

EFFECT OF ESTRUS SYNCHRONIZATION PROTOCOLS ON THE REPRODUCTIVE PERFORMANCE OF BARKI SHEEP

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ABSTRACT

The study aimed to determine the influence of estrus synchronization regimes on reproductive performance in Barki ewes. Forty Barki ewes were randomly allocated into 2 equal groups (A & B). Group A were treated with vaginal sponges impregnated with 25 mg medroxy-progesterone acetate (MAP). On the day of sponge's withdrawal the ewes were reassigned according to eCG dose into three subgroups (A1, A2 and A3). Group A1, served as control, without eCG injection, while groups A2 and A3 were injected IM with 300 and 500 IU/eCG, respectively. The ewes of group B have received vaginal sponge containing 50 mg of MAP. At the day of sponge removal, the ewes were subdivided into 3 subgroups B1, B2 and B3 and treated with eCG as subgroups A1, A2 and A3, respectively. Three fertile rams were subjected to run with ewes for heat detection and natural mating. Blood samples were taken from mated ewes on day 17 post mating, for determination the level of progesterone for pregnancy determination. Results revealed that the incidence of estrus response were 74.60 ± 9.71 and 79.37 ± 9.71 for groups A (25 mg MAP) and B (50 mg MAP), respectively. Irrespective to MAP sponge concentrations, the estrus response in ewes injected with 500 IU/eCG (A3B3) was higher ($92.86 \pm 11.58\%$) than group A2B2 ($71.43 \pm 11.58\%$) which treated with 300 IU/eCG and control group A1B1 without eCG ($66.67 \pm 12.51\%$) with no significant differences. The onset of estrus in ewes of group A was significantly shorter ($P < 0.05$) than group B (36.23 ± 3.18 h vs 46.10 ± 3.41 h). Regardless of MAP, the onset of estrus in control ewes (without eCG) was significantly longer (56.02 ± 4.70 h; $P < 0.01$) than ewes treated with 300 IU/eCG, (36.20 ± 3.82 h) and 500 IU/eCG (31.27 ± 3.49 h). The means of estrus duration were similar with no significant effects of MAP and eCG doses. No significant differences in the pregnancy rates between group A and B, whereas, the pregnancy rate was significantly higher in group A3B3 ($92.86 \pm 11.21\%$) than groups A1B1 and A2B2 (50.00 ± 14.24 and $80.00 \pm 12.74\%$, respectively). Neither MAP nor eCG has been a significant effects on the lambing and fecundity rates. In conclusion, Sponges containing 25mg MAP co-treated with 300-500 IU/eCG is suitable protocol for estrus synchronization and improve pregnancy rate in Barki ewes.

Key words: Barki sheep, Estrus synchronization, Reproductive performance

INTRODUCTION

Estrus synchronization is a valuable management tool that has been accomplished for the last decades and it's one of the major steps towards the enhancement reproductive efficiency and productive performance in ewes with various degrees of success (Akoz *et al.*, 2006 and Abecia *et al.*, 2013). The improvement of estrous synchronization in ewes depends on more effective manipulation of the estrus cycle either during the luteal or the follicular phase (Iida *et al.*, 2004; Menchaca and Rubianes, 2004; Zonturlu *et al.*, 2011). Intravaginal devices containing progesterone or sponges impregnated with different types and concentrations of progestagens are the most

commonly and widely applied treatments for estrus synchronization in small ruminants during breeding (Hashemi *et al.*, 2006; Bitaraf *et al.*, 2007; Ustuner *et al.*, 2007 and Moradikor *et al.*, 2012) and non-breeding seasons (Akoz *et al.*, 2006; Amer and Hazzaa, 2009; Moradikor *et al.*, 2012). Although this method appears to be the most practical for sheep reproductive management programs, but the estrus response and fertility rates recorded are highly variable (Martemucci, 1986 and Wildeus, 2000).

The most important factors leading to depression in the fertility rate following estrus synchronization is the dose level of the used progestagens (Moradikor *et al.*, 2013). Impregnated sponges containing 60 mg of medroxy progesterone acetate (MAP) are commercially available and used for estrus synchronization in small ruminants (Simonetti *et al.*, 2000). Regimes and doses of hormonal treatment,

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beside sheep breeds and its geographic origin to a great extent have an effect on the success of synchronize estrus (Whisnant and Inskeep, 1992; Ataman *et al.*, 2006; Dogan and Nur, 2006; Abu Gazal, 2010; Nasser *et al.*, 2012; Najafi *et al.*, 2014; Silva *et al.*, 2015). There are suggestions that optimal fertility, following synchronization can be achieved with lower doses of progestagen, by halving the intravaginal sponges (Faure *et al.*, 1983 and Greyling *et al.*, 1997).

The administration of an adequate dose of eCG at the time of sponge withdrawal stimulates follicular growth and increases ovulation rate and induces a tighter synchrony of ovulation in both anestrous and cycling sheep (Dogan *et al.*, 2005; Dogan and Nur, 2006; Ustuner *et al.*, 2007 and Lamrani *et al.*, 2008). Furthermore, application of suitable dose of eCG as a co-treatment during estrus synchronization in sheep increase estrus response, conception rate, twinning and lambing rates (Boscós *et al.*, 2002; Zare Shahneh *et al.*, 2006; Ozyurtlu *et al.*, 2008). On the other side, the use high dose of eCG induces multiple gestations and increase fetal or lamb mortality (Ataman *et al.*, 2006). Hence, to avoid non-desirable losses and large litter sizes, the dosage level of such gonadotropin has to be adjusted according to breed, season and the physiological status of the ewes (Simonetti *et al.*, 2002 and Nosrati *et al.*, 2011).

