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The effect of different ram introducing procedures (protocol 66) on fertility parameters in Anatolian Merino ewes during breeding season

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Abstract: In this study, different applications of ram introduction at the beginning of the breeding season were investigated in terms of their effects on some fertility parameters. The rams were introduced to the herd (Farm II), removed for 6 days, and reintroduced again in the experiment group, while the rams stayed within the herd (Farm I) all of the breeding season in the control group. In both farms, ewes were hand mated. These practices were performed in two consecutive breeding seasons of 2014 and 2015. In farm II, in the following 13 days of the reintroduction of rams, 83.4% and 83.7% of the ewes were mated in 2014 and 2015, respectively. In farm I, these ratios were 67.9% and 75.7%. In farm II, the breeding season lasted 18 and 28 days in 2014 and 2015, respectively, while it lasted 28 and 40 days in Farm I. In farm II, 77% of births occurred within 11 days in 2014 and 82% in 15 days in 2015, and the births were completed in 27 and 32 days, respectively. In farm I, 65% of births occurred within 14 days in 2014 and 74% in 17 days in 2015, and the births were completed in 41 and 43 days, respectively. Mating and birth periods were compared, and significant differences (P values?) were found between the farms. Although breeding and offspring parameters were not different, the parameters investigated had positive results for farm II. As a result, when rams are introduced for 6 days, removed for 6 days, and reintroduced into the flocks, the breeding and the birth periods might be shortened, however there is no benefit in terms of production.

Keywords: Ewes, Central Anatolian merino, fertility, ram effect

1. Introduction

Ewes are seasonally polyestrous due to the amount of day light and those animals in the northern hemisphere start their estrus when the length of the day begins to decrease [1]. In addition to daylight, many factors such as environment and management also play a key role in the onset of estrous activity in ewes [2]. Reproductive efficiency is increased by supplementary feeding (flushing) 3–4 weeks before the breeding season [2–5]. Ewes farming enterprises receive their income from meat, milk, and wool. Meat (90%) constitutes the largest share of these revenues, while the others provide the rest (10%) [6–8]. A large segment of ewes' presence in Turkey consists of low-yield breeds [3].

The maintenance cost (feeding, labor, veterinary care, etc.) for sheep breeding should be kept low to achieve high yields [3,9,10]. In sheep, two methods are used to increase yield: the natural route that is to introduce the ram into the flock after the short-period of removing the ram (ram effect) and the chemical route (pharmacological) namely the use of effective drugs. Controlling heat with the effect of ram is more economical than pharmacological methods [11]. Although applications such as ram introduction, artificial lightning, and high energy-diets (feeding) are effective on estrous induction during the breeding season, different applications are recommended during the transition period, inside or outside the breeding season [12]. The pregnancy rate obtained by hormone applications during the anestrus period in sheep is lower and embryonic mortality rates are higher compared to the pregnancy rates during the breeding season [13,14]. Following birth, sheep first enter the lactation anestrous and then seasonal anestrous. Even if the physiological postpartum process is completed, sexual cycles do not occur until the period, when daylight begins to shorten. Since the reproductive activity has been shown by seasonal patterns, the irregular estrous cycles during the transition periods at the beginning or end of the breeding season, and the absence of synchrony at the onset of the breeding season negatively affect the reproductive productivity in the sheep flocks [11,14].

Efforts to increase the reproductive productivity in sheep (especially the applications of the reproductive control programs) should be in accordance with reproductive physiology. It has been reported in many previous studies that the fertility rate obtained after the

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use of eCG, progesterone and prostaglandin F2α and derivatives, which are widely preferred for the induction or synchronization of estrous in sheep, is lower than the fertility rates obtained by mating in the natural estrous [15,16]. In addition, the increasing global awareness for animal products by consumers who form the last link in the sector changes the attitudes and behaviors of the breeders to impose the restrictions on the use of hormonal and chemical agents in the control of reproduction. These results force not only the growers to use natural methods to produce clean, green, and ethical in line with consumers' demands and legal requirements while increasing reproductive efficiency but also the researchers to improve existing natural methods and/or develop new alternative natural applications [11].

