

# A new approach to enhance reproductive performance in sheep using equine chorionic gonadotropin plus royal jelly

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#### **Abstract**

An oestrous synchronization study was conducted on 24 ewes, during the anoestrus season. one dose of eCG administration 300 IU eCG (n = 12) and one doses of 500 mg royal jelly in combination with 300 IU eCG (RJ-eCG, n = 12) were compared regarding synchronization efficiency (oestrous response and time to onset of oestrus) and fertility (pregnancy rate, lambing rate and Multiple birth rate). Behavioral estrus was checked using fertile rams at 6 h intervals for 5 days beginning on d 1. Non-return rates were monitored from 15 day after sponge removal to 20 day with the aid of teaser rams. There were no significant differences in terms of oestrous response due to differences in the dose of eCG and RJ-eCG administration. However, intervals to onset of estrus were shorter (P< 0.05) in RJ-eCG treated (37.16 h) than 300 IU eCG (45.175 h) ewes. First-cycle pregnancy was greater (P< 0.05) in RJ-eCG treated (81.81) than 300 IU eCG (50%) ewes, respectively. Other indicators studied were not different in the groups received 300 IU eCG and RJ-eCG. Results demonstrate that The treatments of RJ- eCG in conjunction with sponge-MAP were more effective in induction of estrus and improvement of fertility.

Keywords: Ewes; Fertility; Progestagen; RJ-eCG; Anoestrus season



## 1. Introduction

One of the most important features of sheep and goat reproduction, is seasonality with alternated periods of anoestrus and sexual activity. In the last decades different reproductive control methods have been developed, allowing significant progress improving of extensive production and is controlling the reproductive process for intensive production. Improving reproductive efficiency through estrus synchronization can be accomplished through hormonal treatments including progestagens, and luteolytic agents as prostaglandin  $F_2\alpha$  (PGF2 $\alpha$ ) (Oliveira et al., 2001). However Use of intravaginal devices impregnated with progesterone without pregnant mare serum gonadotrophin (PMSG) has been previously reported to be ineffective in spring or out of season (Hamra et al., 1989). PMSG is generally administered at the time of progestagens removal during the breeding season (Motlomelo et al.,2002), or 48 h prior to progestagen removal during the non-breeding season (Baril et al.,1993).

Royal jelly (RJ) is a homogeneous substance secreted by the hypopharyngeal glands of worker bees to feed young larvae and the adult queen bee (Krell, 1996). Several studies reported positive effects of RJ on animal reproduction (Husein et al., 1999; Khattab et al., 1989). Treating Awassi ewes with RJ in conjunction with exogenous progesterone has been used to induce estrus and improve the first service conception rate (Husein and Kridli, 2002; Kridli et al., 2003). Husein and Haddad, (2006) reported that RJ exerted its effect in a manner similar to that of a gonadotropin and was as effective as eCG in improving reproductive performance in the ewe. The current study was designed to evaluate the effect of administering royal jelly in combination with low dose of eCG (RJ-eCG) on estrous cycle control, pregnancy rate, and lambing responses in Arabian ewes. Additionally, this study compared the use of RJ-eCG with 300 IU eCG in estrous cycle control.

