RAM EFFECT ON ESTRUS BEHAVIOR, OVARIAN STRUCTURE AND STEROID HORMONE LEVELS IN OSSIMI EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ FOR ESTRUS SYNCHRONIZATION

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SUMMARY

The objective of this study was to assess presence of ram with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on estrus behavior, patterns of follicular growth and steroid hormone production of Ossimi ewes in subtropics. Ewes (n= 20), were isolated from rams 30 days and thereafter, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was used for synchronization by two injections 10 days apart. Ewes were randomly divided into two equal groups (n=10). The first group, $PGF_{2\alpha}$ with ram effect (PGRE), ewes were exposed to two trained teaser rams at the time of the second $PGF_{2\alpha}$ injection until the end of the experiment, whilst the second group (PG), ewes were isolated from rams except at the time for estrus detection. Estrus behavior and time of ovulation were detected after the second prostaglandin injection by noting the responses of the ewes to the male in the pen and by ultrasonography. The number and size of all follicles ≥2 mm and corpus luteum (CL) were monitored by ultrasonography. Blood samples were collected for measuring estradiol-17β (E_2) and progesterone (P_4) hormones. Onset of estrus, time of ovulation and estrous cycle length were significantly shorter ($P<0.05$) in PGRE group compared with PG group. The number of preovulatory follicles and the mean size of ovulatory follicles were higher ($P<0.05$) in PGRE group compared with those in PG group. Moreover, the mean ovulation rate was higher ($P<0.05$) in PGRE group than that in PG group. In addition, the production of P_4 was greater ($P<0.05$) in PGRE group on days 10 and 14 of the estrous cycle compared to the ewes in PG group. While, concentrations of the P_4 were increased numerically in PGRE group during 0, 2, 6 and 8 d of the estrous cycle. E_2 production was almost similar in both groups.

In conclusion, the presence of rams combined with $PGF_{2\alpha}$ treatment, hastens estrus onset, decreased the time of ovulation, increased the ovulation rate and ovarian activity of Ossimi ewes in subtropical areas.

Keywords: Ram effect, estrus behavior, ovarian structure, steroid hormones

INTRODUCTION

Estrus synchronization of ewes is the main element of the reproductive management in sheep breeding. Two injections of prostaglandin ($PGF_{2\alpha}$) are widely used for estrus synchronization in cyclic ewes and control of the reproduction manages in the breeding season (Fierro *et al.*, 2013). The estrus synchronization by $PGF_{2\alpha}$ is a relatively low cost, good, quickly and almost the ewes at the time of the second dose of $PGF_{2\alpha}$ in mid luteal phase and become in estrus (Abecia *et al.*, 2012).

The sudden exposure of rams to seasonal anestrous ewes after period of isolated (phenomenon, ram effect), induces behavior of estrus, ovulations and parturition (Martin *et al.*, 1986; Ungerfeld *et al.*, 2004 and Delgadillo *et al.*, 2009). The release of LH surge in anestrous ewes appears to be due to the presence of ram and resulted in ovulation (Hawken and Martin, 2012; Jorre de St. Jorre *et al.*, 2014; Fabre-Nys *et al.*, 2016 and Ferreira-Silva *et al.*, 2017_{ab}). Also, the LH surge was released after introduction of the ram to cyclic ewes during breeding season of all stages of the estrous cycle (Hawken *et al.*, 2007). The continuous presence of rams during the follicular phase increases the LH surge and hastens the onset of estrus (Fletcher and Lindsay, 1971; Lindsay *et al.*, 1975; Ungerfeld and Rubianes, 1999 and Contreras-Solis *et al.*, 2009). Additionally, the ram effect becomes widespread use in the 'Mediterranean' genotypes (Folch, 1990; Rosa and Bryant, 2002 and Ungerfeld *et al.*, 2004). Although the ram effect widely used in ewes of anestrous season, however, its possible applications in cyclic ewes have scarcely studied. Thus, the $PGF_{2\alpha}$ with presence of the ram may be a useful tool for flock management. Thus, the objective of the present study was to investigate the ram effect combined with $PGF_{2\alpha}$ estrus synchronization on estrus behavior, patterns of follicular growth and estradiol-17β (E_2) and progesterone (P_4) hormones production of Ossimi ewes in subtropics.

