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ENDEAVORS FOR MAXIMIZING THE REPRODUCTIVE PERFORMANCE OF EWES DURING SUMMARY SEASON

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SUMMARY

Trials were conducted to evaluate the influence of vitamin B2, ascorbic acid and melatonin on the reproductive performance of cross ewes during summer.

A total of 42 healthy mature cross ewes (3/8 Finnish X 5/8 Rahamani) was randomly allocated into 4 groups : the first group (G1) served as control (n=11) without any treatment. The second group G2 (n=10) and third group G3 (n=11) were injected daily with 2 cm (15mg) of vitamin B2 and ascorbic acid intramuscular, respectively. Ewes in the fourth group G4 (n=11) received double subcutaneous injections daily of 0.5mg melatonin at 8 A.M and 16 P.M at the dorsal surface of ear. All treated groups (G2,G3,G4) received 6cm of zinc chloride (1gm) and chromium (2.5mg) per os once per week. All animals were checked for the onset of oestrus two times daily using an intact ram. Fertility parameters including lambing rate, frequency of single, twin, triplet and sex ratio (male / female) were recorded. Blood samples were collected weekly and sera were separated for determination of progesterone. Laparoscopic examinations were carried out pre and post-treatment (3weeks). Follicular development and ovulation rates were observed among different groups. Results indicated that, ascorbic acid treated group had a significantly ($P>0.05$) decreased mean interval from treatment to oestrus as compared to control group (15.73 ± 1.83 Vs. 22.64 ± 2.50, respectively). A remarkable improvement in the lambing rate in vitamin B_2, ascorbic acid and melatonin treated groups than that in the control one (80.00, 90.91, 90.00 Vs. 72.72%, respectively). The highest proportion of ewes bearing single lamb was observed in ascorbic acid group (100.00%). Incidence of lamb

mortality found only in vitamin B2 treated group (triplet) in one ewe. Sex ratio was higher in melatonin group (4.0) than those in control, ascorbic acid and vitamin B_2 treated groups (0.80, 1.00 and 1.75, respectively). The ovulation rate was significantly (P>0.05) elevated in melatonin treated ewes than that in vitamin B_2 and control groups (1.60 ± 0.16 vs. 1.20 ± 0.13 and 1.14 ± 0.00, respectively). The follicular development did not vary among different groups. In conclusion, vitamin B_2, ascorbic acid and melatonin have a beneficial effect on the reproductive performance of ewes during summer.

INTRODUCTION

Seasonal reproductive patterns of sheep are dependent largely on latitude and breed and are influenced to a lesser extent by other factors such as strain, age and nutrition (Hafez, 1952). Ewes are typically anestrus in spring after lambing and lactating and remain so until late august or early september. Seasonal anestrous reduces reproductive efficiency and continues to hinder productivity (Wheaton et al., 1990). The present study was therefore conducted to investigate the effect of different treatments (vitamin B_2, ascorbic acid and melatonin) to stimulate fertility during summer (May-June) under local environmental conditions.

MATERIALS AND METHODS

Experimental animals

The study was carried out on 42 mature cross ewes (3/8 Finnish X 5/8 Rahamani). Ewes aged between 3 to 5 years old and weighting 40-50 kg. The ewes were raised in Sakha Experimental Station, belonging to Animal Production Research Institute. The station is located in the northern part of the Nile Delta (31N'). the experiment started in May 2003 and lasted till June 2003.

Feeding

Animals were fed daily a diet of 750g concentrate feed mixture containing 16% crude protein and 250g barseem hay per head. Animals were housed in semi open yards. All ewes were healthy and clinically free of external and internal parasites. Free vitamins were available to ewes all the time. Three experiments were carried out to induce estrus in ewes during anestrus using different treatments according to experimental design.

Experimental design

Relaying on laparoscopic examination and evaluation of blood progesterone as described by Robinson et al. (1991) and Restall et al. (1995), all animals were confirmed as acyclic (true anestrum). No corpora lutea were found on both ovaries by laparoscopy. Ewes were randomly allocated into four groups: the first group (G_1) served as control (n=11) without any treatment. The second group

G_2 (n=10) and third group G_3 (n=11) were injected daily I/M with 2cm (15mg) of vitamin B_2 and 2cm (15mg) of ascorbic acid dissolved in distilled water, respectively. Ewes in the fourth group G_4 (n=10) received a subcutaneous injection of 0.5 mg melatonin dissolved in 20 ml of a mixture (4:1 v / v) of water and ethanol at 8 A.M and 16 P.M at the dorsal surface of ear. All treated ewes were given one gm zinc chloride and 2.5 mg chromium in form of 6 ml oral drench once per week.

