

**Effect of *Nigella Sativa* seeds, Royal jelly and Pregnant Mare Serum Gonadotrophine on Some Reproductive Traits of Ossimi Ewes**

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**ABSTARCT**

Forty-eight Ossimi ewes aged 2-4 years and averaged  $41 \pm 2.5$  kg body weights were randomly assigned into four equal groups (12/ each). All ewe groups were synchronized for estrous by intra - vaginal progesterone-releasing sponges.

Sponges were inserted and remained in situ for 12 days before removal (as estrous synchronization protocol). Control group not drenched anything. First group (T1) daily drenched orally 100 mg *Nigella Sativa* seeds (NSS) powder/kg/head for 12 days starting at the time of sponge insertion until the time of sponge withdrawal. The second group (T2) drenched orally fresh Royal jelly (RJ) 500 mg per day/for 12 days, starting at time of sponge insertion until sponge withdrawal. Royal jelly (RJ) was kept into natural drug capsules. The third group (T3) was intramuscularly injected at time of sponge withdrawal, with 750 IU of Pregnant Mare Serum gonadotropin (PMSG).

A fertile, Ossimi ram ran with the ewes immediately following sponge removal at (day 0). Ewes observed three times daily to identify and detect estrus for 3 days. Ultrasound scanner apparatus was used to count number of corpus luteum and diagnose of pregnancy. Detection of pregnancy depended on presence of embryos and/or embryonic fluid that filled the uterus.

The results indicated that the response of ewes to estrus synchronization did not differ among the four groups. The response of the four groups was 100%. At the same time treatments led to increase percentage of occurred estrus in the first 24 hr and also the estrus duration increased in the control group. Ovulation rate at  $8 \pm 1$  days post mating as indicated by number of corpus luteum (CLs) recorded ( $p < 0.01$ ) the higher values in T3 followed by T2, T1 and then control group for single or twin pregnant ewes. The percentages of improvement in ovulation rate were 150%, 130% and 105% for T3, T2 and T1, respectively compared to the control group (100%). Regardless of treatments, progesterone levels at  $8 \pm 1$  or at  $34 \pm 1$  days were higher in twinning pregnant ewes compared to single pregnant ones. Also, percentages of conception rate were higher in T2 and T3 (91%), while the lowest value was recorded in control group. Litter size per ewe at birth increased as a result of treatment effect. The highest values recorded in T3 (1.63) flowed by T2 (1.54) and T1 (1.50), while the lowest values were recorded in control group (1.22).

According to the net profit percentages, it could conclude that the best and more beneficial effects are occurred with PMSG. However, from the practical point of view, treatment with *Nigella Sativa* seeds could be preferred and effectively applied as it showed the lowest cost.

Key words: *Nigella Sativa* seeds, Royal jelly, PMSG, reproductive performance, Ossimi Ewes.

**INTRODUCTION**

Improving productive and reproductive performance are the main goals of sheep producers. Increasing lambs production can achieved mainly through increasing the number of lambs per ewe and the number of lambing per year.

There are large numbers of feed additives for inclusion in animal diet to improve performance of ewes. An attempt to use natural materials such as medical plants is widely accepted as feed additives. However, the use of chemical products generally may cause unfavorable side effects. The world health organization (WHO) encourages using medicinal

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herbs and plants to substitute or minimize the global use of chemicals to go back to natural nutrients. There are several investigators illustrated that supplementation of *Nigella Sativa* seeds or oil or cake in the diet of sheep (EI-Ekhnawy *et al.*, 1999), goat (Badawy *et al.*, 2001), dairy cattle (EI-Gaafarawy *et al.*, 2003) and buffaloes (Sanad, 2010) had a beneficial effect on reproductive performances.

Under traditional system of sheep breeding (once/year), treating ewes with Pregnant Mare Serum Gonadotrophin led to enhance ovulation rate which can be used and applied widely among sheep flocks (Ozbey and Tatli, 2002 and Abdel-Mageed, 2006).

Pregnant Mare Serum Gonadotrophin has primarily long acting effect and used successfully for estrus induction and super-ovulation in sheep (Kouyncu *et al.*, 2002; Abdullah *et al.*, 2003; Adel, 2004 and Abdel-Mageed, 2006). Techniques of ovarian enhancement before breeding season may have an impact on farm profitability, which could be measured by farm budget under different production systems.

