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Research Article

Efficacy of Various Synchronization Protocols on the Estrus Behavior, Lambing Rate and Prolificacy in Rahmani Egyptian Ewes During the Non-Breeding Season

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Abstract

Although various progestagens are often used to induce and synchronize estrus and ovulation in small ruminants, concerns regarding residues are the impetus to develop alternative approaches, including reduced doses of progestagens. Therefore, this study investigated the effects of various estrus synchronization protocols on fertility of Rahmani ewes during the non-breeding season. In June, 82 ewes were randomly assigned to five groups and each group received 1 of 5 treatments: (1) The ewes (n = 10) intramuscularly (IM) injected with 20 mg progesterone (P4) acetate day after day for 12 days and equine Chorionic Gonadotropin (eCG) on day 12, (2) Two doses of prostaglandin (PG) $F_{2\alpha}$ 9 days apart (n = 10) and eCG on day 9, (3) Ovulation synchronization (OVS, n = 10) with a gonadotropin Releasing Hormone (GnRH, day 0), $PGF_{2\alpha}$ (day 5) and GnRH 48 h later, (4) Whole (20 mg, n = 20), or halved (10 mg, n = 22) Progesterone Releasing Intravaginal Device (PRID) for 6, 8 and 14 days; $PGF_{2\alpha}$ a day prior to PRID removal and eCG at PRID removal and (5) Untreated-ewes (Control, n = 10). Blood samples were taken for serum P4 assay. There were significant differences ($p<0.05$) among treatments for estrus rate, onset of estrus, pregnancy and lambing rates as well as prolificacy. The highest (100%) estrus rate recorded in whole-PRID for 6 days and halved-PRID for 8 days, while the rapid onset (24 h) of estrus was in $PGF_{2\alpha}$. Pregnancy and lambing rates were greater ($p<0.05$) in $PGF_{2\alpha}$ and halved-PRID for 8 days. The highest prolificacy (1.75) was recorded with whole-PRID for 6 days whereas; the lowest (1.0) was recorded with $PGF_{2\alpha}$ and halved-PRID for 8 and 14 days. Serum P4 concentrations were not different among all PRID protocols on 4, 7 and 13 days. In conclusion, all synchronization protocols except OVS had a positive effect on ewe fertility during the non-breeding season.

Key words: Rahmani ewes, synchronization, progesterone, non-breeding season, prolificacy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In small ruminants, several protocols were used to induce and synchronize estrus and ovulation (Kusina *et al*., 2000). Progestagen-based protocols are commonly used worldwide (Abecia *et al*., 2011, 2012) by various methods, routes and doses. The most common route of application in ewe is intravaginal sponges impregnated either with fluorogestone acetate (FGA), or methylacetoxy progesterone (MAP) or with a Controlled Internal Drug Releasing (CIDR) device containing 0.3 g of progesterone P4 (Ungerfeld and Rubianes, 2002). Although progestagen-based protocols are preferred to manage reproduction of the flock (Gordon, 1999), they have the potential for environmental contamination because of the residual P4 in used devices and the addition of antibiotics to avoid vaginitis (Vinoles *et al*., 2011). Using prostaglandin (PG) $F_{2\alpha}$ and/or its analogues are a good alternative, because they are rapidly metabolized in the lung and therefore, not accumulated in tissues (Davis *et al*., 1980). According to the role of $PGF_{2\alpha}$ in luteolysis of Corpus Luteum (CL) (Turk *et al*., 2008), double $PGF_{2\alpha}$ injections is common for estrus synchronization in ewes. Since efficacy of $PGF_{2\alpha}$ is limited to the breeding season where active CL, different protocols using combinations of P4 and gonadotropin Releasing Hormone (GnRH) or human Chorionic Gonadotropin (hCG) have been recommended for estrus synchronization outside the breeding season (Kaya *et al*., 2013; Martinez *et al*., 2015).

Few studies have used GnRH in ovulation synchronization (OVS; El-Saidy *et al*., 2005) of ewes. The GnRH-based protocols used during the non-breeding season aimed at providing a source of P4 for inducing ovulation or luteinization of follicles, moreover, the time required to accomplish this protocol is shorter than other methods (Ashmawy, 2003).

Although, administration of intravaginal progestagens such as FGA or CIDR for 10-16 days followed by intramuscular (IM) injection of equine Chorionic Gonadotropin (eCG) appear to be the most practical method for estrus synchronization (Gomez *et al*., 2006; Swelum *et al*., 2015) in ewes. But a prolonged time of administration could result in low conception rates (Martin *et al*., 2004). Meanwhile, short-term protocols possibly allow for facilitating the managerial tasks, minimizing the vaginal discharge and infection risks and thus increasing the fertility rates. Indeed, short-term sponge treatments (5-7 days) have been successful in sheep regardless of breeding season (Ataman and Akoz, 2006; Oliveira *et al*., 2015).

