

*Full Length Research Paper*

# Improved reproductive response of sheep in intrauterine insemination program with the use of royal jelly

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The objective of the present study was to evaluate estrus and conception rates obtained with the use of progesterone (P<sub>4</sub>) cream in intravaginal sponges in conjunction with equine chorionic gonadotropin (eCG) or eCG plus royal jelly (RJ) given at the time of sponge withdrawal. A total of 365 fat tailed ewes were assigned randomly to two treatment groups: insertion of intravaginal sponge containing 30 mg (LNP; n = 188) or 60 mg (HNP; n = 177) progesterone for 12 days. For each treatment, 300 or 600 IU of eCG were compared with 100 or 300 IU of eCG plus 500 mg of RJ to assess the effect on reproductive parameters of ewes inseminated laparoscopically in the anoestrus season. Ewes in each group were inseminated with fresh diluted semen (10 × 10<sup>7</sup> sperm per insemination dose). Inseminations were carried out 11 to 18 h after the first detection of estrus. Pregnancy diagnosis was done at approximately 55 days after insemination. For the 30 and 60 mg natural progesterone treated groups, estrous (22.3 versus 30.5%) and conception rates (50.0% for both treatments) did not differ significantly. Ewes that received 300 IU eCG plus 500 mg RJ had higher (P>0.05) conception rates (66.7 versus 47.6 and 40.0%) than those treated with only eCG (300 or 600 IU) at sponge removal. There was a significant (P≤0.05) delay in onset of estrus in ewes treated with 100 IU eCG plus 500 mg RJ when compared with the other treatment groups.

**Key words:** Royal jelly, equine chorionic gonadotropin (eCG), natural progesterone, artificial insemination, ewe.

## INTRODUCTION

Royal jelly (RJ) is a viscous substance secreted by the hypopharyngeal and mandibular glands of worker honeybees (*Apis mellifera*) and is utilized as an essential food for the queen bee larva and the queen itself. RJ contains a considerable amount of protein, free amino acids, lipids, vitamins and sugars, and is known to have some diverse nutritional and pharmacological functions in humans such as vasodilative and hypotensive activities, anti hypercholesterolemic activity and antitumor activity

(Fujii, 1995; Mateescu and Barbulescu, 1999). The RJ has been widely used as a means of improving fertility in human males and females (Al-Masri, 1986), quail (Csuka et al., 1978) and rabbits (Khattab et al., 1989). Recently, there have been a number of reports on successful incorporation of RJ into a 12-day progestagen estrus synchronization protocols in sheep (Husein et al., 1999; Husein and Kridli, 2002; Kridli et al., 2003). Estrus synchronization protocols in ewes, utilizing intravaginal devices impregnated with natural progesterone or progestagens (Keefe and Wichte, 2000, Kohno et al., 2005) are the most efficient tools for making a planned reproduction management program. These programs have resulted in more ewes detection in estrus, and reduction in number of days open. Moreover, they are indispensable for inducing estrus and ovulation in artificial

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**Abbreviations:** eCG, Equine chorionic gonadotropin; RJ, royal jelly; P<sub>4</sub>, progesterone.

insemination programs in ewes.

In previous experiments (Hussein and Haddad, 2006, Kridli and Al-Khetib, 2006), RJ treatments combined with intravaginal devices were used to induce estrus in ewes in natural mating program. However, knowledge of the reproductive response of sheep treated with RJ together with exogenous natural progesterone, and then artificially inseminated is still limited. The aim of this study was to examine the dose-response effects of equine chorionic gonadotropin (eCG) treatment with/without a single intramuscular injection of RJ on reproductive response of ewes treated with intravaginal sponges impregnated with natural progesterone and then inseminated laparoscopically.

## MATERIALS AND METHODS

### Animals and management

A total of 365 multiparous fat tailed ewes (Redkaraman, n = 84; Whitekaraman, n = 176; Daglic breeds, n = 105; 50 to 55 kg adult weight) reared in eastern (39° 45'8"N / 39°29'34"E) Turkey were used. Animals were satisfactorily homogeneous in terms of age (4 ± 1 year) and body condition score (BCS = 3 ± 0.5). Scoring was done on a scale of 1 to 5, where 1 is emaciated (extremely thin), and 5 is very fat. All animals were dry and time from the previous weaning was at least 13 weeks. Ewes were fed 1.5 kg alfalfa hay and 0.5 kg concentrate mixture per ewe per day. Water and mineral licks were available *ad libitum*. The research was conducted in the natural anestrus season (mid-February).

### Estrus synchronization treatments

Polyurethane sponges were made using a 33-density polyurethane foam, cut in 2 x 2 x 2 cm cubes, and threaded with a cotton string. Sponges were washed, sterilized by autoclaving and then impregnated with 30 or 60 mg natural progesterone cream (Natural Hormone Balancing Progesterone Cream, Pharmacist Ultimate Health Corporation. PUH, Saint Paul, MN, United States).

