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Estrous activity detection device in mammals

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1. ABSTRACT

Control of estrus is essential for the efficiency of any breeding program in farm animals, artificial insemination, hand mating and prediction of parturition dates. The present study is providing new evidence about determination of silent estrus in *Damascus does.* Since many *D. does* do not show any interest toward the male across seasons compared with spontaneous estrus detected by buck goat (BG). Results revealed that estrus detected by BG has significantly higher in autumn (78 of 86) than spring (3 of 11), while estrus detected by frequent vaginal inspection (FVI) was higher in spring (8 of 11) than autumn (8 of 86). Normal estrous cycles (EC) is significantly higher in *D. does* detected by BG than by FVI, while short and long EC are higher in *D. does* detected by FVI than by BG. Moreover, biochemical and hormonal analysis revealed that BG is an effective tool to measure estrus's quality especially in autumn and winter.

2. INTRODUCTION

Control of estrus may overcome silent estrus (silent ovulation) and increase reproductive performance in farm animals. Silent estrus is an important key factor that responsible for poor reproductive efficiency in farm livestock. During silent estrus a female ovulates without estrous signs and lack of mating behavior. Generally, there are several factors affecting the degree of estrous expression such as ambient temperature, photoperiod, season, age, body weight, productivity, nutrition and hormonal imbalances (1). Interestingly, the presence of a male within female nich stimulates the estrous behavior and promotes silent heat. Studies reported (2) that *Angora does* do not show estrous cycle if the bucks are placed with them, however, they enters into estrus phase by the presence of male. In general, at least 10 days are required to initiate estrus phase in does. A distinct seasonal odor of male goats stimulates female estrus cycle (2). However, failure of estrous detection represents serious problem in breeding efficiency (3) and leads to economic loss for the producer because of extended management practices (4). Silent estrus may be caused by insufficient secretion of estrogen by the ovaries. In some cases, stress may cause excessive progesterone production from the adrenal glands and block the stimulating effects of estrogen. Hence, a proper balance of hormones is necessary to prompt a psychological estrus and the doe's mating response.

In Egypt, about fifty percent of undetected estruses are found in farm livestock. The primary signs of estrus is a doe stands and mounted by a buck goat and consider as a most accurate sign of estrus, while the secondary signs includes discharge of mucus from the vagina before, during, and shortly after the estrus; and also include physical features such as long viscous and clear elastic strands; swelling and reddening of the vulva, and raising tail. In the present study we distinguish the silent estrus by examined the estrus cycle of does in presence or absence of male goat, and determined the physical and chemical characteristics of cervical mucus and arborization. Moreover, we compared progesterone profile at the different phases of the estrous cycles.

3. MATERIALS AND METHODS

3.1. Animals

This study was carried out in goat barn (*Damascus does)*, Faculty of Agriculture, Assiut University, South Egypt. *Damascus does* (n=18) with mean body weight of 32 +/- 0.8 kg were used in the current experimental studies. EA was detected by buck goat (BG) and frequent vaginal inspection (FVI) for a year-round. All does were in good health, free of abnormal genital discharges, and had one or two successive kidding before starting the experiment. Animals were housed in semi-open pens and fed twice daily with 33 percent yellow corn, 21 percent decorticated cotton seeds, 43 percent wheat bran, 2 percent limestone and 1 percent sodium chloride. In addition, *D. does* were fed with Egyptian clover and Darawa from December-March and late summer. Hay and water were provided to all animals throughout the experiment. The amount of dry matter consumed by each animal was between 0.8 – 1.2 Kg/day (5).

3.2. Estrus detection

Estrus was detected by BG and FVI. All *D. does* were randomly divided into two groups and exposed to two sexually active adult bucks (each BG run with each group for 15 min., with the reciprocal) twice daily (8 a.m. and 4 p.m.) for 30 min. A doe that stands and to be mounted by BG was considered in estrus, whereas a doe in estrus phase as detected by FVI (but not detected by BG), is calculated as "silent estrus or silent ovulation". Followed by does were inspected by the frequent vaginal inspection (FVI), however, undetected by BG. If the vaginal secretion was found in these animals, the physical and chemical characteristics of CM were determined. The same thing was done on the estrous *D. does* detected by BG. The primary and secondary estrus signs, presence of cervical mucus (CM), arborization and P_4 concentration less than 1.0 ng/100 ml blood serum were taken into consideration upon determination of estrus (6, 7).

3.3. Estrous cycle (EC) classification

EC length is defined between two consecutive estruses or ovulations, whereas estrus (heat) duration is defined as the period in which a doe accepts a BG. EC was classified into three types based on duration namely, normal (17 – 26 days), long (more than 26 days) and short (less than 17 days) (8-10).

