EFFECT OF OVARIAN SYNCHRONIZATION PROTOCOLS, USING GnRH AND PGF2α, ON OVARIAN RESPONSE AND REPRODUCTIVE TRAITS OF RAHMANI EWES

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SUMMARY

Thirty Rahmani ewes were used to study the effect of ovsynch protocol (GnRH+$PGF_2\alpha$) on the reproductive performance of Rahmani ewes. They were equally divided into three treatment groups. All ewes in the treated groups were intramuscularly injected on Day (0) with 1 ml GnRH analogue, (Receptal®) followed by intramuscular injection of 0.7 ml $PGF_2\alpha$ analogue (Estrumate) either by 5 days (G1), 6 days (G2) or 7 days (G3) . A second dose of GnRH analogue (1 ml) was given on day-7 (G1), day-8 (G2) or day-9 (G3), and artificial insemination was carried out 24 h later.

Results showed that 1 out of 10 ewes exhibited estrous activity in G1 *vs.* 3 out of 10 ewes in both G2 and G3. Ewes in G1 had the highest ($P<0.05$) lambing rate (60%), compared to G2 (50%) and G3 (40%). Litter size and fecundity were higher ($P<0.05$) in G1 (1.67 lambs/ewe and 100%), followed by G2 (1.40/lambs/ewe and 70%) and G3 (1.25/lambs/ewe and 50%). P_4 concentration was high ($P<0.05$) in all treated groups as affected by the 1st GnRH injection. Thereafter, P4 decreased in all treated groups after $PGF_{2\alpha}$ injection. Post-2nd GnRH injections, P_4 levels showed a pronounced increase in all treated groups. At days 21to 24 post-mating, P_4 levels showed the highest values in all treated groups.

Based on the obtained results, using GnRH-$PGF_{2\alpha}$-GnRH (GnRH, 0 d; $PGF_2\alpha$ 5 d laterandGnRH 48 h later) protocol during the breeding season (January) can be used for synchronization of ovulation to reduce service and lambing intervals of Rahmani ewes.

Key words: *Ewes, GnRH, PGF2α, lambing, litter size, progesterone*

INTRODUCTION

Controlled breeding by synchronization of estrus is an important tool to improve lambing percentage to control lamb crops in addition to maximize the return of ewe's investment. Estrous synchronization of ewes has been accomplished using several methods with various degrees of success. Artificial insemination programs provide several advantages (e.g. protection of flock from genital diseases transmitted through mating). Though progesterone impregnated intravaginal sponges is the widely used method, it has disadvantages when applied for a long period (Safdarian*et al.*, 2006). Similar results could be achieved using two injections of $PGF_2\alpha$ at 11-day intervals (Ceddene*t al.*, 2008).

Using intravaginal sponge induces inflammation with adherence to vaginal mucosa, following the withdrawal of the pessary (Larsson *et al.*, 1991). Moreover, after the second $PGF_2\alpha$ administration, there was no signs of estrus because luteolysis or formation of the corpus luteummay be altered after the first $PGF_2\alpha$ injection (Alaçam, 1994).

In cyclic animals, follicular waves are terminated when the dominant follicle either regressed or ovulated, leading to the start of a new wave of follicular growth (Gonzalez-Bulnes*et al.*, 2001). However, early studies demonstrated success of GnRH-based protocols in synchronization induction of ovulation in anestrous cattle (Ganah, 2000). Fewer studies have examined the use of GnRH in ewes (El-Saidy*et al.*, 2005). The GnRH treatment based protocols, either in time, dose or method of treatment, used out-of-breeding season aimed at providing a source of P_4 for inducing ovulation or luteinization of follicles (Ashmawy, 2003).

An injection of GnRH analogues, 6 days prior to injection of $PGF_2\alpha$, enhanced the conception rate (Stevenson *et al.* 1996),

increased the number of synchronized animals and reduced variability in time to estrus (Twagiramungu*et al.*, 1992). These positive results may be explained by the initiation of a new follicular wave following the injection of GnRH and recruitment of a new dominant follicle, being present at the time of $PGF_2\alpha$ injection (Pursley*et al.*, 1998). The time required to accomplish this synchronization protocol is shorter than that in other methods (e.g. progesterone sponges, CIDR, P_4 implantation or double doses of $PGF_2\alpha$).

Therefore, the objective of this study was to determine the effectiveness of GnRH-PGF2*α*-GnRH protocolsto synchronize estrus and/or ovulation of Rahmani ewes during January breeding season.

MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Animals and experimental groups

Thirty Rahmani ewes (averaged 45.61±3.14 kg live body weight, 3-4 years old and had 2-3 parities) were used in this study. The experimental ewes were allocated into three similar groups according to live body weight, age and parity (10 ewes each). On day 0 (of treatment), does in the 1st, 2nd and 3rd groups were intramuscularly injected with 1 ml GnRH analogue, contained 0.004 mg Buserelin (Receptal, Intervet International BV Boxmeer-Holland). A second intramuscularly injection applied either on Day-5 (G1); Day-6 (G2) or Day-7 (G3) by 0.7 ml $PGF_2\alpha$ analogue (Estrumate, Coopers Animal Health LTD, Berkhamsted-England). Each ml of Estrumate contained 250 μg Cloprostenol Acetate). The second dose of GnRH (1 ml) analogue was given two days after PGF2α injection in G1, G2 and G3. Artificial insemination was carried out 24 h after the 2nd GnRH treatment of ewes.

Housing and feeding system

All experimental ewes were housed in semi-open sheds in groups and fed concentrate feed mixture (CFM) and roughages according to NRC (1985) requirements for goats. The diet offered daily to each ewe composed of 1.25 kg CFM (14% CP) plus 4 kg Egyptian berseem during winter or 1.0 kg berseem hay during summer.

Semen dilution and artificial insemination

Semen was collected by using artificial vagina and diluted just before insemination. Immediately after semen collection, the fresh semen was checked to determine sperm motility (Bane, 1982). Only ejaculates of >80% initial motility were diluted by Tris-yolk extender (1:4) according to Leboeuf*et al.* (2000). After dilution, sperm concentration was about 300×10^6 sperm/ml and sperm motility remained over 70%. Insemination was carried out using a simple inseminating pipette with a fine blunt bent end and a vaginal speculum. About 1 cm of diluted semen was deposited into the cervix as far as possible.

Laparoscopic examination

Laparoscope (Wolf/8933/7 mm-made in USA with W German lens system) was used to visualize the ovaries 7 to 12 days post-insemination. Six fastened (16 hours before examination) ewes in each treatment group were examined for follicles diameter and to count the follicle and corpora lutea (CLs). Follicle diameter was determined by the aid of scales on the stainless-steal rode used for the genital tract manipulation.

Reproductive efficiency

Estrous activity in terms of estrous rate, (number of ewes exhibiting estrus by ram within 72 h following PGF2α injection /number of treated ewes) x 100, onset of estrus (estrus occurring within 72 h following PGF2α injection) and estrous duration (the period from onset of estrus to the end of estrus) were recorded in treated groups. Litter size and date of lambing were also recorded. Fecundity rate, (number of born lambs/total number of treated ewes)×100, was calculated.

Blood sampling and progesterone assay

Blood samples were collected at the morning before feeding via the jugular vein from all ewes using evacuated tubes (10 ml). Serum were withdrawn then separated by centrifugation of blood at 2500 rpm for 15 min and stored at −20 ºC until used later for P_4 assay. Blood samples represent; just pre-treatment, one day post-1st GnRH, one day post-$PGF_2\alpha$, one day post-2nd GnRH (Day of AI) and on days 21th and 24th post-insemination (pregnancy diagnosis).

Progesterone concentration was determined in selected serum samples of 5 animals (3 lambed ewes +2 non conceived ewes from each treatment group). Determination of progesterone in serum samples was carried out using radioimmunoassay kit (catalog No. 1188 manufactured by Immunotech, France) as described by the manufacture. The sensitivity of the progesterone assay was 0.03 ng/ml, while coefficient of variation was 4.3% for both progesterone intra- and inter-assay.

Statistical analysis

Data in tables are presented as mean±SE for onset and duration of estrus, litter size, number of CLs and P_4 concentration. Data were analyzed by using SAS/ procedures (2000). Meanwhile, exhibited estrous, lambing and fecundity rates were performed using Chi-square test. All significant differences among groups were set at P<0.05.

The statistical model was:

$$Y_{ij} = \mu + A_i + e_{ij}.$$

Where: Y_{ij} = Observed values, μ= Overall mean, A_i= groups, e_{ij} = Random error.

