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Determination of the Effect of Different Doses of ECG Applications on Reproductive Parameters in Primiparous Akkaraman Kangal Sheep

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ABSTRACT

To increase meat production at a level parallel to meet the demand of the increasing world population in recent years, reproductive management in farm animals must be successful. In order to reach the desired level of fertility in sheep, particularly starting with the non-prolific breeds, anoestrus period applications should be carried out effectively. In this study, different doses of equine chorionic gonadotropin were applied to determine the effective eCG dose for underwent estrus synchronization with progesterone in anoestrus Kangal ewes whose milk and wool yield has been completely abandoned by the breeder for various reasons and whose only economic benefit is lamb production. A total of 225 Kangal Akkaraman Sheep breeds, 2 years old, with a body condition score of 2.5-3.5 and an average weight of 40-50 kg, were included in the study. In line with this purpose, ewes were divided into 3 groups, containing 75 primiparous sheep. A vaginal sponge containing progesterone was inserted into the animals in all groups on day 0. Seven days after this application (on the 7th day), vaginal sponges were removed and Prostaglandin F2α (PGF2α) was applied. When the vaginal sponge was removed and the PGF2α was administered, 400 IU of eCG was given to animals in Group 1 (n:75), 500 IU of eCG was administrated to animals in Group 2 (n:75), and 600 IU of eCG was injected to the ewes in Group 3. The effectiveness of different doses of eCG application on reproductive parameters such as estrous rate, pregnancy rate, multiple pregnancy rate, and the number of offspring was evaluated. While no statistical difference could be determined between the groups in terms of the stated reproductive parameters, numerically more offspring were obtained in the group in which eCG was applied at a low rate (Group 1). In the light of this information, it is concluded that 400 IU eCG can be as effective as 600 IU eCG in sexual stimulation of Kangal ewes in anestrus.

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Introduction

The rapidly increasing population of the world also increases the demand for animal products, mostly demand for animal protein (Hill et al., 2017; Van Zanten et al., 2018). Worldwide cattle breeding has become difficult due to changes in climate and environmental conditions, especially in countries that provide majority of the world's meat supply (Renaudeau et al., 2012; Rust, 2019; El-Sayed et al., 2020; Naranjo Gómez et al., 2021). In this context, modern ewe breeding aims to improve reproductive management to provide more production efficiency (Lindsay, 1991).

Since ewes have a long anoestrus period, they can give birth to one lamb per year and births are concentrated at a particular time of the year. However, the demand for lamb continues throughout the year (Goulet et al., 2002). To implement production modules that aim to increase lamb production at the same level of consumption, it is aimed that the ewe become pregnant as soon as possible, within the physiological limits following delivery (O'Shea et al., 1984; Goulet et al., 2002).

The fact that ewes are not reproductively active during the anestrus period is the most significant problem of the efficiency and profitability of the lamb production (Cizmeci et al., 2022). In terms of follicular dynamism, it is known that the ovaries of ewes are not completely inactive during the anestrus period. It is known that follicle stimulating hormone (FSH) synthesis continues in this period, and the existence of follicular development fluctuations in parallel with FSH fluctuations is also known (Bartlewski et al., 1998). Because follicular activity continues during the anoestrus period, it is possible to achieve a reproduction cycle in which pregnancies can be

achieved with interventions to ovulation (Simões, 2015). Off-season induction methods of estrous are based on the use of progesterone and its analogues with equine chorionic gonadotropin (eCG) (Wildeus, 2000). Recent studies aim to determine the effective dose of eCG by applying different doses of eCG during removal of the progesterone-containing device to optimize progesterone-supported synchronizations (Antunes-Melo et al., 2015; Hameed et al., 2020, 2021).

The Akkaraman Kangal sheep breed, which is one of the most significant genetic assets of Turkey, is extensively and purely bred in the central regions of Anatolia, especially in Sivas and the surrounding provinces. While the Akkaraman Kangal sheep breed was previously considered a variant of the Akkaraman sheep breed due to its resemblance to the Akkaraman sheep breed, it was accepted as a separate breed in 2012 with its superior meat and milk yield, as well as different body sizes, head and wool structure. The Kangal species is centered in Sivas today and is bred purely throughout Central Anatolia. This species is accepted as a significant genetic resource of the country due to its high adaptability and superior productivity (Akçapınar, 2000).