The addition of rams within the herd in the transition period ensures that the heats begin and are synchronized at the appropriate time. The ram effect occurs more prominently during the transition period from the anestrous to the reproductive season [16]. For this purpose, the rams join the herd after being kept separate from the sheep for 4–6 weeks. This situation is described as the ram effect. It is a method that can be applied because the ram effect is cheaper and easier than other methods [9,11]. The ram effect was first applied in 1944 by Australian scientist Underwood et al. The ram effect was described as the participation of rams in sheep kept separate from the males for a certain period during the estrus period and the addition of rams after 18–25 days for the ram's pheromonal and behavioral stimulation that provides synchronized heat of the sheep [1,11].

After the transition to the breeding season following the anestrus period, ovulation is stimulated within 3–6 days, when the rams were introduced into herd and after 17–24 days, estrus activities appear prominently. This is due to a biostimulator effect which is called pheromones existing in the fat tissues of rams [9,11,17]. For pheromone secretion (urine, feces, sniffing, and vision), the rams should be kept separately for at least 4–6 weeks from the sheep. The constant presence of the rams in the herd reduces this stimulating effect [11,18,19].

It is also determined laparoscopically that the sexual stimulation caused by the ram stimulates ovulation in sheep. In the earlier studies, the follicular period was stimulated after the ram effect in sheep in the anestrus period [20,21]. With the application of the ram effect during the breeding season, Hawken et al. [22] have determined that pulsatile LH release increased in cyclic sheep in both follicular and luteal stages. With rams briefly removed from the herd and reintroduced, cyclic activity begins in sheep that were insensitive to the previous ram effect, and estrus were synchronized. In fact, it is stated that with the LH release stimulated by the application of ram effect in the follicular phase during the reproductive season, changes in cycle length and follicular dynamics may occur and a more effective synchronization of LH release also occurs.

Numerous studies have been carried out on hormone application, but studies on direct ram effect and its use in field conditions are very rare [2,11]. Most ram effect studies are carried out on Merino and Romney genotypes, which are grown in large flocks in countries such as Australia and New Zealand [23]. It is reported that the ram effect is more economical than the results obtained from studies with drugs during the breeding season in Turkey [13,24]. Similar results are reported in studies conducted globally [25].

In this study, it was aimed to investigate the effects of different practices (protocol 66) of ram effect on lambing period and fertilization yield in Central Anatolian Merinos by isolating rams from sheep for a short period of time during the breeding season.

2. Material and methods

The study was conducted in Karapınar district of Konya Province within the scope of the National Cattle Breeding Project (project code 42OAM2011-01), which was carried out under the coordination of the Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policies. It was carried out in two sheep farms. Karapınar is a district localized between 37°7′ north latitude (parallel) and 33°5′ eastern longitudes (meridian) in the Central Anatolia Region of Turkey, at an altitude of 1026 m above sea level. According to official data, the average precipitation for many years is 279.5 mm and the temperature average is 10.9 °C. In the district dominated by the typical terrestrial climate, pastures consist of step-character steppes, most of which consist of single-year-old thorny plants.

The study involved sheep aged between 18–72 months, with a body weight of 48–67 kg and a body condition score (BCS) of 2.5–3. Rams used for mating in the previous year were also used in the next year but 50% of these rams were replaced by new rams after their health check. A total of 30 sheep were mated with one ram and natural breeding was allowed and all records were kept in both farms.