The present study is a part of MS Thesis in which estrus synchronization was applied using MAP impregnated sponge with two different concentrations and co-treated with different doses of eCG to improve the reproductive efficiency of Barki ewes under Egyptian condition.

MATERIALS AND METHODS

Location and animal's management

The present study was carried out during spring season at Animal Reproduction Research Institute (ARRI), Giza province (located at latitude of 30°00'29"N, longitude of 31°12'39"E, and altitude of 30 m above sea level). The study was conducted on Barki ewes aging 3-5 years and weighing 35-45 kg. The animals were multiparous, non-pregnant, clinically healthy, free from reproductive disorders and feeds on maintenance ration containing Egyptian clover plus concentrate mixture with 16.6 % crude protein, water and a mineral supplement was available ad-libitum.

Experimental design and treatment schedule

A total 40 Barki ewes were randomly allocated into 2 main groups (A & B) of equal numbers (n=20 for each group). Group A were treated with polyurethane vaginal sponges impregnated with 25 mg medroxy-progesterone acetate; (MAP, DEPO-PROVERA, Pfizer manufacturing, Puurs, Belgium) and left in situ for 14 days. On the day of sponge's withdrawal the

ewes were randomly reassigned according to eCG dose into three subgroups (A1, A2 and A3). Group A1 (n=6), served as control, without eCG injection, while groups A2 and A3 (n=7 ewes for each) were injected intramuscularly (IM) at the time of sponge removal with 300 and 500 IU/eCG (Gonaser, Hipra, Girona, Spain), respectively. Ewes of main group B were received vaginally polyurethane sponges containing 50 mg of MAP for 14 days. On the day of sponge withdraw; the ewes were subdivided into 3 subgroups (B1, B2 and B3). Group B1 (n=6), used as control, without eCG injection, while groups B2 and B3 (n=7 ewes/each group) were injected IM with 300 and 500 IU/eCG, respectively, as groups A2 and A3.

Estrus detection and breeding

The occurrence of estrus was observed 8h after sponge withdrawal for a period of 30 min., three times daily for 5 days, using three fertile rams with highly sexual desire. Rams were introduced and subjected to run with the treated ewes for heat detection and natural mating. Ewes started to have responded to the treatment when they have showing estrus signs during the observation periods and stood to allow the ram to mount, and the end of estrus was defined by the antagonistic behavior of ewes to the rams (Jarquin *et al.*, 2014). The ewes which detected in estrus were mated with fertile ram. The following parameters were recorded:

Estrus response: Number of ewes exhibiting estrus/total ewes in each treatment group x 100 (Yavuzer *et al.*, 2014).

Time to onset estrus (h): It estimated as the elapsed time between the cessation of the treatment (sponge withdrawal) to the first tolerance of rams mounting (Zonturlu *et al.*, 2011).

Duration of estrus (h): It estimated as the time extended from the first positive signs of estrus in which ewes receptive rams for mating to the half time between last positive and first negative signs.

Reproductive performance measuring

Pregnancy diagnosis was performed by determination the level of plasma progesterone of the ewes on day 17 after mating (Shemesh *et al.*, 1979). Additionally, the pregnancy was assured by trans-rectal ultrasonography using a real time B-Mode ultrasound equipped with a stiffened 7.5 MHz linear array trans-rectal probe (Pie Medical LC 100, Netherlands) on day 35-40 following the mating (Yavuzer *et al.*, 2014). On the day of lambing, the number of lambs born per ewe was recorded for each treatment group. The parameters of reproductive efficiency were estimated according to Yavuzer *et al.* (2014) as following:

Pregnancy rate: (pregnant ewes/ewes mated) x 100.

Lambing rate: (lambd ewes/pregnant ewes) x 100.

Fecundity rate: (number of lambs born/number of pregnant ewes) x 100.

Prolificacy rate: (lambs born /lambled ewes) x 100.

Blood sampling and progesterone determination

Blood samples were taken from mated ewes on day 17 post mating, for determination the level of progesterone for pregnancy check. The blood samples (10 mL) were collected via a jugular vein into vacutainers tubes containing EDTA. Blood samples were centrifuged within 30 minutes of collection at 3000 rpm for 15 minutes. Plasma was pipetted into 2 mL Eppendorf tubes using sterilized plastic disposable Pasteur pipettes, and then stored at -20 °C until assayed for progesterone assay.

Progesterone assay: The plasma progesterone (P_4) concentrations were assayed using a commercially available ELISA kit (BIOTECH, Inc. Foster City, USA) according to the instructions provided by the manufacturer.

Statistical Analysis: All data were analyzed using SPSS version 10.0.1, software package statistical analyses. The onset, duration of estrus and hormonal assay were statistically analyzed using analysis of variance (ANOVA), with the GLM-General Linear Model of SPSS and post hoc mean comparisons were performed using Duncan test. Estrus response and reproductive performance were compared between treatment groups using the chi-square test. Results are presented as means \pm SE.

RESULTS

Estrus Response: Generally, 77% of the treated ewes in this study exhibited signs of estrus during the observation period of estrus which extended up to 120h after sponge withdraw. As shown in Table 1, the incidence of estrus response were 74.6 \pm 9.71 and 79.37 \pm 9.71 for groups A and B, respectively. Irrespective to MAP sponge concentrations, the estrus response in ewes group A3B3 was higher (92.86 \pm 11.58) than group A2B2 (71.43 \pm 11.58) and group A1B1 (66.67 \pm 12.51). Neither MAP nor eCG has significant effect on the estrus response (Table 1).

