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Post–Mating Mefepronic acid treatment has no effect on Progesterone levels and fertility in early lactating ewes during the non–breeding season

El tratamiento con ácido mefeprónico después del apareamiento, no tiene efecto en los niveles de progesterona ni en la fertilidad de las ovejas durante la temporada no reproductiva

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ABSTRACT

This study aimed to assess the effects of post–mating treatment with mefepronic acid on serum P4 concentrations and reproductive parameters in early lactating Merino ewes during the non–breeding season. A total of 92 Merino ewes, 40-50 days (d) postpartum, were treated with an intravaginal sponge containing 60 mg of medroxyprogesterone acetate for duration of 7 d during the non–breeding season. On the day the sponge was removed (d 7), an injection of 500 IU eCG was administered. The mated ewes were then randomly divided into two groups: a control group and a treatment group. In the treatment group (MA) (n=28), ewes received an intramuscular injection of 10 mg·kg^{-1} mefepronic acid on d 9 post–mating. In the control group (n=27), the ewes did not receive any drug treatment on d 9 post–mating. The results showed that there were no statistically significant differences (P>0.05) between the Control Group and MA Group in pregnancy rates (33.3% and 39.2%), early fetal mortality rates (22.2% and 18.2), lambing rates (77.8 and 81.8%), twin rates (0 and 33.4%) and litter sizes (1.0 and 1.44). The P4 concentration on d 11 post–mating in the MA group (2.74 ng·mL^{-1}) was not significantly different from that of the control group (3.16 ng·mL^{-1}) (P>0.05). It is concluded that post–mating mefepronic acid treatment did not improve the P4 and fertility in early lactating Merino ewes during the non–breeding season.

Key words: 2-methyl-2-phenoxy propionic acid; ewe; fertility; mefepronic acid; progesterone

RESUMEN

Este estudio tuvo como objetivo evaluar los efectos del tratamiento post–apareamiento con ácido mefeprónico sobre las concentraciones séricas de P4 y los parámetros reproductivos en ovejas Merino en lactancia temprana durante la temporada no reproductiva. Un total de 92 ovejas Merino, entre 40 y 50 días (d) después del parto, fueron tratadas con una esponja intravaginal que contenía 60 mg de acetato de medroxiprogesterona durante 7 d en la temporada no reproductiva. El día que se retiró la esponja (d 7), se administraron 500 UI de eCG mediante inyección. Las ovejas apareadas se dividieron aleatoriamente en dos grupos: un grupo de control y un grupo de tratamiento. En el grupo de tratamiento (MA) (n=28), las ovejas recibieron una inyección intramuscular de 10 mg·kg^{-1} de ácido mefeprónico el d 9 después del apareamiento. En el grupo de control (n=27), las ovejas no recibieron ningún tratamiento farmacológico el d 9 después del apareamiento. Los resultados mostraron que no hubo diferencias estadísticamente significativas (P>0,05) entre el grupo de control y el grupo MA en las tasas de gestación (33,3 y 39,2 %), las tasas de mortalidad fetal temprana (22,2 y 18,2 %), las tasas de parto (77,8 y 81,8 %), las tasas de gemelos (0 y 33,4 %) y el número de crias (1,0 y 1,44). La concentración de P4 en el d 11 después del apareamiento en el grupo MA (2,74 ng·mL^{-1}) no fue significativamente diferente de la del grupo de control (3,16 ng·mL^{-1}) (P>0,05). Se concluye que el tratamiento con ácido mefeprónico después del apareamiento no mejoró los niveles de P4 ni la fertilidad en ovejas Merino en lactancia temprana durante la temporada no reproductiva.