In this study, Kangal ewes, which is a regional species that has been bred for many years within the borders of Sivas province, were used. With an aim to increase lamb production, which has become the only economic gain for Kangal ewe breeders in recent years, the researchers in the current study aimed to determine the effective dose of eCG for sexual stimulation of the breed during the anoestrus period, which has not been previously determined.

Materials and Methods

Localization

The study was conducted in an ewe farm located at the coordinates of39.83371433796894, 36.34688098838113 and an altitude of 1290 meters in Ortaklar Village of Yıldızeli District of Sivas Province (Türkiye).

Materials and Application Methods

This study was approved by the Animal Research Ethics Committee of Cumhuriyet University with the decision no. 293 on 10.02.2020.

This study was conducted in early anoestrus period (in april) with (2 years old) 225 primiparous (has given birth once) ewe who conceived in autumn and gave birth in spring and nursed their lambs for approximately 50-70 days. At the beginning of the treatment, it was determined that the mean body weight was 44±5 kg, and the BCS (Body Condition Score) was between 2.5 and 3.25. At the same time, since the study was carried out in anoestrus period, 21 Kangal rams, aged between 4 and 6 years, with a weight of 102 ± 8 kg and a BCS of 3-3.5, who proved their fertility, were used for mating under sexual stimulation. When the records of the farm are examined, it was seen that although the ewes were kept together with the rams in the anoestrus period, pregnancy was been achieved in the same periods in previous years. Based on this, progesterone measurements were made in the blood samples taken before the treatment. When the progesterone levels were examined, it was determined that there were no ewes with a luteal and higher level (≥ 1 ng/ml) of P4 both in the experimental and the control groups, and the P4 value of the animals in both groups was at Subbasal level (<1 ng/ml). The study group was divided into 3 groups each containing 75 primiparous ewes. Vaginal sponge containing progesterone was inserted into the animals in all groups on day 0. Seven days after this treatment (on the 7th day), the inserted vaginal sponges were removed and PGF2α was administrated. When the vaginal sponge was removed and the PGF2α was administered, 400 IU of eCG was injected to animals in Group 1 (n:75), 500 IU of eCG to animals in Group 2 (n:75), and 600 IU of eCG was injected to the ewes in Group 3 (n:75). Treatment scheme has been showed in Figure 1. Each group had seven rams. The rams were kept with the ewes continuously for 5 days for mating in the fold.

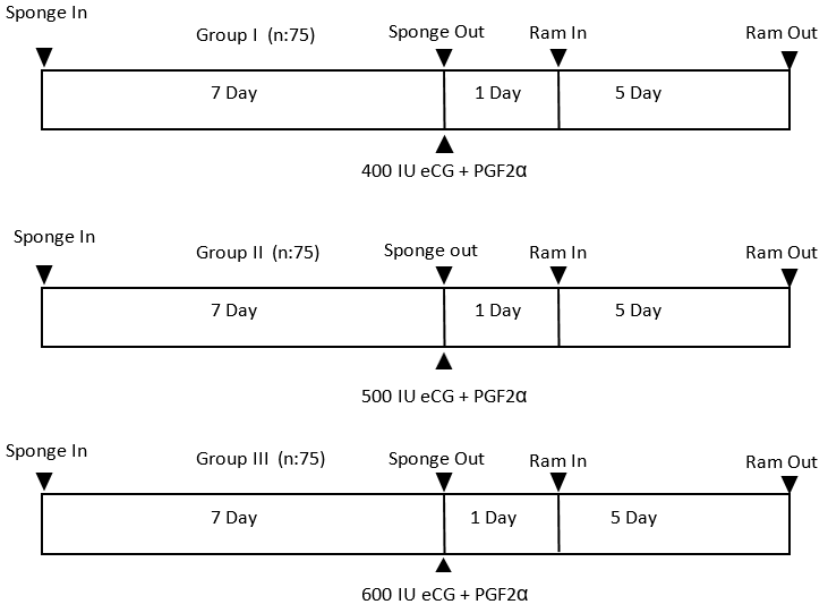

Figure 1. Treatment scheme

Pregnancy Examination

Pregnancies were diagnosed by ultrasonography 28 days after the rams were introduced (on 38th day). Approximately 1 month after the first pregnancy examination (on 60th day), the second ultrasonographic examination was performed and embryonic death rates were determined. The effectiveness of different doses of eCG administration on reproductive parameters such as estrous, conception, multiple pregnancy and embryonic death was determined in groups that underwent sexual stimulation during anestrus.