MATERIALS AND METHODS

Animals and experimental design:

The trial was conducted on the experimental farm of Agriculture Faculty, Assiut University, Assiut, Egypt. Twenty multiparous Ossimi ewes (4–5 years old and average body weight 46.1±1.3 kg),

raised in semi-open pens were used for this experiment. The experiment conducted during autumn season (September-October). Experimental diet used for feeding the ewes was formulated according to NRC (1985). Water and trace mineral salt were available all days.

All ewes were isolated from males for 30 days (Ferreira-Silva*et al.*, 2018) and thereafter were synchronized using two intramuscular injections of 250 µg/mL of $PGF_{2\alpha}$ analog (cloprostenol) 10 days apart. Ewes were randomly divided into two equal groups, 10 ewes each, balanced for body weight and parity. The first group, ewes were injected with $PGF_{2\alpha}$ in association with ram effect (PGRE). Ewes were exposed to two trained teaser rams at the timing of the second $PGF_{2\alpha}$ injection (Day 0 for the trial) until the end of the experiment. While, the second group (PG) ewes were isolated from rams after the second $PGF_{2\alpha}$ injection except at the time where rams were introduced for estrus detection.

Estrus observation:

Estrus behavior was detected twice daily (8.0 a.m. and 4.0 p.m.) after the second prostaglandin injection by noting the responses of the ewes to the male in the pen using trained teaser rams and ultrasonography was carried out daily to monitor the occurrence of ovulation.

The onset of estrus (interval from the second $PGF_{2\alpha}$ injection to the first time ewes were receptive to the males). The estrous cycle length was determined as the interval between two successive heats or ovulations.

Ultrasound monitoring of ovarian structures:

From one day prior the second $PGF_{2\alpha}$ dose, all follicles number and size ≥ 2 mm were monitored daily by transrectal ultrasound scanning (Pie Medical, 100 LC, Holland) having a 6–8 MHz end of rectal probe (Lv513). The probe was turn in the rectum 90◦ clockwise and 180◦ anticlockwise for ovaries scanning in the standing ewe. The number and diameter of larger follicles were detected and evaluation by sketches that given the size for each follicle. The ovulation rate was recorded, when the largest follicles (> 5 mm) identified and followed without constant observation that considered the ovulation have occurred. The CL was examined and an image of the maximum diameter from the largest cross-sectional area was estimated.

Blood sampling and hormonal analysis:

After the second $PGF_{2\alpha}$ dose, blood samples were collected daily from jugular vein during the days of the estrous cycle before feeding on the morning. Samples of blood were centrifuged for 20 minutes at 2000 × g, thereafter, serum was harvested and stored at – 20 ^{0}C. Progesterone and Estradiol-17β hormonal levels were executed by ELISA technique. Laboratory Diagnostic System Co. (Catalogue No. 3900, DSL, USA) gave Kits. The variations coefficients of the intra- and inter assay were 3.6% and 12.43% for progesterone and 4.8% and 9.2%, for Estradiol-17β, respectively. Assay sensitivity was 0.12 ng for progesterone and 2 pg for Estradiol-17β.

Statistical analysis:

Statistical analyses were done using SPSS package (2007). The mean differences between the two groups regarding the estrus onset, ovulation time, the number and size of the ovulatory follicles, length of the estrous cycle, E_2 and P_4 hormones were estimated by independent t-test (Senosy *et al.*, 2017). Values of probability less than 0.05 were considered significant. The results were expressed as means ± SEM.

RESULTS AND DISCUSSION

Estrus behavior:

The results in terms of estrus characters are presented in Table 1. Estrus was detected in all ewes. Similarly, in previous studies Contreras-Solis *et al.* (2009) and Abecia *et al.* (2012) found that, ewes responded to injection of the second $PGF_{2\alpha}$ and showed luteolysis and detected in estrus. Abecia *et al.* (2012) reported that, in tropical breeds, synchronization of estrus required two doses of $PGF_{2\alpha}$ 10 days apart, whereas, most females will be in the mid-luteal phase and will be detected in estrus and occurrence of ovulation.

