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SOME ENZYMATIC ACTIVITIES AND BLOOD PARAMETERS IN FOLLICULAR FLUID AND SERUM OF NON PREGNANT SHE-CAMEL (*Camelus dromadorious*)

(With 4 Tables)

By

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نشاط بعض الخمائر والمعايير الدموية في مصل وسائل الحاثة الجرابية لإنـــــــاث الجمال غير العشـــــــــار

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إستهدفت الدراسة معرفة بعض المكونات الأساسية لمصل الدم وسائل الحاثـــــة الجرابية في مبايض إناث الجمال الغير عشار . وقد أظهرت الدراسة مايلى : ١ .. حـــدوث زيادة معنوية في نشاط خميرة ترانس أمتيز جلوتامك أوكسال أستيك في مصل الحيوانات التى تتميز بحويصلاتها الصغيرة ومتوسطة الحجم ، بينما نشاطه في سائل الحاثة الجرابية للحويصلات كبيرة الحجم كانت قليلة عنها في المصل . ٢ ـ زاد نشاط خميرة ترانس أمينيز جلوتامـــك بيروفيك في الحيوانات التى تتميز بحويصلاتها ذات الحجم الكبير عنه في المصل ، بينمـــا نشاطه في الحويصلات الصغيرة تتمثل في إنخفاضاً معنوياً عنه في المصل . ٣ ـ قد لوحـــظ أن إنخفاض في نشاط خميرة الفوسفات الحامضى في مصل الحيوانات التى تتميز بحويصـــلات كبيرة الحجم ، بينما نشاطه في سائل الحاثة الجرابية تبين أنه كان أعلى نشاطاً فـــــي الحيوانات التى تتميز بحويصلات صغيرة الحجم وكان أقل نشاطاً قد وجد في سائل الحيوصلات متوسطة الحجم . ٤ ـ لايوجد فروق معنوية في نشاط خميرة الفوسفات القاعدى لسائل الحاثـــة الجرابية لمختلف المجاميع بينما كان نشاطه في سائل الحاثة الجرابية عن مثيله في المصـــل في الثلاث مجموعات . ٥ ـ فيما يختص بمستوى عنصرى الصوديوم والبوتاسيوم أظهر زيادة فـــي مصل الحيوانات عن سائل الحاثة الجرابية للحويصلات كبيرة الحجم . بينما كان مستوى كـــل منهم في سائل الحاثة الجرابية في الحويصلات صغيرة ومتوسطة الحجم في زيادة معنوية عنـــه في المصل . لايوجد فروق معنوية لمستوى عنصر الكالسيوم في كل من المصل وسائل الحويصـــلات تبين أن مستوى عنصر الفوسفور في سائل الحاثة الجرابية منخفضاً في الحويصلات ذات الحجـــم المتوسط وكان أعلى مستوى في الحويصلات الصغيرة عنها في المصـــل .

SUMMARY

The present study dealt with some basic information on the follicular fluid constituents of non pregnant She-Camel. The results indicated the following findings and conclusions:

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1. GOT activity appeared to be higher in the follicular fluid than in serum of animals having small and medium size follicles, while its activity in the follicular fluid of large size follicles was significantly lower than in serum.

2. GPT activity increased in those having large size follicles than in the serum, while its activity in the small follicles represents a significant decrease than in the serum.

3. Acid phosphatase activity dropped significantly in the serum of animals having large size follicles, while its activity in the follicular fluid showed fluctuation with highest activity in animals having small size follicles and with lowest activity in medium size ones.

4. There were no significant differences in alkaline phosphatase activity of folliclular fluid of different groups. However its activity in the follicular fluid was significantly higher than that in the serum.

5. Concerning the electrolytes, the sodium and potassium levels were significantly higher in serum than follicular fluid of large size follicles. However, their levels in the follicular fluid of small and medium size follicles were significantly higher than in serum.