3.4. Cervical mucus (CM)

CM was collected from all *D. does* by using vaginal scope into a 10 ml glass tube and transported to the laboratory for further analysis. One drop of the collected mucus was spread on a glass slide, dried using the electrical fan and analyzed under the light microscope (low power of magnification) for arborization (11, 12). The rest of mucus was stored in eppendorf tubes at - 30°C for the mineral elements (Na, K, Cl, P, Cu and Zn) analysis (13,14).

3.5. Analysis of physical properties of CM

Evaluation of pH, volume, color, texture, and elasticity in the cervical mucus were performed as follows:

- pH was measured by using comparative nitrating pH paper.
- Volume (ml) was taken directly from the graded test tube to the nearest 0.1 ml.
- Color was assessed by using scale of 1-3, where 1= watery, 2= milky and 3= creamy.
- Texture was compared with "egg white" using scale of 1-3, where 1= low viscosity, 2= medium viscosity and 3= high viscosity. Thumb and forefinger were used in this measurement.
- Elasticity was assessed by using scale of 1-3, where 1= low elasticity, 2= medium elasticity and 3= high elasticity. Mucous length was measured in centimeters by using glass slide (small drop of mucus was placed onto the microscopic slide and allowed to draw by collecting tube until the mucous thread to cut).

3.6. Analysis of mineral elements of CM

Atomic absorption spectrophotometer was used in determination of Na, K, Cu and Zn in CM (15), whereas Cl and P were determined by colorimetric method (16-18) at Institute of Reproductions, Giza, Egypt.

3.7. Blood collection

Blood samples were collected from ten *D. does* for four seasons. A total of 640 blood samples were withdrawn from does via j.v. at 8.0 a.m. before feed intake (19, 20) and distributed as shown in Table 1.

Blood samples were allowed to clot at 4 °C for 10 hours in the refrigerator, and then centrifuged at 2000 g for 20 minutes The sera were collected and stored in eppendorf tubes (4 ml) at -20 °C until hormonal assay (21, 22).

3.8. Progesterone (P_4) assay

Progesterone was assayed by enzyme-linked immunosorbent (ELISA), DRG Frauenbergstr**,** 18, D-35039

Table 1. A tabular representation of blood samples collection during different phases of EC

Type of EC	Days of blood samples
Normal	1, 3 or 4, 6, 15 and 18 or 21
Short	1, (3 or 4), half and last of the cycle
Long	1, 3 or 4, 6, 12, 18, 24 and 30

Day 1 = estrus (heat) period, Day 3/4 = met-estrus, Days 6, 12 and 15 = di-estrus, Days 18 and 21= pro-estrous

Table 2. A tabular representation of percentage and number of estrus detected by BG and FVI in different seasons

Season*	Number of animals	Number of animals in estrus	Total number of estruses	Number and estrous percent detected by	
				BG	FVI
Spring	12	6 (50 %)[b]	11[b]	3 (27.27 %)	8 (72.73 %)
Summer	18	18 (100 %)[a]	79[a]	41 (51.90 %)	38 (48.10 %)
Autumn	17	17 (100 %)[a]	86[a]	78 (90.70 %)	8 (9.30 %)
Winter	15	14 (93.3 %)[a]	49[ab]	29 (59.18 %)	20 (40.82 %)
Overall			225	151 (67.11 %)	74 (32.89 %)

[a and b] Means in the same column with different superscripts are significantly different (P<0.01). *Significant at 0.001 Chi-square = 39.264

Table 3. Representation of numbers and percentages of short, normal and long EC

ED device*	Number of estruses	Type of EC		
		Short	Normal	Long
FVI	58	27 (46.55 %)	26 (44.83 %)	5 (8.62 %)
BG	112	39 (34.82 %)	70 (62.50 %)	3 (2.68 %)
Total	170	66 (38.82 %)	96 (56.47 %)	8 (4.71 %)

*Significant at 0.05 Chi-square = 6.335

Marburg, Germany, 2005, (23). The method is based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the P_4. Endogenous P_4 of a sample competes with P_4 horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugates were washed off. The amount of bound peroxidase conjugates is reversing proportional to the concentration of P_4 in the sample. After addition of the substrate solution, the intensity of color developed was read at 450+/-10 nm and the intensity was proportioned to the concentration of P_4 in the sample.

3.9. Statistical analysis

Data were statistically analyzed by using General linear model (GLM) and Chi Square test procedure of SAS (24). Whereas, the variance between seasons, months, type of estrous (short, normal and long estrous) and methods of estrus detection were analyzed for the quantitative variable by (GLM) procedure, as well as Duncan's multiple range test (25). The qualitative variables were analyzed by Chi-Square test procedure.

