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Appraisal of different protocols for estrus synchronization in local Rahmani sheep

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ABSTRACT

This study was carried out to compare efficacy of using 4 different protocols on estrus synchronization and reproductive performance of Rahmani ewes during mating season (May, 2015). Seventy-five ewes aged 2.5- 3.0 years and weighed 47.42± 1.35 kg were used in this experiment. Animals divided into 5 equal groups (15 each). Ewes of the first group (G1) served as a control. Group two (G2) was exposed to vasectomized ram one week before start of mating season (ram effect). CIDR device containing 0.3g progesterone was inserted for 12 days into ewes' vagina of group 3 (G3), then ewes were injected with 500 IU PMSG at time of CIDR withdrawal. Meanwhile, intravaginal sponges impregnated with 60 mg medroxyprogesterone acetate (MAP) were inserted for 14 days in ewes of group four (G4) and then injected with 500 IU of PMSG at time of sponges withdrawal. Group five (G5) received double injections of GnRH, the first one (1 ml) on the first day (0 day) and the second injection (1.5 ml) on day 11th, in addition to single injection of 125 µg $PGF_{2\alpha}$ on day 9 prior to the second injection of GnRH (GPG). All ewes groups were naturally mated on time of standing estrus or at 72- 80 hour after treatment administration in case of ewes failed to show estrus.

Results showed that percentages of estrus exhibition in hormonal treated groups G3, G4 and G5 were significantly higher (80, 86.67 and 93.33%, respectively) than those in G1 and G2 (60 and 66.67%, respectively). Moreover, G5 (GPG) showed the highest percent of estrus comparing to G4 (sponges) and G3 (CIDR) groups. Estrus duration for G3, G4 and G5 were significantly longer (43.20±15.92, 45.60±11.00 and 40.00±16.80 hours, respectively) than those of G1 and G2 (24.00±13.14 and 31.20±13.99 hours, respectively). The mean intervals from treatment to the onset of estrus were significantly shorter in hormonal treated ewes of G3, G4 and G5 (3.60±0.87, 2.20±0.49, and 5.40±0.60 days, respectively) than those in G2 and G1 (12.80±2.63 and 17.20±1.65 days, respectively). Moreover, G5 (GPG) and G3 (CIDR) groups showed significant higher percent of non- return to estrus (85.71 and 83.33%, respectively) than that observed in ram effect (G2) and control (G1) groups (70 and 66.67%, respectively). The time to conception was significantly shorter in G3, G4 and G5 than the control group (G1).

The number of large follicles of total ovaries was significantly low in ram effect group (G2) compared to those in G3, G4 and G5, being the highest in G5. Moreover, hormonal treated ewes in groups G3, G4 and G5 showed significantly higher total CLs numbers (1.00±0.32, 1.40±0.24 and 1.67±0.21/ ewe, respectively) than in ram effect and control groups (G2 and G1) (0.75±0.25 and 0.60±0.24/ewe, respectively), where it was the highest in G5 (GPG group).

Progesterone levels, recorded before treatments, were significantly lower in hormonal treated groups G3, G4 and G5 (0.87±0.20, 0.77±0.70 and 0.80±0.59 ng/mL, respectively) than in G1 and G2 (9.19±0.15and 3.86±1.07 ng/mL, respectively). Their levels decreased in all experimental groups reaching minimal values at the onset of estrus (< 0.5 ng/ml). On day 30 post mating, G4 and G5 showed significantly the highest progesterone concentration comparing to other groups.

The pregnancy rate and number of lamb born/ ewe lambed were significantly the highest in G5 (80 and 127%, respectively) while the lowest in G1 (46.67 and 53.33%, respectively). In addition, percentage of ewes lambed twins was higher in ewes treated with GPG protocol (G5, 58.33%) followed by those treated with 500 IU PMSG after MAP (G4) and CIDR (G3) withdrawal (50 and 40 %, respectively).

In conclusion, GnRH– $PGF_{2\alpha}$– GnRH (GPG) protocol found to be more effective for estrus synchronization than CIDR and MAP+ PMSG. Treatment of Rhamani ewes during summer season with GPG protocol increased estrus and ovarian activities, as well as, pregnancy, lambing and multiple birth rates.

Key words: GnRH– $PGF_{2\alpha}$- intravaginal devises -estrus synchronization– lambing rate

INTRODUCTION

Induction of estrus or estrus synchronization is a valuable management tool for increasing pregnancy rate of ewes. Successful estrus synchronization programs have key role on lambing rate efficiency and profitability of sheep holders undersemi-intensive production systems (**Knights *et al.*, 2001).** In ewes, synchronization of estrus focuses on the manipulation of estrus cycle (**Zonturlu*et al.*, 2011**) through extending the cycle with exogenous progesterone or its analog progestagen or reducing length of luteal phase of estrus cycle with prostaglandin (F2α) (**Jainudeen et al., 2000)**.

Several hormonal protocols used for induction of estrus and ovulation during early and late postpartum **(Gordon 1996)**. Ovarian response of sheep to estrus synchronization varies according to the type of intravaginal device, kind of progestagen, nutritional status, stress, environmental aspects, male effect **(Kleemannand Walker, 2005)** and breed **(Boscos et al., 2002)**.

Intravaginal sponges containing 60 mg medroxyprogesterone acetate (MAP) or 40 mg fluoroprogesterone acetate (FGA) and controlled internal drug release (CIDR) device impregnated with 300 mg progesterone are the most commonly applied treatments for estrus synchronization for small ruminants during breeding and non-breeding seasons (**Ungerfeld and Rubianes, 2002**).

