulation, fluid in the uterus at the time of embryo recovery, mare being removed from the herd before two embryos were recovered, recovering an embryo when there was one experimental regimen imposed, however, not when the other experimental regimen was imposed (treatment or control), and failure to detect an embryo after flushing the mare uterus. For both groups, seven of eight embryos were collected on Day 6 and the remaining embryo was collected on Day 6.5 (different mares for the two experimental groups). Because the data sets for Day of embryo

recovery in each group were identical, a statistical analysis was not performed ($P = 1$). All embryos were ≤ 300 microns in diameter. Serum progesterone concentration data were normally distributed. Serum progesterone concentrations were not different ($P \geq 0.05$) for the mares of the control compared with the misoprostol groups (12.5 ± 1.3 ng/ml and 11.8 ± 1.2 ng/ml, respectively; LSM ± SEM) and there was no effect ($P \geq 0.05$) of time or treatment-by-time interaction (data not shown).

3.2. Experiment 2

There were evaluations during seventeen estrous cycles for this experiment; during two estrous cycles, there were unilateral double ovulations, therefore, data from these mares were excluded when there were statistical analyses. Of the remaining 15 estrous cycles during which data were collected, during 12 of these estrous cycles, there was collection of embryos with data being included in the statistical analyses: $n = 6$ for each experimental group. The distribution of Day of embryo recovery was Day 5.5 = three estrous cycles, Day 6 = two estrous cycles, and Day 6.5 = one estrous cycle with there again being the same pattern for mares in both the control and misoprostol-treatment groups (Fig. 1.). A statistical analysis was not performed because the data sets were identical ($P = 1$). All embryos were ≤ 300 microns in diameter.

Serum progesterone concentration data were normally distributed. Serum progesterone concentrations were not different ($P \geq 0.05$) for control and misoprostol-treated mares (20.0 ± 2.8 ng/ml and 15.2 ± 2.5 ng/ml, respectively; LSM ± SEM), there was effect of time ($P \leq 0.01$), but not of treatment-by-time interaction ($P \geq 0.05$; Fig. 2.).

Corpus luteum pixel area data were normally distributed. Corpus luteum pixel areas were not different ($P \geq 0.05$) for the mares of the control or misoprostol-treated group (27,660 ± 3,330 pixels and 34,635 ± 3,044 pixels, respectively; LSM ± SEM). There was an effect of time ($P \leq 0.05$), however, there was not a treatment-by-time interaction ($P \geq 0.05$; Fig. 3.).

Corpus luteum pixel intensity data were normally distributed. Average corpus luteum pixel intensity was not different ($P \geq 0.05$) for the mares of the control and misoprostol-treatment group (58.1 ± 3.3 and 58.1 ± 3.0, respectively; LSM ± SEM). There was also no effect of time nor was there a treatment-by-time interaction ($P \geq 0.05$; Fig. 3.).

4. Discussion

Increasing the collection rate of small Day 6 embryos, combined with vitrification techniques, which do not require sophisticated equipment, would allow more practitioners to cryopreserve embryos in the field, and therefore, more horse owners to access and utilize this technology (Squires and McCue, 2016). In the present study, there was for Experiment 1 the hypothesis that the two doses of misoprostol would complement the PGE_2 being produced by the embryo, and thus hasten embryo transport into the uterus. A greater number of embryos collected from the mares of misoprostol-treatment as compared with control group was expected for Day 6. Due to the equal number of embryos collected on Day 6 in both groups (seven of eight embryos), there was not acceptance or rejection of the hypothesis that misoprostol hastens oviductal transport of horse embryos. There, however, was support of the previous findings from Freeman et

al. (1991) that some embryos may not have been transported into the uterus by Day 6 subsequent to ovulation. Experiment 2 was designed to increase the distribution of possible embryo recovery days to test this hypothesis. In addition, the change in physical location of the mares also was associated with a difference in the genetic composition of mares and stallions used to conduct Experiment 2. Results from the present study are again, as with Experiment 1, consistent with those of Freeman et al. (1991) because half of the embryos were collected on Day 5.5 and most of the embryos were collected by Day 6 (10 out of 12 embryos), and there was collection of the other two embryos at Day 6.5 subsequent to the time ovulation was detected (Fig. 1.). In the present study, however, there was not any difference in the distribution of times of embryo collections among mares of the control and misoprostol-treatment groups. The lack of collections of embryos at an earlier time from mares of the misoprostol-treatment group likely indicates that there was no effect when misoprostol was administered orally at the dose used in the present study on oviductal embryo transport.