4. RESULTS

4.1. Incidence of estrous

As shown in Table 2, seasonal changes have a significant effects (p<0.01) on incidence of estrus. The numbers and percentages of silent estrus of *D. does* examined by FVI were higher in spring (8 of 11, 72.73 percent) and low in autumn (8 of 86, 9.30 percent) compared with BG (3 of 11, 27.27 percent, spring and 78 of 86, 90.70 percent, autumn). Regardless of ED device, EA varied significantly (P<0.01) from month to another and increased gradually from January (73 percent) to September (94.4 percent), then reached 100 percent in October and November, while the lowest value was in April (8.3 percent). Therefore, EA was better in autumn (October and November) than the other seasons. Otherwise, estrus detected by BG was higher in November (100 percent, 27 of 27), December (95.83 percent, 23 of 24) and September (93.10 percent, 27 of 29) than in April (0.00 percent), May (0.00 percent) and June (18.18 percent, 2 of 11) (Figure 1). Generally, *D. does* are exhibited their normal EA from September-January and typified their silent estrus from April-July.

4.2. Estrous cycle (day)

As shown in Table 3, method of ED had a significant (p <0.05) influence on type of EC. Percentages of short and long EC in *D. does* detected by BG were lower (34.82 percent and 2.68 percent) than that examined by FVI (46.55 percent and 8.62 percent), while, the normal EC of *D. does* detected by BG was higher (62.50 percent) than that examined by FVI (44.83 percent).

A significant difference in EC is found due to the seasonal changes. The overall mean EC length allover year-round was 20.39+/-0.22, 38.82 percent of cycles were short, 56.47 percent were normal and 4.71 percent were long. In Table 4, the average EC length detected by BG decreased slightly (16.63+/-0.62) than that examined by FVI (17.43+/-1.20). However, ED device had no a significant effect on the detection of EC length.

4.3. Duration (hour) and timing of estrus

The average estrus duration of *D. does* detected by BG was highly significant (49.27+/-1.79) than that examined by FVI, and most estruses was concentrated in the early morning than in the evening (Table 4). These data also indicate that numerous silent estruses occurred in the early morning of *D. does* are non-detected by BG. This means that Damascus BG failed to detect all *D. does*, and hence FVI device should be used for increasing reproductive efficiency in farm animals.

Table 4. Representation of means +/- SE of estrous duration (hours), EC length (days) and estrus detected in morning and evening by BG and FVI

ED device*	Number of estruses	Mean ±SE Estrus duration	Mean ±SE length of EC	Timing of estrous detection	
				Morning	Evening
BG	151	49.27+/-1.79[a]	16.63+/-0.62	80.79 % n=122	19.21 % n= 29
FVI	74	0.00[b]	17.43+/-1.20	97.30 % n= 72	2.70 % n= 2

Values are least-squares means (L.S.M.) ± standard error of L.S.M. [a and b] Means in the same column with different superscripts are significantly different (P<0.05). *Significant at 0.001 Chi-square = 11.385

Table 5. Representation of CM color, pH and amount of mucus in the does detected by BG and FVI during estrous phase

ED device	Number of estruses	Color*			pH	Volume
		Watery %	Milky %	Creamy%		
BG	137	37.23 n=51	54.01 n=74	8.76 n=12	6.94+/-0.03[3]	0.50+/-0.04[2]
FVI	73	54.79 n=40	45.21 n=33	0.00	7.14+/-0.03[a]	0.35+/-0.05[b]

[a and b] Means in the same column with different superscripts are significantly different (P<0.05). *Significant at 0.05 Chi-square = 10.511

Table 6. Representation of CM texture and elasticity of mucus during the estrous phase of does

ED device *	Number of estruses	Texture			Elasticity**			Length of elasticity (cm)
		Low %	Medium %	High %	Low %	Medium %	High %	
BG	137	10.29 n=14	35.29 n=48	54.41 n=74	13.14 n=18	45.26 n=62	41.61 n=57	5.78+/-0.18
FVI	73	9.59 n=7	53.42 n=39	36.99 n=27	13.70 n=10	57.53 n= 42	28.77 n=21	5.29+/-0.21

* Significant at 0.05 Chi-square = 6.759 ** Significant at 0.01%, Chi-square = 3.574

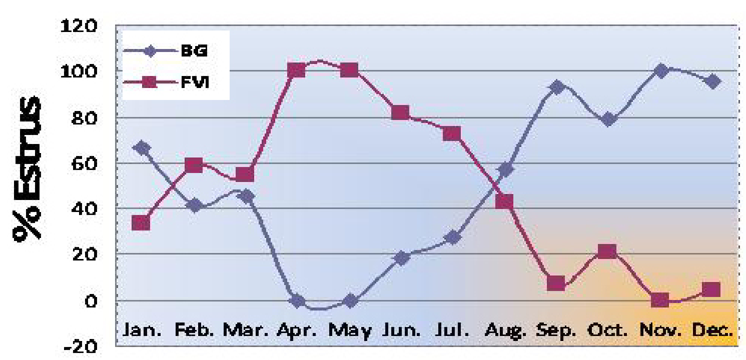

Figure 1. Estrous percentage detected by BG and FVI in one year.