The onset of heat (41.1±2.4 h) and time of ovulation (72.6±2.2 h) in PGRE group were shorter ($P<0.05$) than in PG group (60.0±2.6 and 96.0±2.5 h), respectively. Also, the estrous cycle length (17.4±0.2) was shorter ($P<0.05$) in PGRE group compared with the PG group (20.3±0.6). This agrees with the results of Hawken *et al.* (2007) who mentioned that, exposure of cyclic ewes to rams stimulated their response in the breeding season. The rams continuous presence during the follicular phase, increased the LH surge and hasten onset of estrus (Fletcher and Lindsay, 1971; Lindsay *et al.*, 1975; Ungerfeld and Rubianes, 1999 and Contreras-Solis *et al.*, 2009). Furthermore, induced preovulatory LH surge, estrus onset and ovulation time were more accelerated and their occurrence was earlier in ewes when the rams were introduced at the injection timing of the second $PGF_{2\alpha}$ compared with the use of two $PGF_{2\alpha}$ injections alone (Contreras-Solis *et al.*, 2009; Ungerfeld, 2011 and Meilán and Ungerfeld, 2014). Similarly, in goats, the continuous presence of a male during the breeding season with estrus synchronized females using either progestogens sponges or $PGF_{2\alpha}$ and controlled internal drug release (CIDR), hastened onset of estrus, reduced estrus duration and time of ovulation compared with females not exposed to a male (Romano, 1998 and Romano *et al.*, 2016).

Table 1. Onset of estrus, time of ovulation and estrous cycle length in ewes synchronized by prostaglandin ($PGF_{2\alpha}$) with or without ram effect

Group	Onset of estrus (h)	Ovulation time (h)	Estrous cycle length (d)
PGRE	41.1±2.4[a]	72.6±2.2[a]	17.4±0.2[a]
PG	60.0±2.6[b]	96.0±2.5[b]	20.3±0.6[b]

PGRE; $PGF_{2\alpha}$ with ram effect, ewes were exposed to two trained teaser rams at the timing of the second $PGF_{2\alpha}$ injection (Day 0 of the trial) until the end of the experiment. PG: ewes were isolated from rams after the second $PGF_{2\alpha}$ injection except at the time for estrus detection.
[a–b] Means within the same column differed significantly ($P< 0.05$).

Follicular growth:

The patterns of follicular development are shown in Table 2. The number of preovulatory follicles (4.8±0.1) and the mean size of the ovulatory follicles (6.01±0.0 mm) were higher (P<0.05) in PGRE group than in PG group (3.9±0.1 and 5.20±0.0 mm), respectively. The ovulation rate in PGRE group was higher (P< 0.05) than that in PG group (1.40±0.2 *vs* 1.00±0). These results are in agreement with those of Rubianes *et al*. (1997) and Ferreira-Silva *et al*. (2018) who indicated that, the number and size of the larger follicles and ovulatory follicles were increased after introduction of the rams to ewes. Moreover, FSH level and LH surge increased in ewes as an endocrine response to the presence of the ram (Ungerfeld *et al*., 2000 and Ungerfeld, 2003). The continuous presence of rams with ewes increased LH surge and enhanced the ovarian activity (Murtagh *et al*., 1984 and Folch, 1990). However, the LH pulsatility was the main effect on the growth of preovulatory follicular and it coincided with the increase in the large follicles number and largest follicle diameter (Baird and McNeilly, 1981 and Ungerfeld, 2003). Preovulatory LH peaks occurred in ewes, due to the presence of rams that acts upon this role of the growth of the ovarian follicles (Ferreira-Silva *et al*., 2017_{ab} and Ferreira-Silva *et al*., 2018).

Table 2. Characteristics of the follicular populations and corpus luteum (CL) in ewes synchronized by prostaglandin ($PGF_{2\alpha}$) with or without ram effect

Group	Follicles ≤5 mm	Ovulatory follicles (mm)	Ovulation rate	CL diameter (cm)
PGRE	4.8±0.1[a]	6.01±0.0[a]	1.40±0.2[a]	1.24±0.0
PG	3.9±0.1[b]	5.20±0.0[b]	1.00±0.0[b]	1.10±0.0

PGRE; $PGF_{2\alpha}$ with ram effect, ewes were exposed to two trained teaser rams at the timing of the second $PGF_{2\alpha}$ injection (Day 0 of the trial) until the end of the experiment. PG: remaining ewes were isolated from rams after the second $PGF_{2\alpha}$ injection except at the time for estrus detection.
[a–b] Means within the same column differed significantly ($P < 0.05$).