Sponges impregnated with progesterone provide estrus synchronization by exerting negative feedback on Luteinizing Hormone (LH) secretion that inhibit the endocrine events and lead to the maturation of preovulatory follicles and ovulation **(Wildeus, 1999 and Whitley and Jackson, 2004)**.

Moreover, sponges are used together with PMSG injection at time of sponge withdrawal or 48 h prior to sponge removal particularly during out of season (**Jainudeen *et al.*, 2000).** It reported that PMSG can increase pregnancy and twinning rates in breeds characterized by low litter size (**Boscos *et al.*, 2002)**. However, there are many factors influencing the effect of PMSG, including the dose and administration time of PMSG (**Timurkan and Yildiz**, **2005**) and season (**Zeleke *et al.*, 2005)**. Combination of PMSG and progesterone impregnated intravaginal devices causes readily estrus synchronization **(Romano, 2004**), reduces the interval between the onset of estrus and ovulation (**Dogan and Nur, 2006**). Moreover, one dose of PMSG can stimulate follicular development and increase ovulation rate in ewes (**Koyuncu and Ozis Alticekic, 2010**).

The Controlled Internal Drug Release (CIDR) is an alternative device to progestogen sponges for estrus synchronization in ruminants. The usage of CIDR provides advantages compared with the sponges such as elimination of foul-smelling mucus discharged upon removal of sponges, lower loss rates, increase estrus percentage, earlier exhibited estrus and more compact estrus **(Rhodes and Nathanielsz, 1988**). The effectiveness of CIDR in estrus synchronization can increased by co-treatment with hormones **(Oliviera *et al.*, 2001**). Estrus synchronization protocols using CIDR vary from insertion of CIDR for 5 to 16 days with hormone co –treatment, using 100 to 500 IU of eCG or PMSG and/or 0.05mg $PGF2_{\alpha}$ (**Whitley and Jackson, 2004 and Hashemi *et al.*, 2006**).

Moreover, treatments with a combinations of GnRH plus $PGF2_{\alpha}$ have been used to control ovarian follicular, luteal function and increase the precision of estrus and ovulation synchronization in reproductive management programs (**BO'*et al.*, 2003**). The major reason for using GnRH is induction of follicular growth and ovulation for improving fertility and reproduction (**Britt *et al.*, 1977**). An injection of GnRH analogues 6 days prior to injection of PGF2α, enhances conception rate (**Stevenson *et al.* 1996**), increases number of synchronized animals and reduces variability of time to estrus (**Twagiramungu *et al.* 1995).** This decrease may explained by the initiation of a new follicular wave following injection of GnRH, resulting in a new dominant follicle, being present at the time of $PGF_{2\alpha}$ injection (**Pursley *et al.* 1998**).

Moreover, ram effect is a useful and suitable tool for out of season estrus induction, because its cost is negligible, so it has become widely included in reproductive management (**Martin *et al.*, 1986**). Ram effect leads to a rise of LH concentration in females within a few

minutes and increases LH pulse frequency, which is a prerequisite for this method can improve the efficacy of estrus synchronization or induction as well. In this respect, **Jordan (2005)** reported that introduction of rams to ewes induced ovulation.This effect allows induction of breeding during anestrus and produces some synchrony of cycle among ewes in the flock **(Chanvallon *et al*, 2008**).

The objective of this study was to investigate efficacy of different synchronization protocols on estrus and ovarian activities, as well as pregnancy, lambing and twining rates of Rahmani ewes during summer season.

MATERIALS AND METHODS

Animals and their management

Seventy five Rahmani ewes aged 2.5- 3 years and weighed 47.42±1.35 kg during the period from mid-May to mid-October (2015) were used in this study. The animals raised at El-Gemmaza Experimental Station, Animal Production Research Institute, Ministry of Agriculture, Egypt.

All ewes were healthy and clinically free of external and internal parasites. Animals housed in semi open pens under natural day light condition and fed according to **NRC (2007)**. Animals fed 0.50 kg concentrate mixture, 0.60 kg rice straw and berseem hay. Water and mineral blocks were available all time.

Animal groups and treatment protocols

Ewes used in the present study did not show signs of heat or conceived during mating seasons (September– December). Ewes randomly assigned to five equal groups (15 each) as shown in figure 1. The first group (G1) served as a control, while, ewes in G2 exposed to vasectomized ram one week before start of mating season (May). Group 3, treated with CIDR device containing 0.3g progesterone (Eazi-breed CIDR; PFizer New- Zealand) which inserted intravaginal for a period of 12 days then animals injected with 500 IU PMSG at time of CIDR withdrawal (Fig.2). Ewes of group four (G4), treated with sponges containing 60 mg medroxyprogesterone acetate (MAP) which inserted intravaginal for 14 days then injected with 500 IU PMSG at the time of sponges withdrawal (Fig.2). The fifth group (G5) received two injections of GnRH, 1 ml on the first day (day 0) and 1.5 ml on day 11th, as well as, a single injection of 125 µg $PGF_{2\alpha}$was given on day 9 prior to the second injection of GnRH (Fig.2).

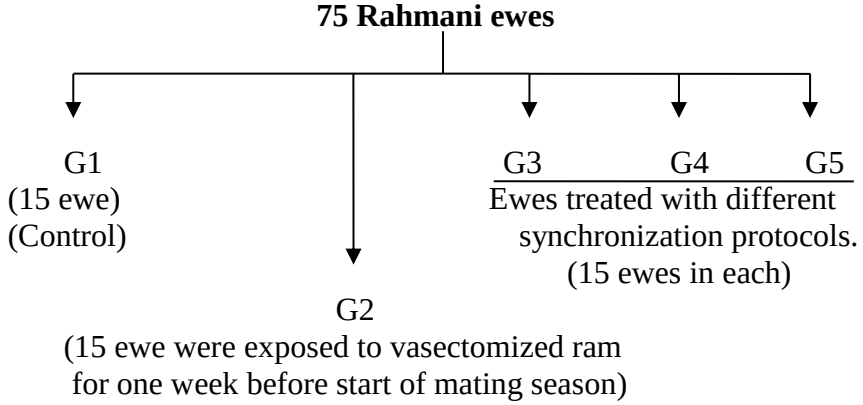

Figure 1: Diagram showing ewe groups used in the experiment.

