Egyptian J. Anim. Prod. (2014) 51(2):89-97

SEXUAL ACTIVITY AND OVARIAN STEROIDOGENIC CAPACITY OF LACTATING AND NON-LACTATING BARKI EWES SYNCHRONIZED FOR ESTRUS DURING SEASONAL ANESTRUS UNDER SUBTROPICAL CONDITIONS

N.M. Hashem

***Animal Production Department, Faculty of Agriculture (El-Shatby) Alexandria University, Alexandria 21545, Egypt, E-mail address*: hashemnesreen@yahoo.com**

SUMMARY

This study was designed to identify the sexual activity and ovarian steroidogenic capacity of lactating ewes during periods of early and mid-lactations compared to non-lactating ewes through the anestrous season under subtropical conditions. Occurrence of uterine involution and resumption of ovarian activity of lactating ewes (n=10) were recorded and confirmed on days 15 and 30 postpartum (PP) using ultrasonography. Both lactating ewes (during early lactation: 40-60 days PP and mid-lactation: 70-90 days PP) and non-lactating ewes (n=10) were subjected to estrus synchronization using a double intramuscular injection of prostaglandin $F_{2\alpha}$ (125 µg of cloprostenol/head), 11 days apart. Blood samples were collected on day 2 (follicular phase) and day 9 (luteal phase) following the second $PGF_{2\alpha}$ injection. Serum concentrations of progesterone (P_4), estradiol (E_2) and their ratios were evaluated as well as concentrations of insulin and some blood metabolites. Ultrasonic examinations revealed that by day 30 PP lactating ewes had mean uterine horn diameters and ovarian structures in line to those of non-lactating ewes. During the experimental period, regardless of the physiological status (lactating or non-lactating) most females failed ($P>0.05$) to exert signs of estrus (100% in early lactation, 80% in mid-lactation and 60% in non-lactating ewes). However, 70% of the ewes in early lactation, 70% of the ewes in mid-lactation and 100% of the non-lactating ewes had corpora lutea (CLs) in the luteal phase of the synchronized estrus. Concentrations of P_4 and E_2 and P_4/E_2 ratios did not significantly differ between both follicular and luteal phases of the synchronized estrous cycles either in lactating (early lactation or mid-lactation) or non-lactating ewes. Lactating ewes in both early and mid-lactation periods had lower ($P<0.05$) concentrations of circulating blood glucose, triglycerides, total protein and albumin compared with non-lactating ewes. Also, the lowest ($P < 0.05$) overall means of P_4 and E_2 concentrations were recorded for ewes in mid-lactation, followed by ewes in early lactation and non-lactating ewes. Concentrations of serum P_4 and E_2 were positively correlated ($P < 0.05$) with each of body weight ($r = 0.3$ for both correlations), concentrations of serum glucose ($r = 0.3$, $P < 0.05$ and $r = 0.4$, $P < 0.01$; respectively) and concentrations of serum triglycerides ($r = 0.4$, $P < 0.05$ and $r = 0.6$, $P < 0.01$; respectively).

Results of the present study revealed that both lactating and non-lactating ewes during seasonal anestrus displayed similar pattern of sexual activity and hormonal disturbances as reflected by unchanged P_4/E_2 ratios on day 2 (follicular phase) and day 9 (luteal phase) of the synchronized estrous cycle, suggesting presence of a persistent corpus luteum in a high proportion of females. This ovarian dysfunction seems to be related to seasonal effects more likely than lactational effects. However, lactation presents an additional load on the reproductive functions by inducing symptoms of negative energy balance (lower body weight and poorer metabolic status).

Keywords: Ewes, seasonality, lactation, persistent CL, sexual activity, subtropics

INTRODUCTION

The profitability of the sheep industry could be maximized by applying accelerated lamb production system. The success of this system depends on the females' ability to breed successfully through an extended period of the year. On the other hand, many sheep breeds exert high varieties in their breeding ability ascribing to many environmental and physiological conditions which presents a great challenge to obtain frequent lambings throughout the year. Under subtropics, female sheep exert a seasonal pattern of reproduction, where maximum sexual activity falls during summer and autumn seasons (Abu-Zanat *et al*., 2005 and Tabbaa *et al*., 2008). On the other hand, out of season falls during late winter and spring and is associated with the peak of parturition season (from November to March; Hashem *et al*., 2011). Ultrasonic evaluation of ovaries of the subtropical female sheep during seasonal anestrus revealed that about 45% of females (lactating or not) had active corpora lutea. However, a high proportion of females failed to exhibit signs of estrus (Hashem *et al*., 2011). The reason of this conflict is not completely clear since both seasonal effect and lactational effect combine during the same time which makes reason of this disorder ambiguous and manipulation of the reproductive process is more difficult. In such conditions, identifying the role of each factor and different mechanisms by which

sexual activity and ovarian functions of subtropical sheep breeds are controlled may facilitate achieving suitable measures for sustaining optimum productivity during off-season. In particular, the reports about the effect of lactation on the reproductive performance of female sheep are not crucially established. Several studies reported that lactation increases the postpartum anestrous interval (Pope *et al*., 1989) and reduces fertility level (Hulet and Foote, 1967). Other studies, however, revealed that lactation or suckling is not the major factor that controls the reproductive activity of ewes or the viability of subsequent offspring (Fogarty *et al*., 1992; Hamadeh *et al*., 1996 and deNicolo *et al*., 2006). Therefore, the aim of the present study was to identify the sexual activity, ovarian steroidogenic capacity and metabolic status of lactating Barki ewes during early (40-60 day PP) and mid (70-90 day PP) lactations compared with non-lactating ewes during seasonal anestrus in the subtropics.