There were no significant differences in the calcium level of both serum and follicular fluid. It showed a lower level in medium size follicles and higher values in small ones rather than in the serum.

INTRODUCTION

It is well established that in all mammals, the female animals after reaching the age of puberty, exhibit their sexual activity and male receiptivity during rhythmic periods known as the Oestrus cycle. In most species of animals ovulation occurs spontaneously at a definite time in the cycle, however, in others it does not occur unless mating takes place (ARTHUR et al., 1982).

The ovary of Dromedorius camel resembles a bunch of grape (ABDALLA, 1966). However, it presents no ovulation fossa and follicular growh takes place at any part of the ovary (TAYEB, 1948). TAYEB (1948) and NAWITO et al. (1967) reported that the cyclic corpus luteum is not present in the ovary except during pregnancy. They stated also that the follicular wave includes four phases which correspond to the four phases of the oestrous cycle of spontanous ovulators; proestrus, oestrus, metastrus and dioestrus. The duration of the follicular wave as well as the oestrus phace was determined by several investigators (MAWITO et al., 1967; MUSA and ABU-SINEINA, 1978). However, the physical and the biochemical composition of the follicular fluid was reported by EDWARDS (1974) who stated that the follicular fluid is a slightly viscous. In some species like cow, rat and hamster the viscous nature is not constant during the follicular growth, it decreases as the follicle matures, however, these changes may be attributed to the alterations in the biochemical composition of the mucopolysaccharides of the fluid (ZACHARIAE and THORSOE, 1966). The two fractions of mucopolysaccharides have been identified in the follicular fluid, the highly viscous is hyalouronic acid while the less one is condroitin sulphuric acid (ZACHARIAE, 1957).

The pH of the follicular fluid is either similar or lower than that of serum or plasma (SHALGI et al., 1972a). The osmotic pressure of human follicular fluid was studied by Manarang Dangan and Menge (1971) who reported that it slightly exceeds that of serum. Old and Van DEMARK (1957) concluded that concentration of sodium, magnesium, zinc, copper, chloride and inorganic phosphate in the follicular fluid are similar to those in serum in most species of animals.

Recently WISE (1987) and YOUNIS et al. (1988) found that these inorganic constituents were increased with the follicular enlargement.

PASCU et al. (1968) reported that the glucose level represents about 80% of the total carbohydrate content in cows while trace amounts of fructose are present. They stated that follicular glucose level varies throughout the ovarian cycle where the highest level being found in the follicles at the oestrous phase. It was also reported that several enzymes are found in the follicular fluid (LIPNER, 1973). Various enzymes including ATPase, lactate dehydrogenase, transaminase, acid and alkaline phosphatases were identified in the follicular and cyst fluid obtained from human ovaries (CERLETTI and ZICHELLA, 1961). Acid and alkaline phosphatases, various isomers of lactate dehydrogenase and other enzymes were determined in bovine and buffalo follicular fluid (STALLCUP, 1970). However, CAUSIG et al. (1972) stated that in women the activity of these enzymes in the fluid from healthy follicles is higher than in fluid from cyatic folliclen or serum.

In bovine follicular fluid the alkaline phosphatase activity was highest in all follicular sizes during the earlier stages of oestrous cycle (Day 1-12) and decreased to its lowest levels on the days (13-21) as the follicular diameter increased. YOUNIS et al. (1988) reported that there was no significant alteration in the activities of GPT, GOT and alkaline phosphatase in the follicular fluid of medium size follicles when compared to those recorded in large one.

MATERIAL and METHODS

One hundred and thirty non pregnant one humped mature She-camels (camelus dromedorius) slaughtered in Cairo abattoir were subjected for this study.