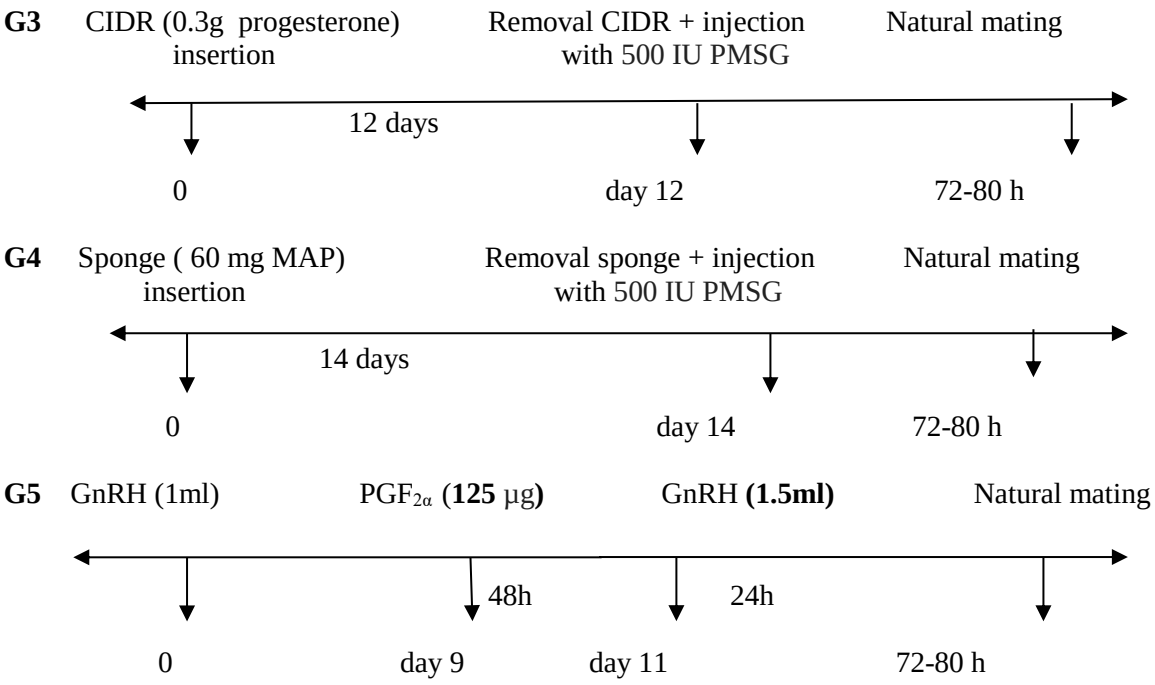

Fig (2): Timeline for administration of different hormonal protocols to experimental ewe groups.

Estrus detection

All ewe groups were observed for behavioral estrus manifestation after the end of the treatments, three times a day. One ram versus 15 ewes was made to ensure adequate detection of heat. Ewes used as a control and ewes exposed to ram effect were naturally mated with mature ram (mating period lasted for 45 and 35 days, respectively). Treated ewes showed estrus behaviors within 72-80 h after the end of the treatment were classified as synchronized and naturally mated.

Blood samples and hormonal analysis

Five ewes, randomly selected from each group, used for blood samples collection through jugular vein puncture into heparinized vacationer tubes (5 ml).Samples withdrawn before treatments, during treatment, at the onset of estrus and post-mating (day 18 and 30). Blood samples centrifuged at 4000 rpm for 15 minutes and plasma were harvested and stored at – 20^{o}C until progesterone assay.

Plasma progesterone concentrations were determined using a radioimmunoassay kit (Beckman Coulter- Czech Republic, catalog No. IM1188). The assay based on competition reaction with sensitivity 0.03 ng/ml and coefficient of variation below or equal to 8.15 and 8.66% for the intra- and inter- assay, respectively (**Meizger, 1992**).

Ultrasonic examinations

Digital ultrasound diagnostic system (Model DP-30 Vet; Germany, 7.5 MHz) used for the examination of ovarian structure changes 7 days post mating. Five fastened (16 hours before examination) ewes of each group were examined for follicles and corpora lutea (CLs) counts. Scans from both ovaries were recorded on high resolution video tapes using a video cassette recorder (Model Fuji S-VSH, ST-120 N). The number, diameter and relative position of all follicles (≥2 mm in diameter) and corpora lutea (CL) were recorded and sketched on ovarian charts to analyze the pattern of growth or/and atresia. The mean diameter was taken when a follicle or CL was not spherical. Follicles were classified into three classes according to its size [small (diameter= 2 mm), medium (diameter 2-4 mm) and large (diameter ≥4 mm)].

Estrus activity

The following measurements were calculated for all ewe groups:

- Estrus response (%): The number of ewes showed standing estrus/ total

number of ewes in each treatment groupX 100.

- Onset of estrus: days interval from end of treatment to the time when ewes expressed standing estrus (heat).
- Estrus duration (heat duration): time (hours) between the first and last accepted mount.
- Non- return to estrus.
- Time to conception: time of non-return to estrus and confirmed at lambing.