Palabras clave: Ácido 2-metil-2-fenoxipropiónico; oveja; fertilidad; ácido mefeprónico; progesterona

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INTRODUCTION

One of the most significant reasons for the failure to achieve targeted reproductive parameters in livestock is embryonic losses [1]. In ewes (*Ovis aries*), it is estimated that 30-40% of fertilized eggs are lost within the initial three weeks of pregnancy [2]. Embryonic loss is often linked to inadequate progesterone (P4) secretion by the corpus luteum (CL) during the pre–implantation period [3].

Many different methods are being tried to prevent/reduce embryonic death. In order to prevent embryonic losses and improve reproductive performance in ewes, gonadotropin–releasing hormone or human chorionic gonadotropin can be injected at various times following sponge removal [4, 5, 6, 7, 8, 9]. Post–mating injections of these hormones can enhance ovulation induction of or luteinization of the dominant follicle, potentially increasing P4 concentrations via through the formation of accessory CL [10]. In addition, non–steroidal anti–inflammatory drugs inhibit prostaglandin production by blocking both cyclooxygenase isoforms, COX–1 and COX–2 [11].

Non–steroidal anti–inflammatory drugs (NSAIDs) have been reported to inhibit luteolytic prostaglandin $F_2\alpha$ ($PGF_2\alpha$) release resulting from insufficient IFN–τ secretion in farm animals and can be administered post–mating to help prevent embryonic losses during early pregnancy [12, 13].

Some researchers have demonstrated that peroxisome proliferator–activated receptors (PPAR) can suppress COX-2 expression [14, 15, 16]. COX-2 also plays a role in the production of $PGF_2\alpha$ [17]. The PPARs, members of the nuclear receptor superfamily, regulates several genes involved in lipid metabolism and adipocyte differentiation [18]. PPAR activation is induced by fatty acids, prostaglandins, and fibrates, which are used in human medicine for their lipid–lowering effects [19]. PPAR activation enhances mitochondrial function, promotes peroxisomal β–oxidation, stimulates hepatic gluconeogenesis, regulates lipoprotein metabolism, and supports choleretic and cholagogic activities [20].

Mefepronic acid (2-methyl-2-phenoxy propionic acid) is a member of the fibrate family of compounds used in the treatment of dyslipidemia [21]. In veterinary practice, it is utilized for the treatment of ketosis, liver diseases, and fat cow syndrome [20, 22]. Limited studies on mefepronic acid have demonstrated its positive effects on hepatic metabolism and reproductive parameters in postpartum dairy cows [22, 23]. Ewes in early lactation have high nutritional requirements [24]. Moreover, uterine venous $PGF_2\alpha$ first increases in ewes between days (d) 11–13 [25], therefore, administering mefepronic acid on d 9 post–mating is considered.

This study hypothesized that administering mefepronic acid to lactating ewes in the early postpartum period could reduce lipomobilization and prolong the lifespan of the CL by suppressing $PGF_2\alpha$ release during maternal recognition. To enhance fertility under these conditions, the study aimed to assess the effects of post–mating mefepronic acid administration on serum P4 levels and fertility in early lactating Merino ewes during the non–breeding season. To date, no studies have explored the effects of post–mating mefepronic acid in early lactating Merino ewes.

MATERIAL AND METHODS

Animals

This study was carried out on a commercial sheep farm located in Konya province, Türkiye (37°86'44.06" N, 34°16'33.55" E, Alt: 1020 m) during the non–breeding season in March 2024. The study included 92 clinically healthy multiparous Merino ewes, aged 2 to 5 years, with weights ranging from 50 to 60 kg (Demirtaş tarti, Tem, Türkiye). They were 40–50 d postpartum. The ewes were allowed to graze on pasture for 12 hours (h) each d and were not given with any compound feed. The ewes had unrestricted access to water, with no nutritional flushing provided before mating. All were lactating and nursing their lambs.