All animals were detected for the onset of oestrus twice daily (30 minute for each) using an intact ram. Ewes came in heat were naturally bred by a proven fertile ram. Fertility measures including the time interval between the treatment to onset of oestrus, lambing rate, number of lambs, sex ratio (male/female) and frequency of single, twin and triplet among different treatments were recorded.

Laparoscopic examination was carried out on pre and post-treatment (about 3 weeks after treatment). Number of corpora lutea on both ovaries and ovulation rate were recorded among different groups as previously reported by Kelly and Allison (1976). Ovarian follicles were counted and grouped according to their diameter into small (>2mm), medium (2-4mm) and large follicles (>4mm).

Hormonal assay

Serum progesterone was measured by enzyme immune-assay using Fertigenix - Prog - Easia (Biosource). This method has been verified by Janssen-Caspers (1986). Antisera for the hormone were highly specific with an extremely low cross reactivity to other steroids. The sensitivity of the assay was 0.20 ng/ml and the intra-and inter-assay CV were 9.70% and 8.80 %, respectively.

Statistical analysis

The data were analyzed using a commercial soft ware Statistica for windows (1993).

RESULTS

Table 1 and figure 1 showed that the incidence of estrous expression among different treatments was statistically non-significant. While, the time elapsed from treatment to 1st estrus was shorter in ascorbic acid treated group than that in the other groups, but the differences were only significant ($P<0.05$) between the ascorbic acid treated group and the control one. There was a remarkable increase in the lambing rate in vitamin B_2, ascorbic acid and melatonin treated groups than in the control one (Table 1).

Table 1: Incidence of estrus (%), onset of estrus (mean ±SEM) and lambing rate (%) among different treatments.

Groups	Number of ewes	Number of ewes showed estrus	Onset of estrus (Days)	Lambed ewes
G_1 (Control)	11	11 (100%)	22.64 a ± 2.50	8 (72.72%)
G_2 (Vitamin B_2)	10	10 (100%)	19.50 ab ± 1.598	(80.0%)
G_3 (Ascorbic acid)	11	11 (100%)	15.73 b ± 1.83	10 (90.91%)
G_4 (Melatonin)	10	10 (100%)	19.80 ab ± 1.41	9 (90.0%)

Values with different superscripts in the same column are significantly different at $P<0.05$.

Table 2 declared that, the mean number of lambs per ewe was not altered significantly among different treatments. The higher percentage of ewes having one lamb was observed in ascorbic acid treated group (100.00%) while the lower one (75.00%) was recorded in vitamin B_2 treated one. The proportion of twinning among different groups (G_1, G_2, and G_4) was not varied. Incidence of lamb mortality (3 out of 11) was found only in a vitamin B_2 treated ewe bearing a triplet (Table 2).

The sex ratio (male/female) was found to be higher in melatonin treated group than in the other groups.