Blood samples were collected from each animal immediately after slaughtering. After evaceration the ovaries were collected from the corresponding animals. The diameter of the growing mature follicles were measured using specific caliber. According to the size of the measured follicles animals were classified into three groups: **Group I:** animals having ovaries with small follicle with a diameter (2 to 5 mm) and constitute 80 in number, **Group II:** animals have ovaries with medium follicle with a diameter (5-10 mm) and constitute 40 in number and **Group III:** animals having ovaries with large follicle with a diameter more than 10 mm and comprises 10 in number.

The follicular fluid was aspirated from the follicles by separate clean dry syrings. Animals having small or medium follicles, the follicular fluid were pooled and considered as one sample. A final pooled sample (10) were obtained in both groups I and II. In the third group the collected follicular fluid was quite enough to fulfill all the required biochemical determination.

Blood samples were collected and the serum was obtained.

The activity of GOT (u/L) and GPT in serum and follicular fluid was determined according to the method of REITMAN and FRANKEL (1957).

The activity of the alkaline phosphate (u/L) in serum and follicular fluid was determined according to the method of BESSEY et al. (1946).

The acid phosphatase activity (u/L) in serum and follicular fluid was determined according to the method of FISHMAN and LERNEY (1953).

Calcium content in mg% in both serum and follicular fluid was measured according to GINDLER and KING (1972).

The phosphorous level in mg% in both serum and follicular fluid was determined according to GOMORRI (1942).

Sodium and potassium (MEq/1) concentration in both serum and follicular fluid was measured according to VARELY (1976) using the flamephotometer 4/0 Corning.

RESULTS

Table (1) illustrate the activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in serum and follicular fluid. It is clear that the activity of SGOT in serum increases significantly (P/ 0.01) with the increase in the folicular size whereas its activities in the follicular fluid were nearly the same in small and medium size follicles and decreased significantly (P/ 0.01) in large size one. concerning the level of SGPT in serum there is no significant difference between the different groups. There was a siignificant decreament in its level in the small size follicles and an increament in those having large follicles.

Data given in Table (2) represent the activities of acid phosphatase and alkaline phosphatase in both serum and follicular fluid. It is obvious that there was a significant drop (P/ 0.01) in its level in the serum of animals having large size follicles. However it can be noticed that the acid phosphatase activity in the follicular fluid was significantly higher (P/ 0.01) than its activity in serum. Similarly the alkaline phosphatase levels in the follicular fluid were containuously significantly higher than its level in the serum.

Table (3) showed the level of sodium in serum and follicular fluid of different groups. It represents a highly significant (P/ 0.01) positive correlation in sodium level in serum and follicular fluid of animals having small size follicles. Moreover, there was a significant difference (P/ 0.01) between sodium level in serum and follicular fluid of animals having large size follicles.

Table (1): Activities of glutamic oxalo acetic transaminase(SGOT) U/L and glutamic pyravic transaminase (GPT) U/L in the serum and follicular fluid of non pregnant she-camels.

Group No.	(SGOT) U/L					(SGPT) U/L				
	Serum		Follicular Fluid		r	Serum		Follicular Fluid		r
	Range	Mean	Range	Mean		Range	Mean	Range	Mean	
I Small size Follicles	89 - 230	a(1) 163.3 + 11.51	520 - 890	a (2) 742.2+50.04	- 0.58	10 - 19	a (1) 14.01+ 1.09	4 - 12	a (2) 6.84 + 0.98	-0.07
II Medium size Follicles	310 - 520	b (1) 417.28+19.9	520 - 890	a (2) 775.3+47.91	- 0.17	12 - 19	a (1) 14.78 + 0.89	4 - 17	b (1) 11.72 + 1.16	+0.18
III Large size Follicles	470 - 890	c (1) 708.06+47.9	270 - 760	b (II) 535.16+55.1	+ 0.11	8 - 17	a (1) 11.9 + 1.1	12 - 19	c (2) 15.74 + 0.79	* + 0.70

± : Standard error
* : Significant at $P \leq 0.05$.

