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Effect of synchronizing estrus with intravaginal progestagen sponges or prostaglandin $F_{2\alpha}$ on estrus behavior, ovarian structures, estradiol-17β and progesterone levels of Ossimi ewes under subtropics.

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ABSTRACT

This study was designed to determine the effect of estrus synchronization by either intravaginal progestagen sponges or prostaglandin $F_{2\alpha}$ on estrus behavior, follicular growth patterns and concentrations of estradiol-17β (E_2) and progesterone (P_4) hormones in blood of Ossimi ewes. A total of 14 Ossimi ewes were randomly divided into two groups, 7 ewes each, balanced for body weight and parity. The first group, G1 synchronized by intravaginal progestagen impregnated sponges (40-mg fluorogestone acetate) for 14 days. While, the second group, G2, was synchronized by injecting two doses of 12.5 mg $PGF_{2\alpha}$ (Dinoprost) 10 days apart. Estrus was observed after removing the vaginal sponges in G1 or after the 2nd $PGF_{2\alpha}$ dose using two trained teaser rams and the ovaries were examined using ultrasonography technique to detect follicles ≥2 mm and corpus luteum (CL). Blood samples were collected via jugular vein to determine E_2 and P_4 concentrations in peripheral blood.

Estrus displaying time after the end of treatment, duration, ovulation time and estrous cycle length were significantly shorter ($P<0.05$) in G1 than G2. Moreover, diameter of ovulatory follicles and CL were larger in G1 ($p<0.05$). Ewes in G2 showed higher ($P<0.05$) number of preovulatory follicles than G1.Ovulation rate was similar in the two groups. E_2 level was higher ($P<0.05$) in G1 during day 0 and P_4 level during days 10 and 14 of the estrous cycle than that in G2.

In conclusion, ewes synchronized by intravaginal progestagen sponges improved estrus expression and ovulation time, in addition to that the estrus duration were shorter. The diameter of ovulatory follicles and CL were larger but the number of the preovulatory follicles was less and not affected on ovulation rate when compared with ewes synchronized by $PGF_{2\alpha}$.

Keywords: intravaginal sponges, $PGF_{2\alpha}$, estrus behavior, ovarian structure, E_2, P_4 levels

INTRODUCTION

Application of modern sheep management and estrus synchronization technique, via controlling lambing period, under intensive production system could increase the efficiency of farm productivity (Lindsay, 1991 and Ozyurtlu *et al.*, 2010). Estrus synchronization also is required for superovulation and embryo transfer (Menchaca and Rubianes, 2004 and Vilariño *et al.*, 2017).

Many protocols used for estrus synchronization, one of them is using intra-vaginal sponges containing progesterone or its analogues progestagen for 12–14 days in ewes, which widely used during breeding or non-breeding season for estrus synchronization of sheep (Garcıa-Palencia *et al.*, 2007; Abecia *et al.*, 2011; Gatti and Ungerfeld, 2012 and Oliveira *et al.*, 2016). There was abundant literature report about the sufficient levels of progestagen required for the ovarian follicles growth and life span of the corpus luteum in females (Bartlewski *et al.*, 2000, 2001; Niswender *et al.*, 2000 and Husein and Ababneh, 2008). The progestagen sponges treatment is basically affect release of gonadotropin hormone and diminish LH secretion, thus control follicles growth and responses to gonadotropins which required for the corpus luteum development (Martinez-Garcia *et al.*, 2007). However, treated ewes may display lower conception rates (Evans *et al.*, 2001 and Evans, 2003) due to either the

abnormalities of sperm viability and transport in female reproductive tract or impaired embryo growth and development (Gonz´alez-Bulnes *et al.,* 2005 and Berlinguer *et al.,* 2007).

Another method for estrus synchronization based on using luteolytic agents (prostaglandin $F_{2\alpha}$). It is clean product and safety for animals, humans and the environment (Macrı *et al.,* 2006). Moreover, for estrus synchronization of climate breeds, two injections of $PGF_{2\alpha}$ is required, whereas almost the female during the mid-luteal phase and will be in estrus by ovulation (Abecia *et al.,* 2012). The objective of this study was to compare estrus behavior, follicular growth, estradiol and progesterone concentrations after estrus synchronized by either intravaginal progestagens sponges or $PGF_{2\alpha}$ under subtropical condition.