There are several possible reasons for the lack of an effect of misoprostol in the present study, including 1) too small a dosage, 2) lack of an efficacious timing of the frequency or route of misoprostol administration; 3) a lack of binding of misoprostol to and/or adequate populations of PGE_2 receptors in the oviduct for binding of misoprostol; and 4) a pharmacological downregulation of PGE_2 receptors.

In horses, the effects of oral misoprostol (0.005 mg/kg) on basal free acid contents of the stomach lasted for the 8 hours monitored in a previous experiment (Sangiah et al., 1989). The same dose of misoprostol when administered orally had a half-life of 40 minutes, similar to human patients (Martin et al., 2019), or a longer interval ranging from

119 to almost 200 minutes (Lopp et al., 2019). If there had been a greater frequency of oral administration of misoprostol, there may have been an effect on enhancing the rate of oviductal transport of the horse embryos in the present experiments. It, however, is possible that the oviductal misoprostol concentration that is needed for an induction of the effects to enhance the rate of horse embryo transport into the uterus cannot be safely attained with a systemic dosage, similar to that reported for PGE_2, where local administration, but not intramuscular, intrauterine, or intraperitoneal administration, resulted in an enhanced rate of embryo transport into the uterus from the oviduct (Weber et al., 1991a).

In humans, PGE_2 has different effects on the circular and longitudinal muscle layers of the oviduct, inhibiting the circular and stimulating the longitudinal musculature *in vitro* (Lindblom et al., 1978). In horses, PGE_2 treatment increased electrical activity of the oviductal musculature *in vivo* (Troedsson et al., 1995), and relaxation of the circular muscle layer *in vitro* (Weber et al., 1995). Prostaglandin E_2 functions through one of four receptors, EP1; EP2; EP3; EP4; signaling via cAMP (EP2 and EP4 receptors) or changes in intracellular Ca (EP1 receptors) concentrations. Furthermore, EP3 receptors have several splice variants, which may signal via cAMP or changes in intracellular Ca concentrations; and there may be synergistic regulations as a result of activation of different EP receptors (Blikslager et al., 2001; Blikslager, 2013). In general, activation of EP2 and EP4 receptors induces smooth muscle relaxation, whereas activation of EP1 and EP3 receptors induces smooth muscle contraction. In the horse oviduct, localization of the EP2 and EP4 receptors in ciliated epithelial cells, secretory epithelial cells, putative lymphocytes, smooth muscle, and vascular endothelium is indicative that PGE_2 has

additional effects beyond muscular contraction and relaxation (Ball et al., 2013). In particular, modulation of ciliary beat and epithelial secretions may also contribute to the PGE_2-stimulated oviductal transport of the early developing embryo. Misoprostol is a PGE_1 analog that binds to EP2; EP3; and EP4 receptors (Blikslager et al., 2001) and mimics many of the PGE_2 effects. Horses, however, may have a different receptor interaction with misoprostol than other species. Misoprostol induces early abortions and uterine contractility in humans (Zhang et al. 2015; Bilgin and Komurcu, 2019), but it has no adverse effects in pregnant mares (Jacobson et al. 2013; Linton and McDonnell, 2014), and its effects when administered locally in substitution for PGE_2 for cervical relaxation is controversial (McNaughten et al., 2014). Blikslager (2013) hypothesized that a variant of the EP3 receptor with low-affinity and of unknown relevance in myometrial cells, may be …"the mechanism whereby misoprostol has less of an effect on contraction of urogenital smooth muscle as compared with PGE_2"… (Blikslager, 2013). This proposed less than optimal (or absent) activation of the EP3 receptor for contractility of the muscularis when misoprostol is administered may very well be the reason why there was not an enhanced oviductal embryo transport when the conditions of the current experiments were imposed.

