




## EFFECT OF KETOPROFEN ADMINISTRATIONS AROUND MATERNAL RECOGNITION OF PREGNANCY ON PROGESTERONE and 12,13-dihydro-15-keto PGF2alpha LEVELS IN AKKARAMAN EWES

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**ABSTRACT.** Ketoprofen is a non-steroid anti-inflammatory drug (NSAID) and acts as an analgesic and antipyretic drug. It is used for treatment of moderate pain and inflammation. The aim of this study was to determine the effects of ketoprofen administrations during maternal recognition of pregnancy (12-13 days post-mating) on progesterone and plasma metabolite of PGF2 $\alpha$ , PGFM, (13,14-dihydro-15-keto-PGF2alpha) in Akkaraman ewes whose estrus were synchronized with intravaginal sponges, cloprostenol and PMSG. Mated sheep (n=42) were randomly divided into two groups as study (n=21) and control (n=21) groups. On the 12th and 13th days following mating, study group were injected with 3 mg/kg ketoprofen, while the control group were injected similar volume of saline. The progesterone and PGFM levels on the 12th and 15th days of the sheep in the study and control groups were compared between the groups, no statistical difference was found ( $p>0.05$ ). There was a statistical difference between both progesterone and PGFM values of the sheep in the study group on the 12th and 15th days and only the PGFM values of the sheep in the control group ( $p<0.05$ ). As a result, it can be concluded that effect of ketoprofen injections (3mg/kg im) at day 12 and 13 after mating on maintaining lower PGFM levels and higher progesterone levels were controversial. Despite the decrease in progesterone and PGFM levels, there was no positive effect on pregnancy rates.

**Keywords:** Ketoprofen, maternal recognition, progesterone, prostaglandin M, sheep.

## INTRODUCTION

Türkiye is one of the important and major sheep domestication center. Among the different breeds of sheep in Türkiye, Akkaraman has the largest population, which is 45% of the sheep population. Akkaraman, which is a fat tailed sheep, has high survival rates and adaptability to various seasons, and has disease resistance, high production in poor nutritional environment and harsh seasons because of its fatty tail [1]. To improve the acceleration of the increase in sheep breeding, it is possible to control the breeding

performance and breeding process by using natural methods and various hormones in addition to traditional sheep and goat breeding with little or no expense [2]. Most of the studies have focused on pre-pregnancy synchronization and twins and triplets [3-5].

Embryonic mortality is a major problem that causes a significant reduction in the fertility performance of farm animals [6, 7]. Early embryonic loss is a major problem and a significant reason of economic loss in animal production [8, 9]. In sheep, 30-40% of fertilized eggs are lost in the first three weeks of pregnancy [7], and 70% of this deprivation happens between 8-16 days after insemination [10]. One of the causes of embryonic death is thought to be inadequate luteal function [7, 11]. To reduce embryonic loss and to improve fertility and reproductive performance, several different studies have been carried out on the administration of GnRH before maternal recognition of pregnancy to compensate for insufficient luteal function [12, 13]. Recently, new studies containing non-steroid anti-inflammatory drugs (NSAID) have been done in ruminants [8, 9, 14, 15].

Maintenance of corpus luteum, recognition and establishment of pregnancy is completed with the help of secretion of IFN- $\tau$  by conceptus in ruminants [16, 17]. Especially, slow-developing embryos may not be able to secrete sufficient IFN- $\tau$  at maternal recognition period when secretion of PGF2 $\alpha$  had to be mitigated to maintain pregnancy and corpus luteum [9]. When there is an asynchrony between the embryo and uterine environment, embryonic losses occur because of luteolytic effect of secreted prostaglandin F2 $\alpha$  that is not inhibited adequately by conceptus [12].

Ketoprofen is a NSAID and acts as analgesic and antipyretic drug. It is used for treatment of moderate pain and inflammation. It has a duration of effectiveness up to a day. It has been used in cattle, sheep, pigs, and goats. Ketoprofen is a propionic acid with a strong anti-inflammatory feature. It reduces the biosynthesis of prostaglandin F2 $\alpha$  through the inhibition of cyclo-oxygenase enzymes [15].

There is not enough available study regarding the effect of ketoprofen on prostaglandin and progesterone levels during maternal recognition period. Therefore, this study was conducted to determine the effects of ketoprofen administrations during maternal recognition of pregnancy (12-13 days post-mating) on progesterone and plasma metabolite of PGF2 $\alpha$ , PGFM, (13,14-dihydro-15-keto-PGF2alpha) in Akkaraman sheep which are synchronized with MAP impregnated sponges.

## MATERIALS AND METHODS

The study was carried out in Kırşehir in October 2018. The study material consisted of 50 Akkaraman sheep, 2-3 years old and having at least one birth. Body condition scores (BCS) were assessed as described in a previous study [18], and animals included in the study had BCS of 2.5-3 (over 5). The animals were fed in the pasture-based system with crushed barley (200 gr/animal) daily.