MATERIALS AND METHODS

Animals and experimental design:

This experiment was carried out on the experimental farm of Faculty of Agriculture, Assiut University during July and August. A total number of 14 Ossimi ewes (4–5 years old) weighed 45.5±1.5 kg, multiparous were used in this study. Ewes randomly divided into two groups, 7 ewes each, balanced for body weight and parity. Ewes in the first group (G1), were treated for estrus synchronization by intra-vaginal sponges enriched with 40-mg fluorogestone acetate (Intervet International, Chronogest, Boxmeer, Netherlands) for 14 days. While, ewes in the second group (G2), were treated for estrus synchronization with $PGF_{2\alpha}$, by two intramuscular successive injections (12.5 mg Dinoprost; Lutalyse (Pfizer Manufacturing, Purts, Belgium), 10 days apart. The second $PGF_{2\alpha}$ injection was at the same time of intra-vaginal sponges withdrawal of the first group.

The experimental ewes were raised in semi-open yards and fed mainly the concentrate mixture according to the NRC (1985) for sheep. The concentrate contained 11.43% crude protein, 2.79 Mcal/kg, 11.55% crude fiber, 1.88% crude fat, 0.6% calcium and 0.43% phosphorous. Beside the concentrate feed mixture, ewes fed on wheat straw *ad libtum*. Water and trace mineral salt were made available all day-time.

Estrus observation:

Estrus behavior was checked twice daily (at 8:0 am and 4:0 pm) after the end of each treatment using two trained teaser rams by keeping them together with ewes of each group for one hour and ultrasonography was conducted daily until incidence of ovulation.

Estrus onset (the time from end of treatment to the first time ewes accepted ram), duration of estrus (the period from onset of estrus to last time ewes were receptive to the male). Length of estrous cycle (the interval between two consecutive estrus or ovulation) were recorded.

Monitoring of follicular development:

A day prior the vaginal sponges withdrawal or the second $PGF_{2\alpha}$ injection, the diameter and number of all follicles ≥2 mm were observed daily by Trans-rectal ultrasound scanning (Holland, Pie Medical and 100 LC) having a 6 to 8 MHz linear transducer. The probe was turn in the rectum 90◦ clockwise and 180◦ anticlockwise for ovaries scanning in the standing ewe. The number and diameter of the largest follicles were detected and evaluated by sketches that gave the alteration in diameter of each follicle. When the dominant follicle (> 5 mm) was identified, its disappearance considered as indicator for ovulation occurrence, which supported also by detection of the new CL.

Blood sampling and steroid hormones analyses:

After the vaginal sponges withdrawal and the second $PGF_{2\alpha}$ injection, daily blood samples were collected via the jugular vein, before feeding and watering at the morning. Samples of blood were centrifuged for 20 minutes at 2000 × g then serum was harvested and stored at –20 ˚C. Both P_4 and E_2 concentrations were measured using direct ELISA technique, using kits from Laboratory Diagnostic System Co. (Catalogue No. 3900, DSL, USA). The variations coefficients of the intra- and interassay were 3.6% and 12.43% for progesterone and 4.8% and 9.2%, for Estradiol-17β, respectively. The

assay sensitivity for progesterone was 0.12 ng and for Estradiol-17β was 2 pg.

Statistical analysis:

Statistical analyses were done by SPSS (2007). The mean variation between the two treatments concerning the time to estrus onset, estrus duration, ovulation time, the number and diameter of the ovarian follicles, estrous cycle length and E_2 and P_4 concentrations were estimated by independent t-test. Values of probability less than 5% reflect significance. Results expressed as means ± SE.