RESULTS AND DISCUSSION

Estrous activity

Results presented in Table (1) show that estrus rate after $PGF_2\alpha$ injection was not affected significantly by treatment, being 10, 30 and 30%in G1,G2 and G3, respectively. This means that exhibiting estrus was higher following $PGF_2\alpha$ injection on day 6th or 7th than on day 5th.

Onset of estrus after $PGF_2\alpha$ injection was shortest (P<0.05) in G1, modest in G2 and longest in G3. Meanwhile, estrous duration was not affected significantly by treatment, ranging between 30-34 h in all treated groups (Table 1).

The present lambing rate of 60% was lower than that reported by Husein and Kridli (2003) in ewes treated with P_4-GnRH-PGF2α (82%) and Beck *et al.* (1996) (88.8%) in ewes treated with 4 μg of GnRH agonist (Buserelin) followed, 5 days later, by an injection of 100 μg of cloprostenol. An injection of GnRH analogues 5 days prior to an injection of PGF2α, enhanced the conception rates and enhance synchronization rate than double PGF2α injection (Mihm *et al.*, 1999).

Lambing performance

Results (Table 1) show that the lowest estrous rate response to $PGF_2\alpha$ injection in (G1) was significantly (P<0.05) associated with the higher lambing rate of G1 (60%), followed by (G2, 50%) and the lowest in (G3, 40%) (Table 2). Lambing period (the period from the 1st to last lambing of ewes in the same group) was considerably short (4-6 days) which almost similar in all groups (Table 2).

Estrous rate was lower than that obtained in previous studies. In this respect, estrus rates measured as 90.9% (Beck *et al.*,

Table 1. Estrous activity (Mean ± SE) observed in different treatment groups *.

Group	N	Ewes in estrus (n)	Estrus rate (%)	Onset of estrus(h)	Duration of estrus (h)
G1 ($PGF_2\alpha$ on day 5)	10	1	10	42.0 ± 0.00^{b}	30.0 ± 0.0^{a}
G2 ($PGF_2\alpha$ on day 6)	10	3	30	46.0 ± 1.63^{ab}	34.0 ± 3.2^{a}
G3 ($PGF_2\alpha$ on day 7)	10	3	30	49.3 ± 1.08^{a}	32.0 ± 1.6^{a}

a and b: Means within the same column with different superscripts are significantly different at 5% level. N: Number of treated ewes.
* Estrus was observed only for ewes in the treated groups, while the control ewes were left with fertile ram during the experimental period.

1996) and 93.3% (Ataman and Aköz, 2006) after GnRH-PGF2α injection (100 µg of busereline) then 5 d laterinjection of 0.294 mg of Triaprosttromethamine (an analogue of PGF2α). While estrous rate were 86.6% (Fritzgerald *et al.*, 1985 and Ataman and Aköz, 2006) or 100% (Öztürkler *et al.*, 2003) after double injection of PGF2α (9 days interval). Observation in the present results may be due to the breed, dose and PGF2α analogue and time of treatment during the year

Litter size and fecundity

Results in Table (3) show significant differences (P<0.05) in litter size (LS) and fecundity rate (FC) among treatment groups. The LS and FC were higher (P<0.05) in G1 (1.67/lambs and 100%), followed by G2 (1.40/lambs and 70%) and lower in G3 (1.25/lambs and 50%), respectively.

In accordance with the present results, Beck *et al.* (1996) found that LS was 1.69 and 1.74 for ewes treated with GnRH-$PGF_2\alpha$-GnRH protocol or double $PGF_2\alpha$ 11 days apart.

Ovarian structure

Average number of CLs and follicles over 2 mm diameter, counted through laparoscopy on the 7th days post insemination on the right and left ovaries of ewes in treated groups are shown in Table (4). Average number of CLs on the right ovary was greater (P<0.05) in G1 than G2 and G3 (1.16 vs. 0.33 and 0.33/ovary). However, there were no differences among the three studied groups concerning number of follicles on both ovaries. It is of interest to note that the significant differences in number of Cls was observed in right ovary while not on left one. It seems that there is a negative effect of CL on number of follicles growing on the same ovary which is supported by the finding of El-Gohary (2006).

El-Gohary (2006) reported that the total number of CLs ranged between 1.1 and 1.4 CL for Rahmani ewes. In pregnant ewes, up to day 26th post-mating, follicular waves have also been documented, based on ultrasonographic observations. It is worth noting that no follicles ≥3 mm was observed on the ovaries in sheep. This may be due to the local inhibition of CL on follicular dynamics, in the ovary that bore the CLs during pregnancy, up to 4 weeks after parturition (Bartlewski *et al.*, 2000).