The optimal fertility can be achieved with lower doses of progestagen by halving the intravaginal sponges (Ungerfeld and Rubianes, 2002). On the other hand, Crosby *et al*. (1991) postulate that a high level of progestagen followed by its rapid withdrawal is a necessary prerequisite for acceptable fertility. The use of gonadotropin especially eCG is routinely incorporated into progestagen-based protocols used to induce ovulation in anestrous ewes. However, there are many factors that can influence the effect of eCG in controlled breeding (Ali, 2007) including the dose of progestagen and eCG (Akoz *et al*., 2006) as well as the duration (Vinoles *et al*., 2001) of progestagen treatment. Recently, Kasikci *et al*. (2011) have concluded that the sponges containing 20 mg FGA can be halved for a more economical estrus synchronization of ewe at the farm level, resulting in higher pregnancy rates, but halving the recommended dose of eCG (600 IU) could decrease the fecundity rate. Therefore, the objectives of this study were to: (1) Investigate the relative efficacy of various estrus synchronization protocols (P4, $PGF_{2\alpha}$, OVS and PRID) on the fertility of ewe during the non-breeding season, (2) Compare between long-term and short-term application of both halved and whole-PRID on the reproductive efficiency of Rahmani ewes during the non-breeding season.

MATERIALS AND METHODS

Animals: A total of 82 Rahmani Egyptian ewes (3-5 years of age, weighing 50-60 Kg, having 1-3 parities and with a body condition score of between 2 and 3) exhibiting estrus in the presence of rams during March, 2013 were selected and separated from rams until June, 2013. The present study was conducted at Riwina Animal Production Station, Agriculture production sector, Agricultural Research Center, Ministry of Agriculture, Egypt during the non-breeding season from June to August (long-day light) 2013. This farm located in Kafrelsheikh (Northern Egypt, Latitude 31° N). This location has a Mediterranean-type climate. The ewes were managed under semi intensive condition, housed in open yard and fed Concentrate Feed Mixture (CFM) and roughages according to National Research Council (NRC., 2007) requirements. Furthermore, the diet offered daily to ewes composed of 1 kg CFM containing 14% crude protein plus 1 kg of alfalfa hay and fresh water was available *ad libitum*. All treatments were performed during the non-breeding season under a natural photoperiod environment. This study was approved by Kafrelsheikh University, Animal Local Ethics Committee of the Faculty of Veterinary Medicine.

Synchronization protocols: All estrus synchronization protocols began on the same day which was defined as day 0. Ewes were randomly allocated into a five major (4 treatments+1 control) groups. The groups were selected such that the body condition scores, age distribution and

parity were even. The ewes in each treatment were marked with different colors. Estrus was synchronized with P4, $PGF_{2\alpha}$, OVS and PRID protocols with some modifications as described below.

Progesterone: Each ewe was IM injected with 20 mg progesterone acetate (Lutone, Misr, Egypt) day after day for a period of 12 days with 500 IU eCG (Folligon, Intervet, Germany) on the last day of treatment according to Hashemi *et al*. (2006).

Prostaglandin $F_{2\alpha}$: Following this method, synchronization was initiated by IM administration of 175 µg $PGF_{2\alpha}$ analogue (Estrumate, Berkhamsted, England). Each mL of Estrumate contained 250 µg cloprostenol acetate; the second dose of $PGF_{2\alpha}$ (175 µg) was administered 9 days later (Turk *et al*., 2008) with simultaneous 500 IU eCG (Folligon).

Ovulation synchronization: In this study OVS protocol was applied according to Ashmawy (2012) where, an initial dose (4 µg) of GnRH analogue (Receptal, Intervet International, Netherlands) was utilized to synchronize ovulation in ewes. The $PGF_{2\alpha}$ (175 µg Estrumate) was given IM 5 days later to remove the resulting CL. The second dose of GnRH analogue (4 µg) was given 2 days after $PGF_{2\alpha}$ (day 7) to increase the synchrony of ovulation.

Progesterone releasing intravaginal device: Whole-PRID (20 mg, Chronogest®; Intervet International, Germany) or halved-PRID (10 mg) was inserted intravaginally to investigate the dose effect of FGA (20 vs. 10 mg; Kasikci *et al*., 2011) on the reproductive efficiency of ewes. Whole or halved-PRID was inserted intravaginally using sterile applicator (Intervet International, Netherlands) for 6, 8 and 14 days with a single dose of 250 µg $PGF_{2\alpha}$ analogue (Estrumate, IM) a day prior to PRID removal. Finally, 500 IU eCG (Folligon) was injected at PRID removal.