Several studies had reported positive effects of Royal Jelly (RJ) on the reproductive performance of sheep (Husein *et al.*, 1999; Husein and Kridli, 2002 and Husein and Haddad, 2006). To our knowledge, the exact mode of action of RJ on reproduction is yet not well identified. It may exert its effect through containing hormone-like substance or through altering hormonal secretion in individuals (Kridli and Khetib, 2005).

Therefore, this investigation was carried out as an attempt to evaluate the effect of *Nigella Sativa* seeds, Royal jelly and Pregnant Mare Serum Gonadotrophine on some reproductive performances of Ossimi ewes.

### MATERIALS AND METHODS

#### Experimental design

Forty eight Ossimi ewes aged 2-4 years and averaged  $41 \pm 2.5$  kg body weight were randomly assigned into four equal groups (12/each). Each group separately housed in a semi-roofed yard ( $4 \times 3 \times 3$  meters) and fed on 75%

concentrate mixture (CFM) and wheat straw (25%) to cover the requirement of CP and TDN according to NRC (1985). Fresh water was freely available during all day. All experimental groups were estrous synchronized by receiving intravaginal progesterone-releasing sponges; contained 20 mg fluorogestone acetate. The sponges were intravaginally inserted and remained in situ for 12 days before removal according to the estrous synchronization protocol.

Ewes in the control group were not drenched anything. First group (T1) was orally drenched daily 100 mg/kg body weight *Nigella Sativa* seeds powder (NSS) for 12 days (at the time of sponge insertion to the time of sponge withdrawal). The second group (T2) was orally drenched for 12 days 500 mg per day fresh Royal jelly (RJ), starting at time of sponge insertion to time of sponge withdrawal. Royal jelly was weighed and packaged into natural drug capsules. Third group (T3) was intramuscularly injected with 750 IU of Pregnant mare serum gonadotropin (PMSG), (Folligon) at the time of sponge withdrawal.

A mature Ossimi ram used as teaser to detect estrus and was run with the ewes following sponge removal (day 0). Ewes were examined three times daily for 3 days to identify and detect those in estrus. A fertile Ossimi ram used to breed all ewes showed estrus. Each ewe bred twice at the beginning of estrus and later after 12 hr.

#### Blood samples

Blood samples were collected from the jugular vein in test tubes without anticoagulant at time of estrus (day 0),  $8 \pm 1$  days from mating and lastly at  $34 \pm 1$  days after mating. Blood samples were centrifuged at 1500 xg for 15 min and the serum samples were collected and stored at  $-20\text{ C}^\circ$  until assayed. Serum progesterone concentration (ng/ml) was determined in single assay by RIA (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, U.S.A). Intra-assay CV was 5%.

#### Ovulation rate and pregnancy

A real-time, B-mode linear array ultrasound scanner was used to count number of

corpus luteum and diagnose pregnancy. The scanner was provided with transected transducer with a dual frequency of 6 and 8 MHz. Number of corpus luteum (CLs) existed on the two ovaries of mated ewes was counted at day  $8 \pm 1$  post-mating as an indicator of ovulation rate (OR). Diagnosis of pregnancy was performed at day  $34 \pm 1$  post-mating. Detection of pregnancy depended on the presence of embryos and/or the embryonic fluid that filled uterus. The fluids appear as dark spot, while embryo appears as relatively lighter spot surrounded by the fluids (dark spot).

### Measured traits throughout the experimental period

- 1- Response to estrous synchronization = Number of ewes respond to estrous synchronization / Number of ewes treated with intravaginal sponges at day 12
- 2- Estrus duration (hrs) = The time between the first and last accepted mounting by the ram.
- 3- Ovulation rate (OR) (%) = The number of CLs per ewe counted at day  $8 \pm 1$ .
- 4- Conception rate (CR) (%) = Number of ewes conceived as confirmed by pregnancy diagnosis at day  $34 \pm 1$  / Number of ewes mated
- 5- Litter size at birth /ewe = Number of lambs born / Number of ewes lambled

### Statistical analysis.

The results statistically analyzed using the General Linear Model (**SAS, 1998**) for complete randomized design. Reproductive and blood parameters were performed by methods of analysis of variance. All statements of significant difference based on the 0.05 or 0.01 level of probability. Significant differences among treatments were analyzed using **Duncan (1955)**.