MATERIALS AND METHODS

The present study was carried out at the Agricultural Experimental Station (31° 20′ N, 30° E), Faculty of Agriculture, Alexandria University, Alexandria, Egypt, during the off-season from November to April (winter and spring, non-breeding season, Hashem *et al*., 2011). The procedures imposed on the animals were carried out meeting the International Guiding Principles for Biomedical Research Involving Animals (1985).

Animals and management:

A total of twenty Barki ewes, aging 4 to 6 years, and weighing 45.7 ± 2.4 kg for lactating ewes (at allocation) and 42.0 ± 1.5 kg for non-lactating ewes were used. Body condition scores were 2.5 ± 0.2 and 2.72 ± 0.1 for lactating and non-lactating ewes respectively, using a scale ranging from 1- emaciated to 5- obese (Jefferies, 1961). Ewes were divided into two groups according to their physiological statuses: non-lactating ewes (n=10) and lactating ewes (n=10; females who had lambed through the third week of November). All ewes were kept outdoors with shelter during the daytime and were housed in a semi-open barn at night. Animals grazed daily from 8:00 a.m. to 1:30 p.m. in addition to supplementation with a pelleted concentrated diet (89% OM, 14% CP and 3.7% EE) at a daily level of 0.5 kg/head (non-lactating ewes) or 0.75 kg/head (lactating ewes) and clover (*Trifolium alexandrinum*; 90.3% OM; 13.7% CP; 2.11% EE) *ad libitum.* Animals were fed the roughage and the concentrated supplement according to NRC (1985) recommendation. Water was offered to animals as a free choice. Animals were disease-free and were clinically normal with a healthy appearance. According to the system followed in the Experimental Station, ewes give one birth during the year and normally nurse their lambs for 4 months (120 days postpartum, i.e. weaning age).

Experimental design:

Transrectal ultrasonography equipment was used with lactating ewes on days 15 and 30 postpartum (PP) and with non-lactating ewes to identify the earlier postpartum time at which uterine involution and resumption of ovarian activity occur. Uterine horn diameters (cm), numbers and diameters (mm) of ovarian follicles (≥2mm) and presence of corpora lutea (CLs) were recorded using a real time B-mode scanner, equipped with 5 and 7.5 MHz linear array (Pie Medical Equipment B.V., Maastricht, Netherlands). Results were used to find out the suitable time for beginning of estrus synchronization, after ensuring the occurrence of uterine involution (uterine horn diameter of the lactating ewes is near that of the non-lactating ewes) and ovulation. Sexual behavior and ovarian steroidogenic capacity of lactating ewes during early lactation (40-60 days PP) and mid-lactation (70-90 days PP) periods were studied and compared with those of the non-lactating ewes. All females were intramuscularly injected with a double injection of 0.5 ml prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Estrumate, 250 µg cloprostenol/ml, Schering-Plough Animal Health, Germany), 11 days apart. This estrus synchronization protocol was selected to identify the normal pattern of non-induced sexual and ovarian activities in specific periods (follicular phase: day 2 of the synchronized estrous cycle and luteal phase: day 9 of the synchronized estrous cycle) of the reproductive cycle in lactating and non-lactating ewes (Hashem and Sallam, 2012). Additional ovarian scanning was carried out during the luteal phase (day 9) of the synchronized estrous cycle to confirm presence or absence of a corpus luteum following estrous synchronization.

Estrous detection:

Ewes were observed for overt signs of estrus twice a day (07:00 h and 14:00 h) for 1 h using teaser rams. Estrus was detected for 2 estrous cycles pre and post- estrous synchronization. Ewes were considered to be in estrus when standing to be mounted by the teaser rams. All females (lactating or not) did not show signs of estrus during the period prior to estrous synchronization.

Animal weighing:

Body weights of lactating ewes were recorded at 2 weeks postpartum and monthly until weaning. Also, body weights of non-lactating ewes were recorded before ewes were subjected to estrous synchronization. Ewes were not allowed any access to feed or water 12 h before weighing. The weights of animals were used to estimate the correlations between body weight, metabolic status and ovarian activity.

Blood sampling, biochemical and hormonal measurements:

Blood samples were obtained from the jugular vein of lactating ewes (during early and mid

lactations) and non-lactating ewes in the morning before access to feed and water. Blood samples (5 ml) were collected using non-heparinized venipuncture collection tubes. Samples were collected on day 2 (follicular phase) and on day 9 (luteal phase) following the second $PGF_{2\alpha}$ injection. Blood samples were centrifuged at 700 × g for 20 min and separated sera were stored at −20 ˚C until later analyses.