Estrus detection and breeding: Signs of induced estrus were observed by both direct visual observation and by using fertile ram (3-5 years old), immediately after PRID removal for the PRID protocol whereas, for P4, $PGF_{2\alpha}$ and OVS protocols, observation of estrus was started 24 h after the last treatment. The rams were introduced to treated ewes twice (one in the morning and another in the evening) daily; one hour each. Observation of estrus by using rams was continued near the ovulation (nearly 4 days) time. The reaction of the treated ewes to rams was noticed for restlessness, seeking out and teasing the rams by tail wagging and nuzzles his scrotum and finally standing to be mounted by rams (Gordon, 1997). When estrus ewe was bred by fertile ram, it returns back to its corresponding treatment group.

Reproductive efficiency: Estrous activity in terms of estrus rate (number of ewes exhibiting estrus/total number of ewes in the treated group×100) and onset of estrus (estrus occurring after hormonal treatment) were recorded in all treated groups. Ultrasonography (Esaote, Europe B.V., Netherlands) was used to diagnose the pregnant ewes between 45 and 50 days post-breeding. The pregnancy rate was defined as the total number of ewes that fell pregnant per number of ewes bred after estrus synchronization whereas, the lambing rate was defined as the total number of ewes which give a live lamb per the total number of ewes bred after estrus synchronization. Also number of ewes carrying twins or triplets was determined to calculate the prolificacy which defined as number of lambs/total number of ewes lambing.

Progesterone assay: To evaluate concentrations of serum P4, blood samples were collected via jugular venipuncture and immediately placed on crushed ice. On day 0, blood samples were collected from all experimental ewes (n = 82). In PRID protocol blood samples were collected on day 4 from all sub-groups but on day 7 from whole and halved-PRID for 8 days. On day 8 samples were collected from OVS group. On day 13 samples were collected from $PGF_{2\alpha}$, whole and halved-PRID for 14 days. On day 19 samples were collected from P4, $PGF_{2\alpha}$ and control groups. Blood samples were centrifuged at 1500×g for 15 min at 4°C and the serum was transferred into 1.5 mL micro-centrifuge tubes and frozen at -20°C until assayed. To detect circulating concentrations of P4, samples were assayed using mini-VIDAS (VIDAS TESTS, BIOMÉRIEUX, France) according to the manufacturer's instructions. Assay sensitivity for a 200 µL sample was 0.1 ng mL^{-1} P4. All samples for a single ewe were analyzed within the same assay and treatments were run in a random order.

Experimental design

Progesterone: Ten ewes were injected with 0.8 mL Lutone containing 20 mg P4 acetate day after day for 12 days with 500 IU eCG (Folligon) on day 12 as depicted in Fig. 1, P4. After 24 h from the last treatment two fertile rams were introduced to the ewes for estrus detection and breeding. The estrus was detected at 12 h intervals for 4 days.

Prostaglandin $F_{2\alpha}$: Ten ewes received two doses of 0.7 mL $PGF_{2\alpha}$ analogue (Estrumate) 9 days interval with 500 IU eCG (Folligon) concurrent with the second dose ($PGF_{2\alpha}$) as shown

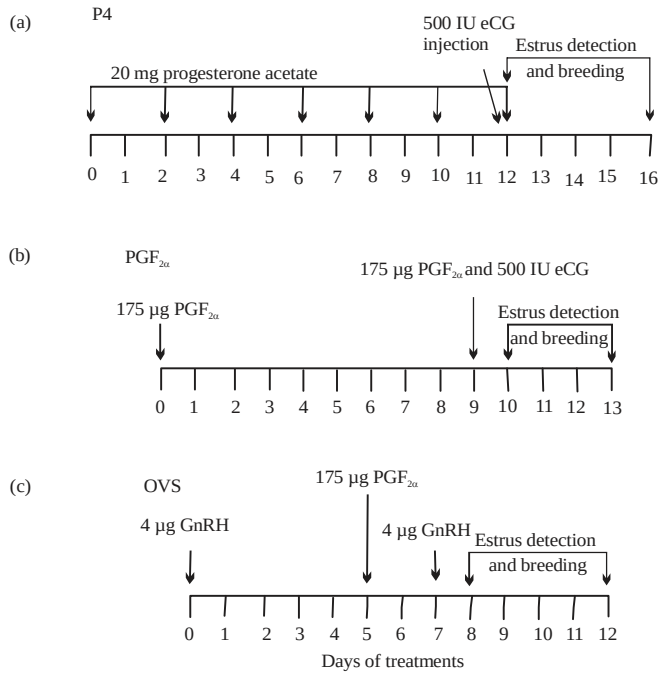

Fig. 1(a-c): Schedule of treatments with (a) P4, (b) $PGF_{2\alpha}$ and (c) OVS protocols, P4: Synchronization of estrus by progesterone, $PGF_{2\alpha}$: Synchronization of estrus using prostaglandin, OVS: Synchronization of estrus by ovulation synchronization

in Fig. 1, PGF_{2a}. Two fertile rams were introduced for estrus detection and breeding after 24 h from the second dose. The estrus was detected at 12 h intervals for 4 days.