Royal jelly paste was obtained from a single local honeybee keeper and transferred into graduated glass tubes and then stored at -20°C until used. Tubes containing frozen RJ paste were taken out and placed at room temperature for thawing and RJ injections prepared on the day of use. One hundred grams of frozen-thawed RJ paste were weighed and placed into a vial to which distilled water was added to bring the total volume to 1000 ml. The vial was gently mixed until the contents dissolved and was filtered using a funnel and glass wool. Then, 200 equal doses (5 ml each) containing 500 mg RJ paste were made. The sterility of the solution was confirmed by incubating for 24 h at 37°C.

Ewes were treated with intravaginal sponges containing either 30 mg (LNP; n = 188) or 60 mg (HNP; n = 177) natural progesterone cream for 12 days. Ewes were divided into four groups and received one of the following intramuscular (i.m.) injections at sponge removal: (1) 100 IU eCG and 500 mg RJ (LNP, n = 46; HNP, n = 47); (2) 300 IU eCG and 500 mg RJ (LNP, n = 38; HNP, n = 38); (3) 300 IU eCG (LNP, n = 47; HNP, n = 40) or (4) 600 IU eCG (LNP, n = 57; HNP, n = 52).

### Estrous observation and laparoscopic insemination procedures

Vasectomized rams fitted with a crayon-marking harness were

introduced (at the rate of 5 rams per 100 ewes) to increase synchrony of the ewes and to mark ewes as they came into heat. The animals were observed for signs of estrus beginning at 24 h after sponge removal and continuing up to 72 h. Observation for onset of estrus was performed every day at 9:00 am and 9:00 pm. Animals that did not show any mating marks at 72 h were not inseminated. Ewes marked by the vasectomized rams received an i.v. injection of anesthetic cocktail containing 2 cc Ketazol (Indus Pharma, Karachi, Pakistan) + 0.04 cc Rompun (Bayer) and were inseminated laparoscopically 11 to 18 h after their estrus were first detected with freshly diluted semen (10 x 10<sup>7</sup> motile spermatozoa/0.5 ml). Pooled semen from three Romanov rams, collected with an artificial vagina was used. Semen was extended at 35°C in OviPro<sup>®</sup> (Minitüb, Tiefenbach, Germany) and maintained at 30°C in a water bath for a maximum of 2 h until intrauterine inseminations. A single experienced laparoscopic AI operator was used for all the inseminations. At the time of laparoscopic AI, uterine tone was scored as intense tone (1), moderate tone (2) or no tone (3). Uterine tone is a measure of quality of heat and conception rate is a measure of the percentage of inseminations that result in conception.

All ewes were scanned transabdominally by using a real-time ultrasound scanner equipped with a 5 MHz linear-array transducer (Pie Medical, 100 Falco. Vet) at day 55 post insemination.

### Statistical analyses

The data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Minitab software version 13 to test for the effect of synchronization treatment, dose of progesterone in the sponge and the different dose of eCG and RJ injection on the time to onset of estrus and uterine tone. The Chi square analysis was used to test the effect of these factors on estrus response and conception rates. A regression equation was computed for uterine tone and conception rates. Ewe breed effect was found not to be significant and therefore it was omitted from the statistical analysis.

## RESULTS

About 26% of ewes exhibited overt signs of estrus during the 72 h observation period (Table 1). No significant difference in percentage of ewes exhibiting estrus, time to onset of estrus, uterine tone at the time of insemination, or conception rates was recorded between ewes synchronized with 30 or 60 mg progesterone sponges. Similarly, different doses of eCG with/without RJ administration did not significantly affect the percentage of ewes exhibiting estrus, uterine tone at the time of insemination, or the conception rates. However, time to onset of estrus differed significantly (P≤0.05) among groups of ewes. Ewes that received 100 IU eCG and 500 mg RJ had the latest onset of estrus as compared to the other groups. The interaction between natural progesterone dose and different dose of eCG with/without RJ administration was not significant for the same parameters. Royal jelly treatment combined with eCG injection tended to influence conception rate favorably and resulted in 66.7% for RJ-300 IU eCG and 52.4% for RJ-100 IU eCG treated ewes that conceived as compared to those that received only eCG (Table 1). The uterine tone at the time of laparoscopic insemination was

**Table 1.** Reproductive parameters in ewes treated with eCG and RJ-eCG following natural progesterone impregnated sponge removal.