Hormonal profile:

Concentration of $P_4$was higher (P <0.05) in PGRE group on days 10 and 14 of the estrous cycle (5.3±0.4 and 4.6±0.4 ng/mL) compared to the PG group (3.9±0.0 and 3.2±0.2 ng/mL), respectively. Moreover, concentrations of P_4 increased numerically in PGRE group during 0, 2, 6 and 8 d of the estrous cycle (0.2±0.0, 1.1±0.6, 2.4±0.5 and 3.3±0.5 ng/ml) than those of the PG group (0.1±0.0, 0.8±0.5, 1.83±0.92 and 2.7±0.2 ng/ml), respectively (Fig. 1). These results are in agreement with those of Scaramuzzi *et al*. (1993) and Godfrey *et al*. (1999) who indicated that, P_4 level was decreased to below 1 ng/mL in estrus period and it decreased after the second injection of $PGF_{2\alpha}$ (Ganaie *et al*., 2009). In addition, Homeida *et al*. (2009) documented that, P_4 concentrations on days 2, 8, 10 and 14 were 0.4±0.1, 1.1±0.3, 4.3±0.3 and 2.3±0.2, ng/ml, respectively in ewes after the second $PGF_{2\alpha}$ injection. These results may be due to the continuous presence of rams after the second $PGF_{2\alpha}$ injection. Its influences were on ovarian follicle development and the life span of the resulting CL. Introduced rams to ewes, led to increased LH and FSH secretion these in turn led to increased follicular size and formation of CL with higher production of P_4 (Ferreira-Silva *et al*., 2018). These notices point out to the importance of follicular growth for P_4 production in the following CL formation. The production and amount of P_4 was connected to the health and number of the granulosa and thecal cells *in vitro* (Niswender *et al*., 2000 and Niswender, 2002). The initiation of P_4 was increased significantly of ewes in estrus after introduction of the rams (Ungerfeld, 2003).

Furthermore, the differences in E_2 in all studied days during the estrous cycle were not significant in both groups (Fig. 1). These results may be due to the high levels of P_4 concentration during days of the estrous cycle, which affected on E_2 by negative feedback mechanism (Lassoued *et al*., 1997). In addition, it is known that, the LH surge increased in ewes, due to the prime step of ram presence with ewes, this caused an increase in preovulatory LH surge and occurrence of ovulation. The real

relationship between E_2 and LH at this time is not well established whereas, in the absence of E_2, the ram introduction can increase LH in ewes. The pulsatility of LH was increased in ovariectomized ewes although the E_2 absence (Rosa and Bryant, 2002).

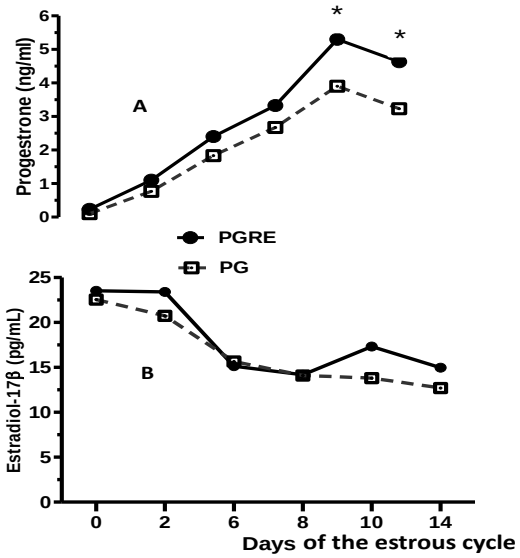

Fig. 1. Changes in serum progesterone (P_4; A) and estradiol-17β (E_2; B) concentrations in the presence or absence of ram effect. PGRE; $PGF_{2\alpha}$ with ram effect, ewes were exposed to two trained teaser rams at the timing of the second $PGF_{2\alpha}$ injection (Day 0 of the trail) until the end of the experiment. PG: ewes were isolated from rams after the injection of the second $PGF_{2\alpha}$.
* Means: The difference between the two groups was significant ($P< 0.05$).