= Values within the same column with different alphabetic superscripts are significantly different at ($P \leq 0.01$)

= Values with different arabic numbers within the same row are significantly different at ($P \leq$ o.ol)

= Values with different Lattin numbers within the same row are significantly different at ($P \leq 0.05$)

Table (2): The activities of Acid Phosphatase (ACP) U/L and Alkaline Phosphatase (ALP) U/L in the serum and Follicular Fluid of non-pregnant she-camels.

Group No.	(ACP) U/L					(ALP) U/L				
	Serum		Follicular Fluid		r	Serum		Follicular Fluid		r
	Range	Mean	Range	Mean		Range	Mean	Range	Mean	
I Small size Follicles	9.1 - 13.6	11.15 ± 0.48 [a (1)]	15.7 - 31.3	24.03 ± 1.43 [a (2)]	-0.54	88 - 110	98.9 ± 2.27 [a (1)]	106 - 142	123.6 ± 3.07 [a (2)]	-0.26
II Medium size Follicles	6.1 - 17.7	11.1 ± 1.06 [a (1)]	11.1 - 26.3	17.73 ± 1.57 [b (2)]	-0.21	102-126.	112.2 ± 2.45 [b (1)]	116 - 142	127.1 ± 2.58 [a (2)]	-0.44
III Large size Follicles	1 - 7.6	4.02 ± 0.71 [b (1)]	11.6 - 36.4	27.37 ± 2.59 [ab (2)]	-0.67 *	98 - 138	115.1 ± 4.04 [b (I)]	116 - 150	128 ± 3.19 [a (II)]	+0.64 *

± Standard error.

* Significant at P ∠ 0.05.

- Values within the same column with different alphabatic supersaripts are significantly different at(P/0.01).
- Values within the same row with different arabic numbers are significantly different at(P ∠ 0.01
- Values within the same row with different Latin numbers are significantly different at(P ∠ 0.05) .
- r = correlation factor.

Table (3) Levels of Sodium (mE g/1) and Potassium (mEd /L) in the serum and follicular fluid of non-pergnant she-camels.

Group No.	Sodium , mEg /L					Potassium mEg /L				
	Serum		Follicular Fluid		r	Serum		Follicular Fluid		r
	Range	Mean	Range	Mean		Range	Mean	Range	Mean	
I Small size Follicles	102.5-223	166.9 ± 14.31 [a] (1)	158 - 261	191.35 ±10.58 [a] (1)	+0.88 **	2.5-15.5	6.8 ± 1.39 a(1)	4.5-13.5	8.4 ± 1.01 A (1)	-0.58
II Medium size Follicles	87.5-188.5	169.65 ± 12.34 [a] (1)	134.5-221.5	193.85 ± 9.09 [a] (1)	+0.22	1.5-9.5	4.6 ± 0.80 [a] (1)	5.5-14	8.6 ± 0.81 [a] (2)	-0.54
III Large size Follicles	180.5-308	227.75 ± 11.54 [b] (1)	61.5-158.5	117.6±10.47 [b] (2)	+0.10	2.5-8.5	6.25±0.57 [a] (1)	3-7.5	5.4 ± 0.48 b B (1)	-0,34

± Standard error

** Significant at (P /_ 0.01).

- Values within the same column with different alphabetic superscripts are significantly different at(P ∠ 0.05 case of Capital letters and(P ∠ 0.01 in case of small letters.
- Values within the same row with different arabic numbers are significantly different at (F ∠ 0.01).
- r = correlation factor.

The potassium level did not vary significantly between the different groups Table (3). Where there was a significant (0.01) difference between its level in serum and follicular fluid in animals having medium size follicles.

Table (4) show the calcium and phosphorus levels in serum and follicular fluid, it is clear that the calcium level was nearly similar in serum and follicular fluid. Concerning the phosphorus level no significant differences was shown of different animals. On the other hand its level in the follicular fluid showed, significant changes with the lowest level in animals with medium size follieles and highest level in animals with small size follicles. However, its level in animals having small size follicles was still significant (P/ 0.01) higher than in serum.