Reproductive traits

The following measurements were calculated for all ewe groups:

- Pregnancy rate: Number of ewes lambed/ number of ewes mated X100.
- Lambing rate: Number of ewes lambed/ number of pregnant ewes in each groupX100.
- Litter size: Number of total lambs/ number of lambing ewes in each group.
- Frequency of single and twins and litter weight at birth.

Statistical analysis

All data were analyzed using analysis of variance procedure (**SPSS, 2012)** by applying the following fixed model:-

$Yij=\mu + Ti + eij$

Where:Yij = observations, μ = Overall means, Ti = Effect of treatments and eij = Standard error.

Duncan's Multiple Range Test **(Duncan, 1955)** was utilized to determine significant differences among means.

RESULTS AND DISCUSSION

Estrus activity

Estrus response, duration of estrus, onset of estrus, non-return to estrus and time of conception for Rahmani ewes in all experimental groups are illustrated in Table (1).

Results showed that percentages of ewes exhibited estrus were significantly ($P<0.05$) higher in hormonal treated groups G3, G4 and G5 (80, 86.67 and 93.33%, respectively) than in G1 and G2 (60.00 and 66.67%, respectively). Meanwhile, no significant differences were detected among hormonal treated groups. Ewes in G5 (GPG) showed the highest percentage of estrus (93.33%). Similarly, **Jobst *et al*. (2000) and Abu El-Ella (2007)** observed that administration of GnRH 9 days prior to $PGF_{2\alpha}$ improved the rate and precision of synchronization of subsequent estrus. In other studies, estrus response found to be 93.3 % (**Ataman and AkÖz, 2006**), 83.3 % (**Abu El-Ella 2007**)

Table (1): Estrus activity of treated and control Rahmani ewes during summer season.

Items	Different protocols				
	G1 (Control)	G2 (Ram effect)	G3 (CIDR)	G4 (MAP)	G5 (GPG)
Number of ewes	15	15	15	15	15
Estrus response number,%	9 (60 %)^{b}	10 (66.67%)^{b}	12 (80%)^{a}	13 (86.67%)^{a}	14 (93.33 %)^{a}
Duration of estrus (h)	24.00^{b}± 13.14	31.20^{b}± 13.99	43.20^{a}± 15.92	45.60^{a}± 11.00	40.00^{a}± 16.80
Onset of estrus (d)	17.20^{a}± 1.65	12.80^{b}± 2.63	3.60^{c}± 0.87	2.20^{c}± 0.49	5.40^{c}± 0.60
Non-return to estrus	6 (66.67%)^{b}	7 (70.00%)^{b}	10 (83.33%)^{a}	10 (76.92)^{ab}	12 (85.71)^{a}
Time to conception (d)	17.83 ^{a}± 1.19	15.67 ^{ab}± 3.01	10.50 ^{c}± 6.51	9.00 ^{c}± 3.79	14.17 $^{b\,c}$± 1.76

$^{a, b \text{ and } c}$,values in the same row with different superscripts are significantly different ($P<0.05$).

And 90.9**% (Beck *et al*., 1996)** after using GnRH-$PGF_{2\alpha}$ protocol, while it was 86.6% (**Ataman and Aköz, 2006; Fritzgerald*et al*. 1985**) and 100% (**Öztürkler*et al*. 2003**) after double injection of $PGF_{2\alpha}$ (9 days interval). **Essam et al. (2016)** reported that injection of $PGF_{2\alpha}$ 5 days after the 1st injection of GnRH failed to induce estrus in Rhamani Egyptian ewes during non- breeding season. Moreover, the higher percentage of estrus observed in G4 (86.67%) than those in G3 (80%), may be due to some losses of CIDR device during treatment time comparing to sponges **(Bitaraf *et***

***al*., 2007 and Hozhabri *et al*., 2007)**. However, the differences between the estrus response recorded in our results and othersmay due to the many factors affect fertility, synchronized estrus and ovulation (**Omontese *et al*., 2010)**.

Heat duration (estrus period) was classified to short (less than 25 hours), normal (25-40 hours) and long (more than 40 hours) according to **Deghady (2000)**. In the present study, estrus durations were significantly longer (P< 0.05) in hormonal treated ewes of G3, G4 and G5 (43.20, 45.60 and 40.00 hours, respectively) than in G1 and G2 (24.00 and 31.20 hours, respectively). Moreover, ewes treated with MAP (G4) showed the longest estrus duration (45.60 hour), while the shortest was in the control group (24.00 hour, table 1). Results of the present study agree with that recorded by **Ola and Egbunike (2005)** and **Omontese *et al*. (2010).** While, **Hashemi *et al*. (2006)** reported shorter duration of estrus (22.11 hours) for ewes treated with MAP with 500 IUeCG out of breeding season.

However, the longer heat duration detected in G3 and G4 (intravaginal devise with 500 IU PMSG protocol) might be due to development of more ova, as the increase in numbers of developed follicles lead to a higher level of plasma estrogen,which may cause the longest duration. **Ptaszynska (2001) and Yildiz et al. (2004)** suggested that a prolonged estrus presumably resulted from elevated concentrations of circulating estrogen (produced by enlarged follicles) would ensure subsequent LH peak to take place, which might increase the chance of a higher rate of ovulation and thus successful fertilization. **El-Shamaa *et al*., (2003**) also, mentioned thatin Romanov crossbred ewes the variation between treatments with regard to duration of estrus might due to the amount of estrogen in the blood produced by induced luteolysis. The rise of estrogen level in blood bring the animal into estrus and has a depressing effect on progesterone levels. Moreover, **Nasser *et al*. (2012)** attributed the longer duration to higher estrogen level in the blood, breed differences, age and geographical location. As, stimulation of follicular growth induce in the ovary by follicle-stimulating hormone (FSH) or exogenous PMSG together with high levels and longer duration of serum estrogen concentrations could be responsible ofa prolonged duration of estrus period**.**