4.4. Physical properties of CM

4.4.1. Color, pH and volume

Color, pH and volume of mucus secreted by the cervix changed dramatically during the estrous cycle, and are varied between spontaneous and silent estrus (Table 5). CM color observed in the mucus of does detected by BG was significantly milky (p<0.01) than that examined by FVI. No creamy color has been observed in the mucus of *D. does* examined by FVI. The table indicated also that amount of CM in *D. does* examined by FVI dropped significantly (P<0.05) as pH value became slightly alkaline compared with *D. does* detected by BG.

4.4.2. Texture and elasticity

Texture of CM during the estrous phase was significantly (p<0.05) higher (54.41 percent) in *D. does* detected by BG than that examined by FVI (36.99 percent) (Table 6). Variability among scores of elasticity has been found in both *D. does* detected by BG and FVI, but the elasticity fluctuated between medium and high scores in both *D. does* detected by BG and FVI. These data also indicated that the average length of elasticity was insignificant in both *D. does* detected by BG and FVI. It is noted from the results that if the color of CM is watery/milky then amount of CM is high and texture of the mucus resemble the egg white with higher elasticity score and P_4 < 1.0 ng/ml blood serum. These data suggest that estrus and arborization patterns are in same phase in reproductive stage. Arborization patterns during EC phases are mentioned in Figures 2-5 below.

4.4.3. Mineral elements of CM

The average concentrations of Na, K, Cl, P, Cu and Zn in the mucus during pro-estrus, estrus and post-estrus phases of the estrous cycle of Damascus does is present in Table 7. The concentrations of Na (243.13+/-11.32 mm/L) and P (9.89+/-0.39 mg/dl) in the mucus were significantly higher (P<0.05) in post-estrus phase than in

Table 7. Representation of least-square means ± SE of Na, K, Cl, P, Cu, and Zn concentrations in CM during pro-estrus, estrus and post-estrus phases

Estrous phases	Number of observations	Na	K	Cl	P	Cu	Zn
Pro-estrus	19	207.76±18.74^{b}	12.41±0.84^{a}	112.57±4.21^{b}	7.85±0.97^{b}	0.28±0.03	3.67±0.51^{a}
Estrus	48	222.67±10.70^{ab}	10.88±0.60^{b}	121.57±3.05^{a}	8.50±0.43^{ab}	0.30±0.02	2.94±0.21^{ab}
Post-estrus	48	243.13±11.32^{a}	11.60±0.43^{ab}	111.86±2.78^{b}	9.89±0.39^{a}	0.29±0.02	2.72±0.24^{b}

Values are least-squares means (L.S.M.) ± standard error of L.S.M. $^{a\ and\ b}$ Means in the same column with different superscripts are significantly different (P<0.05).

Table 8. Representation of least-square means ± SE of Na, K, Cl, P, Cu, and Zn concentration in CM during the estrous cycle of does detected by BG and FVI.

Ions	BG*			Overall mean	FVI			Overall mean
	Pro-estrus n=12	Estrus n=36	Post-estrus n=30		Pro-estrus n=7	Estrus n=12	Post-estrus n=18	
Na	224.82±18.88	229.94±12.85	254.26±13.27	238.50±8.37*	178.53±38.90	200.88±17.98	224.59±20.29	208.19±13.53^{b}
K	11.15±0.49	10.61±0.62	12.02±0.55	11.23±0.37	14.94±2.07	11.69±1.55	10.90±0.69	11.84±0.73
Cl	120.37±4.85	121.70±2.90	115.20±3.34	118.99±2.00^{a}	99.20±4.84	121.19±8.90	106.30±4.72	109.79±3.95^{b}
P	5.92±0.92	8.78±0.48	10.40±0.37	8.99±0.34	12.10±0.04	7.72±0.88	9.02±0.81	9.01±0.56
Cu	0.25±0.02	0.30±0.03	0.28±0.02	0.28±0.01	0.37±0.10	0.32±0.06	0.30±0.05	0.32±0.03
Zn	4.15±0.62	3.12±0.25	2.77±0.29	3.15±0.19^{a}	2.22±0.30	2.35±0.38	2.64±0.44	2.49±0.26^{b}

Values are least-squares means (L.S.M.) ± standard error of L.S.M. n= number of observations. $^{*\ a\ and\ b}$ Means in the same column with different superscripts are significantly different (P<0.05).