An alternative hypothesis as to why there were not effects of the misoprostol treatment is the downregulation of PGE_2 receptors as a result of administration of misoprostol. Large doses of PGE_2 downregulate receptors for this prostaglandin in the corpus luteum of cattle when there is treatment with intra-luteal implants containing the hormone (Weems et al., 2012). It, however, is unlikely that the oral administration of

misoprostol at the dose and frequency administered in the present study will result in inhibition of contractile functions of the oviduct.

Prostaglandin E_2 is a known luteotropic factor in different domestic species because when administered there is a resulting stimulation of progesterone production by luteal cells *in vitro*. Conversely, $PGF_{2\alpha}$ is the primary luteolytic factor, and it is predominantly secreted by the endometrium (Del Vecchio et al., 1995; Ferreira-Dias et al., 2006; Weems et al., 2012). The enzymes PGE_2-9-ketoreductase (9k-PGR) and aldoketoreductase (AKR1B5) can have functions in conversion of PGE_2 to $PGF_{2\alpha}$ or vice versa, and this may be a common occurrence in the horse corpus luteum (Ferreira-Dias et al., 2006). Serum progesterone concentrations, corpus luteum pixel area and pixel intensity, however, were similar for the mares of the misoprostol-treated and control groups in the present study, leading to the conclusion that there was no effect of misoprostol on progesterone production and corpus luteum echogenic characteristics when the conditions of the current study were imposed.

There was not an enhanced oviductal transport of horse embryos as a result of administration of misoprostol orally in the present study; however, most of the embryos from mares in both experiments were recovered at Day 6 or earlier subsequent to the time when ovulation was detected to have occurred. Nevertheless, there were a few embryos collected on Day 6.5, and there may not be collection of these small embryos accomplished when there are once-a-day assessments for time of ovulation occurrence. In the present study, there was collection of embryos following a second or even a third uterine lavage 12 hours apart. It has been previously reported that 63% of mares from which there were no embryos collected as a result of conducting lavages on day 5 had

embryos collected when there were uterine lavages on day 8 subsequent to the time ovulation was detected to have occurred (reviewed in Allen, 2001). Practitioners could use this as a strategy to optimize rates of collection for small embryos that are going to be cryopreserved by performing embryo collection procedures on Day 6 and repeating the lavage on Day 6.5 after the time of detection when ovulation occurred if the first attempts at embryo collections were unsuccessful.

5. Conclusion

Oral administration of misoprostol at the dose used in the present study did not enhance the rate of embryo transport from the oviduct into the uterus of mares and does not affect progesterone concentration or corpus luteum echogenic characteristics.

CRediT author statement

Celina M Checura: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization, Project administration, Funding acquisition; **Harry W. Momont:** Writing - Review & Editing; **Catalina Castañeira:** Investigation; **Ana Flores-Bragulat:** Investigation; **Luis Losinno:** Methodology, Investigation, Resources, Writing - Review & Editing, Project administration, Funding acquisition.

Funding

This research was supported by an American Quarter Horse Foundation grant; by the Department of Medical Sciences, SVM, University of Wisconsin-Madison; and the Laboratorio de Producción Equina, Universidad Nacional de Río Cuarto.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors would like to thank Claudia Hirsch, Alexis Powers, Abby Van Haren, and all the personnel and students that collaborated with this project at Charmany, UW-Madison; and all the students that collaborated at the Universidad Nacional de Río Cuarto.

