

Effect of royal jelly on puberty of ewe lambs

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Summary

In this study, we investigated the effect of royal jelly on the puberty of female $\frac{1}{4}$ Romanov $\times$ $\frac{3}{4}$ Morkaraman crossbred lambs. Royal jelly was applied in two ways: orally and intravaginally. The rate of estrus as well as the day and time (in hours) of estrus were observed and determined by real-time ultrasonography (for pregnancy) and laparoscopic surgery (ovary examination). Three groups of lambs were observed. The first group, consisting of 15 female lambs, was treated intravaginally; in the second group, 15 female lambs were treated orally; and the third group, which served as control, consisted of 10 ewe lambs that were not treated with royal jelly. Estrus rates in the intravaginally and orally treated groups were 13% and 20%, respectively, whereas the control group showed no estrus activity. However, estrus interval in both groups was observed 3-5 days after the withdrawal of the treatment. All the animals had an active ovary when observed during estrus activity by ovary examination. Yearling $\frac{1}{4}$ Romanov-bred ewes respond positively to royal jelly application. Increasing the doses and extending the duration of royal jelly application might yield better results.

Keywords: follicle, honey bee, reproduction, sheep

Royal jelly, an apicultural product secreted from the mandibular and hypopharyngeal glands of 5-to-15-day-old worker bees (*Apis mellifera* L.), is rich in nutrients (4, 10-14, 23, 25). Royal jelly is secreted by young worker bees to feed the queen. It is a pearlescent jelly-like product with an organic acid character composed of nectar, pollen, and bee enzymes. It is produced by worker bees during the digestion of pollen and is used as food by larvae in the hive during the first days of their lives.

The composition of royal jelly is complex and changes depending on the diet of bees, season, age of larvae, method of production (14), area (23), type of plant used by bees, and health of the plant. Fresh royal jelly contains 60-70% of water, 12-16% of raw protein, 3-6% of fat, 10-18% of carbohydrates, and 1.5% of mineral substances. It has a pH level of 3.5-4.5 and a density of 1.1 g/mL (5, 8, 22). The main constituent, approximately 17-45%, of the dry matter of royal jelly is protein (17). The fat content in the dry matter is 3-19% (2). Carbohydrates, which are the third largest component, comprise fructose, glucose, and sucrose (16, 24). Royal jelly contains vitamins A, C, D, and E and is also a natural source of B-complex vitamins. One of the most important components of royal jelly is pantothenic acid, which plays a basic role in the synthesis and metabolism of protein, fat, carbohydrates, and various hormones.

In this study, we investigated the effects of royal jelly on the sexual maturity of lambs and how early they can reach sexual maturity with the aid of this product. For this purpose, we examined the effects of oral and intravaginal administration of royal jelly on the onset of the reproductive cycle.

The purpose of using crossbred lambs is to combine the reproductive ability of the Romanov breed and the characteristics of the native Morkaraman breed, which is more resistant to the local climatic and environmental conditions. In addition, Morkaraman is the second most common sheep breed in the country.

Material and methods

This study was carried out in a semi-open sheep pen in a sheep raising enterprise in April-May 2014. Forty 7-8-month-old $\frac{1}{4}$ Romanov $\times$ $\frac{3}{4}$ Morkaraman lambs were used in the experiment. The animals used in the study were selected from lambs with a live weight of 40-45 kg.

Lambs were fed mixed forage containing 12% crude protein and 2750 kcal/kg ME and dry alfalfa. The animals were divided into groups, and each ewe was fed 300 g of mixed forage and 1-2 kg of dry alfalfa per day during the trial. The royal jelly used in the trial was obtained from a commercial firm.

When the ewe lambs (n = 40) turned 7-8 months old, they were separated into three groups at random. The first, second, and third groups consisted of 15, 15, and 10 lambs

of homogeneous weight, respectively. The lambs were sheltered in a separate paddock. The 1st, 2nd, and 3rd groups were the intravaginal, oral, and control groups, respectively.

Each animal in the 1st group (the intravaginal group) was injected a total of 4.5 g of royal jelly for nine days with controlled internal drug release (CIDR) devices inserted into the vagina. The injection of royal jelly into the CIDR devices and the insertion of implants were repeated every three days, and each animal was inserted a CIDR device with 1.5 g royal jelly three times in total. For the 2nd (oral) group, a total of 22.5 g of royal jelly, 1.5 g per animal, was mixed homogeneously in 900 cm³ of distilled water. The solution was separated into doses of 60 cm³ for each animal to be used for three days (the solution was kept in a refrigerator at $\pm 4^{\circ}\text{C}$). Later, the 60 cm³ of royal jelly solution was given to each animal orally at daily doses of 20 cm³ per day. Each animal received a total of 180 cm³ (4.5 g) of royal jelly. The animals in the control group were not subjected to any procedure. The doses used in previous studies on the subject were taken into consideration (6).