Ovulation synchronization: Ten ewes were injected with 1 mL GnRH analogue, contained 0.004 mg buserelin acetate (Receptal). On day 5 ewes received 0.7 mL $PGF_{2\alpha}$ analogue (Estrumate) and on day 7 ewes were given a second dose of GnRH analogue (OVS) as shown in Fig. 1, OVS. After 24 h from the second dose of GnRH, two fertile rams were introduced for 4 days for estrus detection and breeding.

Progesterone releasing intravaginal device

Whole-PRID: Twenty ewes of this group were sub-divided into 3 sub-groups where the whole-PRID was inserted intravaginally for 6 (n = 6), 8 (n = 7) and 14 (n = 7) days. All ewes were injected with 1 mL $PGF_{2\alpha}$ (Estrumate) a day prior to PRID removal and received a 500 IU eCG (Folligon) on PRID removal as shown in Fig. 2. Fertile rams were introduced for 1 h at morning and evening starting from PRID removal for 4 consecutive days.

Halved-PRID: Twenty two ewes of this group were also sub-divided into 3 sub-groups where, the halved-PRID was inserted intravaginally for 6 (n = 8), 8 (n = 6) and 14 (n = 8) days. Likewise, all the ewes were received $PGF_{2\alpha}$ a day prior to PRID removal and a 500 IU eCG on PRID removal (Fig. 3). Fertile rams were used for estrus detection and breeding.

Control: Ten ewes were set aside as a control group and no hormones were administered to them. Fertile rams were joined to ensure that the ewes in estrus mated.

Statistical analyses: All data analyses were performed using a statistical software program (GraphPad Prism Version 5.0, GraphPad Software, San Diego, CA, USA). The obtained data were subjected to repeated measures ANOVA. When difference was significant by ANOVA, individual means were further tested by Tukey's multiple comparison test (Motulsky, 1995). Estrus, pregnancy and lambing rates as well as prolificacy were compared using a chi-square test. Effects were considered to be significant when the level of probability was less than 5%.

RESULTS

Estrous activity: The obtained results revealed that estrus rate was significantly ($p<0.05$) different among all synchronization protocols where, it was maximum (100%) with whole-PRID for 6 days and halved-PRID for 8 days (Table 1). Estrus rates were 90, 85.7, 62.5, 57.1, 50, 30 and 10% for P4, whole-PRID for 14 days, halved-PRID for 6 days, whole-PRID for 8 days,