#### 2. Materials and methods

This study was conducted at the research farm of Ramin Agriculture and Natural Resources University, Ahwaz, Iran. A total of 24 multiparous, 2-5 years old Arabic ewes were used in the present study. Ewes were placed in a single open front barn and fitted with intravaginal flourogestone acetate (MAP, 60 mg, hypra, Spanish) sponges for 12 days and subsequently devices removed in 12 days later. Immediately after the removal of the progestagen sponge, ewes were divided into 2 subgroups according to the dose of eCG (Avda. La selva, 135-17170 Amer (Girona) Spain) and RJeCG. Treatments were: 300 IU eCG and 500 mg RJ plus 300 IU eCG (RJ-eCG). RJ injections were prepared on a daily basis, as described by Gimenez-Diaz et al (2012). Estrus was detected with six fertile Arabian rams (one ram:six ewes). The ewes were checked for signs of estrus after sponge removal. The reproductive variables measured in experimental groups were: Oestrus response (number of ewes showing oestrus/total ewes treated in each group × 100). Time to onset of estrus (h) (when ewes allowed a ram to mount and this was registered as the onset time of estrus), Pregnancy rate (number of pregnant ewes/number of ewes showing oestrus and mated in each group  $\times 100$ ), Lambing rate (number of ewes lambing/number of pregnant ewes in each group ×100) and Multiple birth rates (number of multiple lambing/total lambing in each group ×100). Data were analyzed as a completely randomized design with the GLM procedure of SAS (Statistical Analysis Systems Institute, 1998). Data for estrus responses, pregnancy rate, lambing rate and multiple birth rates were analyzed using chi-square test. Time to onset of estrus, was analyzed using PROC GENMOD. All results are given as mean  $\pm$  SEM. Mean values were compared by the Duncan test. The values of less than 0.05 (P < 0.05) were declared significant.

### 3. Results

## 3.1. Oestrus response

Incidence of oestrus was detected in 10/12 300 IU eCG treated and 11/12 RJ- eCG treated ewes within 5 days and occurrence of estrus did not differ (P> 0.5) significantly among groups (Table 1). Interval from device withdrawal to estrus onset were shorter (P< 0.05) in RJ- eCG treated than 300 IU eCG treated ewes.

Table 1

Effect of one dosage of eCG and RJ-eCG on oestrous response, Intervals to onset of oestrus and ewes rebred in Arabian ewes

	Parameter		
Treatment	oestrous response (%)	Intervals to onset of oestrus (h)	Ewes rebred (%)
300 IU eCG	10/12 (83.33) ns	$45.62 \pm 3.76^{\text{ a}}$	7/12 (58.33) <sup>a</sup>
Rj-eCG	11/12 (91.67) ns	$39.97 \pm 3.06^{b}$	3/12 (25) <sup>b</sup>
Overall	21/24 (87.5)	$42.79 \pm 3.41$	10/24 (41.66)

a.b: Means in the same column with different superscripts differ significantly (p<0.05). ns: Not significant.

## 3.2. Pregnancy, lambing and multiple birth rate

Results for the pregnancy, lambing and multiple birth rate are presented in Table 2. The lambing and multiple birth rates for groups 300 IU eCG and RJ-eCG were 80, 0 and 88.88, 25 respectively. No significant differences in the results for lambing rates and multiple birth rates were noted between the 300 IU eCG and RJ-eCG groups. However, the pregnancy rate was found to be significantly different in the 300 IU eCG and RJ-eCG groups (p < 0.05).

Table 2

Effect of one dosage of eCG and RJ-eCG on Pregnancy, lambing and multiple birth rate in Arabian ewes

Treatment			
	Pregnancy rate (%)	Lambing rate (%)	Multiple birth rate (%)
300 IU eCG	5/10 (50) a	4/5 (80) <sup>ns</sup>	0/4 (0) <sup>ns</sup>
Rj-eCG	9/11 (81.81) <sup>b</sup>	8/9 (88.88) <sup>ns</sup>	2/8 (25) <sup>ns</sup>
	·		
Overall	14/21 (66.66)	12/14 (85.71)	2/12 (16.66)

a.b: Means in the same column with different superscripts differ significantly (p<0.05). ns: Not significant.



#### 4. Discussion

The results of this study demonstrate the ability of RJ treatment together with exogenous natural progesterone and low dose of eCG to improve reproductive responses in Arabian ewes. Results reported herein confirm previous reports, that RJ is capable of producing high level incidence of oestrus, shorter intervals to onset estrus and greater pregnancy and lambing rates than controls (Husein et al., 1999; Husein and Kridli, 2002; Kridli et al., 2003; Mostafa et al., 2008; Gimenez-Diaz et al., 2012).