Serum estradiol, progesterone and insulin concentrations during the follicular phase and the luteal phase were determined using commercial solid-phase enzyme immunoassay (ELISA) kits (DRG International Inc., USA). Sensitivity of the methods was 0.38pg/ml, 0.10ng/ml and 1.8 µlU/ml for estradiol, progesterone and insulin, respectively. The corresponding intra- and inter- assay coefficients of variations were 6.3- 9.2 %, 5.1- 7.5% and 2.1- 4.4%, respectively. Concentrations of P_4 and E_2 were used to estimate P_4/E_2 ratios following transforming the values of $P_4$from ng/ml to pg/ml. The concentrations of P_4 for each ewe during the follicular phase and the luteal phase, in addition to the data of estrus occurrence were used to describe the different reproductive statuses of ewes as following: cyclic females (shown signs of estrus with P_4 concentrations ≥ 1 ng/ml during luteal phase); complete anestrous females (no signs of estrus with P_4 concentrations < 1ng/ml during both phases); females with silent ovulations (no signs of estrus with P_4 concentrations ≥ 1ng/ml during luteal phase); females with persistent CL (no signs of estrus with P_4 concentrations ≥1ng/ml during both phases) and others (no signs of estrus with P_4 concentrations ≥1ng/ml during follicular phase).

Serum biochemical parameters including: glucose, cholesterol, triglycerides, total protein, albumin and globulin (estimated by subtraction albumin values from the corresponding total protein values) were determined during the same phases using commercial kits (BioSystems S.A. Costa Brava 30, Barcelona, Spain).

Statistical analysis:

Body weights of lactating ewes, uterine involution and ovarian structures, total number of follicles and diameter of largest follicles of lactating (during days 15 and 30 PP) and non-lactating ewes were analyzed using GLM procedure of SAS (1999). Data of ovarian steroidogenic capacity following estrus synchronization and metabolic status were analyzed for the physiological status (early lactation, mid-lactation and non-lactating) and the phases (follicular and luteal) of the reproductive cycle and their interaction effects using the Split Plot ANOVA of SAS (1999). The physiological status of ewes were included in the main plot using the ewe term within physiological status to calculate the error, while the phase of the reproductive cycle and physiological status by phase of the reproductive cycle interactions were included in the subplot and were tested using the residual mean square. When significant F values were observed, mean separation was considered by LSMEANS-PDIFF option of the PROC GLM. Data that were expressed as percentages were analyzed by the chi-square test. Pearson correlation coefficients between body weight, metabolic status and concentration of sex hormones were estimated using the CORR procedure of SAS (1999). All results were expressed as the least square means (±SEM). The statistical significance was considered at $P < 0.05$.

Results

Uterine involution and ovarian structures on days 15 and 30 postpartum (PP):

Uterine involution of the lactating ewes was almost completed (2.1 cm) and being near that of non-lactating ewes (1.9 cm) by day 30 PP. Similarly, by day 30 PP ovarian structures (total number of follicles, diameter of largest follicles and percentage of ewes with CL) of the lactating and the non-lactating ewes did not differ significantly (Table 1).

Sexual activity and ovarian steroidogenic capacity of lactating and non-lactating ewes:

As shown in Table (2), estrous rates and percentages of ewes with CL (during luteal phase) did not differ significantly between females with different physiological status. Estrus rates were 40, 20 and 0.0% and the percentages of ewes with CL were 100, 70 and 70% for the non-lactating ewes and those in mid and early lactations, respectively. On the other hand, the physiological status of ewes significantly (P<0.05) affected the overall means of P_4 and E_2 concentrations. Also, results in (Table 2) indicated that the lowest (P<0.05) overall means of P_4 (±0.5) and E_2 (±3.7) concentrations were recorded for ewes in mid-lactation (2.0 ng/ml and 31.6 pg/ml, respectively), followed by those in early lactation (3.4 ng/ml and 35.7 pg/ml, respectively) and non-lactating (5.7 ng/ml and 66.2pg/ml, respectively). Ratios of P_4/E_2 were not significantly affected neither by the physiological statuses (non-lactating-lactating) nor by the phases of the estrous cycle. Regardless to the physiological status (non-lactating-lactating) of ewes, females those displayed overt signs of estrus had a significant (P<0.05) lower P_4/E_2 ratios during the follicular phase compared with those recorded during the luteal phase. On the other hand, no differences in P_4/E_2 ratios were observed in anestrous females in both phases (Table 3). Classification of the ewes according to their progesterone concentrations (<1 or ≥1 ng/ml) during both follicular and luteal phases and their sexual activity (Table 4) revealed that 50% of the ewes in early lactation, 20% of the ewes in mid-lactation and 60% of the non-lactating ewes had persistent corpora lutea.

Table1. Least square means (±SEM) of uterine horn diameter and ovarian structures of lactating ewes on days 15 and 30 postpartum compared with non-lactating ewes

Parameter	Physiological status			SEM
	Lactating (n=10)		Non-lactating (n=10)	
	15 PP	30 PP		
Uterine horn diameter, cm	2.7[a]	2.1[b]	1.9[b]	0.1
Total number of follicles (≥2 mm)/ewe	1.0[a]	1.9[b]	2.4[b]	0.5
Diameter of largest follicles, mm	2.5[a]	5.9[b]	5.3[b]	0.1
% of females with CL*	10 (1)	50 (5)	50 (5)	-

* Figures in brackets indicate the number of ewes with corpus luteum (CL).
[a,b] Means with different superscripts within the rows differ significantly ($P < 0.05$).