Time to estrus (h): The overall mean of the time extended from sponge withdrawal to onset estrus was 41.16 \pm 2.33 h. As shown in Table 1, the onset of estrus in ewes of group A was significantly shorter ($P<0.05$) than group B (36.23 \pm 3.18 h vs 46.10 \pm 3.41 h). Contrary, the onset of estrus in ewes without eCG, A1B1 (56.02 \pm 4.70 h) was significantly longer ($P<0.01$) than animal groups treated with 300 IU/eCG, A2B2 (36.20 \pm 3.82 h) and 500 IU/eCG, A3B3 (31.27 \pm 3.49 h). This means of both MAP concentrations in the vaginal sponge and eCG doses have a significant effect on the time of onset estrus. Along the same lines and as shown in Figure 1, more

percent of ewes in group A (25 mg MAP) exhibited estrus signs up to 50 h of sponge withdraw than group B (50 mg MAP), (84% vs 63%). Likewise, only 64% of ewes in-group A1B1 (0 IU/eCG; control) exhibited estrus signs up to 50 h of sponge withdrawal, whereas 76% and 80% of ewes in groups A2B2 (300 IU/eCG) and A3B3 (500 IU/eCG), respectively exhibited estrus for the same period (Figure 2).

Duration of estrus: Regardless of MAP sponge concentration and eCG doses, the overall mean of the time extended from first to last signs of estrus was 31.56 \pm 1.30 h. As shown in Tables 1, the means of estrus duration in ewes of group A were slightly longer (33.03 \pm 1.72 h) than group B (30.10 \pm 1.96 h) with no significant differences between values. Similarly, no significant effect of eCG on the estrus duration for ewe groups A1B1, A2B2 and A3B3, and the mean values were 32.13 \pm 2.68, 31.53 \pm 2.25 and 31.04 \pm 1.74 h, respectively (Table 1).

Pregnancy rate: As illustrated in Table 2, although the pregnancy rate was higher in ewes of group A (83.33 \pm 10.55) than group B (65.24 \pm 10.34) but no significant differences between values. On the other side and as illustrated in Table 2, the pregnancy rates of ewes in groups A2B2 (80.00 \pm 12.74) and A3B3 (92.86 \pm 11.21) were significant higher ($P<0.05$) than group A1B1 (50.00 \pm 14.24).

Lambing rate: Away from the effect of MAP and eCG, the overall of the lambing rate was 88.33 \pm 8.14. The lambing rates were 87.78 \pm 11.09 and 88.89 \pm 11.92 for ewe groups A and B, respectively, and were 100.00 \pm 17.87, 73.33 \pm 13.05 and 91.67 \pm 10.32 for groups A1B1, A2B2 and A3B3, respectively. Neither MAP nor eCG has a significant effect on the lambing rate (Table 2).

Fecundity rate: The overall of the fecundity rate was 99.44 \pm 12.74. As shown in Table 2, the fecundity rates were 93.33 \pm 17.36 and 105.56 \pm 18.65 for animal groups A and B, respectively, and were 100.00 \pm 27.97, 90.00 \pm 20.43 and 108.33 \pm 16.15 for groups A1B1, A2B2 and A3B3, respectively. Neither MAP nor eCG has been a significant effect on the fecundity rate.

Prolificacy rate: Aside the effect of MAP and eCG, the overall prolificacy rate was 114.44 \pm 9.14. Neither MAP nor eCG has been a significant effect on the prolificacy rate. Whatever the case, the prolificacy rates were 106.67 \pm 12.25 and 122.22 \pm 13.58 for animal groups A and B, respectively, and were 100.00 \pm 18.86, 125.00 \pm 16.33 and 118.33 \pm 11.42 for groups A1B1, A2B2 and A3B3, respectively (Table 2).

Plasma progesterone (P_4) assay: As shown in Figure 3, concerning to pregnancy diagnosis through measuring plasma progesterone, the mean level of

plasma P₄ on day 17 of mating were significant lower ($P<0.05$) in non-pregnant ewes (0.65 ± 0.10) than ewes pregnant in single (6.56 ± 1.36) and twins (8.00 ± 0.36).

However, no significant difference was detected between single and twins pregnant ewes.

Table 1: Effect of medroxy-progesterone acetate (MAP) concentration in the vaginal sponge and eCG doses on estrous response, time to onset estrus and duration in Barki ewes synchronized estrus.

Factors	n	Estrus Response (%)	Onset estrus (h)	Estrus duration (h)
MAP Concentrations				
25 mg (Group A)	20	74.60 ± 9.71^a	36.23 ± 3.18^b	33.03 ± 1.72^a
50 mg (Group B)	20	79.37 ± 9.71^a	46.10 ± 3.41^a	30.10 ± 1.96^a
eCG Doses				
0 IU (group A1B1)	12	66.67 ± 12.51^a	56.02 ± 4.70^a	32.13 ± 2.68^a
300 IU (group A2B2)	14	71.43 ± 11.58^a	36.20 ± 3.82^b	31.53 ± 2.25^a
500 IU (group A3B3)	14	92.86 ± 11.58^a	31.27 ± 3.49^b	31.04 ± 1.74^a
Overall	40	76.98 ± 6.69	41.16 ± 2.33	31.56 ± 1.30

Means for the same factor in the same column followed by different superscripts are significantly different at $P<0.05$ for MAP factor and at $P<0.01$ for eCG.