CONCLUSION

In conclusion, the present results indicate that, the ram presence combined with $PGF_{2\alpha}$ two injections10 days apart, hasten estrus onset, decreased the time of ovulation, increased the ovulation rate and ovarian activity of Ossimi ewes in subtropical areas.

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تأثير وجود الكبش على السلوك الشبقى والتراكيب المبيضيه ومستويات الهرمونات الأستيروديه فى النعاج الأوسيمى المعامله بالبروستاجلاندين ($PGF_{2\alpha}$) لتزامن الشبق

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أجريت هذه الدراسه على عشرين من النعاج الأوسيمى منتظمة دورة الشبق بهدف دراسة تأثير وجود الذكر مع برنامج تزامن الشياع بإستخدام $PGF_{2\alpha}$ على صفات وخصائص الشياع (بداية ظهور الشياع ، الوقت اللازم للتبويض وطول دورة الشبق)، نمو وتطور الحويصلات المبيضيه ومستوى هرمون الإستراديول والبروجستيرون أثناء أيام دورة الشبق.

تم عزل النعاج عن الذكور قبل إجراء التجربه بمده لا تقل عن ٣٠ يوماً وبعد ذلك تم تنفيذ برنامج تزامن الشياع بإستخدام $PGF_{2\alpha}$ الحقن مرتين المده بينهما ١٠ أيام. ثم تم تقسيم الأغنام عشوائيا إلى مجموعتين متساويتين (n=10)، المجموعه الأولى (PGRE) ويتم فيها وجود الذكور الكشافه بعد الحقنه الثانية من البروستاجلاندين حتى نهاية التجربه، بينما المجموعه الثانيه (PG) كانت النعاج منعزله عن الذكور ما عدا الوقت اللازم لإكتشاف الشياع. وتم تحديد بداية ظهور علامات الشياع ووقت التبويض بعد الحقنه الثانية من البروستاجلاندين بملاحظة إستجابة النعاج للكباش الكشافه وبإستخدام الموجات فوق الصوتيه (السونار). وأثناء التجربه تم متابعة نمو وتطور الحويصلات المبيضيه وظهور الجسم الأصفر وأخذ قياساته بالموجات فوق الصوتيه. وتم تجميع عينات الدم أثناء أيام دورة الشبق لتقدير هرمونى الأستراديول والبروجستيرون.

أظهرت النتائج المتحصل عليها أن بداية ظهور علامات الشياع ، الوقت اللازم للتبويض وطول دورة الشبق فى المجموعه الأولى (PGRE) والتى تم فيها وجود الذكور الكشافه بعد الحقنه الثانية من البروستاجلاندين أقصر ($P<0.05$) مقارنه بنعاج المجموعه الثانيه (PG) والتى كانت فيها النعاج منعزله عن الذكور ما عدا وقت إكتشاف الشياع لها. كما أظهرت النتائج أن متوسط العدد الكلى من الحويصلات المبيضبه الناميه ومتوسط قطر حويصلات التبويض زادت زياده معنويه ($P<0.05$) فى المجموعه الأولى (PGRE) مقارنه بنعاج المجموعه الثانيه (PG). بالإضافه إلى أن معدل التبويض أثناء التجربه كان أكبر ($P<0.05$) فى نعاج المجموعه الأولى (PGRE). كما وجد أن متوسط تركيز هرمون البروجستيرون فى الأيام ١٠ و ١٤ من دورة الشبق زاد زياده معنويه ($P<0.05$) فى نعاج المجموعه الأولى (PGRE) وزاد زياده رقميه فى أيام ٠، ٢، ٦، ٨ من دورة الشبق مقارنه بنعاج المجموعه الثانيه (PG). ولم يوجد تأثير معنوى لمستويات هرمون الأستراديول بين المجموعتين أثناء أيام دورة الشبق.

وخلصت نتائج هذه التجربه أن وجود الذكر مع برنامج تزامن الشياع بإستخدام $PGF_{2\alpha}$ الحقن مرتين المده بينهما ١٠ أيام أدى الى الإسراع من حدوث الشياع ، تقليل الوقت اللازم للتبويض مع زيادة معدل التبويض وزيادة النشاط المبيضى للأغنام الأوسيمى فى المناطق شبه الحاره.