## RESULTS AND DISCUSSIONS

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Gonadotrophine (T3) on reproductive performances of Ossimi ewes presented in Tables (1, 2 and 3).

Data in Table (1) illustrate that, estrus responses of ewes in the four studied groups (control, T1, T2 and T3) showed no significant differences due to estrous synchronization. The response of the four groups was 100%. Also, results indicated that ewes treated with PMSG had the best response (100%) to estrus synchronization during the first 24 hr. followed by RJ treatment (75%) and NSS treatment (66%) then the control group (50%). Also, data in Table (1) indicated that mean percentages of ewes that had estrus duration less than 30 hr. were 58, 83, 83 and 92% and those for ewes had estrus duration of more than 30 hrs. were 42, 17, 17 and 8% for control, T1, T2 and T3, respectively. These results indicate that ewes treated with PMSG had the shortest estrus duration followed by RJ and NSS then the control.

Data in Table (2) showed that ewes treated with PMSG (T3) had the highest number of corpus luteum as indicator to ovulation rate (OR) followed by Royal jelly (T2) and *Nigella Sativa* seeds (T1) compared to control. The improvement percentages in ovulation rate at  $8 \pm 1$  days were 150 %, 130 % and 105 % for T3, T2 and T1, respectively compared to the control (100%).

Also, data in Table (2) illustrate that progesterone concentration at  $8 \pm 1$  or  $34 \pm 1$  days post-mating in ewes had single or twin lambs recorded higher ( $P < 0.01$ ) values with PMSG treatment (T3) followed by RJ treatment (T2) and NSS treatment (T1). At the same time, the lowest values of progesterone concentrations recorded in the control group. Higher values of progesterone concentration at  $8 \pm 1$  or  $34 \pm 1$  days in treated groups compared to control one could be due to increasing ovulation rate (number of corpus luteum at  $8 \pm 1$  days post-mating).

A strong linear relationship was found between blood P4 concentration and the number of corpus luteum (**Amiridis et al., 2002**). P4 starts to increase 3 days after ovulation with gradual increase from day 5th reaching its peak between days 7 and 13 (**Stenbak et al., 2003** and **Abdel-Mageed, 2006**).

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**Table (I): Effect of *Nigella Sativa* seeds (NSS), Royal jelly (RJ) and Pregnant Mare Serum Gonadotrophine (PMSG) on some reproductive characteristics of Ossimi ewes.**

Items	Control	T1 (NSS)	T2 (RJ)	T3 (PMSG)	
Number of ewes treated	12	12	12	12	
Number of ewes showed estrus	12	12	12	12	
Response to estrus synchronization (%)	12/12= 100	12/12= 100	12/12= 100	12/12= 100	
Time of estrus post-treatment *	First 24 hr	6/12=50%	8/12=66%	9/12=75%	12/12= 100 %
	2 <sup>nd</sup> 24 hr	6/12=50%	4/12=34%	3/12=25%	-----
Estrus duration **	0<30 hr	7/12=58%	10/12=83%	10/12=83%	11/12=92%
	0>60 hr	5/12=42%	2/12=17%	2/12=17%	1/12=8%

Control=Estrus synchronization with progesterone sponges.

T1= Estrus synchronization with progesterone sponges + drenching \ 100 mg/kg/B. W /daily N.S.S for 12 day.

T2= Estrus synchronization with progesterone sponges + drenching 500 mg RJ/daily for 12 day.

T3= Estrus synchronization with progesterone sponges + injected BY PMSG intramuscular at sponge remove.

\*Time of estrus (the ewes was observed to stand and mounted by the ram).

\*\* Estrus duration (the time between the first and last accepted mount).

Progesterone concentration at peak was reported to be 2.4 -4.3 ng/ml (Yassen *et al.*, 2000 and Stenbak *et al.*, 2003).

Values of conception rate (percentage) were also, higher in PMSG and RJ treatments (91%) followed by NSS (83%) and the lower values recorded in control group (75%) as shown in Table (2). The high values of conception rate in treated groups compared to the control one resulted to higher number of conceived ewes at 34±1 days.