Table 2. Least square means (±SEM) of sexual activity and ovarian steroidogenic (progesterone: P_4, ng/ml and estradiol: E_2, pg/ml) capacity of lactating ewes during early (40-60 days PP) and mid (70-90 days PP) lactations compared with non-lactating ewes

Physiological status*	Parameter				
	Estrous rate,%**	% of females with CL**	P_4	E_2	P_4/ E_2 ratio
Early lactation					
F†	-	-	3.1	41.7	120.7
L	-	70 (7)	3.7	29.8	118.8
Overall	0 (0)	-	3.4[b]	35.7[b]	119.8
Mid-lactation					
F	-	-	1.2	30.3	35.4
L	-	70 (7)	2.8	33.0	84.2
Overall	20 (2)	-	2.0[b]	31.6[b]	59.8
Non-lactating					
F	-	-	4.2	65.6	61.0
L	-	100 (10)	7.1	66.8	109.6
Overall	40 (4)	-	5.7[a]	66.2[a]	85.3
SEM	-	-	0.5	3.7	10.9

*Results of lactating ewes were taken twice during early and mid-lactations for 10 lactating ewes.
**Figures in brackets indicate the number of ewes exhibiting estrus and those with corpus luteum (CL).
†Phase: F- means follicular phase (2 days following the second $PGF_{2\alpha}$ injection); L- means luteal phase (9 days following the second $PGF_{2\alpha}$ injection).
[a,b]Means with different superscripts within the column differ significantly ($P<0.05$).

Table 3. Least square means (±SEM) of serum concentrations of progesterone (P_4, ng/ml), estradiol (E_2, pg/ml) and their ratios (P_4/E_2 ratio) during the follicular phase and the luteal phase in estrous and anestrous ewes

Sexual activity	Steroid concentration/ ratio		
	P_4	E_2	P_4/E_2 ratio
Estrus (n=6/30; 20%)*			
F**	0.9[b]	51.2	20.8[b]
L	7.1[a]	50.5	148.5[a]
Anestrus, (n=24/30; 80%)*			
F	3.2	46.6	82.9
L	3.9	44.8	88.0
SEM	0.5	3.7	10.9

*Numbers between packets indicate total number of observations and their percentages.
**Phase: F- means follicular phase (2 days following the second $PGF_{2\alpha}$ injection); L- Means luteal phase (9 days following the second $PGF_{2\alpha}$ injection).
[a,b] Means with different superscripts within the column differ significantly ($P<0.05$).

Table 4. Classification of reproductive status (%) of lactating ewes during early (40-60 days PP) and mid (Mid lac., 70-90 days PP) lactations compared with non-lactating ewes according to their sexual activity and progesterone concentrations (P_4, ng/ml) during the follicular (F-phase) and luteal (L-phase) phases

Reproductive status	P_4 conc.*		Sexual activity**	Physiological status			
	F-phase	L-phase		Early lac.	Mid-lac.	Non-lactating	Overall†
Cyclic	-	+	+	0	20	40	20.0 (6)
Complete anestrus	-	-	-	20	20	0	13.3 (4)
Silent ovulation	-	+	-	20	30	0	16.6 (5)
Persistent CL	+	+	-	50	20	60	43.3 (13)
Other	+	-	-	10	10	0	6.6 (2)

*(+) means $P_4 \geq 1$ ng/ml; (-) means $P_4 < 1$ ng/ml.
** (+) means females expressed overt signs of estrus; (-) means females did not express signs of estrus.
†Overall was calculated as no. of each reproductive status observed following estrous synchronization/total no. of observations (30).

Table 5. Least square means (±SEM) of some blood serum metabolic attributes of lactating ewes during early (40-60 days PP) and mid (70-90 days PP) lactations compared with non-lactating ewes

Parameter*	Physiological status			
	Early lactation	Mid lactation	Non-lactating	SEM
Glucose (mg/dl)	48.1^{b}	50.7^{b}	57.0^{a}	1.6
Cholesterol (mg/dl)	65.7	65.3	66.4	1.7
Triglycerides (mg/dl)	5.7^{b}	5.4^{b}	13.5^{a}	2.5
Total protein (g/dl)	7.2^{b}	7.4^{b}	7.8^{a}	0.9
Albumin (g/dl)	3.9^{b}	4.2^{b}	4.9^{a}	1.5
Globulin (g/dl)	3.3	3.2	3.0	0.2
Insulin (µlU/ml)	43.3	47.7	43.1	4.5

*Values of all blood serum biochemical parameters are the average of two readings (samples of the follicular and luteal phases).
[a,b]Means with different superscripts within the row differ significantly ($P < 0.05$).

Table 6. Correlation coefficients (r) between body weight (BW), metabolic status and steroid hormones (progesterone, P_4 and estradiol, E_2)

Variables*	r	*P*- value
BW- P_4	0.3	0.04
BW-E_2	0.3	0.04
BW- Total protein	0.63	0.001
Glucose- P_4	0.3	0.04
Glucose-E_2	0.4	0.01
Triglycerides- P_4	0.4	0.01
Triglycerides- E_2	0.6	0.001

*Correlation coefficients shown in the table are only the significant correlations.

Metabolic status and body weight changes of lactating ewes:

Data shown in Table (5) revealed that lactating ewes during early and mid-lactation periods possessed less metabolic statuses compared with non-lactating ewes. The overall means of serum glucose, triglycerides, total protein and albumin declined significantly ($P < 0.05$) in lactating ewes during early and mid-lactation periods compared with non-lactating ewes. However, the overall means of serum cholesterol, globulin and insulin did not show differences between lactating and non-lactating ewes (Table 5). Positive correlations ($P < 0.05$) were observed between concentrations of glucose or triglycerides and both progesterone and estradiol concentrations (Table 6).