Table 9. Representation of least-square means +/- SE of Na, K, Cl, P, Cu, and Zn concentrations in CM in different seasons

Season*	Number of observations	Na	K	Cl	P	Cu	Zn
Spring	8	208.05±33.37^{b}	13.36±0.64^{a}	109.94±5.63^{b}	9.65±1.07	0.44±0.09^{a}	3.42±0.86^{a}
Summer	33	208.78±15.51^{b}	12.22±0.86^{a}	110.59±4.32^{b}	8.81±0.55	0.30±0.04^{b}	2.34±0.18^{b}
Autumn	45	208.63±7.58^{b}	9.69±0.36^{b}	122.85±2.89^{a}	9.13±0.46	0.28±0.01^{b}	3.49±0.27^{a}
Winter	29	288.40±12.13^{a}	12.70±0.54^{a}	113.32±2.61^{ab}	8.84±0.61	0.25±0.01^{b}	2.61±0.26^{ab}

Values are least-squares means (L.S.M.) ± standard error of L.S.M. $^{*a\ and\ b}$ Means in the same column with different superscripts are significantly different (P<0.05).

estrus phase (222.67+/-10.70 mm/L and 8.50+/-0.43 mg/dl, respectively) or even pro-estrus phase (207.76+/-18.7 mm/L and 7.85+/-0.97 mg/dl, respectively). In contrast, K concentration in pro-estrus phase (12.41+/-0.84) was significantly (P<0.05) higher than in post-estrus (11.60+/-0.43) and estrus (10.88+/-0.60) phases. Cl concentration was significantly (P<0.05) higher (121.57+/-3.05) during the estrous phase than in pro-estrus (112.57+/-4.21) and the post-estrus (111.86+/-2.78) phases. Cu concentration in all the phases of the estrous cycle was similar (0.28 - 0.30 mg/dl), and did not show any effect of the estrous cycle. Zn concentration in the mucus was significantly (P<0.05) higher (3.67+/-0.51) during pro-estrus than in estrus (2.94+/-0.21) and post-estrus (2.72+/-0.24) phases.

The overall mean Na, Cl and Zn concentrations in the mucus of does detected by BG (238.50+/-8.37, 118.99+/-2.00 and 3.15+/-0.19, respectively) were significantly (P<0.05) higher than that examined by FVI (208.19+/-13.53, 106.30+/-4.72 and 2.49+/-0.26, respectively) (Table 8). Does were detected by BG displayed higher Na concentration during the estrous cycle compared with that examined by FVI. Cl concentration was higher in the estrus phase than in the pro-estrus and post-estrus phases of both does detected by BG and by FVI. Zn concentration was higher in pro-estrus and estrus phases than that in the post-estrus phase of does detected by BG, while it was slightly higher in the post-estrus phase than in pro-estrus and estrus phases of does examined by FVI. Cu concentration was similar throughout the estrous cycle of both does detected by BG and FVI. Therefore, there is an insignificant differences among K, P and Cu concentrations of does detected by BG and FVI. In contrast, K (14.94+/-2.07 mm/L) and P (12.10+/-0.04) in the mucus of doe detected by FVI were slightly higher in the pro-estrous phase than that detected by BG (11.15+/-0.49 mm/L and 5.92+/-0.92, respectively).

Correlations among Na, K, Cl, P, Cu, and Zn concentrations in the mucus showed significant variations. The correlation was significantly negative (r = - 0.37, P<0.01) between K and P during the estrus phase and K and Cl (r = - 0.57, P<0.01) during the pro-estrus phase. Whereas, there were significant positive correlations between K and Cu (r = + 0.36, P<0.05) during the estrus phase, between P and Zn (r = + 0.32, P<0.05) during the estrous phase and between Na and K (r = + 0.30, P<0.05) during the post-estrus phase. These results suggest significant differences in ionic composition of CM during heat phase and the ovulation time.

Season had a significant effect on the all mineral elements except P. The average Na concentration was significantly (P<0.05) higher in winter (288.40+/-12.13 mm/L) than in the other seasons (Table 9). Mean K concentration in autumn (9.69+/-0.36 mm/L) was significantly (P<0.05) lower than the other seasons. Furthermore, K value was higher in spring than in summer and winter. Mean concentrations of Cl and Zn was significantly (P<0.05) higher in autumn than the other seasons. The minimum value of Cl was in spring (109.94+/-5.63 mm/L) and summer (110.59+/-4.32 mm/L), while the minimum value of Zn was in winter (2.61+/-0.26 mg/dl). Mean Cu concentration was significantly (P<0.05)

Figure 2. Representation of an incomplete arborization during pro-estrus phase.