Results in Table (3) illustrated that twinning rate increased as a result of treatments. The highest value of twins (7) was recorded in PMSG treatment followed by RJ treatment (6), then NSS treatment (5) compared to control group (2). Also, results indicated that mean values for litter size, (number of lambs born out of number of ewes lambed) were, 1.22, 1.50, 1.54 and 1.63 for control, T1, T2 and T3, respectively. Thus, the highest value was found in PMSG followed by RJ and NSS, while the lowest was recorded in control group. The higher values of litter size per ewe at birth with PMSG, RJ and NSS in comparison with

control might due to the higher values of lambs born in the different treated groups as a result of increasing twinning in these groups compared with control one. Generally, from the present result it could conclude that, treatments with PMSG, RJ and NSS during breeding period lead to improve the reproductive performance of ewes.

Improvement of reproductive performance of ewes as result of PMSG injection may due to the positive action of FSH in PMSG on ovarian functions. PMSG has primarily long acting FSH activity and can be successfully used for estrus induction and super ovulation in sheep (Squires, 2003). Romano *et al.*, (1996) reported that, PMSG (4000 IU/head) increased ovulation rate in ewes from 1.9 to 5.2. Also, Gonzalez- Reyna *et al.*, (1999) and Koyuncu *et al.*, (2002) studied the effect of different doses of PMSG (500 and 700 IU) at sponge removal on fertility and prolificacy of Kivircik ewes. They indicated that twinning rate was increased from 21.6 % in control group to 44.8 and 61.5 % in groups treated by 500 and 700 IU PMSG, respectively.op0

**Table (2): Effect of *Nigella Sativa* seeds (NSS), Royal jelly (RJ) and Pregnant Mare Serum Gonadotrophine (PMSG) on some pregnancy characteristic of Ossimi ewes.**

Items		Control	T1 (NSS)	T2 (RJ)	T3 (PMSG)
Number of treated ewes		12	12	12	12
Ovulation rate at 8±1 days	No	20	21	26	30
Post-mating (1)	%	100%	105%	130%	150%
Progesterone level at 8±1 days Post-mating	Single **	2.89 <sup>c</sup> ± 0.06	2.94 <sup>c</sup> ± 0.07	3.49 <sup>b</sup> ± 0.07	4.40 <sup>a</sup> ± 0.08
	Twins **	3.03 <sup>c</sup> ± 0.11	2.94 <sup>c</sup> ± 0.07	3.73 <sup>b</sup> ± 0.06	4.93 <sup>a</sup> ± 0.06
Progesterone level at 34±1 days Post-mating	Single **	5.13 <sup>b</sup> ± 0.05	6.37 <sup>a</sup> ± 0.06	6.35 <sup>a</sup> ± 0.06	6.38 <sup>a</sup> ± 0.08
	Twins **	5.39 <sup>c</sup> ± 0.08	6.60 <sup>b</sup> ± 0.05	6.71 <sup>ab</sup> ± 0.04	6.81 <sup>a</sup> ± 0.04
Conception rate (%) – (2)		9/12= 75%	10/12=83%	11/12=91%	11/12=91%

1) Ovulation rate are the number of corpus luteum at 8±1 day on post- mating.

2) Conception rate (CR) =  $\frac{\text{Number of ewes conceived as confirmed by pregnancy diagnosis at day 34}\pm 1}{\text{Number of ewes mated}} \times 100$

**Table (3): Effect of *Nigella Sativa* seeds (NSS), Royal jelly (RJ) and Pregnant Mare Serum Gonadotrophine (PMSG) on lambing traits of Ossimi ewes.**

Traits		Control	T1 (NSS)	T2 (RJ)	T3 (PMSG)
Number of treated ewes		12	12	12	12
Number of ewes lambled	Single	9	10	11	11
	twins	7 2	5 5	5 6	4 7
Number of lambs born		11	15	17	18
Litter size at birth /ewe		1.22	1.50	1.54	1.63
Twining rate (%)		22%	50%	54%	63 %

In addition, **Abdel-Mageed (2006)** reported that using one dose of PMSG (750 IU) increased ovulation rate from 1.55 in control group to 3.38 in treated group and increased litter size from 1.21 in control group to 1.73 in treated group but it had insignificant effect on conception rate.