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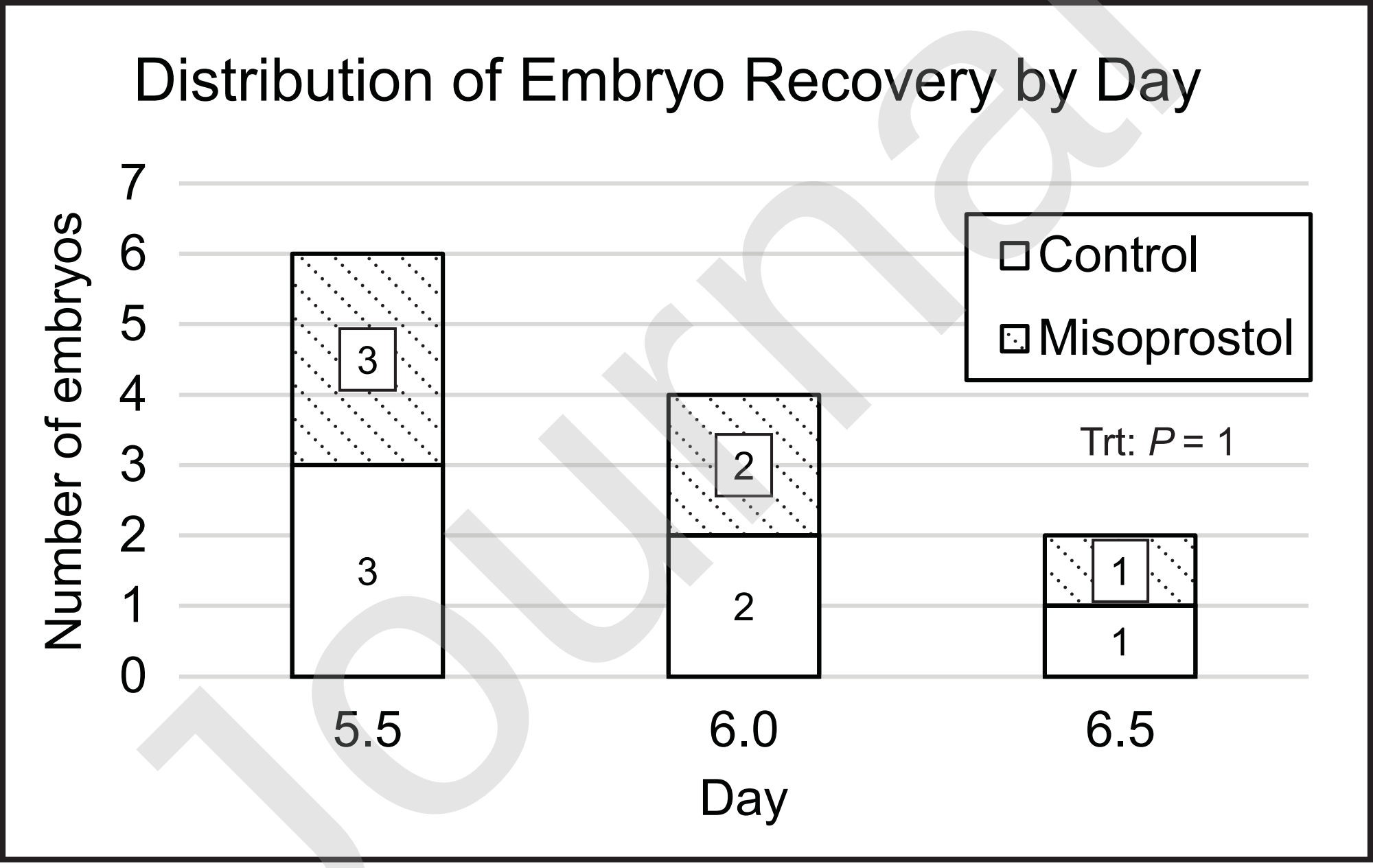
Fig. 1. Day of embryo recovery for the 12 embryos in Experiment 2; treatment was initiated on Day 4.5; untreated control or misoprostol treatment (0.009 mg/kg, per os, two doses 12 h apart); first embryo collection attempt was on Day 5.5, if no embryo was collected, second attempt on Day 6, third attempt on Day 6.5. Trt: treatment effect.

Fig. 2. Serum progesterone concentration for Experiment 2; treatment was initiated on Day 4.5; untreated control (C) or misoprostol (M) (0.009 mg/kg, per os, two doses 12 h apart), *n* = 6 per group; Trt: treatment effect; Time: time effect.