RESULTS AND DISCUSSION

Estrus behavior:

All treated ewes in G1 and G2 displayed estrus by the end of treatment. The onset of estrus (55.20±5.60 h) and time of ovulation (80.20±0.20 h) in G1 were significantly shorter (P<0.05) compared with ewes in G2 (68.4±2.05 and 104±0.34 h, respectively) (Table 1). The result in the present study is similar to findings of Godfrey *et al.* (1997) that, the estrus onset in tropical ewes synchronized with double injections of $PGF_{2\alpha}$ 10 d apart was 69.6±9.6 h. Also, most of the ewes showed estrus between 48 to 72 h after the second $PGF_{2\alpha}$ injection (Fierro *et al.*, 2016) and it was within 2 to 3 d after vaginal sponges removal (Killian *et al.*, 1985 and Koyuncu and Ozis Altıcekic, 2010). Moreover, Wheaton *et al.* (1993) reported that, after the vaginal sponges removal, the onset of estrus was 50±2h. However, the time to estrus onset was shorter in ewes synchronized by vaginal sponges than $PGF_{2\alpha}$ (Godfrey *et al.*, 1999). The pituitary endocrine may responded faster in ewes synchronized by vaginal progestagen sponges rather than $PGF_{2\alpha}$ (Martinez-Garcia *et al.*, 2007).

Moreover, estrus duration (36.20±2.74h) and estrous cycle length (17.80±0.58d) were significantly shorter (P<0.05) in G1 compared with that in G2 (48.10±1.60h and 21.36±0.47d, respectively). Similarly, a previous study by Godfrey *et al.* (1999) reported that, time from estrus to ovulation was 34.3±2.8h in ewes synchronized by vaginal sponges. However, onset of estrus and the interval from the $PGF_{2\alpha}$ injection to ovulation were different among ewes. When the second $PGF_{2\alpha}$ injection was applied, during the midluteal phase, progesterone levels declined slowly to subluteal values, thus, symptoms of estrus and ovulation time were delayed (Rubianes *et al.*, 2003 and Contreras-Solís *et al.*, 2009).

Table 1. Estrus onset and duration, ovulation time and estrous cycle length in ewes synchronized by intravaginal progestagen sponges (G1) or prostaglandin ($PGF_{2\alpha}$) (G2).

Group	Onset of estrus (h)	Estrus duration (h)	Ovulation time (h)	Estrous cycle length (d)
G1	55.20±5.60[a]	36.10±2.74[a]	80.20±0.20[a]	17.80±0.58[a]
G2	68.4±2.05[b]	48.10±1.60[b]	104±0.34[b]	21.36±0.47[b]

[a–b] within a same column, means differed significantly ($P < 0.05$).

Follicular dynamics:

The patterns of follicular growth are shown in Table 2. Ewes synchronized with $PGF_{2\alpha}$ (G2) showed more significant (P<0.05) preovulatory follicles number (5.66±0.12) compared with ewes synchronized with vaginal sponges (G1) (4.26±0.26). In contrast, the diameter of the ovulatory follicles (6.01mm) grew during the present study and the diameter of corpus luteum (1.29±0.06 cm) were larger (P<0.05) in G1 than those in G2 (5.30 mm and 1.07±0.01 cm, respectively). These results are in agreement with Fernandez-Moro *et al.* (2008) who indicated that, the number of preovulatory follicles were lower (P<0.05) in does synchronized with vaginal sponges than does synchronized with $PGF_{2\alpha}$. Also, the diameter of largest follicles developed by vaginal sponges showed a larger mean diameter than does with $PGF_{2\alpha}$. Moreover, after the vaginal' sponges

withdrawal from ewes, the mean diameter of follicle was 6 mm at the time of ovulation (Gonz´alez-Bulnes *et al.,* 2005 and Gonzalez-An˜over *et al.,* 2007).

In addition, the recorded mean of ovulation rate was not significantly different between ewes of the two groups (Table, 2). The comparative study of Gonz´alez-Bulnes *et al.* (2005) recorded similar ovulation rate and oocytes/embryos number in ewes synchronized by vaginal progestagen sponges or $PGF_{2\alpha}$. Fernandez-Moro *et al.* (2008) found the same results on female goats.

Table 2. Ovarian follicles, ovulation rate and corpus luteum (CL) in ewes synchronized by intravaginal progestagen sponges (G1) or prostaglandin ($PGF_{2\alpha}$) (G2).