Figure 3. Representation of an abundant, complete and regular arborization during estrous phase (1st day of estrus).

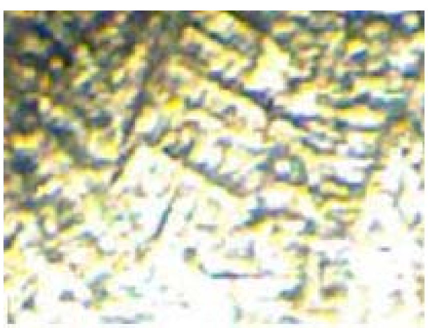

Figure 4. Representation of little and irregular arborization during the 2nd day of estrus.

Figure 5. Representation of absence of arborization in post-estrous phase of estrus.

higher in spring (0.44+/-0.09) than other seasons, but the minimal value of Cu was in winter (0.25+/-0.01 mg/dl). However, the data did not show any significant influence of season on P concentration in the mucus.

4.5. P_4 Profile (ng/ml blood serum)

The overall mean P_4 concentration was influenced significantly (P<0.05) by season. P_4 level was higher in winter (3.21+/-0.50) and autumn (3.13+/-0.33) than in spring (0.31+/-0.06) and summer (0.86±0.30) (Figure 6). Further, P_4 concentration was apparently higher in does detected by BG than that detected by FVI.

The average P_4 concentration during the estrus detected by both BG and FVI was less than 1 ng/ml, thereafter level rose to over 1 ng/ml in the post-estrus phase of does detected by BG, but not by FVI (Figure 7). The maximal level of P_4 concentration was in di-estrous phase of both does detected by BG and FVI, but the increase was much more in the does detected by BG than that detected by FVI. Similarly, level of P_4 during pro-estrous phase was over 1 ng/ml in the does detected by BG than that detected by FVI (< 1 ng/ml) (Figure 7). Further, P_4 concentration in the does detected by BG was significantly (P<0.05) higher (3.16+/-0.25) than that examined by FVI (0.68+/-0.22).

5. DISCUSSION

The present study indicates that silent estrus is common in spring than the other seasons. Interestingly, BG is unable to detect all the estrous phases in *D. does* in spring, hence, FVI device should be aided in detection of estrus phases along with BG. Previous studies have demonstrated that ovulation is associated with estrus signs and seasonal variation has a direct effect on incidence of estrus**.** In Criollo and Granadina does goats have highest incidence of estrus in autumn and winter, and anestrous periods occur in spring and summer (26, 27). The maximal and minimal periods of sexual activity of Creole goats are associated with fall/winter and spring/summer respectively (28). Breeding season in goats start in summer, and extend up to autumn-winter, and incomplete in spring that reared in medium and high latitudes (above 40°N) (29, 30). The present results also demonstrates that EA varied significantly (P<0.01) from month to month, with very high (100 percent) in October and November, and very low in April (8.3 percent). BG detected higher estrus in September to December than April to June. These results are in agreement with previous studies (31) as indicated a significant seasonal effect on Barbary goats EA, higher percentages of EA during September-October, and lowest percentages of EA during February-March. Other studies (32) revealed that dairy goats (Alpine, Saanen and Lamanche) exhibit anestrous from February to May; whereas in Saanen, Alpine and Toggenburg goats have EA during late September to early March (33). Interestingly, the highest EA in Payopya does goat is from August - February and lowest EA from March – June (34). Most Egyptian Baladi goats mate allover year- round, but their EA fluctuates from month to month, with highest percentage (100 percent) of estrus from October to February, while the minimal percentage of estrus from

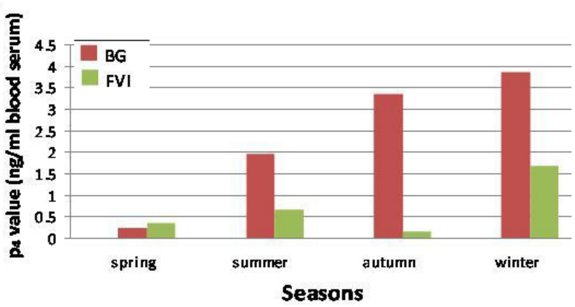

Figure 6. Mean P_4 concentration in EC of does detected by G and FVI across seasons.