Table (1), showed that the mean intervals from treatment to the onset of estrus (time of estrus) were significantly shorter inhormonal treated ewes in G3, G4 and G5 (3.60± 0.87, 2.20± 0.49 and 5.40± 0.60 days, respectively) than that in G1 and G2 (and 17.20± 1.65 and12.80± 2.63 days, respectively). Moreover, ewes in the control group (G1) had significantly longer onset of estrus (17.2± 1.65) comparing to other groups (Table 1). Although, no significant differences were detected among hormonal treated groups (G3, G4 and G5), Rahmani ewes in G3 (CIDR) and G4 (MAP) showed shorter onset of estrus than GPG group (G5). **Das *et al.* (2000); Simonetti *et al*. (2000) and Vinoles *et al*. (2001)** reported that the onset of estrus occurred within 24–144 hours following progestagen or progesterone withdrawal. In addition, **Dogan and Nur 2006)** recorded that ewes came on heat between 18 and 96 hour after sponge withdrawal, with the highest incidence of estrus occurring between 30 and 60 hours. These differences may be explained by variation in breed, lactation, nutrition, season, use of gonadotropins and presence of male after progestagen removal **(Romano, 2002 and Omontese *et al*., 2010).** In the present study, the use of CIDR and MAP with PMSG shorten the intervals to onset of estrus, which may attributed to the action of PMSG on follicular growth by mediating faster pituitary endocrine responses and estradiol secretion. These results are in agreement with **Amar and Hazzaa (2009) and Abdalla et al. (2014)** who reported that PMSG reduce the intervals from sponge withdrawal to estrus and improve the efficiency of synchronization of estrus and ovulation in sheepduring the breeding season.On the other hand, **Nandy *et al*. (1990)** observed that the time to onset of estrus and duration of estrus were not affected by the treatment of PMSG/ hCG gonadotropin.

The percentage of ewes that did not return to estrus after mating was significantly (P<0.05) higher in G5 (85.71%) and G3 (83.33%) than in G2 (70.00%) and untreated ewes (66.67%) (Table1). A similar trend was obtained also by **El-Shamaa *et al*., (2003)** and **Abu El-Ella (2006).** The sign of non-return to estrus, due to

pregnancy, is not physically different from anestrous at the end of the breeding season **(El-Shamaa *et al.*, 2003);** therefore pregnancy diagnosis based on non-return to estrus is not reliable in sheep and goats due to the seasonality of estrus behavior (**Sallam, 1999).**

In the present study, time to conception after treatment was significantly shorter in groups G3 and G4 than other groups (Table 1). Using CIDR and sponges in combine with PMSG shorten the time to conception (10.5±6.51 and 9.00±3.79 days, respectively). **Godfrey *et al*. (2003**) and **Abu El-Ella (2006)** reported that hormonal treatment had no significant effect on time to conception.

Ovarian activity

Ultrasonic examinations on day 7th after mating in term of the number of follicles and Cls on the ovaries of ewes in different experimental groups are shown in table (2).No significant differences were detected between the numbers of small and medium follicles among different groups, however, group 5 (GPG) showed the highest numbers (6.33± 0.42 and 2.83± 0.31, respectively). In agreement, **Samartzi et al. (1995) and Ali (2007**) reported that gonadotropins administration had no effect on the number of small and medium follicles.Moreover, results showed that ewes exposed to ram effect (G2) had significantly (P< 0.05) lower number of large follicles(0.75± 0.25) than hormonal treated groups (G3, G4 and G5). Ewes treated with GPG protocol (G5) showed the highest number of large follicle (1.67± 0.21) comparing to G1, G3

Table (2): Number of follicles and corpora lutea (CLs) on ovaries of treated and control Rahmani ewes during summer season.

Items	Different protocols				
	G1 (Control)	G2 (Ram effect)	G3 (CIDR)	G4 (MAP)	G5 (GPG)
Small follicle (2 m.m)	5.20 [a] ± 0.58	5.50 [a] ± 0.64	5.60 [a] ± 0.51	5.80 [a]± 0.20	6.33 [a]± 0.42
Medium follicle (2-4 m.m)	2.20 [a]±0.21	2.00 [a] ±0.41	2.00 [a]± 0.32	2.20 [a]± 0.20	2.83 [a] ± 0.31
Large follicle (4 m.m)	1.00 [a b]± 0.45	0.75 [b]± 0.25	1.20 [a]± 0.20	1.20 [a]± 0.20	1.67 [a]± 0.21
CLs	0.60 [b]± 0.24	0.75 [b]± 0.25	1.00 [a]± 0.32	1.40 [a] ± 0.24	1.67 [a]± 0.21

[a and b],values in the same row with different superscripts are significantly different (P<0.05).

and G4 (1.00± 0.45, 1.20 ± 0.20 and 1.20 ± 0.20, respectively) with no significant differences detected among the treated groups. The increase in the follicular numbers in GPG and intravaginal devisesgroups was as reported by **Ashmawy (2011)** in Rahmani ewes**,** who suggested that **it** may associate with the presence of 2-4 follicles on the right ovary, as the right ovary may respond better to PMSG treatment than the left **(Moakhar *et al.*, 2010)**. Gonadotropin such as GnRH and PMSG stimulate the number of follicles, which lead to more follicular development **(Dogan and Nur, 2006** and **Sirjani *et al.*, 2011).** Using GnRH based protocols during the non- breeding season aimed at providing a source of P4 for inducing ovulation or luteinization of follicles (**Ashmawy, 2003**). Moreover, PMSG may enhance the entry rate of small and medium follicles into larger follicles and it may prevent the occurrence of natural follicular atresia (**Mandiki *et al*.2000).**