The research was carried out between 2014 and 2016 in two Central Anatolian Merino enterprises with the same environmental conditions (pasture, corral, and courtyard structure). Routine vaccination (ectima, enterotoxemia, brucellosis, foot and mouth disease, pox, and sheep-goat plague) and antiparasitic applications of sheep and rams were completed at least two months before the ram's introduction. Additional feeding was performed fourteen days before the ram introduction and during the season. The diet was calculated as 50 g/day/head for 7 days and

increased to 250 g/day/head on the seventh day in both enterprises. Rams were introduced on the same day in both farms. The estrus was detected by search rams (1 ram for 50 sheep) in the morning and evening cool hours. Ewes showing estrus were mated with fertile rams in separate compartments.

The data on hand mating were recorded. The rams were not allowed to mate more than 3 times per day in the breeding season (September–October). On the sixth day, the rams were removed from the herd for 6 days in farm II (Experiment group, Intermittent Ram Introduction). The estrous behavior of Ewes in this group was repeatedly identified with search rams on day 13. In farm I (Control group, Continuous Ram Introduction), the search continued without any interruption with the search rams from the beginning of the first ram introduction. On the 13th day of the application, estrus was detected with search rams and inseminated with fertile rams. We named this procedure as 66 protocol because the rams were added to the herd for 6 days and suspended for 6 days. Descriptive statistics are given in Table 1 and Table 2. Estrus rate (number of ewes showing estrus/number of ewes available), birth rate (number of ewes giving birth after the first mating/total number of ewes), lamb yield (number of lambs born/number of ewes mated), average number of lambs at one birth (lamb born/number of ewes giving birth), single birth rate (number of ewes giving birth to singleton/number of ewes giving birth), multiple lambing rates (number of ewes giving birth to multiple lambs/number of ewes giving birth), infertile ewes ratio (number of ewes not lambed during the birth season/ number of total ewes) are shown in Table 3 and Table 4. Minitab, version 16, chi-square test was used to compare the data.

3. Results

The study revealed the findings of the ram effect applications during the breeding season in 2014 and 2015. The findings obtainedin 2014 showed that 83.41% (342/410) of the ewes were mated 14 days after the ram reintroduction. A total of 67.98% of ewes (656/965) were mated coincidentally in the other farm, where the rams were not removed (Table 1). The mating period was completed in 18 days in Farm II, while it was completed in 28 days in the control group (Farm I). In farm II, 77% (299/388) of lambing were within 11 days and the lambing period lasted 27 days. In farm I, 65% of ewes (592/911) gave birth in 14 days and the lambing period was completed in 41 days (Table 1).

In 2015, 83.7% (389/465) of the ewes were mated 14 days after the ram reintroduction. A total of 75.7% (782/1033) of ewes were mated coincidentally in the other farm, where the rams were not removed (Table1). The mating period was completed in 28 days in Farm II, while it was completed in 40 days in the control group (Farm I). In farm II, 82% (363/433) of lambing were within 15 days and the lambing period lasted 32 days. In farm I, 74% (723/977) gave birth in 17 days and lambing period was completed in 43 days (Table 2).

Fertility (insemination, birth, twin birth, single birth, nonbreeding) did not differ between the seasons (Table 3 and Table 4) ($p \geq 0.05$).

4. Discussion

Small ruminant farming in the region where the study is carried out is based on pasture feeding. Therefore, animals should be used at a maximum level for the profitability of the enterprises and to keep costs at a minimum. For this purpose, it is important to increase the yield of offspring in a short period of time. Therefore, it is important that the mating and lambing periods are as short as possible. This can be achieved either by using hormones or ram effects which is a more feasible method because of its ease of application, low cost, and naturalness.

To take advantage of the ram effect, Mc Dougall [26] reported that search rams are introduced to the herd for 16 days. On day 16, search rams are removed from the herd, and fertile rams are introduced. A total of 17 days are needed for intensive estrus behavior [9,11,26].

Table 1. Data for insemination rates in 2014 and number and birth rates in 2015.