The positive effect of Royal jelly on the reproductive performance of ewes was studied by many investigators. **Vittekk (1995)** and **Kridli et al. (2003)** attributed the positive effect of RJ to its ability to stimulate the release of certain liver enzymes that speed up progesterone clearance and facilitate the endogenous production of GnRH,

which improves follicular development. **Husein and Kridli (2002)** treated ewes with a total of 3g Royal jelly given in 12 equal doses of 250 mg/ewes/days starting on the time of sponge insertion and found that the rate of progesterone was greater ( $P < 0.01$ ) in Royal jelly treated ewes than in the control. Furthermore, results indicated that treatment with Royal jelly resulted in greater ( $P < 0.05$ ) incidence of estrus and shorter ( $P < 0.05$ ) intervals to onset of estrus after lambing than control. In another study, **Kridli and Khetib (2005)** studied the effect of different levels of Royal jelly (250, 500 and 750 mg RJ/day) on

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reproductive performance of ewes. They found that lambing rate was higher ( $P < 0.05$ ) in 500 /mg RJ group compared with other levels. Lambing rates from mating of induced estrus were 28.5% , 50% ,100% and 57% in control, 250, 500 and 750 RJ mg daily groups, respectively.

The positive effect of *Nigella Sativa* seeds on reproductive performance may due to presence of high contents of fatty acids specially unsaturated fatty acids in this seeds. **Houghton et al., (1995)** reported that the unsaturated fatty acids constitute 84%, 82% of total acids of *Nigella Sativa* seeds, especially palmitic (12.07) Oleic (23.46%) and linoleic acids (58%). Polyunsaturated fatty acids, especially linoleic supplement increased medium size follicles during folliculogenesis in cattle (**Ryan et al., 1992**). Moreover, linoleic and archidonic acids are considered essential fatty acids that act as a precursor in the biosynthesis of prostaglandin (**Baiomy, 1999**) and it increase circulating gonadotropin normal reproductive function (**Hafez, 1987**). **Youssef et al., (1998)** found a significant decrease in days for uterine involution, number of days open, number of services per conception and calving interval by 32.9%, 41.6%, 49.5 % and 12.4 %, respectively in the *Nigella Sativa* cake treated groups than those in the control group in Egyptian buffaloes.

Also, **EI-Ekhnawy et al., (1999)** found that *Nigella sativa* oil supplementation to Barki ewes caused a significant increase of ewes lambed/exhibit out of those estrus. In addition, **Badawy et al., (2001)** indicated that there was a marked increase in the reproductive characteristics of female goats supplemented with *Niglla Sativa* meal. Moreover, **Sanad (2010)** in buffaloes found that, dietary *Nigella Sativa* seeds supplementation in the ration led to reduce the time needed for fetal membrane withdrawal post calving, date of the first calving and days open.

**Economical evaluation of reproductive characteristics**

Results in Table (4) show the economic evaluation of the reproductive characteristics of Ossimi ewes treated with *Nigella Sativa* seeds, Royal jelly and PMSG. The total outcome of lambs born were higher in all treated groups (T1, T2 and T3) compared to the control. Also, results indicated that the total cost of treatments were higher with PMSG treatment followed by Royal jelly treatment, while the lowest cost was recorded in *Nigella Sativa* seeds treatment. Generally, the best net profit percentage (155%) was obtained when PMSG was used followed by Royal jelly (150%) and *Nigella Sativa* (136%) compared with the control (100%).

**Table (4): Economic evaluation of reproductive characteristics.**

Items	control	T1	T2	T3
Number of ewes	12	12	12	12
Number of lambs born	11	15	17	18
Price/ lamp	400 LE	400 LE	400 LE	400 LE
Total price of lambs (1)	4400 LE	6000 LE	6800 LE	7200 LE
Excess in the cost of treatments (2)	.....	13.82 LE	216.00 LE	324.00 LE
Net price of lambs	4400 LE	5986 LE	6584 LE	6876 LE
*** Net profit (LE)	100 %	136 %	150%	155%

\*Total price of lambs = Number of lambs born x price/ lambs (LE)-Price in 2011.

\*\*\*Net profit (LE) % = 1-2

LE= Egyptian Libra

In conclusion, supplementation with 100 mg/kg/BW/day *Nigella Sativa* seeds or 500 mg/head/day Royal jelly as a natural feed additives, as well as, hormonal treatment

improved conception rate, lambing rate and twinning rate of Ossimi ewes. However, the best value of the net profit percentage as an indicator for economic evaluation for the treatment was measured in the hormonal treatment group compared with other treatments.

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