After the rams were put in the groups, the first pregnancy examination was performed with the transrectal ultrasonographic method 1 month later (on the 38th day), and the second pregnancy examination was performed with the transabdominal ultrasonographic method 2 months later (on the 68th day). To determine early pregnancies and offspring counts in the pregnancy examinations, B-mode, linear-array was performed with an ultrasonography device (Mindray DP50/Vet/US) entailing 5.0-7.5 MHz rectal probe in the supine position or via the transabdominal route to determine embryonic and fetal losses that may occur in the days following pregnancy. During the transabdominal application, the probe was preferably placed in the hairless area just above the breast to the ventral of the right fossa paralumbalis for the placement of the probe. Depending on whether signs of pregnancy could be seen in this region, the breast was scanned completely in the dorso-caudal direction. After the detection of the gestational sac, if the embryo/fetus, offspring membranes, fluids, heartbeat, and placentomes were observed during the gestational period in the ultrasonic examination, it was decided that the animal was pregnant. To follow up the pregnancies observed during these examinations, the farm was visited monthly. One week before the probable delivery date, daily visits were made to the facility and birth records were kept.

Statistical Analysis

The statistical analysis of the data obtained in the study was evaluated using the SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program. Initially, normality and homogeneity of the data was checked with the Shapiro wilk and Levene tests. Estrous rates, pregnancy rates, multiple pregnancy rates, and conception rates across groups were evaluated using chi-square analysis.

Results

Reproductive parameters such as estrous rate, pregnancy rate, multiple pregnancy rate, embryonic death rate, number of births, number of offspring and fertility were compared among the groups and results have been displayed in Table 1-5. There was no statistical difference between group for reproductive parameters following use of different dose of eCG treatment at the progesterone impregnated sponge removal P=(0.52).

The following parameters were used in the interpretation of the reproductive results:

Estrous Rate: Number of animals in group showing estrous/Total number of animals in group

Pregnancy Rate: Number of pregnancy in group/Total number of animals in group

Multiple Pregnancy Rate: Number of animals with multiple pregnancy / Total number of pregnant animals in the group

Embryonic Death Rate: Number of embryonic deaths in the group/ Total number of pregnant animals in the group

Number of Births: The number of animals that completed the pregnancy and gave birth

Number of Offspring: The number of offspring given birth by the pregnant animals

Fertility: Total number of offspring/total number of pregnant animals

Table 1. Overall reproductive outcomes in the study

Parameters	Groups		
	Group 1 (n:75)	Group 2 (n:75)	Group 3 (n:75)
Number of Estrous	64	61	67
Number of Pregnancy	52	49	54
Number of Twin Pregnancy	24	17	21
Number of Triplets	2	3	2
Number of Multiple Pregnancy	26	20	23
Number of Births	52	49	54
Number of Embryonic Deaths	0	0	0
Number of Offspring	80	72	79
Fertility	80/52 (400 IU PMSG)	72/49 (500 IU PMSG)	79/54 (600 IU PMSG)

Table 2. Statistical results of estrous rates among groups

Estrous Rates		Groups		
		First	Second	Third
Positive	Number of Animals (%)	64_a (85.3%)	61_a (81.3%)	67_a (89.3%)
Negative	Number of Animals (%)	11_a (14.7%)	14_a (18.7%)	8_a (10.7%)

$_{a, b, c}$: Different letters in the same row and column indicate significant difference (P<0.05)

Table 3. Statistical evaluation of pregnancy rates among groups

Pregnancy Rates		Groups		
		First	Second	Third
Positive	Number of Animals (%)	52_a (69.3%)	49_a (65.3%)	54_a (72.0%)
Negative	Number of Animals (%)	23_a (30.7%)	26_a (34.7%)	21_a (28.0%)

a, b, c : Different letters in the same row and column indicate significant difference (P<0.05)

Table 4. Statistical evaluation of multiple pregnancy rates among groups

Multiple Pregnancy Rates		Groups		
		First	Second	Third
Positive	Number of Animals (%)	26_a (41.9%)	20_a (40.8%)	23_a (42.6%)
Negative	Number of Animals (%)	36_a (58.1%)	29_a (59.2%)	31_a (57.4%)

a, b, c : Different letters in the same row and column indicate significant difference (P<0.05)

Table 5. Statistical evaluation of conception rates among groups

Conception Rates		Groups		
		First	Second	Third
Positive	Number of Animals (%)	52_a (81.3%)	49_a (80.3%)	54_a (80.6%)
Negative	Number of Animals (%)	12_a (18.8%)	12_a (19.7%)	13_a (19.4%)

Discussion

During the breeding season, estrous and ovulations spontaneously occur (Boscos et al., 2002). However, even during the breeding season, eCG should be administrated for estrous and ovulations to occur in a shorter time and more intensely (Cline et al., 2001).