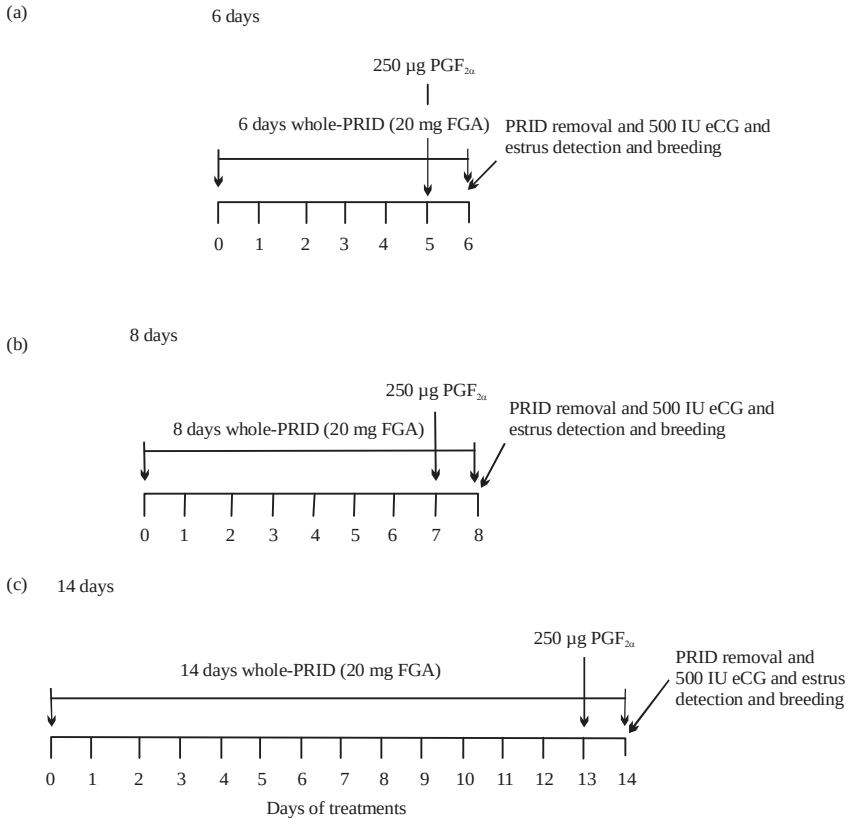

Fig. 2(a-c): Schedule of treatments with whole-PRID for (a) 6, (b) 8 and (c) 14 days

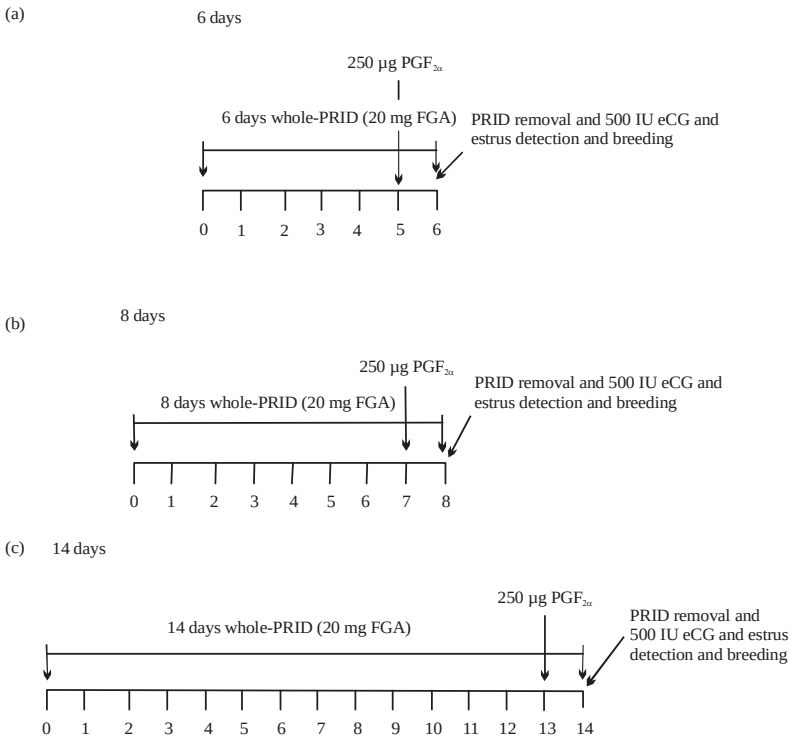

Fig. 3(a-c): Schedule of treatments with halved-PRID for (a) 6, (b) 8 and (c) 14 days

Table 1: Productive efficiency of treated and control ewes during the non-breeding season

Treatments	No. of ewes	No. of estrus ewes	Estrus rate (%)	Onset of estrus (h)* (mean±SEM)	Pregnancy rate (%)**	Lambing rate*** (%)	Prolificacy****
P4	10	9	90^{b}	37.3 ± 4.2^{ce}	44.4^{e}	44.4^{e}	1.5^{b}
$PGF_{2\alpha}$	10	3	30^{g}	24.0 ± 0^{d}	100^{a}	100^{a}	1.0^{d}
OVS	10	0	0^{i}	0^{f}	0^{h}	0^{h}	0^{e}
PRID							
Whole-PRID (20 mg FGA)							
6 days	6	6	100^{a}	64.0 ± 5.0^{a}	66.6^{c}	66.6^{c}	1.75^{a}
8 days	7	4	57.1^{e}	49.7 ± 1.7^{be}	75^{b}	75^{b}	1.3^{c}
14 days	7	6	85.7^{c}	28.0 ± 4.0^{cd}	33.3^{f}	33.3^{f}	1.5^{b}
Halved-PRID (10 mg FGA)							
6 days	8	5	62.5^{d}	62.4 ± 5.8^{ab}	60^{d}	60^{d}	1.3^{c}
8 days	6	6	100^{a}	56.0 ± 5.0^{ab}	100^{a}	100^{a}	1.0^{d}
14 days	8	4	50^{f}	30.0 ± 6.0^{cd}	75^{b}	75^{b}	1.0^{d}
Control	10	1	10^{h}	0^{f}	0^{h}	0^{h}	0^{e}