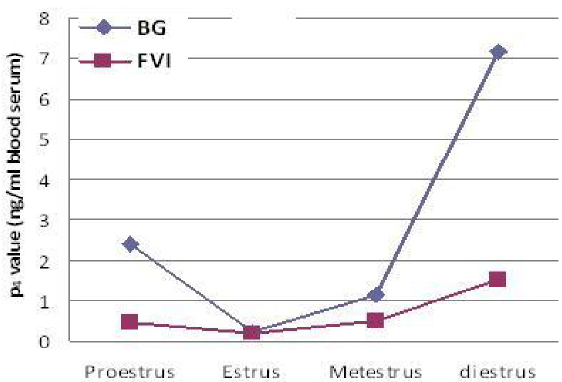

Figure 7. Mean P_4 concentration during EC phases of does detected by BG and FVI.

March (50 percent) to June (58.3 percent) (10). The present study suggested that *Damascus does* normal EA from September-January and silent estrus from April-July.

The current results further revealed that percentages of short (46.55 percent) and long (8.62 percent) EC are higher in does examined by FVI than that detected by BG (34.82 percent and 2.68 percent, respectively; Table 3). Causes of short EC are not well understood, but many previous studies indicated that short EC is usually observed in seasonally breeding goats at the initiation of breeding season (34-37). While, the long EC could be due to an alteration in the pulsatile release of LH and decrease in estrogen levels or may be due to the silent heat (ovulation without estrous signs) or occurrence estrus without ovulation. Other studies also indicated that the silent or very short duration of estrus is the main factor responsible for occurrence of long EC (38). Additionally, short EC may refer to a rupture of more than one follicle that escaped from the previous ovulation in the mid-cycle to induce short EC (39, 40**).** In contrast, long EC in domestic mammals is always at the end of breeding season (41, 42). However, studies in *Nubian does* indicated (43) that 21 of 48 estrous cycles are very short cycles (3 - 15 days) with average 6.5+/-0.5 days, whereas other cycles (27) are normal with average 21.5+/-0.8 days and initiated during the early breeding season. In Argentina, the proportion of abnormal cycles of Creole goats (either short or long) is higher at the beginning and ending of breeding season (28). The cause silent estrus may refer to the absence of progesterone of the previous cycles which prime the behavioral center in the brain for estrogen effects (44). Thus, without progesterone, estrogen produces during ovulation unable to induce estrous signs. Other causes of silent estrus may refer to the emotional status. Studies have shown that the emotional heifers had a higher percentage of silent ovulations than unemotional heifers (45). Furthermore, in buffalo, the hot or cold environment and food intake prolonged the length of estrous cycle with shorter duration of heat phase (1).

Our results also revealed that estrus duration of does detected by BG are significantly higher than that examined by FVI, and the most estruses are concentrated in the early morning than in the evening. Additionally, most silent estruses observed in the does by FVI are in the early morning than evening (Table 4), suggesting Damascus BG failed to detect all the EC in does. These results are in agreement with previous findings (10, 46, 47,) and suggested that estrus may occur at any time of the day but high in the first hours of the morning. The relative humidity might be responsible for the high estrus incidence during morning (47). Other causes may be multiple hormones (growth hormone, cortisol, epinephrine and norepinephrin) that secretes in the early morning and raises blood glucose. However, further detail studies are required.

Physical properties of CM play an important role in the estrous activity. CM volume, color, pH, texture, elasticity of mucus and ionic (Cl, K and Zn) concentrations may be used as an indicator for the prediction of the pro-estrus and estrous phases. Cl, K and Zn are significantly higher in CM of does detected by BG than that detected by FVI. Based on previous studies (48), full arborization preceded the time of ovulation by one day, and then regressed dramatically in its original form in the following day. In CM, total solids and sodium chloride increase with the reduction in sugars as a typical 'fern' pattern" when mucus is dried over a flame (51). Properties of mucus changes dramatically during ovulation, and volume of mucus increase with less viscosity (profuse and thin) (49). Similar CM characteristics have been observed in human and cattle, due to accumulation of water in CM (50, 52, 53). Thus, the mucus collected from spontaneous estrus was copious and clear (14). Moreover, during the post-ovulatory phase, CM turns into thick and cheesy shape (54). Hence, the results of current study indicated that the variability in mucus production is well established for instance, lack of CM volume observed in does examined by FVI, and refer to the hormonal imbalance (estrogen: progesterone ratio during mating), while increasing P_4 during the pro-estrus phase may assist in producing mucus with low pH value as in case of does detected by BG. Many previous studies reported that the physical and chemical properties of CM are regulated by hormones (55-59). The increase estrogen promotes the production of thin and isotonic mucus with high molecular weight glycoproteins, while increase P_4 promotes viscous mucus (60). In women, CM contains 98% water at mid-cycle, metallic ions, enzymes (such as alkaline phosphatase, etc.), soluble proteins and salts. Also, treatment with exogenous progesterone in ewes decreases mucus significantly at estrus with low fertility than untreated ewes (61); whereas ovariectomized heifers injected with 400 µg oestradiol benzoate i.m. showed estrus within 15- 20 hr after treatment with higher flow of mucus similar to the natural estrus. In contrast, exogenous hormonal treatment of cows did not influence CM characteristics (62).