Lactating ewes showed significant ($P < 0.05$) decreases in their body weights during lactation period (Figure 1). The average body weight was 45.7 ± 2.5 kg on day 15 PP and reached the lowest value

(32.9 ± 1.4 kg) on day 120 PP. The greatest loss of body weight was noted (about 8 kg) during the first 60 days PP (45.7 ± 2.5 on day 15 and 37.7 ± 1.9 on day 60). Results in Table 6 revealed that significant positive correlations were observed between body weight and both serum concentrations of progesterone and estradiol (r = 0.3, P = 0.04; Table 6). Also, a significant positive correlation was observed between body weight and concentration of total protein (r = 0.63, P = 0.001).

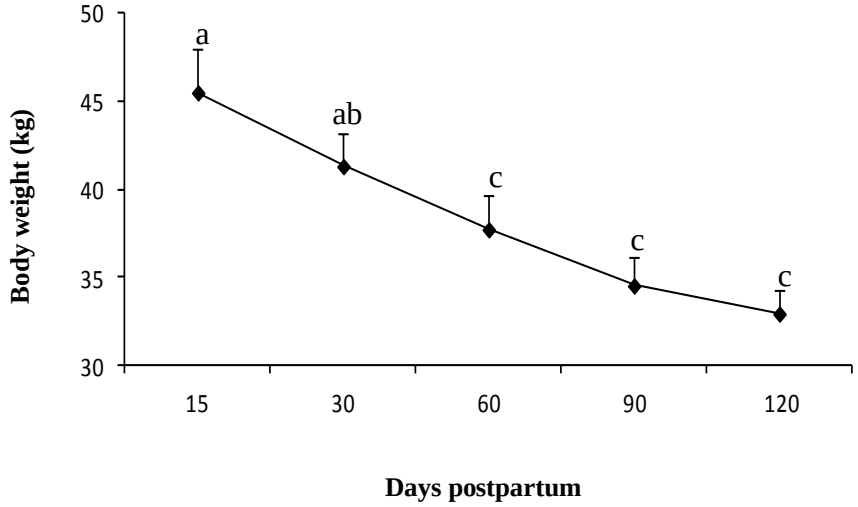

Fig.1. Body weight changes of lactating Barki ewes throughout lactation period.
a,b,c indicate significant differences (P < 0.05).

DISCUSSION

Opposite of what is expected during seasonal anestrus, high concentrations of P_4 were obtained in the anestrous female sheep used in this study. Furthermore, ovarian ultrasonic evaluation and hormonal assessments performed in the present study showed that most lactating (70%) and all non-lactating ewes (100%) had corpora lutea during the luteal phases of the synchronized estrous cycles with concentrations of P_4 more than 1 ng/ml (up basal concentration) that reflects presence of active corpra lutea. These findings are in agreement with those obtained in similar studies performed in the subtropical sheep breeds. Ali *et al*. (2006) observed the presence of ovarian activities (ovulations) in Ossimi ewes during different seasons of the year (spring, autumn and winter). Also, an ovulation activity was observed in Barki × Rahmani crossbred ewes during non-breeding season (Hashem *et al*., 2011). The current results suggest that subtropical female sheep could not be classified as truly anestrous females (lack of estrous signs and ovulation), but the nature of seasonality in ewes under the subtropics tended to be due to inability of ewes to displaying behavioral estrus rather than absence of ovarian activity (ovulation). However, similar studies performed in the temperate zones revealed that anestrous ewes are characterized by lack of ovulations (Goodman, 1994) with basal concentrations of progesterone (Caraty *et al*., 2002).

In the present study, ovarian activity was not associated with occurrence of overt signs of estrus. In the normal estrous cycle, P_4 is the predominant hormone during the luteal phase and reaches the basal level during the follicular phase so P_4/ E_2 ratio tracks the same trend (Scaramuzzi *et al*., 1971). This pattern of hormonal change during estrous cycle was only observed for females that exhibited signs of overt estrus (Table 3). On the other hand, vast majority of ewes were anestrous (100% in early lactation, 80% in mid-lactation and 60% in non-lactating ewes). Also, the present results denoted that, despite the variations in the E_2 and P_4 concentrations due to different physiological statuses of ewes, high proportions of ewes whether they were lactating or not suffered from hormonal disorders as reflected by stable P_4/E_2 ratios (dominancy of P_4) during the follicular phase and the luteal phase of the synchronized estrous cycle. This stability in P_4/E_2 ratios could be mainly ascribed to the high proportion of females that had P_4 concentrations more than 1 ng/ml during the follicular phase, even after treatment with $PGF_{2\alpha}$, suggesting presence of persistent CLs that leads to a prolonged luteal phase. Shrestha *et al*. (2004) reported that about 11-35% of cows that had irregular estrous cycles after resumption of ovulation activity tend to be predominantly due to prolonged luteal phases. It was suggested that prolonged luteal phases are associated with an abnormal uterine environment that disrupts $PGF_{2\alpha}$ production (Crowe, 2008). In ewes and cows, the establishment of the positive feedback mechanism between endometrial $PGF_{2\alpha}$ and ovarian oxytocin terminates the life of the corpus luteum, allowing a new cycle to begin (Flint *et al*., 1992). The release of the luteolytic $PGF_{2\alpha}$ from the endometrium is regulated by E_2 and P_4 (Okuda *et al*., 2002 and Goff, 2004), which modulate $PGF_{2\alpha}$ secretion by regulating the number of oxytocin

receptors; P_4 down-regulates the oxytocin receptors, delaying the time of luteolysis, while E_2 up-regulates the oxytocin receptors, advancing luteolysis (McCracken *et al*., 1999). The latency of endocrine and behavioral estrus events depends upon the balance between the triggering effect of E_2 and the inhibitory role of P_4 (Fabre-Nys and Martin, 1991).