Facilities	Over exceeding dense days in the year 2014					Births are intensive in the year 2015				
	Insemination		Total	%	P	Birth		Total	%	P
	+	-				+	-			
Farm I (NI)	656	309	965	97,9	0.000	592	319	911	65	0.000
Farm II (6DI)	342	68	410	83,4		299	89	388	77	
Total	**998**	**377**	**1375**			**891**	**408**	**1299**		

Farm I: Continuous Ram Introduction, NI: not interrupted Farm II: Intermittent Ram Introduction, 6DI: 6 day Interrupted Insemination and birth times were found to be significant.

Table 2. Data for insemination rates in 2015 and number and birth rates in 2016.

Facilities	Over exceeding dense days in the year 2015					Births are intensive in the year 2016				
	Insemination		Total	%	P	Birth		Total	%	P
	+	-				+	-			
Farm I (NI)	782	251	1033	75.7	0.001	723	254	977	74	0.001
Farm II (6DI)	389	76	465	83.7		363	80	443	82	
Total	1171	327	1498			1086	334	1420		

Farm I: Continuous Ram Introduction, NI: not interrupted Farm II: Intermittent Ram Introduction, 6DI: 6 day Interrupted
Insemination and birth times were found to be significant.

Table 3. The data of fertility in the year 2015 (number and percentages of mating, single, twin, nonbreeding sheep and lamb yield).

Facilities	Inseminated ewes	Breeding ewes		Twin birth		Single birth		Nonbreeding		Lamb yield
		number	%	number	%	number	%	number	%	
Farm I (NI)	965	911	94.41	142	15.58	769	84.42	54	5,59	1.15
Farm II (6DI)	410	388	94.63	43	11.08	345	88.92	22	5.37	1.11
Total	**1375**	**1299**		**185**		**1114**		**76**		

Farm I: Continuous Ram Introduction, NI: not interrupted Farm II: Intermittent Ram Introduction, 6DI: 6 day Interrupted
In 2015, there is no statistical difference between the farms in terms of the number and rates of sheep mating, single, twin, and nonbreeding sheep and lamb yield. However, in farm 2, where protocol 66 was applied, the mating and birth season were completed in a numerically shorter time.

Table 4. The data of fertility in the year 2016 (number and percentages of mating, single, twin, nonbreeding sheep, and lamb yield).

Facilities	Inseminated	Breeding ewes		Twin birth		Single birth		Nonbreeding		Lamb yield
		number	%	number	%	number	%	number	%	
Farm I (NI)	1033	977	94.6	189	19.35	788	80.65	59	5.4	1.19
Farm II (6DI)	465	446	95.27	65	14.17	378	85.33	22	4.73	1.15
Total	**1498**	**1420**		**254**		**1166**		**78**		

Farm I: Continuous Ram Introduction, NI: not interrupted Farm II: Intermittent Ram Introduction, 6DI: 6 day Interrupted
In 2016, there is no statistical difference between the farms in terms of the number and rates of sheep mating, single, twin and nonbreeding sheep and lamb yield. However, in farm 2, where protocol 66 was applied, the mating and birth season were completed in a numerically shorter time.

Intensive estrus behaviors began to appear after 12 days in the present study (Table 1 and Table 2). In 6 days of those 12 days, the rams were removed from the herd, so the herd management was easier, and costs were reduced. It is seen that the method used in the present study provides an advantage of 4 days compared to the known ram effect application. In the efforts to benefit from the ram effect, fertile rams are introduced immediately after the search rams are removed from the herd. In the present study, it differs from the ongoing practice due to the introduction of fertile rams in the herd six days after the search rams are removed from the herd. It was observed that ewes with low body condition scores either did not show standing heat or showed later than expected. Since the additional feeding was performed on a herd basis but not individually, the heat of ewes with low BCS was delayed. As a matter of

fact, Yılmaz et al. [27] reported that BCS is an important influence on heat as well as fertility.