The total numbers of CLs counted from both ovaries of ewes was significantly (P<0.05) higher in hormonal treated ewes (G3, G4, G5) comparing to ewes in G1 and G2 (Table 2), being the highest in ewes treated with GPG protocol (G5, 1.67± 0.21). **EL-Gohary (2006) and Ashmawy (2011)** recorded that total number of CLs for Rahmani ewes ranged 1.1- 1.4 and 1.17 - 1.50, respectively, which are in line with our findings**. Twagiramungu *et al*. (1995)** reported thatthe mode of action for application of GnRH is to reset the follicular wave cycle leading to selection of dominant follicle 1 to 2 days after GnRH treatment. The variation in response might related to the degradation of large follicles and subsequent formation of accessory CL following luteinization (**Sallam *et al.*, 2004**). However, the effects of GnRH on the corpus

luteum present at the time of treatment are equivocal (**Macmillan and Thatcher, 1991**).

Progesterone profile

Mean values of plasma progesterone (P4) concentrations of the five experimental groups are shown in Table (3). Progesterone level was significantly (P<0.05) the highest in G1 (9.19 ng/mL) and the lowest in G3, G4 and G5 (0.87, 0.77 and 0.80 ng/mL, respectively) at pre-treatment period. Progesterone levels detected in ewes of G1 (9.19 ng/mL) and G2 (3.86 ng/ml) indicate that most ewes in those groups were in the luteal phase, while those in G3, G4 and G5 were in the follicular phase. Moreover, during treatment progesterone level increased significantly (P<0.05) in ewes treated with sponges G4 (MAP, 5.89 ng/ml) comparing to G1, G2 and G3 (2.05, 2.75 and 2.58 ng/mL, respectively). Also, ewes in GPG group (G5) showed non-significant higher progesterone level (3.97 ng/ml) than those in G1, G2 and G3. Such trend may indicate higher response of post progestagen device insertion (MAP) in ewes of G4and post-1st GnRH injection of

Table3: Plasma progesterone concentration (ng/ml) in treated and control Rahmani ewes during summer season.

Items	Different protocols				
Ewes groups	G1 (Control)	G2 (Ram effect)	G3 (CIDR)	G4 (MAP)	G5 (GPG)
P4 level prior treatment	$9.19^{a}\pm0.15$	$3.86^{b}\pm1.07$	$0.87^{c}\pm0.20$	$0.77^{c}\pm0.70$	$0.80^{c}\pm0.59$
P4 level during treatment (post insert device or post 1st GnRH)	$2.05^{b}\pm0.82$	$2.75^{b}\pm0.41$	$2.58^{b}\pm0.81$	$5.89^{a}\pm2.89$	$3.97^{ab}\pm1.23$
Onset of estrus (post-removal or post- $PGF_{2\alpha}$)	$0.23^{a}\pm0.08$	$0.32^{a}\pm0.04$	$0.41^{a}\pm0.02$	$0.38^{a}\pm0.03$	$0.37^{a}\pm0.03$
Afterinjection (PMSG or post- 2nd GnRH)	$2.68^{b}\pm1.14$	$2.05^{b}\pm0.82$	$2.70^{ab}\pm1.12$	$4.58^{a}\pm0.86$	$4.83^{a}\pm1.34$
18 days postmating	$1.34^{c}\pm0.81$	$1.76^{bc}\pm0.18$	$2.83^{abc}\pm0.81$	$3.93^{a}\pm0.79$	$3.49^{ab}\pm0.63$
30dayspost mating	$2.37^{b}\pm0.48$	$2.07^{b}\pm0.63$	$4.33^{ab}\pm0.46$	$4.99^{a}\pm0.91$	$5.15^{a}\pm0.85$
Averages	2.98±0.75	2.13±0.52	2.29±0.57	3.42±1.03	3.10±0.78

a, b and c,values in the same row with different superscripts are significantly different (P<0.05).

ewes in G5, being in the luteal phase and reflecting nearly synchronization of the reproductive status of ewes in those groups. Similar trend observed by **Beck *et al.* (1996)** and **Ashmawy (2011)**, who reported that treatment with GnRH resulted in higher plasma P4 concentration. Moreover, at the onset of estrus (after removal of intrvaginal device (G3, G4), post $PGF_{2\alpha}$ injection (G5) and effect of other treatment) P_4 concentrations decreased to the minimal values in ewesof all experimental groups (<0.5 ng/ml) with no significant differences detected. Meanwhile, after injection (post PMSG injection (G3 and G4) and post 2nd GnRH injection (G5) and effect of other treatments) P_4 levels showed againa significant (P<0.05) increase in all treatment groups. Ewes treated with MAP and GPG (G4 and G5) showed significantly (P<0.05) higher progesterone level (4.58 and 4.83, respectively) than other groups. The elevation in P4 level in all ewes was associated with the initiation of new CLs. The higher progesterone levels observed on ewes of groups treated with progestagen devices+ injection of GPG comparing togroups of ram effect and control. This may due to that injection with gonadotropin hormones induces the release of both LH and FSH, which causes maturation of the ovarian follicles and ovulation. Similar results were recorded by **Barkawi and Abul-Ela (1987)** and **(Örsan*et al.*, 2007)** in cattle and sheep, respectively. The domestic species show two stages of ovarian antral follicle development **(Mihm and Bleach, 2003),** first, a slow growth phase, which believed to be independent of gonadotropins (**Cahill, 1981; Lussier*et al.***

1987) and the second, is a fast growth phase that requires gonadotropin support, which described as a follicle wave **(Sunderland *et al*. 1994)**.