Interestingly, in the present study, $PGF_{2\alpha}$ exogenously administrated failed to induce luteolysis of persistent CL and thus to reduce progesterone production. It is known that $PGF_{2\alpha}$ can be metabolized to the inactive prostaglandin, 13, 14-Dihydro-15-Keto-PGF2a (PGFM) by the enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH). This enzyme is found in abundance in the lung which clears >95% of circulating $PGF_{2\alpha}$ in one pass (Davis *et al*., 1980). Local metabolism of $PGF_{2\alpha}$ has been reported in many tissues including the CL. In the sheep CL, PGDH activity is greatest in the early CL and during maternal recognition of pregnancy, both times when the CL is relatively resistant to $PGF_{2\alpha}$ action (Silva *et al*., 2000). This may be also true for some CLs formed during seasonal anestrus.

Collectively, lack of signs of estrus observed in the present study could be attributed to three reproductive disorders including complete anestrous, silent ovulation and formation of persistent CL. It could also be said that last symptom is the major reason for lack of behavioral estrus in many females.

Concerning lactational effects, data revealed that by day 30 PP lactating ewes had completely involuted uteri and ovarian structures in line to those of the non-lactating ewes. Similarly, Gonzalez *et al*. (1987) reported that follicles are present on the ovaries in the majority of ewes 10 days PP and corpora lutea were observed in 67% and 75% of the ewes by day 20 PP and day 30 PP, respectively. Also, Hayder and Ali (2008) reported that the complete uterine involution in Farafra ewes (local Egyptian breed) that had lambed in February was achieved on day 29 PP. Thus, according to the ultrasonography evaluation of uteri and ovaries of lactating ewes, female sheep could be subjected to rebreeding following 30 days PP, since the uterine involution and ovulation activity can be resumed within this period, particularly in the absence of other deleterious factors (seasonal effects or negative energy balance).

In the current study, lactating ewes had lower overall concentrations of estradiol and progesterone compared with the non-lactating ewes. In addition, as the stage of lactation advanced, the lactating ewes showed severe loss in body weights with retarded metabolic status when compared to the non-lactating ewes indicating a symptom of negative energy balance. Generally, under-nutrition and unbalanced feeding during pre-partum and postpartum periods lead to the mobilization of body reserves resulting in weight losses up to 20%, and lactation in particular, accounts for about 50% of daily energy expenditure in grazing sheep (Blache *et al*., 2008). Therefore, decreased ovarian steroidogenic capacity recorded in the present study in lactating ewes may be due to the symptom of negative energy balance induced by lactation. Specially, most energy sources during lactation are directed to cover milk production requirements, whereas different body tissues such as muscles, fats and reproductive organs, unlike mammary glands, become less sensitive to peripheral insulin saving available energy sources (mainly glucose) for milk production (Bell and Bauman, 1997). At the same time, several studies (Downing *et al*., 1999 and Scaramuzzi *et al*., 2010) reported the necessity of glucose as an important source of energy for adequate ovarian activity. Further, glucose has many biological roles correlated with reproductive functions, i.e., it promotes cholesterol uptake by ovarian cells which is used by the ovarian cells for steroid bio-synthesis (Rabiee and Lean, 2000).

CONCLUSION

Results of the present study suggest that one of the important reasons for lack of estrous signs in the subtropical female sheep is the presence of a persistent corpus luteum. This ovarian dysfunction could be mainly attributed to the seasonal effects more likely than lactional effects since a high proportion of lactating and non-lactating ewes had the similar disorders. On the other hand, lactation impacted negatively ovarian steroidogenic capacity via inducing symptoms of negative energy balance. Future research must address the response of the corpus luteum formed during seasonal anesrous to $PGF_{2\alpha}$ as a luteolytic agent and the main seasonal factor(s) that controls the reproductive activity of the subtropical sheep breeds during off-season.

ACKNOWLEDGEMENT

The author presents sincere gratitude to Prof. Dr. M. Salem and Prof. Dr. M. Zeitoun, for revising the language of the manuscript, Professors of Animal Physiology, Faculty of Agriculture, Alexandria University.

REFERENCES

Abu-Zanat M.M., H.A. Mekdadi and M.J. Tabbaa, 2005. Production systems of small ruminants in middle badia of Jordon. Dirasat, 32: 205–13.

Ali A., R. Derar and H. Hussein, 2006. Seasonal variation of the ovarian follicular dynamics and luteal functions of sheep in the subtropics. Theriogenology, 66: 463-46.

Bell A.W. and D.E. Bauman, 1997. Adaptations of glucose metabolism during pregnancy and lactation. Journal of Mammamry Gland Biology and Neoplasia, 12: 265-278.