It is known that eCG has a long LH-FSH effect on the follicles (Somanjaya et al., 2021). eCG enables the selection of more follicles during the recruitment phase, decreases the number of atretic follicles, increases E2 and P4 secretion, and consequently increases ovulation, and multiple pregnancies rate in ewes (De Rensis et al., 2014). Low doses (100-400IU) of eCG are thought to have a limited effect on ovulation, and its main effect is to increase the development of the dominant follicle and initiate estrous at an early stage (Bartlewski et al., 1999; Barrett et al., 2004). It is assumed that high concentrations (1000 IU) cause a higher estrogen concentration in the early luteal phase, thus damaging the oviduct and reducing the Pregnancy Rate.

In a similar fashion, previous research on the effectiveness of different doses of eCG administrated on ovarian functions, suggest that eCG administrated at doses higher than 750 IU increased the ovulation rate but decreased the pregnancy rate (Riesenberg et al., 2001; Kermani Moakhar et al., 2012). When eCG was administered at doses of 550-650 IU, it was found that pregnancy rates were high with multiple pregnancies (Kermani Moakhar et al., 2012).

It is generally accepted that doses of eCG ranging between 500 and700 IU is the optimal dose for increasing conception and multiple pregnancy through sexual stimulation or synchronization of estrous and ovulations depending on the reproductive period (Gordon, 2004). In addition, this general opinion was confirmed by studies revealing that 550 IU-650 IU eCG administration maximized ovarian response, estrogen level and pregnancy rates (Kermani Moakhar et al., 2012). In another study, similar reproductive results were obtained by applying different doses of eCG as 500, 600, and 750 IU (Abdullah et al., 2002). Although our data shows lower dose eCG at the end of progesterone treatment as effective as higher dose of eCG there are also studies emphasizing that the higher doses (750 IU) increase the rate of conception (Timurkan et al., 2005).

In another study using different doses (300, 500 and 700 IU) of eCG and of progesterone-impregnated sponges (30 and 40 mg Flugestan acetate) during the anoestrus period, no difference was found in the rates of estrous, but a positive increase was found in the pregnancy rates in Groups who were administered 700 IU eCG (Ataman et al., 2006). The study's material being a prolific crossbreed and mating of almost all animals in all Groups with estrous response was associated with the fact that animals may be cyclical when synchronization is started. For this reason, it was accepted by our authors that pregnancy and multiple pregnancies may occur at a high rate.

In addition, meta-analysis research on synchronization in the breeding season in Turkey revealed that 300-700 IU eCG administrations have similar economic profitability (Burak et al., 2021). Our data supports this hypothesis. Because a similar number of lambs have been yielded with the use of low and high doses of eCG, profitability can be increased by using 400IU eCG in estrus synchronization.

Another study, conducted outside the breeding season just as the current study, similar reproductive efficiency was obtained in Groups administered different doses (300-600 IU) of eCG. Regardless of the dose, Groups that were administered eCG had a significantly higher estrous response and Pregnancy Rate than Groups that did not receive any eCG (Atalla et al., 2018).

In another study, in which different doses of eCG (300, 400 and 500 IU) were administrated in Angora goats, estrous synchronization and pregnancy rates were found to be similar, similar to the current study. It was reported that administration of higher-doses of eCG changed only the intensity and reflections of estrous behaviors (Tirpan et al., 2019).

Conclusion

When evaluated in terms of reproductive parameters such as estrous rate, pregnancy rate, multiple pregnancy

rate and number of offspring, administration of low dose of eCG (400 IU) yielded similar reproductive fertility as the administration of medium (500 IU) and high (600 IU) doses. When evaluated from this perspective, it is significant for the study that lower dose of the eCG creates less antihormone and achieves the same effect with lower cost.

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