Mean values of progesterone levels started to decrease during post mating (day 18) in all experimental groups (Table 3). Ewes in the control and ram effect group had significantly (P< 0.05) the lower P4 level (1.34± 0.81 and 1.76± 0.18 ng/ml, respectively), while it was the highest in ewes in MAP group (3.93± 0.79 ng/ml). **EL-Gohary (2006)** observed that progesterone concentrations were associated with the number of corpora lutea counted on the ovaries of ewes after mating**.** Thirty day post mating, P_4 level increased in all experimental groups to above 1ng/mL, as result of pregnancy incidence. Ewes in G4 (treated with MAP+ 500 IU PMSG) and G5 (treated with GPG protocol) showed significantly (P<0.05) higher progesterone level (4.99± 0.91 and 5.15± 0.85 ng/ml, respectively) comparing to other groups. Moreover, P4 of ewes in group G3 treated with CIDR + 500 IU PMSG (4.33± 0.46 ng/ml) was higher than that in the control (2.37± 0.48 ng/ml) and the ram effect group (2.07± 0.63 ng/ml). The increase of progesterone levels at day 30 of pregnancyin ewe groups treated with PMSG or GnRH was similar to the previous findings of **Ghader *et al*, (2014).**

In the present study, plasma P_4 concentrations were different among pregnant ewes in the different experimental groups, but were almost at the normal P_4 profile observed by **Wallace *et al*. (1992).** Corpora lutea (CLs) secrete P_4 later with respect to the LH surge and at a lower rate than CLs formed after subsequent ovulations (**Schirar *et al*. 1989).** In sheep, this rise takes the form of an increase of frequency of the pulsatile LH discharges to hourly **(Barid, 1978)**, thus producing a progressive four to five fold increase in mean serum LH concentrations which persists for 2-3 days **(Karsch and Foster, 1981).** Progesterone is essential for pregnancy maintenance and one of the important functions of the blastocyst is to ensure that uterine luteolytic mechanism is counteracted. Progesterone and estrogen determine the proper function of the uterus in preparation for embryo development and implantation. Therefore, increasing P_4 level during early pregnancy reduces embryonic losses and increases pregnancy rate and fertility **(Ataman *et al*., 2013).**

Reproductive performance

Results in table (4) revealed that pregnancy and lambing rates were significantly (P> 0.05) the highest (80 and 127%, respectively) for ewes treated with GPG protocol (G5) and the lowest for those in the control group (G1) (46.67 and 53.33%, respectively). Moreover, ewes treated with the progestagen devices plus an injection of 500 IU PMSG showed more pregnancy and lambing rates (66.67 and 93.33%, respectively) for group (G3) and (66.67 and 100%, respectively) for group (G4) than that in ram effect (G2) and control groups (60, 80% and 46.67%, 53.33%, respectively).

The highest pregnancy rate (80%) recorded in GPG group (G5) was associated with the highest percent of estrus (93.33%), while the increase in lambing rate may reflect higher ovulation rate.

Table (4): Reproductive performance of treated and control Rahmani ewes during summer season.

Items	Different protocols				
Ewes groups	G1 (Control)	G2 (Ram effect)	G3 (CIDR)	G4 (MAP)	G5 (GPG)
Pregnancy rate (%)	46.67 ^{b}± 0.13	60.00 ^{ab}± 0.13	66.67 ^{ab}± 0.13	66.67 ^{ab}± 0.13	80.00 ^{a}± 0.11
Lambing rate (%)	53.33^{c}± 0.17	80.00 $^{b\,c}$± 0.20	93.33^{ab}± 0.21	100.00^{ab}± 0.22	127.00^{a}± 0.21
No. of lamb born/ ewe lambed	01.14^{b}± 0.14	01.33 ^{ab}± 0.17	1.40 ^{a}± 0.16	1.50 ^{a}	1.58 ^{a}± 0.15
Ewes lambing single (%)	6 (85.71)	6 (66.67)	6 (60)	5 (50)	5 (41.67)
Ewes lambing twins (%)	1 (14.86)	3 (33.33)	4 (40)	5 (50)	7 (58.33)
Litter weight at birth (kg)	3.53 ^{a} ± 0.54	4.36 ^{a}± 0.55	4.71 ^{a}± 0.58	4.45 ^{a}± 0.77	4.37 ^{a}± 0.74

$^{a, b \text{ and } c}$values in the same row with different superscripts are significantly different(P<0.05).

Monika (2001) reported that hormones such as GnRH, PMSG, FSH, and LH might use to increase pregnancy rate and numbers of lambs. **Boscos *et al*. (2002)** observed that use of PMSG