For estrus synchronization, intravaginal sponges impregnated with 60 mg medroxyprogesterone acetate (MAP) (Esponjavet, HIPRA®, Spain) were applied for 12 days for the synchronization of estrus. Cloprostenol (Estropur, Interhas, Türkiye) injection of 125  $\mu$ g was administered intramuscularly 24 hours before the sponges were removed, and 300 IU Pregnant Mare Serum Gonadotropin (PMSG) (Oviser, HIPRA®, Spain) administered right after removal of sponges. Rams were added 24 hours after the sponge removal, and matings were followed and recorded. All animals were observed four times daily (at 6, 12, 18 and 24 o'clock), and matings were followed. The sheep which were obviously mated by the ram which had marking harness, were kept in the

same compartment with the rams for following 12 hours. At the end of the period, color-marked sheep were separated from the herd and considered as day 0. Since three animals lost their sponges and five animals did not show estrus signs, they were excluded from the study, and the 42 mated sheep formed the study material.

Mated sheep (n=42) were randomly divided into two groups: study (n=21) and control (n=21) groups. On the 12th and 13th days following mating, the study group received 3 mg/kg ketoprofen (Rifen®, Interhas, Türkiye) at the same time of the day (in the mornings) intramuscular, while the control group received a similar volume of saline intramuscular on the same days and at the same time of the day (in the mornings). From all animals in both groups on the 12th (just before injections) and 15th days, blood was drawn into the serum tubes via the jugular vein. Collected blood was centrifuged at 3000 rpm for 15 minutes, and sera were collected. The sera were stored at -20 °C until analysis (progesterone and PGFM). Pregnancy diagnosis of animals included in the study was performed by ultrasonography (HS-2000, Honda, Japan) in the 35-40 days following mating.

### ***Laboratory analysis***

Commercial ELISA kits were used for the measurement of progesterone (sensitivity: 0.048 ng/mL; assay range: 0.015 ng/mL to 15ng/mL) and PGFM (sensitivity: 2.848 pg/mL; assay range: 3 pg/mL to 900 pg/mL) (SunRed Biotechnology Company®, Shanghai, China) hormones in the obtained blood sera. Analyses were performed by the protocols recommended by commercial ELISA kits' manual. Using the double-antibody sandwich-based kits, the measurements of the standard solutions were made at five different concentrations. Standard and streptavidin HRP samples were added to the standard wells of the ELISA plates. Sera were added to the sample wells, and the plates were incubated at 37 °C for 60 min. After incubation, the plates were washed, and chromogen solutions were added to the wells. Another incubation was performed again at 37 °C for 10 mins. The reaction was stopped with the stop solution, optical density (O.D.) values were measured using a plate reader spectrophotometer (Wavelength 450nm; MultiskanGO, Thermo, USA), and the results were calculated.

### ***Statistical analysis***

The obtained results were tested for normality with Shapiro-Wilk for each group. The data were pre-analyzed in terms of meeting the parametric test assumptions beforehand, it was resulted that our data were not normally distributed. Day 12 and day 15 values (progesterone, PGFM) within the groups were measured with the Wilcoxon test, which is a non-parametric dependent test. Progesterone and PGFM comparisons of the study and control groups on the same days were made with the Mann-Whitney U test, which is an independent non-parametric test.

## **RESULTS AND DISCUSSION**

The progesterone and prostaglandin M levels of the study and control groups on the 12th day are summarized in Table 1. Similarly, the prostaglandin M and progesterone levels on the 15th day of the study and control groups are given in Table 2. When the progesterone and PGFM levels on the 12th and 15th days of the sheep in the study and

control groups were compared between the groups, no statistical difference was found ( $P>0.05$ ).

The comparison of progesterone and PGFM levels on the 12th and 15th days of the study and control groups within the groups is shown in Tables 3 and 4. There was a statistical difference between both progesterone and PGFM values of the sheep in the study group on the 12th and 15th days and only the PGFM values of the sheep in the control group ( $P<0.05$ ).

Ultrasonographic examination performed 35-40 days after mating revealed that 90,47% (19/21 in each group) of animals in both groups were pregnant. All animals were observed till the end of pregnancy; no abortion case was recorded.

**Table 1.** Progesterone and PGFM levels of animals in the study and control groups on the 12<sup>th</sup> day.

	Progesterone (ng/ml)				PGFM (pg/ml)			
	Mean	Median	Mean Rank	P	Mean	Median	Mean Rank	P
Study	4.82±0.49	4.10	24.74	0,087	165.49±23.67	130.26	23.98	0,191
Control	3.76±0.33	3.25	18.26		117.87±11.84	96.15	19.02	

**Table 2.** Progesterone and PGFM levels of animals in the study and control groups on the 15<sup>th</sup> day

	Progesterone (ng/ml)				PGFM (pg/ml)			
	Mean	Median	Mean Rank	P	Mean	Median	Mean Rank	P
Study	4.05±0.45	3.11	23.67	0,252	139.30±21.85	109.93	24.05	0,178
Control	3.45±0.36	2.82	19.33		106.07±15.37	80.06	18.95	