Table 2: Effect of medroxy-progesterone acetate (MAP) concentration in the vaginal sponge and eCG doses on rates of pregnancy, lambing, fecundity and prolificacy in Barki ewes synchronized estrus.

Factors	n	Pregnancy rate (%)	Lambing rate (%)	Fecundity rate (%)	Prolificacy rate (%)
MAP Concentration					
25 mg (Group A)	20	83.33 ± 10.55^a	87.78 ± 11.09^a	93.33 ± 17.36^a	106.67 ± 12.25^a
50 mg (Group B)	20	65.24 ± 10.34^a	88.89 ± 11.92^a	105.56 ± 18.65^a	122.22 ± 13.58^a
eCG Doses					
0 IU (group A1B1)	12	50.00 ± 14.24^b	100.00 ± 17.87^a	100.00 ± 27.97^a	100.00 ± 18.86^a
300 IU (group A2B2)	14	80.00 ± 12.74^{ab}	73.33 ± 13.05^a	90.00 ± 20.43^a	125.00 ± 16.33^a
500 IU (group A3B3)	14	92.86 ± 11.21^a	91.67 ± 10.32^a	108.33 ± 16.15^a	118.33 ± 11.42^a
Overall	40	74.29 ± 7.38	88.33 ± 8.14	99.44 ± 12.74	114.44 ± 9.14

Means for the same factor in the same column followed by different superscripts are significantly different ($P<0.05$).

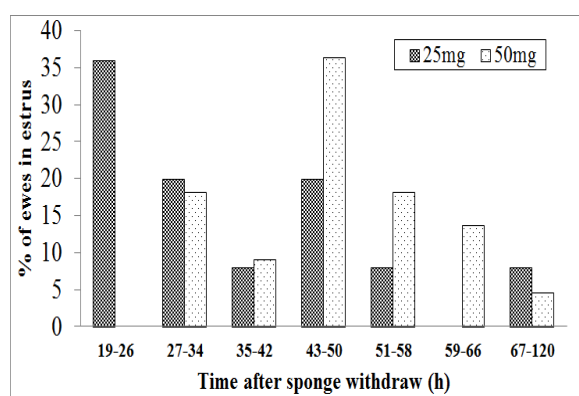


Fig. 1: Effect of medroxy-progesterone acetate (MAP) concentration in the vaginal sponge on the frequency of estrus in Barki ewes synchronized estrus.

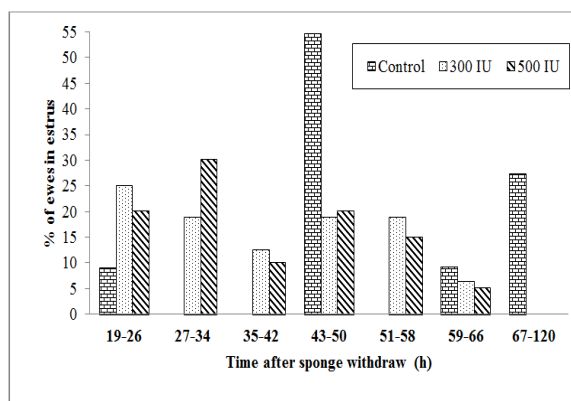


Fig. 2: Effect of eCG doses injected at sponges with draw on estrus frequency in Barki ewes synchronized estrus.

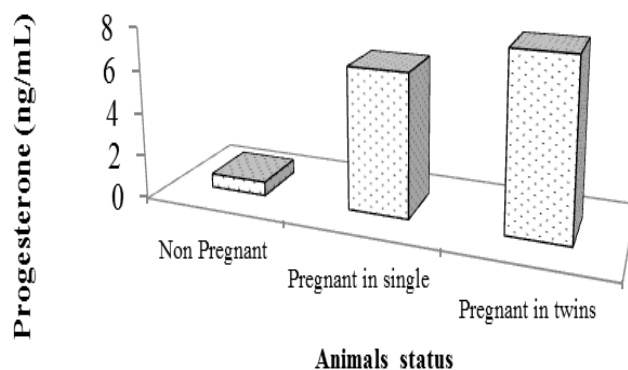


Fig. 3: Plasma progesterone concentration on day 17 after mating of pregnant and non-pregnant ewes.

Mean values above chart column followed by different superscripts are significantly different ($P < 0.05$).

DISCUSSION

Irrespective of MAP and eCG, the overall mean of estrus response was 76.98 ± 6.69 (Figure 1). This technique (MAP+eCG), has an estrous response nearby to 84.5% obtained by Stellflug *et al.* (1994) in Targhee ewes, but higher than 66% obtained with Romney Marsh ewes (Gatica and Correa, 1993), and lower than 96.7% reported in Chios ewes (Tsakalof *et al.*, 1981). Regardless of eCG treatment, the concentration of MAP containing vaginal sponges (25 and 50 mg) in this study have no significant effects on the incidence of estrus response. These results are consistent with Greyling *et al.* (1994) and Ungerfeld (2003) they concluded that, the amount of MAP in intra vaginal sponges could be reduced from 60 to 20 mg without affecting the estrus response. In the same pattern, Simonetti *et al.* (2000) reported that no significant different in estrus rate in ewes treated with sponges impregnated with different doses of MAP 40, 50 and 60 mg. Furthermore, Sareminejad *et al.* (2014) found no significant differences in estrus response in ewes received vaginal sponges impregnated with 60 mg MAP for short (6 days) and long terms (14 days). In addition, the estrus rates were not affected by the type of progestagen treatment and were 95.9% and 93.2% in ewes treated with Chronigest and Eazibreed (CIDR), respectively, (Fleisch *et al.*, 2012).