Synchronization and groups

An intravaginal sponge containing 60 mg of medroxyprogesterone acetate (Esponjavet®, Hipra, Spain) was inserted into the ewes for 7 d (D 0). On the d the sponge was removed (D 7), each ewe received an intramuscular injection of 500 IU of eCG (Oviser®, Hipra, Spain) administered into the neck muscles. Estrus detection was conducted with a teaser ram, introduced twice daily for 1 h each time over the 24–h period following sponge removal. Ewes showing signs of estrus were hand–mated with proven rams, maintaining a ewe–to–ram ratio of 7:1. The mated ewes were then randomly assigned to one of two groups: Control and Treatment. In the treatment group (Mefepronic acid) (n=28), ewes were given an intramuscular injection of 10 $mg \cdot kg^{-1}$ mefepronic acid (100 $mg \cdot mL^{-1}$, 2–methyl–2–phenoxy propionic acid, Hepagen®, Fatro Günesli) on d 9 post–mating. The Control group (n=27) received no drug treatment on d 9 post–mating.

Blood collection and hormonal assessment

Blood samples were collected from all ewes on d 9 and 11 post–mating through jugular venipuncture to measure serum P4 levels. The samples were centrifuged at 2514 g for 15 min (Elektromag, M815 M, Türkiye), and the resulting serum was stored at -20°C until analysis (Uğur Soğutma, UED 280D/S R65, Turkey). Serum P4 concentrations were measured using the ELISA method (BioTek ELx800 microplate reader USA) with a species–specific commercial kit (Sheep Progesterone Sunred 201–07–0084). The assay had a detection limit of 0.048 $ng \cdot mL^{-1}$ and was sensitive to P4 levels ranging from 0.05 to 15 $ng \cdot mL^{-1}$.

Ultrasonography examination

Pregnancy examination was performed transabdominally using real–time B–mode ultrasonography on the 50th d after mating (Hitachi EUB–405, Japan, 3.5 MHz convex probe). Litter size was recorded at the time of parturition.

Determination of reproductive performance

The following reproductive parameters were calculated;

$$\text{Estrus detection rate} = \frac{\text{ewes showing estrus behaviours}}{\text{ewes receiving sponge}} \times 100$$

$$\text{Pregnancy rate} = \frac{\text{pregnant ewes}}{\text{mated ewes}} \times 100$$

$$\text{Early fetal mortality rate} = \frac{\text{detected death fetus}}{\text{pregnant ewes}} \times 100$$

$$\text{Lambing rate} = \frac{\text{lambing ewes}}{\text{pregnant ewes}} \times 100$$

$$\text{Twin rate} = \frac{\text{twin lambing ewes}}{\text{lambing ewes}} \times 100$$

$$\text{Litter size} = \frac{\text{total lambs}}{\text{lambing ewes}} \times 100$$

Statistical analysis

Statistical analyses were conducted using SAS 9.4 software. Reproductive parameters were evaluated with the Chi–squared test, Fisher's exact test, and the PROC GENMOD procedure. The Shapiro–Wilk test assessed the normality of serum P4 concentrations within each group. The GLIMMIX procedure analyzed the effects of main factors (treatment and day) and their interaction (Treatment × Day) on P4 concentrations. Results were presented as percentages or means ± standard error of the mean (± SEM), with statistical significance set at $P<0.05$ [26].

RESULTS AND DISCUSSION

TABLE I presents the estrus detection rate, pregnancy rate, early fetal mortality rate, lambing rate, twin rate, number of lambs, and litter size. Statistical analysis showed no significant differences between the groups regarding these reproductive parameters ($P>0.05$).

Mefepronic acid treatment resulted in no significant change in serum P4 levels between days 9 and 11 post–mating ($P>0.05$) (see FIG. 1).