Progesterone profile

Results in Table 5 revealed that P_4 level, before treatment was higher (P<0.05) in G2, and G1 than G3. These levels indicate that most ewes in G1 and G2 were in luteal phase, while those in G3 were in follicular phase. Post-1st GnRH injection, P_4 level increased (P<0.05) in all treated groups, being the highest in G2, moderate in G3 and the lowest in G1. This trend may indicate higher responses to GnRH injection of ewes in luteal phase and reflecting

Table 2. Lambing rate (%) and lambing period (day) in ewes of studied groups.

Group	N	Lambed ewes(n)	Lambing rate (%)	Lambing period	
				Date (from-to)	Duration (day)
G1 ($PGF_2\alpha$ on day 5)	10	6	60^{a}	01/06 - 06/06	6
G2 ($PGF_2\alpha$ on day 6)	10	5	50^{ab}	01/06 - 04/06	4
G3 ($PGF_2\alpha$ on day 7)	10	4	40^{b}	01/06 - 06/06	6

a and b: Means within the same column with different superscripts are significantly different at 5% level. N: Total number of ewes

Table 3. Litter size and fecundity of ewes in studied groups.

Group	N	Lambed ewes (n)	Born lambs		Fecundity(%)
			Number	Litter size	
G1 ($PGF_2\alpha$ on day 5)	10	6	10	1.67^{a}	100.0^{a}
G2 ($PGF_2\alpha$ on day 6)	10	5	7	1.40^{ab}	70.0^{b}
G3 ($PGF_2\alpha$ on day 7)	10	4	5	1.25^{b}	50.0^{c}

a and b: Means within the same column with different superscripts are significantly different at P<0.05. N: Total number of ewes.

Table 4. Average number of CLs and follicles over 2 mm diameter on ovaries of treated ewes, 7-12 days post-insemination.

Group	Right ovary		Left ovary		Total	
	CLs	Follicles	CLs	Follicles	CLs	Follicles
G1 ($PGF_2\alpha$ on day 5)	1.16±0.43^{a}	1.66±0.30	1.00±0.23	1.16±0.59^{a}	2.17±0.36^{a}	2.50±0.73
G2 ($PGF_2\alpha$ on day 6)	0.33±0.19^{b}	2.00±0.52	1.17±0.15	0.16±0.15^{b}	1.50±0.20^{ab}	2.16±0.64
G3 ($PGF_2\alpha$ on day 7)	0.33±0.19^{b}	1.33±0.30	1.00±0.23	1.00±0.33^{a}	1.33±0.28^{b}	2.33±0.45

a and b: Means within the same column with different superscripts are significantly different at P<0.05.
G1: $PGF_2\alpha$ on day 5. G2: $PGF_2\alpha$ on day 6. G3 $PGF_2\alpha$ on day 7.

Table 5. Progesterone (Mean±SE) concentration (ng/ml) during different treatment periods in treated groups.

Treatment time	Progesterone concentration (ng/ml)		
	G1 ($PGF_2\alpha$ on day 5)	G2 ($PGF_2\alpha$ on day 6)	G3 ($PGF_2\alpha$ on day 7)
Pre-1st GnRH	1.50±0.260^{Cb}	3.12±0.142^{Ca}	0.73±0.012^{Dc}
Post-1st GnRH	3.27±0.311^{Bb}	5.40±0.489^{Ba}	4.13±0.866^{Bab}
Post-PGF2α^{*}	0.45±0.001^{Da}	0.47±0.002^{Ea}	0.45±0.001^{Da}
Post-2nd GnRH	1.00±0.581^{CDa}	1.68±0.383^{Da}	1.14±0.218^{Ca}
Post-mating (21-24 d)	4.13±0.017^{Ab}	7.58±0.443^{Aa}	6.56±0.422^{Aa}

a, b and c: Means within the same row with different superscripts are significantly different at P<0.05
a, b,…and e: Means within the same column with different superscripts are significantly different at P<0.05. * Pre-2nd GnRH.

nearly the synchronization of reproductive status of ewes in the three groups (Table 5). Similar trend was observed by Beck *et al.* (1996), who showed that treatment with GnRH resulted in higher plasma P_4 concentration.