Starting at the 24th hour following the termination of royal jelly administration, estrus was checked with teaser rams at eight-hour intervals. In the ewes whose estrus days and hours were recorded, the ovulation rates were determined by performing an ovarian examination via ultrasound on the 6th day of estrus. Estrus screening started on the 10th day following the first estrus and continued until the 21st day. We determined whether cycles of estrus were composed of sequential cycles.

Prior to laparoscopic examination, the donor ewes were deprived of feed for 12-18 h so that the size of their rumens and bladders would decrease. Before the examination, the ewes were given 2 mL of an anesthetic cocktail (200 mg ketamine HCL + 4 mg xylazine) intravenously. The ewes were laid back on the laparoscopy table, hair on the belly and around the rear flank was sheared, and the hide was disinfected.

Later, by tilting the laparoscopy table in the surgical position to an angle of 40°, the animal was positioned upside-down. The abdominal cavity was entered via 10.5-mm and 5.5-mm trocars with safety actuators at four fingers below the nipples and through the right and left sides of the mid-line. Shortly after the abdominal wall was drilled via trocars, chucks were removed, and an endoscopic probe and a palpation probe were inserted into the abdominal cavity through their cannulas. A small amount of carbon monoxide was pumped into the abdominal cavity to separate the uterus from the other tissues around it and make it easier to find. Shortly after the uterus was placed in the appropriate position for ovary examination, the right ovary was examined using the palpation probe. The same procedure was repeated in the uterine horn on the other side. Shortly after the completion of ovary examination in both uterine horns, the trocar cannulas were removed from the abdominal wall, and the remaining small lesions on the abdominal wall were closed with titanium clips onto which a local antibacterial spray was applied. To prevent a possible infection after the examination, oxytetracycline was applied intravenously. Pregnancy was checked with a Real-Time B Ultrasonography machine (5-7.5 MHz, Draminski).

At the end of the study, one-way and two-way Chi-square tests (χ^2) and Fisher's Exact test were used to analyze the data obtained (19). The significance level adopted in the study was $P < 0.05$.

Results and discussion

In the first stage of the study, we examined the effect of the oral and intravaginal administration of royal jelly on the time of onset of estrus in the ewe lambs according to the number of animals producing follicles. The two-way chi-square (χ^2) test indicated that the effect of royal jelly on the rate of estrus was not statistically significant.

Though not statistically significant, 20% of the estrus hours were obtained with 4.5 g of royal jelly given orally for nine days, and 13% of estrus hours were obtained with the same amount of royal jelly administered intravaginally. The onset of estrus occurred at the 69th and 100th hours after the termination of royal jelly administration. With intravaginal application, the earliest occurrence of estrus was observed at the 80th hour, whereas in the animals receiving royal jelly orally estrus was observed at the 48th hour. Likewise, while the latest estrus was observed in the animals treated intravaginally at 120th hour, the animals treated orally showed it at the 102th hour.

On the 6th or 7th day following estrus, the ovary was examined by the semi-laparoscopic method, but no active corpus luteum and/or follicle were observed. However, in the following cycle, ovary examination was repeated by the semi-laparoscopic method, and an average of 2.7 follicles was obtained in a total of three animals by initiating ovary activity with royal jelly given orally. When royal jelly was used intravaginally, an average of 2 follicles was obtained in a total of 15 animals. These data were subjected to the one-way χ^2 test, and the results were statistically significant ($P < 0.05$).

The animals set for estrus with teaser rams were later inseminated artificially, but no pregnancy was obtained. Their inability to get pregnant was attributed to the coincidence of the small number of inseminated animals and their young age (6).

Some producers adopt the strategy of making male or female lambs complete their development within a period shorter than normal and mating them for impregnation, but the effectiveness of this approach depends greatly on the animals' ability to develop early. In order for this hereditary ability to appear, appropriate care and feeding should be provided to lambs during their development period.