In this present study, the efficacy of post–mating MA injection in early lactation Merino ewes was investigated for the first time. There were no difference was observed between the groups in reproductive performance parameters. PPARα is predominantly

TABLE I
Reproductive performance of ewes with Mefepronic acid treatment

	Control Group (n=27)	Mefepronic acid Group (n=28)	*P*
Estrus detection rate (%)	59.8 (55/92)		
Pregnancy rate (%)	33.3 (9/27)	39.2 (11/28)	0.647
Early fetal mortality rate (%)	22.2 (2/9)	18.18 (2/11)	0.822
Lambing rate (%)	77.8 (7/9)	81.8 (9/11)	0.822
Twin rate (%)	0 (0/7)	33.4 (3/9)	0.999
Number of Lambs	7	13	–
Single	7	5	–
Twin	–	4 (8)	–
Litter Size	1.0 (7/7)	1.44 (13/9)	0.756

*Statistical analysis showed no significant difference between the groups ($P>0.05$). The numbers in parentheses are number of animals used for the calculation

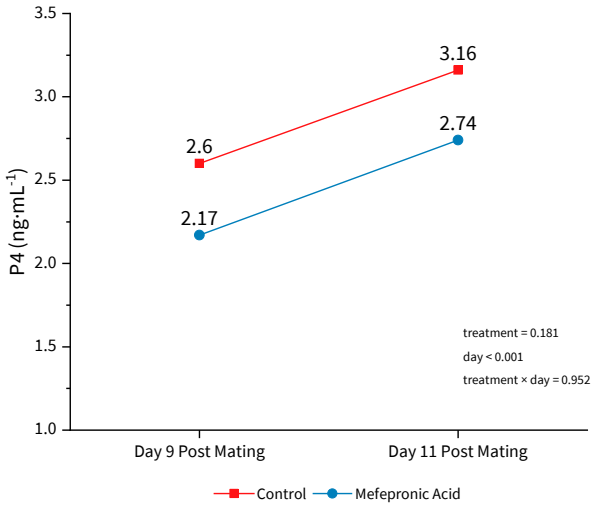

FIGURE 1. Serum P4 concentrations in mated ewes

expressed in the sheep endometrium during the early stages of pregnancy, with its levels declining between days 7 and 17. PPARβ/δ is consistently expressed throughout pregnancy, while the PPARγ is irregularly expressed [27].

Numerous studies have demonstrated that PPARs are functionally expressed throughout the female reproductive system and suggest they may play a significant role in reproduction, largely due to their involvement in energy homeostasis [28]. Furthermore, PPARs have been implicated in ovarian dysfunctions associated with obesity [29, 30], dyslipidemia [31, 32], hyperandrogenemia [33, 34, 35, 36] and insulin resistance [37, 38, 39, 40]. Only a few studies have examined the reproductive effects of mefepronic acid (MA) in farm animals. In the study by Rizzo *et al.* [22] reported that control cows exhibited their first estrus 74.36 ± 6.2 d after calving, while in the MA cows, the first estrus occurred 50 ± 3.7 d after calving ($P<0.05$). Additionally, they reported that the pregnancy rate in the first and second inseminations of dairy cows treated with the MA group increased by 7% numerically compared to the control group. Kutlu *et al.* [41] have reported that no significant difference was observed in the estrus rate, pregnancy rate, lambing rate, and litter size compared to the control group but numerically increased 15% pregnancy rate at first service in the early group when the sponges were removed during both the early and late postpartum periods following the application of MA in Hungarian Merino ewes. The results of the presented study are partially aligned with findings from previous studies. The variation in results may be attributed to differences in animal subjects, breeds, timing of MA administration, and drug dosage used.

In the presented study, early postpartum ewes were selected during the transition period when they are faced with metabolic and hormonal rearrangements. Also, the MA injection time was chosen as the period when maternal recognition is critical which a PPAR agonist is. In this study, post–mating MA injection did not increase P4 concentration compared to the control group. Maternal recognition is crucial for establishing pregnancy, with this essential process occurring in ewes between d 11 – 16 post–mating, known as the critical period [3]. During this period, if the embryo is unable to synthesize interferon tau (IFN–τ), which is essential for maternal

recognition, for various reasons, it cannot inhibit the secretion of luteolytic $PGF_{2}\alpha$ from the endometrium [42, 43]. This disrupts the necessary P4 secretion and causes embryonic death in early pregnancy [44].