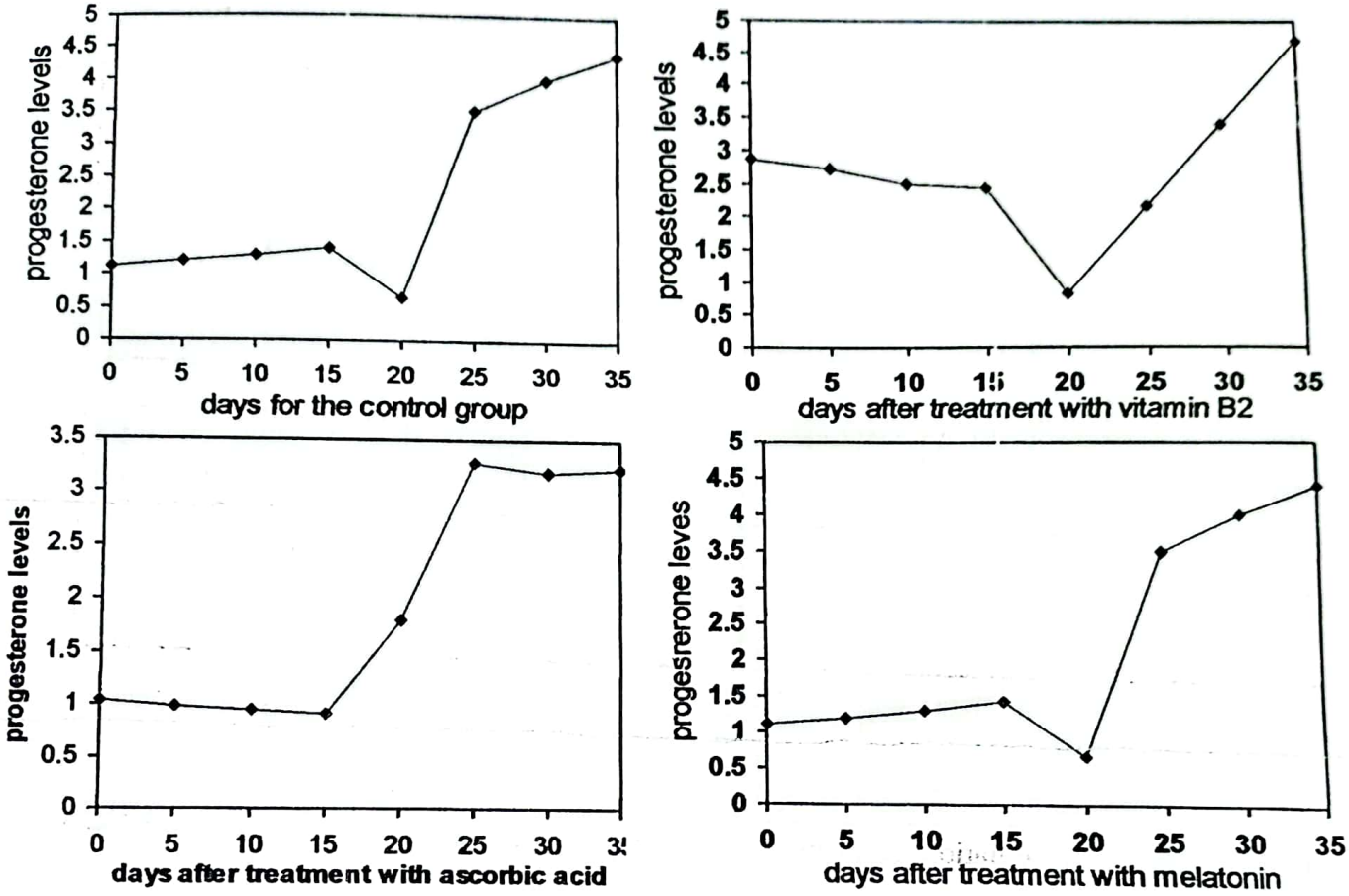

Fig.1: Serum progesterone concentrations (ng/ml) at different intervals post-treatment.

Table 2: Survey of studied ewes among different treatments.

Groups	Total number of lambed ewes	Born lambs				Number of ewes bearing			
		Total number	number of lamb per ewe (mean ± SEM)	Sex		M/F ratio	Single (%)	Twin (%)	Triplet (%)
				Male (M)	Female (F)				
G_1 (Control)	8	9	1.13 ± 0.13	4	5	0.80	7 (87.50)	1 (12.50)	1 (12.50)
G_2 (Vitamin B_2)	8	11	1.38 ± 0.26	7	4	1.75	6 (75.00)	1 (12.50)	1 (12.50)
G_3 (Ascorbic acid)	10	10	1.00 ± 0.00	5	5	1.00	10(100.00)	-------	-------
G_4 (Melatonin)	9	10	1.11 ± 0.11	8	2	4.00	8 (88.89)	1 (11.11)	------

* The born lambs died.

According to laparoscopic examination of ewes after 3 week post-treatment (Table 3), it was observed that, a higher percentage of ovulated ewes was found in G_1, G_2, and G_4 as compared to G_3. On other hand, melatonin treated group has a significant ($P<0.05$) increase in ovulation rate than that in vitamin B2 and control groups (Table 3).

Table 4 showed that the follicular development was equivalent without any significant difference in ewes among different groups after 3 weeks post treatment.

Table 3: Laparoscopy of ovarian activity in ewes three weeks post-treatment.

Groups	Number of examined ewes	Ovulated ewes (%)	Number of corpora lutea			Ovulation rate (mean ± SEM)
			Left ovary	Right ovary	Total	
G_1 (Control)	7	7 (100.00)	4	4	8	$1.14^{a} \pm 0.00$
G_2 (Vitamin B_2)	10	10 (100.00)	5	7	12	$1.20^{a} \pm 0.13$
G_3 (Ascorbic acid)	6	44 (66.67)	5	2	7	$1.75^{ab} \pm 0.25$
G_4 (Melatonin)	10	10 (100.00)	5	11	16	$161.60^{b} \pm 0.16$

Values with different superscript in the same column are significantly different at $P<0.05$.