**Table 3.** Progesterone and PGFM levels on days 12 and 15 of the study group.

	12 <sup>th</sup> day	15 <sup>th</sup> day	P
	Mean + standard error	Mean + standard error	
Progesterone (ng/ml)	4.82±0.49	4.05±0.45	0.011
PGFM (pg/ml)	165.49±23.67	139.30±21.85	0.005

**Table 4.** Progesterone and PGFM levels on days 12 and 15 of the control group.

	12 <sup>th</sup> day	15 <sup>th</sup> day	P
	Mean + standard error	Mean + standard error	
Progesterone (ng/ml)	3.76±0.33	3.45±0.36	0.054
PGFM (pg/ml)	117.87±11.84	106.07±15.37	0.007

Estrus synchronization of sheep can be accomplished by the help of different methods with various degrees of success. According to one meta-analysis, different researchers reported various pregnancy rates ranging between 22-96% of ewes that were synchronized in Türkiye between 1995-2020 [19]. This wide range is affected by several different factors like season, genetics, and protocol. So, the results obtained from the presented study were consistent with this.

It is indisputable that progesterone is essential for the maintenance of pregnancy [20]. It has the important function of ensuring the uterine luteolytic mechanism is counteracted [21]. Progesterone is one of the important hormones that determines the convenient function of the uterus for embryo development and implantation [22, 23]. In one study, the effect of one of the NSAIDs, flunixin meglumine (FM), on corpus luteum and progesterone levels was investigated. In that study, it could be seen that FM injections positively contributed to attaining higher levels of progesterone. Similarly, it was observed that the progesterone values on the 15th day in the control group decreased compared to the 12th day, but this decrease was slowed down in the FM-treated group and remained at a higher level on the 15th day compared to the control group. In that study, pregnancy rates in sheep treated with FM were not affected by NSAID use [24]. Our results were not consistent with that study. Administration of ketoprofen was not apparently effective in attaining higher progesterone concentrations at day 15 than 12. However, as seen in table 3 and 4, comparing the decrease of progesterone levels in the study and control groups from day 12 to 15, the alteration of progesterone in the study group is statistically ( $p=0.011$ ) significant, but in the control group, it was limited significant ( $p=0,054$ ). In the view of such information, it can be concluded that ketoprofen injections had no effect on breaking the fall of progesterone. Also, it was observed that it did not provide a higher progesterone level on the 15th day. However, this decrease in progesterone did not negatively affect pregnancy rates.

In a study, it was reported that synchronized ewes had markedly higher reproductive losses than untreated ones in season. It is reported that one of main reason for this loss was embryonic mortality which is associated with asynchronies of timing of ovulation and fertilization [25]. The critical period of maternal recognition of pregnancy are days 12 and 13 after mating. Those days correspond to the beginning of corpus luteum regression in the estrus cycle [21]. Progesterone may elevate IFN- $\tau$  production [26] which prevents PGF<sub>2</sub> $\alpha$  secretion by this mean prevents luteolysis. Hence, increase in progesterone prevent the synthesis of PGF<sub>2</sub> $\alpha$ , providing pregnancy maintenance [27]. The presented study aimed to take benefit of anti-prostaglandin effect of ketoprofen to attain lower levels of prostaglandin to support pregnancy by preventing luteolysis. In the presented study, from day 12 to 15 after ketoprofen injections, PGFM levels decreased significantly ( $p=0,005$ ) in study group, however, decrease in PGFM levels in control group is statistically significant too ( $p=0,007$ ). Higher levels of PGFM resulted in higher progesterone levels in both groups at 12th and 15th day. The decrease in PGFM in both groups is thought to be likely due to interferon tau. Hereby, it is thought that ketoprofen injections had no effect on PGFM levels. Although it was aimed to have higher pregnancy rates with ketoprofen injections pregnancy rates of both groups were identical in the presented study (19/21; 90,47%). Even though it is reported that ketoprofen concentration in plasma is maximum at 12 hours and reside for 24 hours [28], Ali et al.[29] reported that ketoprofen was eliminated from body at 12 hours. As can be concluded from the above studies, there is controversy over the pharmacokinetics of ketoprofen. It has been reported that differences in pharmacokinetics may be affected by many factors such as

genetics, environment, blood flow rate and metabolic rate [29]. Despite the progesterone and PGFM levels, it was thought that the reason why the pregnancy rates, PGFM and progesterone levels were not affected may be ketoprofen dose and repetition.

## CONCLUSION

As a result, it can be concluded that effect of ketoprofen injections (3mg/kg im) at day 12 and 13 after mating on maintaining lower PGFM levels and higher progesterone levels were controversy. So, it was not effective on to improve pregnancy rates.

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**Conflict of Interest.** The authors declared that there is no conflict of interest.

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**Ethical Statement.** This study was carried out after the animal experiment was approved by Kırıkkale University Local Ethics Committee (Decision number: 1809/51).

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