Irrespective to MAP sponge concentrations in this study, although the estrus response in ewes group injected with 500 IU/eCG was higher than injected with 300IU (92.86 ± 11.58 vs 71.43 ± 11.58) and control (66.67 ± 12.51) but no significant different. Similarly, various researchers used different doses of eCG ranged from 300 to 850IU followed different progestagens treatment and they found no significant differences in estrus response between treatment animal groups (Husein *et al.*, 2007; Kasikci *et al.*,

2011; Nosrati *et al.*, 2011 and Moakhar *et al.*, 2012). Moreover, Barrett *et al.* (2004) indicated no advancement of estrus was noted in eCG-treated ewes compared to controls. Furthermore, the proportions of ewes displaying estrus did not differ significantly between seasons, breeds or eCG doses (Rekik *et al.*, 2002 and de Nicolo *et al.*, 2008). However, 33.33%, 28.57% and 7.14% for ewes control, 300 and 500 IU/eCG not exhibit estrus, respectively, and this may be resulting from inadequate estradiol secretion by ovarian follicles, indicating incomplete follicular growth and development (Baird and McNeilly, 1981).

The onset of estrus in ewes of group A was significantly shorter ($P < 0.05$) than group B (Table 1). Similarly, Simonetti *et al.* (2000) and Kasikci *et al.* (2011) has been found that the time extended to onset estrus in a lower dose of progestagens was earlier than high doses. These reasons may be attributed to the high dose of progestagens leads to increases for progesterone absorbed by female reproductive tract; this increases in the blood progesterone level extended in the interval to onset estrus after sponge with drawal. This interpretation is confirmed by the mean level of plasma progesterone of ewes group B at the day of sponge withdraw was higher than group A (1.61 ± 0.33 vs 1.08 ± 0.20 ng/mL).

In the same concept, as showed in Table 1, the onset estrus time in this study was significantly shorter ($P < 0.01$) in ewes treated with 500 and 300 IU/eCG than control group. However, no significant differences between groups 300 and 500 IU. Similarly, many researcher had reported that the low doses of eCG (300-400IU) either concomitant with or before progesterone removal included in estrus synchronization protocols reduce and accelerate the interval to the onset estrus than non eCG treated ewes (Barrett *et al.*, 2004; Husein *et al.*, 2007 and Amer

and Hazzaa, 2009). In the same line, McMillan (1994) reviewing the interval to the onset of estrus in synchronized ewes during breeding season, ewes synchronized by CIDR with eCG injection at doses of 0, 400 and 800 IU had similar mean intervals to the onset of estrus (33 vs 31 vs 30 h, respectively). The shorter intervals in the eCG treated ewes groups are attributed to the action of exogenous eCG which stimulate the follicular growth by mediating faster pituitary endocrine responses and subsequently increase follicular activity which promotes higher levels of estrogen and improve the occurrence of estrus. This effect is not found in control group (without eCG), so the ewes come into estrus late (Vinoles, 2003; Kridli and Al-Khetib, 2006; Ali, 2007).

The synchronized ewes which treated with low level (25mg) of MAP (group A) exhibited more precise (84%) of estrus behavior between 19 to 50 h of sponge with draw than group B (63%) which treated with 50 mg (Figure 1). Similarly, Abdul Rashid and Jamsuri (2005) cited the distribution pattern of ewes showing estrus signs over the observation period up to 55 h after FGA or CIDR removal were 85% and 70%, respectively. The injection of eCG at the end of the progestagens treatment causes more precise synchronization of estrus and tighter synchrony of ovulation in small ruminants (Ustuner *et al.*, 2007; Quintero-Elisea *et al.*, 2011). Furthermore, Lamrani *et al.* (2008) reported that, the administration of eCG following progestagen withdrawal increasing the ovulation rate and decreases the negative effects of progestagens through the recruitment of new follicles. In our study, the administration of 500 or 300 IU/eCG at the end of the progestagens treatment induces a tighter synchrony of estrus in 80% and 76% of treated ewes, respectively, between 19 to 50 h of sponge withdrawal than ewes without eCG treatment (64%) (Figure 2). Similarly, Zarkawi *et al.* (1999) observed 82% of Awassi ewes showed estrous behavior within 48 h post Progestagens vaginal sponge withdrawal and co-treatment with 600 IU/eCG at the sponge removal. Contrary, Robinson (1988) reported early onset of estrus in eCG-administrated ewes which has a normal distribution, with a range of 24 h, centered on a mean of 33 h. However, the earlier observation of estrous behavior in ewes may be related to the large number of preovulatory follicles at the time of sponge removal (Habibizad *et al.*, 2015). It is worth mentioning that in the present study, estrus was not observed in any ewe before 19h after sponge withdrawal.

The estrus duration means in ewes of groups A (25 mg MAP) and B (50 mg MAP) were slightly similar with no significant differences between values (Table 1). In the same accordance, Fuentes *et al.* (2001), Dogan and Nur (2006); Hashemi *et al.* (2006); Ozyurtlu *et al.* (2008); Ozyurtlu *et al.* (2010);

Kulaksiz *et al.* (2013); Tamer and Al-Hamedawi, (2013); Jarquin *et al.* (2014) they recorded the average duration of estrus were 24 to 36 h, with no significant differences between high and low doses of progestagens or between different progesterone analogues. Furthermore, Nasser *et al.* (2012) recorded that, the durations of estrus were 34.4 and 36.7 h following a short (6 days) and a long (12 days) term CIDR application, respectively, with no significant differences between periods.