Some researchers have shown that PPAR can inhibit the expression of COX–2, which also plays a role in the production of $PGF_{2}\alpha$ [14, 15, 16, 17]. COX–2 also plays a role in the production of $PGF_{2}\alpha$, a crucial factor in regulating the regression of the CL [17]. Previous studies have shown that female rats with COX–2 deficiency are infertile due to defects in ovulation, fertilization, implantation, and decidualization [45]. Mefepronic acid injection may help reduce lipomobilization and extend the lifespan of the CL by inhibiting the release of $PGF_{2}\alpha$ during the maternal recognition period.

Additionally, MA elevates cholesterol levels, which are essential for the formation of P4. Steroid synthesis typically starts with cholesterol, which can originate from dietary sources or be synthesized within the body. This cholesterol is then transported to the ovaries by lipoproteins, primarily high–density lipoprotein and low–density lipoprotein (LDL) [46]. P4 is crucial for regulating blastocyst implantation [47] and must be maintained at an optimal concentration following mating. Ewes with reduced P4 levels are at a greater risk of experiencing embryo loss [48]. In previous studies, Zerani *et al.* [49] reported that *in vitro* studies on CL treated with PPARγ agonists revealed an increase in P4 and 3–beta–hydroxysteroid dehydrogenase activity during the early and mid–luteal stages, while concurrently decreasing prostaglandin–endoperoxide synthase 2 activity and $PGF_{2}\alpha$ levels at these same stages in pseudopregnant rabbits.

Bogacka and Bogacki [50] demonstrated through quantitative analysis of PPAR mRNA expression in the porcine endometrium during the estrous cycle and early pregnancy that all three PPAR isoforms are present in this tissue. Notably, a significant increase in PPARγ mRNA levels occurred on d 13–15 of the estrous cycle, while PPARβ/δ levels decreased on d 11–12 of pregnancy, suggesting that PPARs are involved in luteolysis and maternal recognition of pregnancy in pigs. Additionally, PPAR ligands have been shown to influence the secretion of P4 and 17β–estradiol by the porcine CL during pregnancy [51]. PPARβ/δ appears to be essential in embryo implantation, with several lines of evidence indicating that the effects of PGI2, the key prostaglandin for fixation and decidualization, are mediated through PPARβ/δ [45].

Furthermore, Kang *et al.* [52] demonstrated through molecular, pharmacological, and genetic methods that PGI2–induced activation of PPARβ/δ enhances blastocyst hatching in mice. Rizzo *et al.* [22] demonstrated that administering three doses of mefepronic acid to dairy cows on the 1st, 3rd, and 5th d postpartum increased cholesterol and high–density lipoprotein levels, thereby supporting hepatic metabolism and lipogenesis, while showing a reverse trend in non–esterified fatty acids. Additionally, they reported that P4 concentrations (on the 13th d after insemination) were higher in cows treated with MA (6.47 ± 0.37 ng·mL^{-1}) compared to the control cows (4.24 ± 0.37 ng·mL^{-1}). The difference from previous *in vitro* studies may be attributed to the use of mefepronic acid, a PPARα agonist, in the current study. Furthermore, the discrepancy with Rizzo *et al.* [22] could be attributed to the lower intensity of liver metabolism in ewes compared to cows.

CONCLUSIONS

In conclusion, post–mating treatment with mefepronic acid did not enhance serum P4 levels or fertility in early lactating Merino ewes during the non–breeding season. However, further research is recommended to evaluate the efficacy of mefepronic acid, especially in ewes with high lactation.

Ethics approval

The present study was conducted with approval from Selçuk University Animal Experiments Local Ethics Committee, Konya, Türkiye (2024/065).

Conflict of interest

The authors declare no competing interests.

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