*Hours after the end of hormonal treatment, **Number of pregnant ewes/number of bred ewes, *** Number of lambed ewes/number of bred ewes, ****Number of lambs born/number of ewes lambing, means bearing at least one common superscript in a column did not differ statistically ($p\geq0.05$), otherwise significant at 5% level ($p<0.05$)

halved- PRID for 14 days, $PGF_{2\alpha}$ and control, respectively (Table 1). Whole-PRID treated ewes for 6 and 14 days had a significantly ($p<0.05$) greater estrus rate than the halved-PRID for 6 and 14 days. On contrary, halved-PRID for 8 days had a significant greater estrus rate than that of the whole-PRID for 8 days as shown in Table 1. Onset of estrus showing significant differences among the majority of all synchronization protocols and it was the shortest 24 h, ($p<0.05$) for $PGF_{2\alpha}$ and the longest (64 h) for whole-PRID for 6 days. The mean onset of estrus were 28, 30, 37.3, 49.7, 56 and 62.4 h for whole-PRID for 14 days, halved-PRID for 14 days, P4, whole-PRID for 8 days, halved-PRID for 8 days and halved-PRID for 6 days, respectively (Table 1).

Pregnancy and lambing rates: The results presented in Table 1 revealed that pregnancy and lambing rates were significantly ($p<0.05$) different among all treatments and the lowest (30%) estrus rate of $PGF_{2\alpha}$ was significantly ($p<0.05$) associated with the highest (100%) pregnancy rate. Likewise, the highest pregnancy rate was recorded in halved-PRID for 8 days. Pregnancy rate was 75% for both whole-PRID for 8 days and halved-PRID for 14 days. For other treatments it was 66.6, 60, 44.4 and 33.3% for whole-PRID for 6 days, halved-PRID for 6 days, P4 and whole-PRID for 14 days, respectively. No abortion or fetal death was recorded in this study so that both lambing and pregnancy rates were identical for each synchronization protocol. The FGA dose (whole or halved-PRID) affected the pregnancy rate, lambing rate and the prolificacy. Halved-PRID for 8 and 14 days has greater ($p<0.05$) pregnancy and lambing rates than that of whole-PRID. On contrary, all insertion periods of the whole-PRID have greater ($p<0.05$) prolificacy than that of the halved-PRID as presented in Table 1. Prolificacy was the highest (1.75) with whole-PRID for 6 days and equal to 1.5 in P4 and whole-PRID for 14 days and was 1.3 in whole-PRID for 8 days and halved-PRID for 6 days. Lastly, prolificacy was 1 for $PGF_{2\alpha}$, halved-PRID for 8 and 14 days synchronized ewes.

Progesterone assay: Table 2 revealed that P4 level, on day 0 (basal level) of treatment was non-significantly different among all treatment groups but on day 4, P4 level was significantly increased ($p<0.05$) in halved-PRID for 6 (1.8 ± 0.9 ng mL^{-1}) days. Also on day 7, P4 level significantly increased ($p<0.05$) in halved-PRID for 8 day (1.9 ± 0.4 ng mL^{-1}) than the basal level (0.9 ± 0.5 ng mL^{-1}). On day 8 there was no significant difference in P4 level of OVS (0.5 ± 0.2 ng mL^{-1}) group than basal level (0.6 ± 0.1 ng mL^{-1}) similarly, on day 13 no difference in P4 level of $PGF_{2\alpha}$ (1.5 ± 0.9 ng mL^{-1}), whole (2.0 ± 0.2 ng mL^{-1}) and halved-PRID (1.7 ± 0.3 ng mL^{-1}) for 14 day. Whereas, on day 19, P4 level significantly increased ($p<0.05$) in P4 (2.6 ± 0.4 ng mL^{-1}) and $PGF_{2\alpha}$ (2.4 ± 0.7 ng mL^{-1}) groups than its basal level. Notably, there were no significant differences in P4 concentrations between whole and halved-PRID on days 4, 7 and 13. Progesterone level of control group on day 19 was significantly lower (0.7 ± 0.2 ng mL^{-1}) than both P4 and $PGF_{2\alpha}$ synchronized ewes.