### 4.1. estrus responses

Estrus responses was numerically greater in RJ plus eCG-treated than in the 300 IU eCG-treated group. Diffrent in occurrence of estrus responses between two groups reflect in actions of Royall Jelly. In fact, RJ has been shown to stimulate ovarian activity and enhanced follicular growth in the ewe due to its rich dietary contents and subsequently increasing oestradiol secretion (Husein and Haddad, 2006). Oestrus responses observed in the groups received RJ plus eCG resembled those previously reported in sheep, utilizing the traditional progesterone-600 IU eCG. Incidence of estruses was similar to that reported in previous studies utilizing 300 IU eCG and long term progesterone treatment (Ungerfeld and Rubianes, 2002; Moradi kor et al, 2012; zonturlu et al, 2008) or 600 IU eCG (Domingues Fdez-Tejerina et al.,1991; Zare Shahneh et al., 2006).

Estrus responses occurred about 6 h earlier in RJ-eCG treated than 300 IU eCG-treated ewes. Intervals to onset of estrus resembled those reported in previous studies in which ewes were treated with progesterone-RJ (Husein and Kridli, 2002; Husein and Haddad, 2006) and progesterone-300 IU eCG (Zonturlu et al., 2008). There were significant difference in time to onset of estrus between Groups recived RJ plus eCG and 300 IU eCG. Present difference among groups in intervals from progesterone device withdrawal to onset of oestrus may be attributed to the rate of follicular development and therefore oestrogen secretion necessary for behavioral oestrus and luteinizing hormone (LH) surge (Evans, 1988). In numerous studies reported that administration of 300IU PMSG and less than was not sufficient to stimulate additional follicular development (Koyuncu et al. 2008; Fallah et al., 2007; Oyedipe et al., 1989; Zonturlu et al., 2011). The significant reduction in interval to onset of estrus as a result of RJ treatment combined with low dose of eCG compared with 300 IU eCG can be attributed to the stimulating effects RJ.

## 4.2. Pregnancy, lambing and the multiple birth rate

In our study, the doses of 300 IU eCG (80 and 50%) compared to 500 mg RJ plus 300 IU of eCG (88.88 and 81.81%) were found to be less effective to induce lambing and pregnancy rate.

Ewes in the RJ-eCG group tended to have greater pregnancy and lambing rate than those in the 300IU eCG group. Similar results were reported in the study by Gimenez-Diaz et al. (2012) in which 71.4 and 45.5% conception rates were obtained in RJ-eCG-treated and 300IU eCG- treated ewes, respectively. The lower pregnancy and lambing rate observed in the 300IU eCG may be attributed to

not sufficient 300IU eCG to stimulate additional follicular development (Zonturlu et al., 2011). In comparison with previous studies the higher estrus response in RJ-eCG group, is believed to be the result of combination RJ with low dose of eCG. RJ worked in a manner similar to that of eCG causing improved follicular growth and development.

Multiple lambing in group with injection dose of RJ-eCG was the highest value between two groups, but there was no difference between 300 IU eCG-treated and RJ-eCG-treated groups. Results of multiple births was in agreement with previous report in which Kurdi Ewes were treated with progesteronee 300,400,500 and 600 IU of PMSG, Multiple birth in group with injection dose of 600 IU (58.9%) was the highest value between groups (Nosrati et al., 2011). Akoz et al (2006) reported the dose of 300 IU to be less effective to induce multiple births. Increase in multiple-birth rates following added RJ to the 300 IU eCG may be due to the action of RJ. Mostafa et al (2008) reported oral RJ administration was appeared to have a good effect in increase multiple births in Ossimi breed.

In conclusion, The practical value of RJ plus eCG treatment upon termination of progesteronee treatment was greater than eCG. However, RJ plus eCG treatment still needs further investigation in terms of appropriate dosage and mechanism of action also greater animal numbers are needed to investigate comparison between different doses of eCG and RJ plus eCG treatments during the nonbreeding season.

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