Neither MAP nor eCG have an effect on the estrus duration and the overall mean of estrus duration in the herein studied Barki ewes was 31.56 ± 1.30 h (Table 1). This finding is consistent with Elias (1987) who reported that the average duration of estrus in Barki ewes was 27-29 h. However, the duration of estrus in our results is more longer than reported by Ekiz and Ozcan (2006) in Kivircik ewes (18 h); Nasser *et al.* (2012) in Dammar ewes (8.5h); Sareminejad *et al.* (2014) in Arabian ewes (14.77 h) and shorter than that reported by Cavalcanti *et al.* (2012) in Dorper crossbred ewes (36.0 h).

The short duration of estrus may be attributed to lower estrogen level in the blood, on the other hand, Stimulation of follicular growth by indigenous FSH and/or by exogenous eCG together leads to high levels and longer duration of serum estrogen concentrations, could be responsible for a prolonged duration of the estrus period (Nasser *et al.*, 2012). The differences in estrous duration between the present study and those of others could be ascribed to the breed differences, period of progesterone treatment (short or long term), time of eCG administration (before, after or at the time removal), season of the treatment, ewes age, geographical location of the experiment and overall managerial conditions (Zonturlu *et al.*, 2011 and Nasser *et al.*, 2012). However, Hafez (1993) reported that the duration of estrus is dependent and varies slightly from one female to another within the same species and breed.

MAP levels in the vaginal sponge of groups A and B have no significant effect on the pregnancy rates (Table 2). Similarly, Husien and Ababneh (2007); Ozyurtlu *et al.* (2010) and Kasikci *et al.* (2011) reported that there was no significant differences in the pregnancy rates between high or low doses of progesterone when used during estrus synchronization in ewes. Not only, Greyling *et al.* (1994) and Simonetti *et al.* (2000) observed that the absorbed levels of MAP were similar among intra-vaginal sponges impregnated with different doses of MAP and no significant effect on the blood progesterone level during treatment. Moreover, the pregnancy rates following treatment with 30, 40, 50 or 60 mg MAP were no significant differences. Indeed, the low pregnancy rate (65.24%) in our study

is achieved in-group B which treated with sponge containing high amount of MAP (50mg), while the group A which treated with 25 mg MAP recorded higher percentage of pregnancy rate (83.33%). These findings indicate that, the dose of MAP has played somewhat role and can effects the fertility rate. Its seems that, not only progestagens doses containing vaginal sponge effects the reproductive performance in the sheep, but also source of the progestagens and its duration of application can do. Wilson and Maxwell (1989) compared CIDR and FGA sponges in Merino ewes, and found significantly more ewes treated with FGA sponges became pregnant than those treated with CIDR. Moreover, Vinales *et al.* (2001); Ungerfeld and Rubianes (2002); Sareminejad *et al.* (2014) they recorded that, pregnancy rates were significantly higher in short-term group than long-term group. However, the progestagens treatment causes impair sperm transport and reducing the survival number of fertilized ova (Allison and Robinson, 1970; Hawk and Conley, 1972 and Pearce & Robinson, 1985). Such alterations in the quality of ovulated oocyte and sperm viability reduces fertility rate (Simonetti *et al.*, 2000; Ungerfeld and Rubianes, 2002; Zeleke *et al.*, 2005).

The occurrence of fertility rates is influenced by eCG, and as eCG doses increased the pregnancy rate increased (Table 2). Accordingly, low pregnancy rates were recorded in ewes synchronized by vaginal devices containing different doses of progestagens without eCG (Husien and Ababneh, 2007; Quintero-Elisea *et al.*, 2011; Najafi *et al.*, 2014 and Martinez *et al.*, 2015). Regarding to the use of different doses of eCG, herein study showed that, although the pregnancy rates were significant higher ($P<0.05$) in eCG treated ewes than control but no significant differences between eCG treated groups. This finding accordance with Nosrati *et al.* (2011) where they observed no significant difference in pregnancy rates for different eCG doses (300, 400, 500 and 600 IU). However, in this study, there is a trend for improve the pregnancy rate by 30% and 42% for groups administered with 300 and 500 IU/eCG, respectively than control (0eCG). Likewise, the use of 300-600 IU/eCG at sponge withdrawal was reported to increase the fertility rate by more than 35% (Zaiem *et al.*, 1996; Zeleke *et al.*, 2005).

The improvement in the fertility rates by eCG treated ewes probably due to eCG supports follicular growth and increases ovulation rates (Boscós *et al.*, 2002; Dogan and Nur, 2006) with enhance the recruitment of small follicles (Noel *et al.*, 1994) and improve the pregnancy rate (Akoz *et al.*, 2006; Ronquillo *et al.*, 2008). On the other side, the ovulatory follicles from ewes treated with progestagens without eCG have showed deficiencies in estradiol secretion during the preovulatory phase and low capacity to ovulate an

oocyte that is capable of being fertilized, as well as diminished secretion of progesterone by the subsequent corpora luteum (Gonzalez-Bulnes *et al.*, 2005). Contrary, Ali (2007); Nasser *et al.* (2012) and found no significant differences in the pregnancy rate between ewes treated with 300 IU eCG or without eCG. Barrett *et al.* (2004) concluded that, no eCG application or even low doses given at the end of progestagens treatment had a limited effects on the dynamics of ovarian follicular waves and resulted in some hormonal imbalances in ewes.