DISCUSSION

Reproduction of small ruminants can be controlled by several methods developed in recent decades. Some of these involve administration of hormones that modify the physiological chain of events involved in the sexual cycle (Abecia *et al*., 2012). Methods which utilize P4 or its analogues are based on their effects in the luteal phase of estrous cycle, simulating the action of natural P4 produced by the CL after

Table 2: Concentration (Mean±SEM) of treated and control ewes*

				Whole-PRID			Halved-PRID			
Sampling days	P4	$PGF_{2\alpha}$	OVS	6 days	8 days	14 days	6 days	8 days	14 days	Control
0	0.7±0.4[a]	1.1±0.5[a]	0.6±0.1[a]	1.3±0.2[a]	0.9±0.7[a]	1.4±0.1[a]	1.2±0.8[a]	0.9±0.5[a]	1.6±0.6[a]	1.1±0.3[a]
4	-	-	-	1.7±0.9[a]	1.9±0.6[a]	1.9±0.7[a]	1.8±0.9[b]	1.7±0.2[ab]	1.5±0.5[a]	-
7	-	-	-	-	2.1±0.5[a]	-	-	1.9±0.4[b]	-	-
8	-	-	0.5±0.2[a]	-	-	-	-	-	-	-
13	-	1.5±0.9[a]	-	-	-	2.0±0.2[a]	-	-	1.7±0.3[a]	-
19	2.6±0.4*[b]	2.4±0.7*[b]	-	-	-	-	-	-	-	0.7±0.2[b]

*P4 concentration expressed as ng mL^{-1}, means bearing at least one common superscript in a column did not differ statistically ($p \geq 0.05$) otherwise, significant at 5% level ($p<0.05$), means bearing asterisk were significantly differed from other means within the same row ($p<0.05$), P4: Synchronization of estrus by progesterone, PGF_{2a}: Synchronization of estrus using prostaglandin, OVS: Synchronization of estrus by ovulation synchronization

ovulation, which is responsible for controlling LH secretion from the pituitary gland. The results of our study not only emphasized and extend the findings that estrus can be synchronized successfully by using halved-PRID as reported previously (Greyling *et al*., 1997; Ungerfeld and Rubianes, 2002; Kasikci *et al*., 2011). But also showed the potential for the use of halved-PRID for 6, 8 and 14 days with eCG to improve the reproductive efficiency of Rahmani ewes during the non-breeding season.

Based on our findings which revealed that, the halved-PRID for 8 and 14 days yielded greater ($p<0.05$) pregnancy and lambing rates than the corresponding insertion periods of whole-PRID. Also, there were no significant differences in P4 concentrations among all insertion periods of whole and halved-PRID on days 4, 7 and 13. Taken together, it is obvious that the progestagen dose of the PRID used to synchronize estrus in ewes, after a 14 days treatment was not entirely utilized which are in close agreement with the findings of Greyling *et al*. (1997). Those revealed only 23.4% of progestagen from the whole sponge and 50.7% from the halved sponge were absorbed by the animal during the insertion time. Similarly, the differences among the initial doses (40, 50 and 60 mg) of MAP correlated with differences among the residual MAP remaining in the sponges but not the absorbed levels (Simonetti *et al*., 2000). In addition, there was no significant difference for the onset of estrus between the two corresponding insertion periods of halved and whole-PRID which discloses no benefit of the whole-PRID over the halved-PRID at least for estrus synchronization in sheep farm during the non-breeding season.

Consequently, the most reasonable explanation can be that depending upon the differences of FGA dose in the PRID, blood P4 level during the treatment or residual P4 following PRID removal could cause little differentiation in the pre-ovulatory LH surge which may have resulted significant variations in ovulation and fertility. Although, we could not prove this by measuring LH in our study but Greyling *et al*. (1997), found that the halved-sponge has slightly higher mean LH and significantly greater conception and lambing rates in comparison with the whole-sponge, which is in close agreement with our findings. Furthermore, sub-luteal concentrations of P4 (1-2 ng mL^{-1}) were effective in suppressing the occurrence of pre-ovulatory surge of LH (Stock and Fortune, 1993) in cattle. Interestingly, P4 was shown to have these inhibitory effects at much lower concentrations where, the mean P4 concentrations in follicular phase of cow were 0.13, 0.30, 0.70 and 1.20 ng mL^{-1} and the percentage of cows that ovulated were 100, 42.9, 0.0 and 0.0% (Hatler *et al*., 2008), respectively.

Regarding our findings although, P4 and whole-PRID for 6 and 14 days have acceptable estrus rates (90, 100 and 85.7, respectively), but have low pregnancy rates (44.4, 66.6 and 33.3, respectively). This might be due to the high serum P4 concentration which has inhibitory effect on pre-ovulatory LH surge so that some ewes showing non ovulatory estrus and the other showing ovulatory estrus. Both P4 and PRID protocols provided more exogenous P4 as evident by elevated P4 ($\geq 1.5\pm0.5$ ng mL^{-1}) concentrations on days 4, 7 and 13 in all PRID-treated and P4 injected ewes regardless of their pregnancy status. Similar to Bartlewski *et al*. (1999) they reported that the concentration of P4 begin to increase between 3 and 7 days after estrus and then reach peak at day 12, we found the basal level (day 0) of P4 in P4 injected ewes was 0.7±0.4 ng mL^{-1} and increased (2.6±0.4) on day 19 which means after estrus by nearly 5 days.