Parameter	Estrus response (%)	Onset of estrus (Mean±SE)	Uterine tone (Mean±SE)	Conception rate (%)
<b>Dose of Natural Progesterone</b>				
30 mg	(22.3) (42/188)	33.5 ± 1.81	1.6 ± 0.15	21/42 (50.0)
60 mg	(30.5) (54/177)	30.1 ± 1.52	1.8 ± 0.13	27/54 (50.0)
<b>Gonadotrophin administration</b>				
RJ +100 eCG	(22.5) (21/93)	37.7 ± 2.55 <sup>a</sup>	1.8 ± 0.21	11/21 (52.4)
RJ +300 eCG	(31.5) (24/76)	31.0 ± 2.28 <sup>b</sup>	1.6 ± 0.19	16/24 (66.7)
300 eCG	(24.1) (21/87)	28.1 ± 2.56 <sup>b</sup>	1.8 ± 0.22	10/21 (47.6)
600 eCG	(27.5) (30/109)	30.6 ± 2.04 <sup>b</sup>	1.7 ± 0.18	12/30 (40.0)
<b>Interactions</b>				
30 mg x RJ+100 eCG	(15.2) (7/46)	41.1 ± 4.16	1.9 ± 0.36	4/7 (57.1)
30 mg x RJ+300 eCG	(26.3) (10/38)	31.2 ± 3.48	1.7 ± 0.29	5/10 (50.0)
60 mg x RJ+100 eCG	(29.8) (14/47)	34.3 ± 2.94	1.8 ± 0.25	7/14 (50.0)
60 mg x RJ+300 eCG	(36.8) (14/38)	30.9 ± 2.94	1.5 ± 0.25	10/14 (71.4)
30 mg x 300 eCG	(21.3) (10/47)	30.0 ± 3.89	1.6 ± 0.33	5/10 (50.0)
30 mg x 600 eCG	(26.3) (15/57)	32.0 ± 2.84	1.4 ± 0.24	7/15 (46.7)
60 mg x 300 eCG	(27.5) (11/40)	26.1 ± 3.32	2.0 ± 0.28	5/11 (45.5)
60 mg x 600 eCG	(28.8) (15/52)	29.1 ± 2.94	2.0 ± 0.25	5/15 (33.3)

Means within the same column with different letters differ significantly,  $P \leq 0.05$ .

found to have a linear (negative) association with conception rates. The regression equation computed indicated that uterine tone was significantly ( $P < 0.01$ ) related to conception rates. There was significant ( $P < 0.01$ ) difference in the conception rate in ewes recorded with uterine tone score 1 (64.7%), 2 (44.4%) and 3 (20%).

## DISCUSSION

The results of this study demonstrate the ability of RJ treatment together with exogenous natural progesterone and low dose of eCG to shorten the time to onset of estrus, and increase conception rates in fat tailed ewes. Royal jelly, a secretion from worker mandibular and hypopharyngeal glands, stimulates honey bee queens to lay eggs. It was underlined that intensive royal jelly feeding drastically increases the queen's egg-laying rates (Winston, 1987). Lin and Winston (1998) also reported that high levels of royal jelly increased ovarian development of worker bees. In the current study, higher conception rates in ewes were achieved with RJ treatment combined with low dose of eCG as compared to low and high doses of eCG alone.

In comparison with previous studies (Kridli et al., 2003; Husein and Kridli, 2002; Husein and Haddad, 2006), the low estrus response in all groups, is believed to be the result of factors related to the breed, time of year and climatic conditions. Chemineau et al. (2007) reported that

in some species (sheep and goat), some reproductive traits including the onset, offset and duration of the breeding season were found to be heritable and that climate has an effect on these traits. The breeding season of domestic sheep is affected by the geographic origin of individual breeds, with those originating from higher latitudes and colder climates having a more restricted season than those originating near the tropics (Santiago-Moreno et al., 2001). The breeds used in our current study are strongly seasonal, while the breeds such as Awassi used by other researcher are less seasonal. This study was also conducted in conditions of higher latitude and colder climate. Even though ovarian response was not checked, the absence of estrus in these ewes could be attributed to factors including silent heats accompanied by inadequate or insufficient estradiol secretion by the ovarian follicles, resulting in incomplete follicular growth and development (Baird and McNeilly, 1981; Quirke et al., 1981).

Husein and Ghazlan (2007) evaluated the effects of RJ and/or equine chorionic gonadotropin (eCG) on the reproductive responses of ewes that were out-of-season to estrus using CIDR-G as the source of exogenous progestagen. In that study, ewes that received 12 equal doses of RJ (total of 4.8 g) showed similar reproductive performance with the ewes treated with RJ- eCG (500 I.U) and eCG alone. The conception rates were reported as 25% for RJ treated and 37.5% for RJ-eCG treated ewes which is lower than our current study results in

which a single 500 mg RJ injection was used. On the other hand, estrus response induced with CIDR use was 82%, which is higher than our results. CIDR-G treatment could be the source of the increased estrus response as compared to the use of natural progesterone impregnated sponges in our study. The interval of onset of estrus was similar with that observed by Husein and Ghozlan (2007). Konho et al. (2005) used intravaginal cream containing 500 mg progesterone to induce estrus in Suffolk ewes in the non-breeding season and obtained 100% estrus rate. They reported the pregnancy rates of ewes inseminated at detected estrus as 25.0% which was lower than our results.

## Conclusion

Estrus induction with vaginal sponges impregnated with natural progesterone and RJ-eCG combined treatment resulted in acceptable conception rates in ewes inseminated laparoscopically. A single injection of 500 mg RJ was found to be an important and simple strategy to increase overall reproductive performance of highly seasonal sheep breeds in the anoestrus season. Additional research needs to be done to examine ovarian activity in RJ-eCG treated ewes.

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