after progestagens devices treatment, increases ovarian response, conception rate and percentage of multiple births from the induced ovulations. Injection of 500 IU PMSG following the treatment of ewes in the breeding season with vaginal sponges containing 30-40 mg of FGA resulted in 90% and 85% estrus and conception rates, respectively **(Miljkovic *et al*., 1989).** Moreover, administration of GnRH prior mating increased yield of fertilized ova from ewes and initiated a new wave of follicular development (**Walker *et al*., 1989 and Őrsan *et al*., 2007)**. Improving of pregnancy rate following GnRH treatment has attributed to the prevention of ovulation failure or reducing variation in the interval to ovulation **(Coulson *et al*., 1980 and Nakao *et al*., 1984)**. In addition, the treatment led to release of LH surge resulting on ovulation or luteinization of ovarian follicles or alternatively prolongation of luteal function **(Beck *et al*., 1996 and Őrsan *et al*., 2007)**. **Scaramuzzi *et al*. (1988)** and **Ibrahim (1993)** reported that effect of gonadotropin in enhancing fertility is probably a direct consequence of its action in increasing ovulation rate. Also, an injection of GnRH analogues 9 d prior to injection of PGF2α enhanced the conception and synchronization rate. This result agrees with that reported by **Mihm *et al*., (1999) and Ashmawy (2011). Abu El-Ella (2007) and Ataman and Akőz (2006)** recorded lambing rate to be116.67 and 85.3%, respectively in ewes treated with GnRH- $PGF_{2\alpha}$ protocol. Different values of lambing rates recorded after using different hormonal protocols. **Zarkawi *et al*. (1999) and Zeleke *et al*. (2005)** reported lambing rates 80 and 94.6% for Awassi ewes treated with 600 IU PMSG after 60 mg dose of MAP and sponges with 300 IU PMSG, respectively during out of breeding season. Moreover, **Al-Merestani *et al*. (1999)** recorded lambing rates 78 % in ewes treated with intravaginal sponges combined with 400 IU of PMSG. **Hozhabri *et al*. (2007)** reported lambing rates 63.60, 54.50 and 54.50 % for ewes treated with 300, 450 and 600 IU PMSG, respectivelyafter CIDR withdrawal. **Zarkawi *et al*., (1999) and Ustuner *et al*., (2007)** reported that PMSG administration improved the efficiency of synchronization of estrus and ovulation during and outside the breeding season. However, differences in pregnancy rate can ascribed to differences in mating systems, breed, age, body condition, season, duration of treatment and overall managerial conditions **(Safdarian *et al*. 2006).**

In the present study, the embryonic losses were higher in the control ewes than that in the treated groups. **Mohammed *et al*. (2000) and El-Shamaa *et al*. (2003)** reported that PGF2α injection followed by GnRH (48hr) produced high incidence of conception and low early embryonic losses. This may because ovine and bovine conspectuses secrete protein, prostaglandin and steroids, which together with ovarian steroid modify uterine biochemistry and morphology, which may lead to embryo mortality **(Ashworth, 1992)**. **Dowing (1980)** suggested that luteal inadequacy is of factors lead to early embryonic losses. Embryonic losses in Rahmani ewes ranged from 25 to 40%, which normally occur during early pregnancy period in domestic animals, or it may also contributed to the treatment protocol **(Jainudeen and Hafez, 2000)**.

Number of lambs born per ewe lambed was significantly (P> 0.05) higher in treated ewe groups G3 (1.40), G4 (1.50) and G5 (1.58) than in the control group (1.14), where ewes treated with GPG protocol showed the highest values (Table 4). Meanwhile, no significant differences detected between hormonal treated groups and ram effect group (1.33). The use of GnRH in G5 increased ovulation rate and thus incidence of multiple births. This may due to that administration of GnRH prior mating increased yield of fertilized ova from ewe **(Walker *et al*., 1989)**, which initiate a new wave of follicular development and improved the number of ovulations (**Cognie, 1990**). **Beck *et al*. (1996) and Abu El-Ella (2007)** recorded that number of lambs born for ewes treated with GnRH-PGF2α - GnRH protocol was 1.69 and 1.56, respectively. In addition, mean values of litter size for ewes treated with 500 IU PMSG at time of progestagen devises removal (G3 and G4) was significantly (P> 0.05) higher than the control group. Using PMSG increased ovulation rate, multiple births and number of lambs born per ewe lambed as reported by **Akoz*et al*. (2006**). Also, **Ibrahim (1993)** reported that number of lambs born per ewe lambed was greater in ewes received PMSG than non-treated ewes.

In the present study, percentage of ewes lambed twins was higher for ewes treated with GnRH-$PGF_{2\alpha}$- GnRH protocol (58.33%) followed by ewes injected with 500 IU PMSG at MAP and CIDR withdrawal (50 and 40 %, respectively) comparing to ram effect and control groups (33.33 and 14.86%, respectively) (Table 4). The pervious results showed clearly that administration of GnRH followed by PGF2α (48 hrs) increased the number of twin lambs born per ewe, which is a direct reflection of the induced multiple ovulations. In agreement, **Sallam *et al.* (2004)** reported that using GnRH in conjunction with injection of $PGF_{2}\alpha$ led to increase incidence of twins. Moreover, PMSG injection increased twinning rate to 40 and 50% in ewes treated with CIDR and MAP protocol with an injection of 500 IU PMSG. **Gulyuzand Kozat (1995)** pointed out that administration of PMSG increased the number of follicles and therefore raised the twinning and triplet rates, which ofgreat value to sheep holders. **Zarkawi (2001)** detected twinning rate of 50% in Syrian Awassi ewes treated with sponges plus PMSG comparing to 20% for sponge-treated ewes without PMSG. **Nosrati *et al.* (2011)** recorded twining rate 33.5% for Kurdi ewes synchronized with CIDR for 14 days and super ovulated by 500 IU of PMSG injection, which was lower than that detected in the present study.

Litter weight at birth was not significantly differed among all experimental groups; however, it was the lowest in the control group (G1) and the highest in G3 (Table 4).

CONCLUSION

Results of the present study showed that using GnRH- $PGF_{2\alpha}$-GnRH protocol for estrus induction and synchronization for Rahmani ewes during summer season could increase estrus percentage, ovulation rate and in consequence pregnancy and lambing rates, as well as multiple births. It is a good alternative because they are rapidly metabolized and not accumulated in tissues (**Davis *et al.*, 1980**). Moreover, the time required to accomplish this protocol is shorter than other methods.

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