Table 4: Ovarian follicular development in different groups (mean ± SEM)

Groups	Number of examined ewes	Number of follicles		
		Small (<2mm)	Medium (2-4mm)	Large (>4mm)
G_1 (Control)	7	4.67 ± 1.05	1.33 ± 0.61	0.33 ± 0.21
G_2 (Vitamin B_2)	10	4.71 ± 1.21	1.29 ± 0.36	0.57 ± 0.30
G_3 (Ascorbic acid)	6	2.83 ± 0.98	0.67 ± 0.33	0.50 ± 0.34
G_4 (Melatonin)	10	5.00 ± 2.48	0.50 ± 0.29	0.25 ± 0.25

DISCUSSION

The data obtained in the present work demonstrated that, there was no difference between treatments and control group on the incidence of oestrus. Similar finding was obtained by El-Maghraby (2003) in mature Finn crossed ewes. However, Stellflug et al. (1988) found that, the incidence of oestrus was significantly improved in melatonin treated ewes (75%) compared to control ones (59%). Our finding revealed higher incidence of oestrus (100%) in untreated ewes (control group). In this respect, Aboul-Naga et al (1987) reported that, about 33% of Rahmani ewes showed continuous ovarian activity throughout the year, whilst almost all ewes had periods of behavioral anoestrus.

The mean interval from treatment to the onset of estrus observed herein was significantly shorter for ewes received ascorbic acid than those in control ones (15.73 ± 1.83 Vs. 22.64 ± 2.50 respectively). Ascorbic acid has many biological actions of particular relevance to role as a reducing agent : it is required for the biosynthesis of collagen which is essential for follicular growth, for repair of the ovulated follicle (Himeno et al.,1984), for corpus luteum development (Luck and Zhao, 1993), for biosynthesis of steroid and peptide hormones, and to prevent or reduce the oxidation of biomolecules (Sebrell and Harris, 1967).

Regarding the influence of vitamin B_2, ascorbic acid and melatonin on the reproductive performance of ewes, we observed a pronounced effect of the above mentioned treatments on the lambing rates. These results confirm with those reported in Merino ewes (Haliloglu and Serpek, 2000) who concluded that exogenous vitamin C increases the fertility rate along with body weight of pregnant sheep and newborn. In addition, the reported lambing rate in the melatonin treated group (90.00 %) is close to that recorded (88.89%) by El-Maghraby (2003). Furthermore, Abecia et al. (2002) observed that, melatonin treatment in sheep can increase both fertility and prolificacy by improving luteal function. The beneficial effect of vitamin B_2 on reproductive performance of ewes observed in the present study was described early by Hurley and Doane (1989) who reported that, all B-vitamins are required for the growth and development of fetus.

Ascorbic acid and melatonin treatment had almost no effect on the occurrence of triplet in the present work. This is a desirable outcome in ewes with low fecundity under local conditions where mortality of triplet is very high. This agreed with the opinion of William et al. (1992) in Merino ewes.

As shown in the above results the sex ratio (♂ / ♀) in melatonin treated ewes (4.00) was higher than vitamin B_2 , ascorbic acid and control ones (1.75, 1.00 and 0.80, respectively. In ocn-

trast, Williams et al. (1992) found that there was no significant difference between melatonin and control groups (1.063 Vs. 1.026 respectively).

The ovulation rate was significantly ($P<0.05$) improved in melatonin treated ewes than those treated by vitamin B_2 and control ones (1.60 ± 0.16 Vs. 1.20 ± 0.13 and 1.14 ± 0.00, respectively). These results go in parallel with the findings of Forcada et al. (1995) and El-Maghraby (2003) in sheep during anestrous periods. Melatonin may regulate cyclic activity in ewes by affecting the hypothalamo-hypophesial axis (Arendt et al., 1983). It plays a key role in the transfer of photoperiodic information to the neuro-endocrine axis (David and Helmut, 1992). Follicular activity as indicated by the presence of medium and large sized follicles, was noted in ewes among different groups in the present work without any significant difference. The same finding was reported by Wheaton, et al. (1990) in Suffolk ewes.

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