The overall mean of the lambing rate in Barki ewes used in this study is 88.33% (Table 2). Similar values ranging from 85 to 100% were reported as the normal range of lambing rate for Barki ewes (Ahmed *et al.*, 1992; Abdalla *et al.*, 2014). Regardless of eCG treatment, no significant differences in the lambing rates between ewe groups A and B. Also, our results showed that, no differences in the lambing rates between ewes treated with or without eCG, as well as between ewes treated with different doses (300 or 500 IU) of eCG. These results are in agreement with Abdullah *et al.* (2002); Rekik *et al.* (2002); Aköz *et al.* (2006); Balios (2008); Kasikci *et al.* (2011); Zonturlu *et al.* (2011); Nasser *et al.* (2012) they observed that, the lambing rates ranged from 75% to 100% with no significant differences among ewes groups synchronized by MAP, FGA or CIDR with/without different doses of eCG as co-treatment.

In the current study, irrespective to eCG treatment 12.22% and 11.11% of pregnant ewes in groups A and B, respectively failed to lambing. On the other side, regarding to eCG effects 26.67% and 8.33% of pregnant ewes in groups A2B2 (300 IU/eCG) and A3B3 (500 IU/eCG), respectively showed prenatal mortality. However, all pregnant ewes in control group (A1B1, 0 eCG) success to lambing. Similarly, Lunstra and Christenson (1981) mentioned that, the synchronization of ewes with progestogen followed by eCG were associated with high embryonic mortality (29%) than did untreated ewes (15%).

The variation between the obtained results and which mentioned by the other researchers can be attributed to early embryonic loss which can be due to problems with the embryo itself (Hasler *et al.*, 1983), the uterine environment or interactions between the embryo and the uterus (Almeida *et al.*, 1984). The maternal environment may be inadequate for the support a normal pregnancy as a result of an inappropriate hormone pattern (Wilmot *et al.*, 1986). Progesterone and estrogen determine the proper function of the uterus in preparation for embryo development and implantation (Bindon, 1971; Miller and Moore, 1976; Miller *et al.*, 1977). Embryonic mortality occurred among synchronized ewes was associated with increased variation in stage of embryo

development within ewe and advanced stage of embryo development, which indicated that asynchronies of timing of onset of estrus, ovulation and fertilization (Lunstra and Christenson, 1981). In addition, complete embryonic or fetal losses that occur in sheep throughout the gestation period were associated with inadequate luteal function and subsequently low concentrations of progesterone in maternal serum (Dixon *et al.*, 2007).

The current results revealed, neither MAP concentrations nor eCG doses has been a significant effect on the fecundity and prolificacy rates (Table 2). Similarly, Kasikci *et al.* (2011) observed that the fecundity rates were similar in ewes treated with 10 and 20 mg FGA (152.9 vs 152.5). Moreover, Rodriguez -Iglesias *et al.* (1997) concluded, no differences in the ovulation rate in Corriedale ewes synchronized by intravaginal sponges containing 15, 30, 45, or 60 mg of MAP and authors suggest that MAP doses of 25% of the commercial formulation (60 mg) is still be sufficient to induce estrus in this breed. Also our results are consistent with Rekik *et al.* (2002); Husein *et al.* (2007); Martemucci and D'Alessandro (2010 and 2011); Quintero-Elisea *et al.* (2011); Nasser *et al.* (2012) they found that the fecundity and/or prolificacy rates were similar in ewes treated with different doses of eCG followed progestagens removal sponges with no significant differences. Contrary, Akoz *et al.* (2006); Anilkumar *et al.* (2010); Kasikci *et al.* (2011) they concluded as eCG doses increased a significant improvement in the litter size, fecundity rate and ovulation rates were observed.

The twinning rates in Barki ewes used in our study is consider lower than those recorded in other breeds injected with the same doses of eCG, this difference may be related to breed differences. The eCG doses (300 and 500 IU) used in this study may be insufficient to increase the multiple birth rates for Barki ewes. Similarly, Karagiannidis *et al.* (2001); Akoz *et al.* (2006); Koyuncu *et al.* (2008); Zonturlu *et al.* (2011) have been suggested the response to eCG doses are different and dependent among various breeds, hence, the doses must be adapted to breeds, season, age and physiological status of the animals. However, further studies with greater doses than those used in this study are needed to investigate the comparison between treatments of eCG with higher doses in Barki ewes.

In the current study, the progesterone level on day 17 after mating was measured as a guide for early pregnancy diagnosis. Similarly, Yotov (2007) and Ganaie *et al.* (2009) showed that measuring the progesterone levels on days 18-20 after mating is a reliable indicator of the success of fertilization in sheep. As shown in Figure 3, the mean values of

plasma progesterone on day 17 of mating was significant lower ($P < 0.05$) in non-pregnant ewes (0.65 ng/mL) than ewes pregnant in single (6.56 ng/mL) and twins (8.00 ng/mL). These differences in blood progesterone level among pregnant and non-pregnant ewes due to the functional role of CL, which is responsible for the major source of progesterone soon after fertilization and during the following days of early pregnancy (Mukasa-Mugerwa and Viviane, 1992). This result is consistent with Husein and Kridi (2002); Ganaie *et al.* (2009); Marco-Jimenez *et al.* (2014) they suggested, the progesterone values remained above 4 ng/mL post 14-19 days of mating in pregnant animals, while it declined to less than 1.0 ng/mL in cases of unsuccessful fertilization.