In New Zealand sheep enterprises, 80% of Romney breed ewes reportedly mated in the first 6 days after the ram introduction and 55%–68% of ewes gave birth in the first week of the lambing period [28]. In the present study, it was observed that the duration of the mating and births was longer. It is estimated that this is due to the characteristics of pasture, management, and breed. We observed a significant difference between the farms in terms of mating and lambing periods. In the present study, both estrus and mating rates were 95.27%. In the similar studies, estrus and mating rates were 50% [29], 94% [4], 83.75% [30], 85.71% [31], 92% [32].

Every mated animal is expected to give birth, but a healthy birth does not occur in all animals due to embryonic death, abortion, and premature birth. Kose et al. [32] reported that the birth rate was 68% in Akkaraman sheep, where they applied flushing during the season. Ozbey and Tatlı [33] stated that the birth rate of Awasi breed ewes with hormonal applications in November was 86.67%. Demiral and Iscan [6] reported that the birth rate was 27.5% following artificial insemination in Kangal Akkaraman breed ewes synchronized with hormone applications during the breeding season. This study shows that the desired result could not be achieved with hormone application during the season. In the present study, a higher rate of pregnancy was obtained compared to previous reports [6,32,33].

In addition, Khaiseb et al. [4] report that flushing application negatively affects fertilization efficiency, if the body condensation score is ≥3. In September, there was a significant increase in fertilization efficiency in Akkaraman breed ewes which had 3≤ BCS and were subjected to flushing+hormonal applications, compared to nonflushing group. In previous studies where the ram effect and hormonal application were performed, birth rates were 86% [4], 40% [29], 80.95% [31] and 89.6% [34]. In the present study, a higher pregnancy rate (94.63%) was obtained compared to similar studies.

In sheep breeding, it is desirable for business profitability to obtain more lambs from each ewe. To achieve this, the rate of multiple births must be high. For this purpose, different methods are applied, such as increasing twinning within the seasonal or three lambing in two years. In studies to increase twinning [4, 6, 29, 32, 34], twinning ratios were ranging from 6% to 26.7%. In the present study, the twinning rate was 14.17% in the experiment group and was lower than those detected in previous studies (range 22.4%–26.7%) [6,34]. This is thought to be due to the environmental impacts such as maintenance, nutrition, and pasture. The geographical structure and pasture vegetation (due to precipitation) of the places where those studies were carried out might have been of much better quality. Bulbul et al. [13] reported a "three lambings in two years" study in sheep of the Central Anatolian Merino breed that application of ram effect (1.13) and flushing+ram effect (1.33) in the season yielded a higher lambing rate compared to hormonal applications and because of economic analysis, they stated that it was more accurate to increase twinning in a season. Ozbey and Tatlı 33] reported that lamb yield was 0.79 in Awassi breed sheep with a hormonal application during the breeding season, whereas Alkan et al. (2012) obtained lamb yield of 1.1–1.2 in Tahirova breed sheep with ram effect, flushing and exogenous hormone application in August–September. Tarhan [30] indicated that lamb yield was 0.4 in beef sheep in March (out of season) with hormonal administrations. This clearly suggests that in the climatic conditions of Turkey, out-of-season applications are not successful enough to satisfy the breeders. Nowers [25] reported that merino sheep in the out of season had lamb yields of 1.13 following either hormone applications or flushing+ram effects. Aktas et al. [34] stated that the highest lamb yield between 2007 and 2009 in Central Anatolian Merinos was 1.13. Compared to the studies where lamb yield is evaluated [6,13,33], lower lambing rate was obtained as compared to flushing+ram effect groups and higher lambing rates [4,13,24,25,30,32,34].

In conclusion, the removal of rams for 6 days from ewes and reintroduction shortened the mating period which consequently shortened the lambing period in the breeding season. This practice resulted in the similarly aged lambs for the market, which eventually reduces labor costs in the farm. When evaluated in terms of labor and the production of lamb, the conclusion was that ram removal was advantageous. This ram effect regimen should be investigated in different breeds and regions to evaluate its possible benefits on fertility.

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