Formerly, in developed countries, breeders viewed this strategy with skepticism, assuming that the use of yearling ewes in breeding at an early age might shorten their lifespan ("usable" period). Today, however, in areas where breeding requirements and environmental conditions are appropriate, early breeding (of some breeds) is often practiced. In recent years, increasing

Tab. 1. Number and proportion of follicle-forming animals

Groups	Follicle-forming		Follicle-free		Total
	Number	Rate (%)	Number	Rate (%)	
Control	0	0 (0/10)	10	100 (10/10)	10
CIDR	2	13 (2/15)	13	87 (13/15)	15
Oral	3	20 (3/15)	12	80 (12/15)	15

Explanation: $\chi^2 = 2.21$, $P = 0.331$

Tab. 2. Number of follicles in particular groups

Groups	Number of follicles
Control	0
CIDR	6
Oral	8

Explanations: $\chi^2 = 7.43$, $P = 0.024$

the sexual development and reproductive performance of lambs has come to the fore, especially in order to use ewe lambs in early breeding. As a result of the gradual intensification of production systems, studies on the capacity of female lambs for early development and pregnancy have been under the spotlight (3). Today, it is possible to have female lambs at the age of 6-7 months mate naturally, conceive, and successfully give birth to their first offspring at the age of one year.

Many studies have recently been undertaken with a view to shorten the time between generations and increase the reproductive rate, especially in sheep, and several treatments have been experimented with for this purpose. In recent years, the investigation of the possibility of using royal jelly in many areas has also been placed on the agenda.

Royal jelly has been used in recent years as a natural alternative to synthetic hormones to increase reproductive efficiency and solve reproductive problems in sheep (6). A literature review demonstrates that, although there are studies on the start of the post-natal reproductive cycle, estrus synchronization, and increasing ovulation rates, limited research exists on the start of the reproductive cycle in young, mature, and yearling lambs.

In female rats receiving royal jelly orally, the product had an estrogenic effect. The findings of the current study are in line with those obtained with rats. The hormonal effects of royal jelly increased pregnancy and lambing rates in sheep (15).

In this study, young animals were selected, and we attempted to advance their sexual maturity by using royal jelly. However, no pregnancy was obtained in animals that showed estrus. In recent years, many studies have been carried out to determine the possible effects of royal jelly on early reproduction in animals of different species (4, 6, 9). In some of them, similarly to our results, it was reported that the application of royal jelly alone or in combination with different preparations had an effect on the reproductive performance, conception, reproductive activity, estrus, and

pregnancy rates (4, 6, 9). A comparison of results reported by other researchers and those obtained in this study suggests that the administration of royal jelly together with PMSG, GnRH, or ecG hormones, rather than alone, had positive effects on these parameters and increased the effect of royal jelly considerably. The limited results obtained in the present study might have resulted from the use of royal jelly alone.

The estrus rates obtained in this study with 4.5 g of royal jelly applied by two different methods, which ranged from 13% to 20%, were lower than those obtained in a study by (7), who used yearling ewes in breeding at an early age, applying a total of 4.8 g of royal jelly via intramuscular injection, and reported an average estrus rate of 82%. This difference indicates that the intra-muscular injection of royal jelly is more effective than oral or intravaginal application. In addition, (7) found similar rates in a royal jelly group and a synthetic preparations (PMSG) group.

According to our results, estrus rates were low – 13% and 20% – and estrus occurred at the 100th and 69th hours in the intravaginally and orally treated groups, respectively. In a study by (15), royal jelly and progesterone hormone were used intravaginally at different doses to stimulate estrus in Awassi sheep, and estrus rate was increased by 100% whereas the onset of estrus occurred at 49.6-59.6 h. In the present study, the low rate of estrus might have resulted from the young age of the animals (7-8 months) and the absence of progesterone in the royal jelly (5, 8, 22). In the present study, the onset of estrus was earlier than reported by (15), which might have been due to the method of administration of progesterone to the yearling ewes (intravaginally).

In mature lambs used in early breeding, infertility rates were reported as high as 20% (1) and 60.6% (18) in Romney, 48.6% in Targhee (21), and 41.5, 51.0, and 48.7%, in Malya, Anatolia Merinos, and Akkaraman sheep, respectively (20). While (26) obtained pregnancy rates between 46% and 91% in various crossbred mature lambs in different years, (27) obtained 27.3% in mature lambs giving birth at one year of age, which is close to the rates obtained with the mature Romanov crossbred lambs in the present study.

In terms of the onset of estrus and start of ovary activity, the results obtained in the present study in female lambs aged 7-8 months by the application of royal jelly were promising. However, studies on this subject are not sufficient; there is a special need for studies investigating the effects of royal jelly administered at different doses and for longer periods. It is of great importance to determine the ability of sheep to give birth within the first year of age by using them as breeding animal at a much earlier age under optimum care and feeding conditions. It is also important to determine the genetic abilities of young male lambs in

the same way by using them in breeding and selection on a herd basis.

The administration of royal jelly to yearling $\frac{1}{4}$ Romanov ewes did not produce a sufficiently positive effect, but increasing the doses and extending the duration of royal jelly application might yield better results.

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