The plasma progesterone concentration on day 17 of pregnancy tended to be higher in ewes carrying twin fetuses than single with no significant differences (Figure 3). Similarly, Boscos *et al.* (2003) did not distinguish any significant variations in the blood progesterone in sheep carrying one or more fetuses. However, Mukasa-Mugerwa and Viviani (1992) and Yotov (2007) cited the level of progesterone in sheep carrying two fetuses was higher compared to carrying one, and there is a positive correlation between the stage of pregnancy and the concentrations of blood progesterone as an extra ovarian source of progesterone (placental synthesis) was appeared.

CONCLUSION

The present study shows that the application of sponges impregnated with 25mg MAP for 14 days and co-treatment with 300-500 IU/eCG at the day of sponge withdraw is more suitable protocols for estrus synchronization of Barki ewes under Egyptian conditions whereas resulted in a high estrus response within 50h after sponge withdraw and increase the pregnancy rate.

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تأثير النظم المختلفة للتزامن الشبقي على الكفاءة التناسلية في الأغنام البرقي

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استهدف البحث دراسة تأثير النظم المختلفة للتزامن الشبقي على الكفاءة التناسلية للأغنام البرقي ، وأجريت الدراسة على ٤٠ نعجة برقي تم تقسيمها الى مجموعتين رئيسيتين (أ ، ب)، المجموعة الأولى (أ) تمت معاملتها بالاسفنجات المهبليّة المشبعة بتركيز ٢٥ ملجم من هرمون ميديروكسي بروجسترون استيات، بينما المجموعة الرئيسية الثانية (ب) تمت معاملتها بالاسفنجات المهبليّة المشبعة بتركيز ٥٠ ملجم، عند سحب الاسفنجات المهبليّة تم حقن الهرمون الحاث على نمو حويصلات المبيض والمستخلص من مصل الأفراس العشار (eCG) في العضل وتبعاً لجرعة الهرمون تم تقسيم نعاك كل مجموعة رئيسية الى ثلاث مجموعات فرعية ١ ، ٢ ، ٣ الأولى مجموعة ضابطة (لم يتم حقنها) والثانية تم حقنها بـ ٣٠٠ وحدة دولية والثالثة بـ ٥٠٠ وحدة دولية. تمت مراقبة النعاك لمعرفة بداية الشياح ومدته من وقت سحب الاسفنجية وتم تلقيح النعاك الشائعة وكذلك تم قياس مستوى هرمون البروجسترون في الدم بعد ١٧ يوم من التلقيح لمعرفة النعاك العشار ثم تأكيد العشار بكشف السونار بعد مرور ٤٠ يوم من التلقيح، وعند الولادة تم تسجيل البيانات الخاصة بعدد الطليان المولودة. وقد أوضحت النتائج أن معدلات الاستجابة للشياح كانت ٧٤.٦% و ٧٩.٣٧% للمجموعتين الأساسيتين أ ، ب على التوالي بدون وجود فروق معنوية بين المجموعتين، وللمجموعات الفرعية أ١ب ، أ٢ب٢ ، أ٣ب٣ بصرف النظر عن جرعة البروجسترون في الاسفنجات كانت ٦٦.٦٧% ، ٧١.٤٣% ، ٩٢.٨٦% على التوالي ولم توجد اختلافات معنوية بين النسب الثلاثة. الفترة الفاصلة بين سحب الاسفنجات وبداية الشياح كانت أطول بزيادة معنوية في المجموعة الرئيسية أ عن المجموعة ب (٤٦.١ مقابل ٣٦.٢٣ ساعة)، وبغض النظر عن جرعة البروجسترون في الاسفنجات كان الوقت في المجموعة الضابطة (أ١ب١) أطول بزيادة معنوية عن المجموعتين (أ٢ب٢) و (أ٣ب٣)، وكانت مدته ٥٦.٠٢ ، ٣٦.٢ ، ٣١.٢٧ ساعة للمجموعات الثلاثة على التوالي. مدة الشياح أظهرت النتائج أن مدة الشياح لجميع المعاملات لم تتأثر معنوياً بجرعة البروجسترون في الاسفنجات المهبليّة ولا بجرعة هرمون الأفراس العشار (eCG). معدلات الحمل للمجموعتين أ ، ب الرئيسيتين لم تتأثر معنوياً بجرعة البروجسترون في الاسفنجات المهبليّة ولكنها تأثرت معنوياً بجرعة هرمون الأفراس العشار (eCG)، حيث كانت نسبة العشار أعلى في المجموعة الفرعية أ٣ب٣ (٩٢.٨٦%) عن المجموعتين أ١ب١ (٥٠%) ، أ٢ب٢ (٨٠%). معدل الولادات وحجم البطن لجميع المعاملات لم تتأثر معنوياً بجرعة البروجسترون في الاسفنجات المهبليّة ولا بجرعة هرمون الأفراس العشار (eCG). مستوى هرمون البروجسترون لبلازما الدم في اليوم ١٧ بعد التلقيح كان منخفضاً بدرجة كبيرة للنعاك الغير عشار مقارنة بالنعاك العشار في جنين واحد أو توأم ولم تظهر النتائج وجود فروق معنوية بين النعاك العشار في حنين واحد أو العشار في توأم.