Fig. 3. Ultrasonic-image analysis of the corpus luteum for Experiment 2; treatment was initiated on Day 4.5; untreated control (C) or misoprostol treatment (M) (0.009 mg/kg, per os, two doses 12 h apart), *n* = 6 per group; Trt: treatment effect, Time: time effect.



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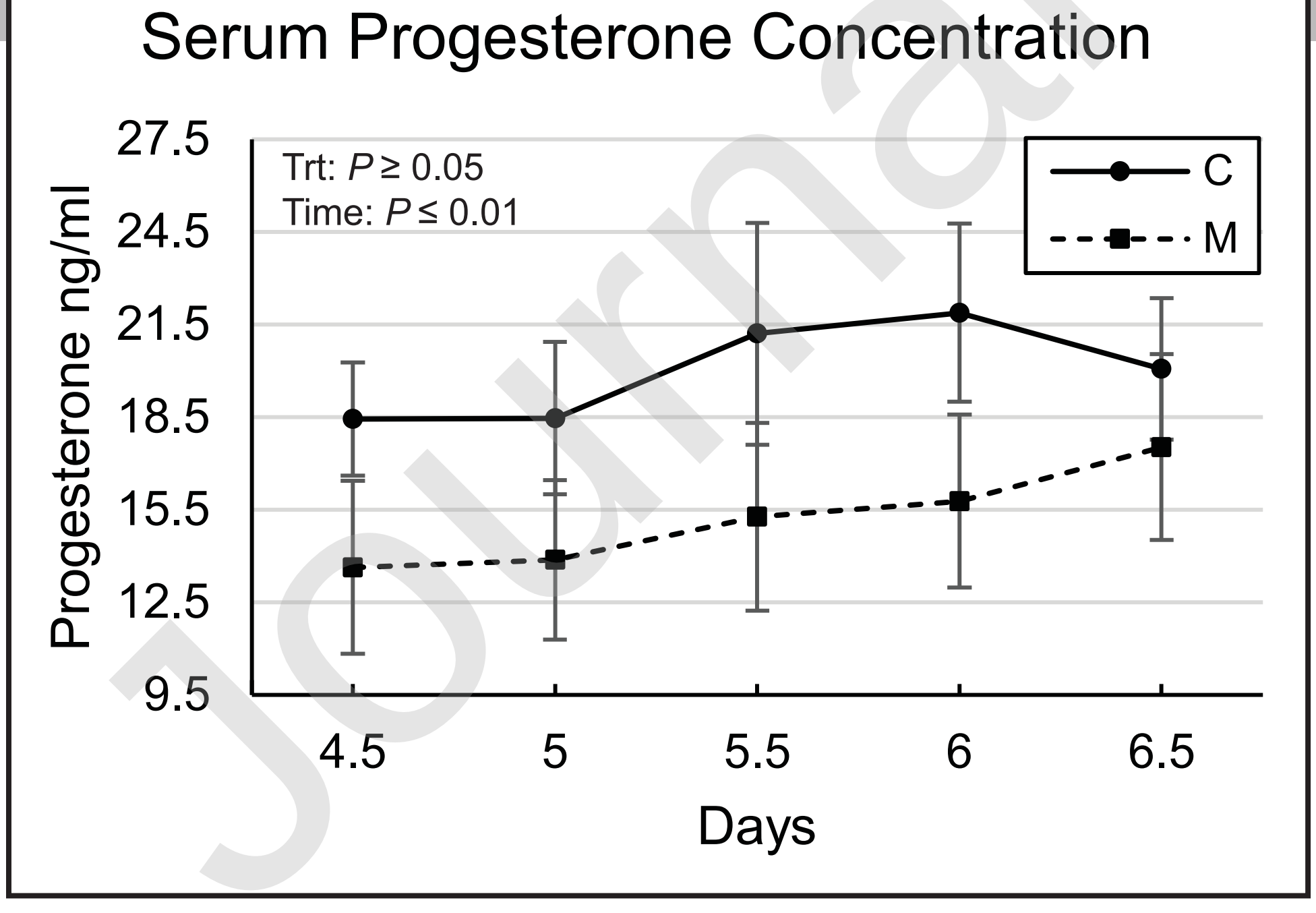