Blache D., S.K. Maloney and D.K. Revell, 2008. Use and limitations of alternative feed resources to

sustain and improve reproductive performance in sheep and goats. Animal Feed Science and

Caraty A., B. Delaleu, D. Chesneau and C. Fabre-Nys, 2002. Sequential role of E_2 and GnRH for the expression of estrous behavior in ewes. Endocrinology, 143: 139-145.

Crowe M.A., 2008. Resumption of ovarian cyclicity in postpartum beef and dairy cows. Reproduction in Domestic Animals, 43: 20-28.

Davis A.J., I.R. Fleet, F.A. Harrison and F.M.M. Walker, 1980. Pulmonary metabolism of prostaglandin $F_{2\alpha}$ in the conscious non-pregnant ewe and sow. Journal of Physiology, 301: 86P.

deNicolo G., S.T. Morris and P.R. Kenyon, 2006. Effect of weaning pre- or post-mating on performance of spring-mated ewes and their lambs in New Zealand. New Zealand Journal of Agricultural Research, 49: 255– 60.

Downing J., J. Joss and R.J. Scaramuzzi, 1999. The effect of a direct arterial infusion of insulin and glucose on the ovarian secretion rates of androstenedione and oestradiol in ewes with an autotransplanted ovary. Endocrinology, 163: 531-541.

Fabre-Nys C. and G.B., Martin, 1999. Roles of progesterone and oestradiol in determining the temporal sequence and quantitative expression of sexual receptivity and the preovulatory LH surge in the ewe. Journal of Endocrinology, 130: 367-379.

Flint A.P., H.J. Stewart, G. Lamming and J.H. Payn, 1992. Role of the oxytocin receptor in the choice between cyclicity and gestation in ruminants. Journal of Reproduction and Fertility, 45: 53-58.

Fogarty N.M., D.G. Hall, S.T. Dawe, W. Atkinson and C. Allan, 1992. Management of highly fecund ewe types and their lambs for 8-monthly lambing. 1. Effect of lamb weaning age on ewe reproductive activity in spring. Australian Journal of Experimental Agriculture, 32: 421–428.

Goff A.K., 2004. Steroid hormone modulation of prostaglandin secretion in the ruminant endometrium during the estrous cycle. Biology of Reproduction, 71: 11-16.

Gonzalez A., B.D. Murphy, J. de Alba and J.G. Manns, 1987. Endocrinology of postpartum period in the Pelibuey ewe. Journal of Animal Science, 64: 17–24.

Goodman R.L., Neuroendocrine Control of the Ovine Estrous Cycle, In: Knobil E, Neill JD, editors. The Physiology of Reproduction, New York, Raven Press Ltd, 1994; p. 659–709.

Hamadeh S.K., E.K Barbour, M. Abi Said and K. Daadaa, 1996. Reproductive performance of postpartum Awassi ewes under different lambing regimes. Small Ruminant Research, 19: 149-154.

Hashem N.M., S.Z. El-Zarkouny, T.A. Taha and Z.R. Abo-Elezz, 2011. Effect of season, month of parturition and lactation on estrous behavior and ovarian activity in Barki × Rahmani crossbred ewes under subtropical conditions. Theriogenology, 75: 1327–1335.

Hashem N.M. and S. Sallam, 2012. Sexual and ovarian activity of crossbred ewes fed different types of roughage during seasonal anestrus. Small Ruminant Research, 107: 136-140.

Hayder, M. and A. Ali, 2008. Factors affecting the postpartum uterine involution and luteal function of sheep in the subtropics. Small Ruminant Research, 79:174–178.

Hulet C.V. and W.C. Foote, 1967. Induction of fertile estrus in lactating and dry anestrous ewes using oral progestogens and repeated PMSG treatment. Journal of Animal Science, 23: 545– 548.

International Guiding Principles for Biomedical Research Involving Animals, 1985: issued by the Council for International Organizations of Medical Sciences (CIOMS).

Jefferies B., 1961. Body condition scoring and its use in management. Tasmanian Journal of Agriculture, 32: 19–21.

McCracken J.A., E.E. Custer and J.C. Lamsa, 1999. Luteolysis: a neuroendocrine-mediated event. Physiology Review, 79: 263-323.

NRC, 1985. Nutrient Requirement of Domestic Animals, sixth ed., National Academy of Science, Washington, D.C., USA.

Okuda K., Y. Miyamoto and D.J. Skarzynski, 2002. Regulation of endometrial prostaglandin $F_{2\alpha}$ synthesis during luteolysis and early pregnancy in cattle. Domestic Animal Endocrinology, 23: 255-264.

Pope W.F., K.E. McClure, D.E. Hogue and M.L. Day, 1989. Effect of season and lactation on postpartum fertility of Polypay, Dorset, St. Croix and Targhee ewes. Journal of Animal Science, 67: 1167–1174.

Rabiee A.R. and I.J. Lean, 2000. Uptake of glucose and cholesterol by the ovary of sheep and cattle and the influence of arterial LH concentrations. Animal Reproduction Science, 29: 199-209.

Scaramuzzi R.J., B. Campbell, C. Souza and D. Baird, 2010. Glucose uptake and lactate production by the autotransplanted ovary of the ewe during the luteal and follicular phases of the oestrous cycle. Theriogenology, 73: 1061-1067.