Although, a recent study (Jackson *et al*., 2014) found that, regardless of season inclusion of GnRH and $PGF_{2\alpha}$ into 5 days CIDR protocols did not improve ewe fertility. On contrary, in our PRID protocols eCG and $PGF_{2\alpha}$ improved the fertility of treated ewes during the non-breeding season. This finding proved that PRID is better than CIDR at least for improvement of the reproductive efficiency of Rahmani ewes during the non-breeding season. These results were consistent with that of Ali (2007) due to eCG on PRID removal increases ovulation rates and fecundity. Furthermore, our findings were consistent with the findings of Titi *et al*. (2010), they found an increase in

prolificacy in P4 treated ewes when coupled with GnRH and $PGF_{2\alpha}$. Thereby, in our report the prolificacy was 1.75 with short-term (6 days) of whole-PRID vs. 1.5 with long-term (14 days). Moreover, prolificacy of 8 days insertion of the whole-PRID was 1.3 vs. 1 of the halved-PRID for 8 and 14 days.

Statistical differences were detected among treatments for estrus rate in the current study, which is inconsistent with the results of Wheaton *et al*. (1992). They reported no differences in ewes overall exhibiting estrus between untreated ewes and those receiving a CIDR insert for 12 days. The reason might be the different forms of intravaginal device, different breeds of ewe and synchronization protocols. Although, gonadotropin is routinely incorporated into the intravaginal devices used in ewe during the non-breeding season to induce ovulation but the effect of eCG is dose (Akoz *et al*., 2006) dependent which might be responsible for this discrepancy.

It is worth noting that, some ewes responded to our protocol of $PGF_{2\alpha}$ during the non-breeding season which proved that some Rahmani ewes were cyclic out of season and this could be explained by the Mediterranean climate of Northern Egypt (shorter day light). Interestingly, the lowest (30%) estrus rate of $PGF_{2\alpha}$was associated with the highest (100%) pregnancy and lambing rates which confirm that double $PGF_{2\alpha}$ injection enhanced fertility of the synchronized Rahmani ewes during the non-breeding season. Perhaps longer (9 days) interval between $PGF_{2\alpha}$ injections could yield better pregnancy rates. These findings consider a novel because $PGF_{2\alpha}$-based protocols generally achieve poor reproductive outcomes (Fierro *et al*., 2013) but our protocol using double doses of $PGF_{2\alpha}$, 9 days interval plus eCG on day 9 achieved maximum pregnancy and lambing rates, especially when there is no reference to the stage of the estrous cycle at the time of the first treatment. Further research under similar experimental conditions is required to determine the best protocol of $PGF_{2\alpha}$ to achieve both greater estrus and pregnancy rates.

Inducing ovulation out of season would provide the opportunity to produce lambs all-year-round in temperate areas of the globe. Unfortunately, our OVS protocol failed to improve the fertility or even to induce estrus during the non-breeding season which might be due to OVS treated ewes may have previously experienced a false heat or actually expressed a full estrous cycle prior to the administration of $PGF_{2\alpha}$ according to Titi *et al*. (2010). Since P4 concentration on day 0 (0.6 ± 0.1) was not significantly differed from that on day 8 (0.5 ± 0.2), then it is obvious that all OVS treated and almost all untreated-ewes (control) may have been in a deep anestrous. Consequently, hormonal treatments and/or ram effect were not powerful enough to bring them out of this seasonal anestrous. In addition, the dose and type of GnRH and $PGF_{2\alpha}$ analogue as well as the season of year might be implicated in this negative response because recently it has been confirmed that OVS protocol improved the fertility of Rahmani ewes (Ashmawy, 2012) and goat (Bowdridge *et al*., 2013) during the breeding season.

Our findings were consistent with the results of Rekik *et al*. (2014), where, both $PGF_{2\alpha}$ and GnRH synchronization revealed lower estrus response in comparison with progestagens treatment during the transition period to the breeding season. Similarly, all control ewes failed to show estrus and did not respond to the ram effect although these ewes might be cyclic due to the elevated (1.1 ± 0.3) concentrations of P4 on day 0.

CONCLUSIONS

In the current study, all synchronization protocols except OVS had a positive effect on the reproductive efficiency of Rahmani ewes during the non-breeding season. Moreover, our study suggest that for more economical estrus synchronization under field conditions, the whole-PRID sponge can be split into 2 and used successfully for 8 days to achieve greater pregnancy and lambing rates. However, further studies with greater animal numbers are needed to investigate the efficacy of halved-PRID for 6 and 8 days with higher doses of eCG in order to achieve both greater lambing and prolificacy rates.

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