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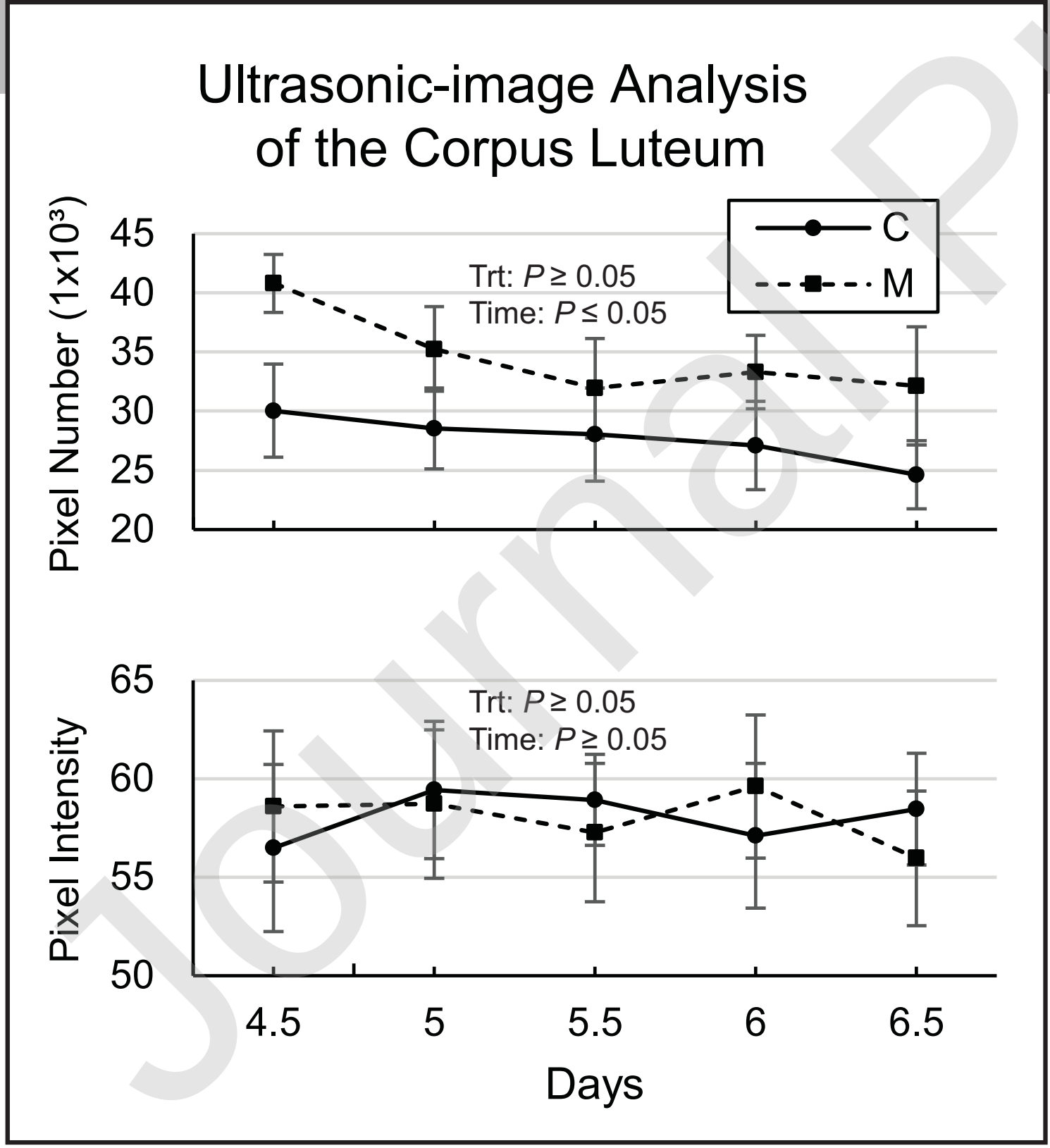

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