P_4 levels were decreased (P<0.05) to the minimal values Post-$PGF_2\alpha$ injection in all treated groups, being less than 0.5 ng/ml without significant differences among groups. Such reduction may indicate higher response to $PGF_2\alpha$ injection in term of CLs regression induced after ovulation by the 1st GnRH injection. Post-2nd GnRH injection, P_4 levels showed again significant increase in all groups (P<0.05). This elevation in P_4 level was associated with the initiation of new CLs as affected by 2nd GnRH injection, but differences in P_4 levels among the three groups were not significant (Table 5).

The pulsatile or tonic mode of LH release is generated in response to pulsatile GnRH release from the hypothalamus (Levine *et al.,* 1982). Pulsatile LH release prevails at all reproductive status in ewes, including the periods before, during and after the preovulatory surge of gonadotropins (Rawlings and Cook, 1993), and it is also present in ovariectomized ewes. Therefore, the marked increase in P4concentration in ewes of all treated groups following GnRH administration could be due to the sudden release of LH, leading to ovulation or luteinization of dominant follicles of the present wave (Örsan *et al.*, 2007).

On days 21-24 post-mating, P4 level was significantly higher (P<0.05) in G2 (7.58 ng/ml) and G3 (6.56 ng/ml) than in G1 (4.13 ng/ml) indicating the pregnancy incidence in each group (Table 5).

Since Ovsynch protocol is now applicable for cows, studying the implementation of this protocol for ewes is valuable. Based on the foregoing results, using GnRH-PGF2α-GnRH (GnRH, 0 d; PGF2α 5 d later and GnRH 48 h later) protocol can be used for synchronization of ovulation during the breeding season to reduce service period and lambing intervals of ewes in large flocks.

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الملخص العربى

تأثير تنظيم التبويض بإستخدامالجونادوتروفينوالبروستاجلاندين على النشاط المبيضى والصفات التناسلية فى النعاج الرحمانى

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إستخدمت 30 نعجة رحمانى لدراسة تأثير تنظيم التبويض بإستخدام(GnRH+PGF2α) على الاداء التناسلى فى النعاج. قسمت النعاج الى ثلاثة مجموعات متماثلة: ج1 وج2 و ج3 (مجموعات معاملة). حقنت كل النعاج المعاملة فى العضل فى اليوم (صفر) بـ 1 مل جونادوتروفين (رسبتال) اعقبت بالحقن بـ 0.7 مل بروستاجلاندين (استروميت) فى اليوم 5 (ج1) ، 6 (ج2) ، 7 (ج3). تم الحقن بالجرعة الثانية من الجونادوتروفين 1مل اعطيت فى اليوم 7 (ج1) ، 8 (ج2) ، 9 (ج3) وتم التلقيح صناعيا للمجموعات الثلاثة بعد 24 ساعة من اخر حقنة جونادوتروفين.

اوضحت النتائج ان نعجة واحدة من 10 نعاج اظهرت مظاهر الشياع فى المجموعة الاولى مقارنة بـ 3 نعاج من 10 نعاج فى كلا من المجموعتين الثانية والثالثة. نعاج المجموعة الاولى اظهرت اعلى معدل ولادة (60%) مقارنة بالمجموعتين الثانية (50%) والثالثة (40%). كان معدل التوائم ومقياس الخصوبة عاليا فى المجموعة الاولى (1.67 مولود و100%) وبفروق معنوية عن المجموعة الثانية (1.40 مولود و70%) وكانت اقلها المجموعة الثالثة (1.25 مولود و50%). زاد مستوى هرمون البروجسترون معنويا فى جميع النعاج المعاملة تحت تأثير الحقن بالجونادوتروفين اول مرة ثم تناقص فى جميع المعاملات بعد الحقن بالبروستاجلاندين. بعد الحقنة الثانية من الجونادوتروفين زاد تركيز هرمون البروجستيرون فى كل المعاملات. فى اليوم 21-24 يوم وبعد التلقيح زادت قيم هرمون البروجستيرون فى جميع المعاملات.

أوضحت النتائج المتحصل عليها ، أن استخدام نظام GnRH-$PGF_{2\alpha}$-GnRH (اليوم صفر جونادوتروفين ، اليوم الخامس بروستاجلاندين ، حقن جونادوتروفين بعد 48 ساعة) خلال موسم التناسل (يناير) يمكن استخدامه لتنظيم التبويض وتقليل فترات التلقيح والولادة فى النعاج الرحمانى.