CM of Damascus does have shown a significant variability in mineral elements (Na, Cl, k, P, Cu, and Zn). Cl ion was detected in mucus by both BG and FVI during the estrus. Na and Zn concentrations are higher in CM of does detected by BG than that detected by FVI, while the other elements are similar to each other. Also Na ion was significantly higher in post-estrus than in pro-estrus and estrous phases. Present study is in agreement with previous reports where chloride was used as an indicator of ovulation in cows (63), and Na/K ratio was used for timing of ovulation in pigs (64). In cow, Cl concentration in CM increased sharply in the estrus phase and ranged from 526 mg/dL (65), 430.66 mg/dL (66), 535.6 mg/dL (67) and 869.2 mg/dL (68) compared with that found in the post-estrus phase. Therefore, occurrence of arborization may rely on the high level of Cl ion during the estrus phase. NaCl is one of the major molecules present in CM that support the arborization. Arborization may be observed in all secretions containing proteins, electrolytes (sodium chloride) and mucoproteins (69). Similar studies have shown in CM of different mammalian estrus phase (Na concentration in cow = 272.9 - 280.5 mg/dL; buffalos 287.0-289.14 mg/dL) (70, 71, 72, 73). Other studies have shown a significant Na variation in CM during estrus, fertile heat (estrus with ovulation) or infertile heat (estrus without ovulation) phases (74, 75). Interestingly, K, Cu and Zn concentrations in Buffalo CM are significantly lower in pro-estrus and estrus phases than in the post-estrus phase. The present results also showed a positive correlation between K and Cu ($r = + 0.36$, $P<0.05$), and between P and Zn ($r = + 0.32$, $P<0.05$) during the estrus phase; and between Na and K ($r = + 0.30$, $P<0.05$) in the post-estrus phase. In contrast, there is a negative correlations ($r = - 0.37$, $P<0.01$) between K and P during the estrus phase, and between K and Cl ($r = - 0.57$, $P<0.01$) in the pro-estrus phase. Previous studies also demonstrated a negative correlations ($r = - 0.59$, $P<0.05$) between N_a and Cl and between N_a and K ($r = - 0.53$, $P<0.05$) in CM of cow during the estrus phase, while, the correlation is positive ($r = 0.53$, $P<0.05$) between K and Cl in cow's CM in the estrus phase (76). Thus analysis of mineral elements in CM can help us to predict the time of ovulation and resolve the issue of silent estrus in farm animals.

The current study indicate that a male goat affect P_4 level significantly in post-estrus and di-esturs phases. P_4 concentration is < 1.0 ng/ml during the estrus phase of both does detected by BG and FVI, and higher during the pro-estrus phase of does detected by BG than that examined by FVI as shown in Figure 7. Our study suggest that P_4 level is higher in autumn and winter than other seasons, whereas, P_4 concentration in the estrous cycle of Anglo-Nubian does is higher in autumn (0.50+/-0.15 ng/ml) than spring (0.25+/-0.04 ng/ml (77). The average P_4 concentration in the estrous cycle of Baladi goats detected in winter was 2.9+/-0.6 ng/ml blood serum, while it was 1.8+/-0.3 ng/ml in autumn of crossbred doe goat (10). In contrast, lowest P4 concentrations of both breeds are occurred in summer. Moreover, ovarian activity of Serrana goats resulted in remarkable variation in the P_4 level allover year-round (78). Thus, present results suggest that the conception rate will be higher if *D. does* estrus phases are detected by BG than by FVI, and silent estrus can be detected by measuring P_4 level in pro-estrus phase.

6. SUMMARY

Our experimental study could be summarized as follows:

- All Damascus does do not displays estrus expression in all seasons, and perfect season of estrous activity is autumn and winter.
- ED requires presence of a male, and all estruses can not be detected by BG.
- FVI device can be used as a supporting aid in detecting silent estrus.
- Color, texture, elasticity, pH, Cl of CM, full pattern of arborization and P_4 level (<1.0 ng/ml) should be used for timing of estrus phase and ovulation.
- Higher Cl concentrations during the estrus phase and Na concentration during post-estrus phase can be used in detection of timing of estrous phases.
- About one-third of the P4 quantity found in the spontaneous estrus may be used in determination of pro-estrus phase of silent estrus.
- Finally, the present study suggest that if the numbers of silent estruses detected by FVI device should be added to the spontaneous estruses for enhancing reproductive efficiency in farm animals.

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