Scaramuzzi R.J., S.A. Tillson, I.H. Thorneycroft and B.V. Caldwell, 1971. Action of exogenous progesterone and estrogen on behavioral estrus and luteinizing hormone levels in the ovariectomized ewe. Endocrinology, 88: 1184-1189.

Shrestha H.K., T. Nakao, T. Higaki, T. Suzuki and M. Akita, 2004. Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. Theriogenology, 61: 1559-1571.

Silva P.J., J.L. Juengel, M.K. Rollyson and G.D. Niswender, 2000. Prostaglandin metabolism in the ovine corpus luteum: catabolism of

prostaglandin $F_{2\alpha}$ coincides with resistance of the corpus luteum to $F_{2\alpha}$. Biology of Reproduction, 63: 1229-1236.

Statistical Analysis Systems Institute Inc, (SAS), 1999. User's Guide, version 8. SAS Institute, Cary, NC, USA

Tabbaa M.J., M.A. Alnimr, M. Shboul and H.H. Titi, 2008. Reproductive characteristics of Awassi ewes mated artificially to Jordanian or Syrian Awassi rams. Animal Reproduction, 5: 23–29.

النشاط الجنسي والقدرة على إنتاج الإسترويدات المبيضية في النعاج الحلابة و الجافة بعد تنظيم الشياع خارج موسم التناسل تحت ظروف البيئة شبه الإستوائية

نسرين محمد هاشم

قسم الإنتاج الحيواني، كلية الزراعة، جامعة الأسكندرية، مصر

صممت هذه الدراسة للتعرف على النشاط الجنسي و قدرة المبيض على تخليق الاسترويدات الجنسية للنعاج الحلابة في بداية ومنتصف فترة إنتاج اللبن مقارنة بالحيوانات الجافة وذلك خارج موسم التزاوج تحت ظروف البيئة شبه الإستوائية. أستخدم جهاز الموجات فوق الصوتية للتأكد من حدوث التبويض ورجوع الرحم لحجمه الطبيعي وذلك في النعاج الحلابة (ن=10) في اليوم ال 15 و 30 بعد الولادة. ثم تم تنظيم الشياع للنعاج الحلابة مرتان وذلك بعد 40-60 أو 70-90يوم من الولادة وكذلك النعاج الجافة (ن=10) باستخدام حقنتان من البروستاجلاندين (٥ , مل /رأس) بالعضل بينهم 11 يوم. تم تجميع عينات دم بعد تنظيم الشياع وذلك بعد يومان من إنتهاء تنظيم الشياع (الطور الحويصلي) و اليوم التاسع (الطور الليوتيني). تم تقدير تركيز هرموني الإستروجين و البروجسترون و حساب النسبة بينهما. كما تم تقدير تركيز هرمون الإنسولين و بعض الصفات البيوكيميائية في السيرم. أوضحت النتائج أن النشاط المبيضي و رجوع الرحم لحجمه الطبيعي بعد الولادة يكتمل بعد مرور 30 يوم من الولادة ويكون مماثل للنعاج الجافة. لوحظ إرتفاع في نسبة النعاج التي لم تظهر علامات الشياع حيث بلغت 100% في النعاج في بداية موسم الحليب و 80% للنعاج في منتصف موسم الحليب و 60%للنعاج الجافة.على الرغم من وجود جسم أصفرفي 70% من النعاج في مراحل الحليب المختلفة و 100% في النعاج الجافة وذلك بعد تنظيم الشياع. كما لوحظ أن تركيزات البروجسترون و الإستروجين و النسبة بينهما لم تختلف معنويا في الطور الحويصلي و الطور الليوتيني وذلك لكل من النعاج الحلابة التي لم تظهر الشياع في بداية و في منتصف موسم الحليب و كذلك النعاج الجافة. أظهرت النعاج الحلابة في كلتا مرحلتي الحليب انخفاض معنوي في تركيز الجلوكوز و الجلسريدات الثلاثية والبروتين الكلي و الألبيومين مقارنة بالنعاج الجافة. كما لوحظ نفس الإتجاه بالنسبة لتركيزات هرموني البروجسترون و الإستروجين. الإنخفاض في تركيز الهرمونات الإسترويدية إرتبط إيجابيا بالإنخفاض في وزن الجسم و كذلك تركيز الجلوكوز و الجلسريدات الثلاثية بالسيرم. وفقا للنتائج المتحصل عليها في هذا البحث فإن كلا من النعاج الحلابة والنعاج الجافة أظهروا نفس النشاط الجنسي و كذلك الخلل الهرموني حيث لم تختلف نسبة البروجسترون/ الإستروجين في الطور الحويصلي و الطور الليوتيني (سيادة البروجسترون في كلا الطورين). هذه النتائج تؤدي لإقتراح أن أحد الأسباب المؤدية الى غياب الشياع في موسم خارج التناسل يرجع لوجود نسبة كبيرة من الأجسام الصفراء المتحوصلة و يرجح أن يكون هذا الخلل راجع إلى تأثير فصل السنة وليس لإنتاج اللبن. على الرغم من أن إنتاج اللبن يؤثر سلبيا على مقدرة المبيض على انتاج الإسترويدات الجنسية عن طريق خفض وزن الجسم وخفض تركيز المواد الميتابولزمية الهامة لتوليد الطاقة.

الكلمات الدالة: النعاج- الموسمية- إدرار اللبن- الجسم الأصفر الدائم- النشاط الجنسي- المنطقة شبه الإستوائية