Group	Number of follicles ≤5 mm	Ovulatory follicles diameter (mm)	Ovulation rate	CL diameter (cm)
G1	4.26±0.26[a]	6.01±0.01[a]	1.13±0.1	1.29±0.06[a]
G2	5.66±0.12[b]	5.30±0.01[b]	1.00±00	1.07±0.01[b]

[a–b] within a same column, means differed significantly ($P < 0.05$).

Serum estradiol 17-β and progesterone levels:

Level of P_4 on days 10th and 14th of the estrous cycle in ewes of G1 was significantly higher (7.96±3.03 and 5.10±1.74 ng/mL) compared to that in ewes of G2 (3.23±0.09 and 2.10±0.25 ng/ml), respectively (Fig 1). Moreover, it was not significantly differed on day 0 between the two groups (0.83±0.40 vs. 0.56±0.23 ng/mL), respectively. These results are in agreement with Godfrey *et al.* (1999) who indicted that, P_4 level decreased to below 1 ng/mL in ewes synchronized with $PGF_{2\alpha}$ or vaginal sponges at the estrus time while not differed between the two treatments. Moreover, within 24 h after sponges removal, P_4 concentrations decreased to the basal levels in all ewes while reached the highest level (7.6±0.3 ng/mL) between days 10 and 14 then began to decline within 24 h to reach the lowest value during the estrous cycle (Husein and Kridli 2002). Also, the levels of P_4 in ewes during 2, 10 and 14d after the second injection of $PGF_{2\alpha}$ were 0.4±0.1, 4.3±0.3, and 2.3±0.2 ng/ml, respectively (Homeida *et al.,* 2009). However, the P_4 levels were higher ($P<0.05$) in ewes synchronized with vaginal sponges. This result may be due to that progestagen sponges' treatment basically affect follicle growth which in turn respond to the gonadotropins required for development of the corpus luteum and P_4 production (Martinez-Garcia *et al.,* 2007).

Serum E_2 concentration was significantly higher in G1 at day 0 (40.73±6.28 pg/ml) compared to that in ewes of G2 (23.83±2.79 pg/ml) (Fig 1). E_2 concentrations at days 10th and 14th of estrous cycle were not different between the two groups. These results are in agreement with Campbell *et al.* (1995) who found lower values of E_2 in ewes synchronized by $PGF_{2\alpha}$, Applying the protocol of progestagen vaginal sponges in ewes caused development of the persistent follicle and enhance levels of E_2 within a longer time (Flynn *et al.,* 2000 and Fierro *et al.,* 2016).

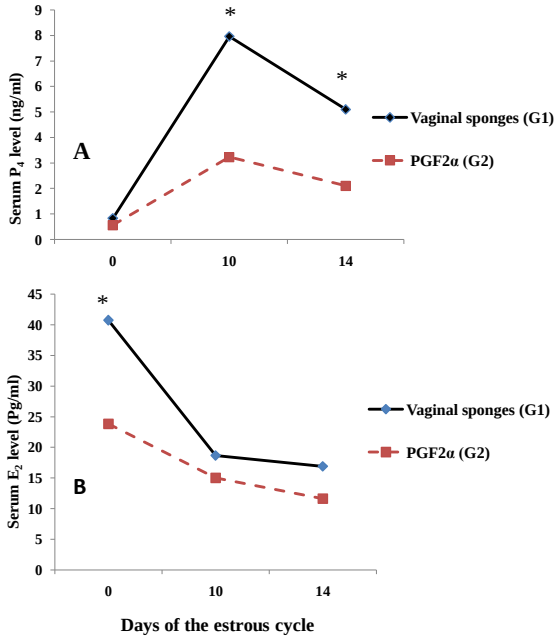

Fig. 1. Changes in serum progesterone (P_4; A) and estradiol-17β (E_2; B) concentrations in ewes synchronized by intravaginal progestagen sponges (G1) or prostaglandin ($PGF_{2\alpha}$) (G2).
* Means: The difference between the two groups was significant ($P< 0.05$).

CONCLUSION

Synchronizing ewes by intravaginal progestagen sponges could improve estrus expression, ovulation time but shorten estrus duration. Diameter of ovulatory follicles and CL were larger but lower in the number of the preovulatory follicles thus lightly affect ovulation rate when compared with ewes synchronized by $PGF_{2\alpha}$.

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السلوك الشبقى والتراكيب المبيضيه ومستويات هرمونى الإستراديول والبروجستيرون فى النعاج الأوسيمى المعامله بالإسفنجات المهبليه أو بالبروستاجلاندين ($PGF_{2\alpha}$) لتزامن الشبق فى المناطق شبه الحاره

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1- قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة أسيوط ، مصر 2- قسم التوليد والتناسليات والتلقيح الإصطناعى ، كلية الطب البيطرى جامعة الوادى الجديد ، مصر

أجريت هذه الدراسه على أربعة عشر من النعاج الأوسيمى منتظمة دورة الشبق بهدف دراسة تأثير برنامج تزامن الشياع بإستخدام الإسفنجات المهبليه أو بإستخدام $PGF_{2\alpha}$ على صفات وخصائص الشياع (بداية ظهور الشياع ، فترة الشياع ، الوقت اللازم للتبويض وطول دورة الشبق) ، نمو وتطور الحويصلات المبيضيه ومستوى هرمونى الإستراديول والبروجستيرون أثناء أيام دورة الشبق.

تم تقسيم النعاج عشوائيا إلى مجموعتين متساويتين (n=7) ومتجانسين من حيث العمر، الوزن وعددالولادات. المجموعه الأولى (G1) تم فيها تنفيذ برنامج تزامن الشياع بإستخدام الإسفنجات المهبليه المعاملة بالبروجستوجين progestagen (40 ملجرام فلورجستون أسيتات، fluorogestone acetate) لمدة 14 يوما. بينما المجموعه الثانيه (G2) تم فيها تنفيذ برنامج تزامن الشياع بإستخدام $PGF_{2\alpha}$ الحقن مرتين 12.5 ملجرام من البروستاجلاندين (dinoprost) المده بينهما 10 أيام. وتم تحديد بداية ظهور علامات الشياع ووقت التبويض بعد نزع اللإسفنجات المهبليه فى المجموعه الأولى وبعد الحقنه الثانية من البروستاجلاندين فى المجموعه الثانيه 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 ، طول فترة الشياع، الوقت اللازم للتبويض وطول دورة الشبق فى المجموعه الأولى (G1) أقصر ($P<0.05$) مقارنه بنعاج المجموعه الثانيه (G2). كما وجد أيضآ أن متوسط قطر حويصلات التبويض والجسم الأصفر زادت زياده معنويه ($P<0.05$) فى المجموعه الأولى(G1) مقارنه بنعاج المجموعه الثانيه (G2). كما أظهرت النتائج أن متوسط العدد الكلى من الحويصلات المبيضبه الناميه زادت زياده معنويه ($P<0.05$) فى المجموعه الثانيه المعامله البروستاجلاندين (G2). ولم يوجد تأثير معنوى عند تنفيذ برنامج تزامن الشياع بإستخدام الإسفنجات المهبليه أو بإستخدام $PGF_{2\alpha}$ على معدل التبويض. كما وجد أن متوسط تركيز هرمون الإستراديول عند وقت التبويض (اليوم صفر من دورة الشبق) وتركيز هرمون البروجستيرون فى الأيام 10 و 14 من دورة الشبق زاد زياده معنويه ($P<0.05$) فى نعاج المجموعه الأولى (G1) مقارنه بنعاج المجموعه الثانيه (G2). وخلصت نتائج هذه التجربه أن النعاج التى تم فيها تنفيذ برنامج تزامن الشياع بإستخدام الإسفنجات المهبليه أدى الى الإسراع من حدوث الشياع ، تقليل فترة الشياع والوقت اللازم للتبويض وزياده قطر حويصلات التبويض والجسم الأصفر وإنخفاض فى عدد الحويصلات المبيضيه الناميه مع عدم التأثير على معدل التبويض مقارنه بالنعاج التى تم فيها تنفيذ برنامج تزامن الشياع بإستخدام البروستاجلاندين.