DISCUSSION

The present study is a trial to establish some basic information on the follicular fluid constituents of non pregnant one humped she-camels (Camelus deomedoerious). The correlation between these constituents in the serum and follicular fluid as well as their relationship during follicular development was also an objective.

The results of the present study revealed that the follicular fluid of non pregnant She-camels contains high levels of glutamic oxalcacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) (Table 1). These findings come in agreement with earlier reports, which recorded the presence of transaminase in the follicular fluid of human ovaries (CERLETTI & ZICHELLA, 1961 and CAUSIG et al., 1971) and also in the recent study on buffalo (YOUNIS et al., 1988).

The present results showed that the levels of GOT in the follicular fluid decreased significantly (P/ 0.01) in mature or large size follicles. The latter findings pass parellel with the results of YOUNIS et al. (1988) on buffaloes. It is obvious that the content of follicular GOT was significantly higher than its level in serum in animals having ovaries with growing of a small or medium size. These results indicate that the active granulosa cells share to some extent in adding GOT to the follicular fluid. The most striking finding in the present study was the tremendous increase in the serum content of GOT which go in parellel with the advanced increase in the follicular size. It has been reported that the ovarian follicle in She-camels reaches the mature size rapidlly; within siz days and persists as mature follicle for a relatively long period; 13 days (MUSA and ABU-SINCINA, 1978). Moreover, the accumlation of estradiol in the follicle and the increase in its level in the blood has been also reported (TAHA et al., 1984). ABDEL-KADER et al. (1979) found that the long acting steriods induce an adverse effect on the liver which results in an increase in the levels of transaminases. Based on these previous findings, the noticed increase in serum GOT levels in the present study, can be acceptable.

On the other hand the level of follicular GPT, in the current work, increased significantly with the increase in the follicular size. The previous study in buffaloes (YOUNIS et al., 1988) disagree with the present results. This disagreement can be attributed partially to species difference, however, since level of GPT of mature

Table (4) The levels of calcium (mg%) and inorganic phosphate (mg%) in the serum and follicular fluid of non-pregnant she-camels.

Group No.	Calcium mg%					Inorganic phosphate mg%				
	Serum		Follicular Fluid		r	Serum		Follicular Fluid		r
	Range	Mean	Range	Mean		Range	Mean	Range	Mean	
I Small size follicles	9.8 - 16.8	13.52 ± 0.79 a (1)	7 - 19.6	14.42 ± 1.39 a (1)	-0.10	6.95-8.85	8 ± 0.19 a (1)	7.4 - 11.2	9.15 ± 0.32 aA (2)	-0,75 *
II Medium size follicles	5.6 - 16.8	11.56 ± 1.39 a (1)	7 - 16.8	13.22 ± 0.77 a (1)	-0.09	6.55-9.5	7.89 ± 0.28 a (1)	6.35 - 8.85	7.51 ± 0.31 b (1)	+0.08
III Large size follicles	11.2 - 18.2	14.28 ± 0.62 a (1)	12.6-16.8	14.29 ± 0.37 a (1)	+0.25	5.9-10.35	8.39±0.41 a (1)	6.55 -9.3	8.11 ± 0.28 B (1)	-0.31

\+ Standard error

* Significant at ($P < 0.05$)

\- Values within the same column with different alphabetic super scripts are significantly different at ($P < 0.05$) in case of captial letters and ($P < 0.01$) in case of small letters.

\- Values within the same row with different arabic numbers are significantly different at ($P < 0.01$)

\- r = Correlation factor.

follicles was significantly higher than its level in serum, it is thought that granulosa cells are contributing to GPT content of the follicular fluid.

During follicular development the granulosa cells are more active and so it has a synthetic activity for steriod production. The precusor of steriod synthesis is the acetate which form cholesterol then cholesterol leads to steroid synthesis (PETERS and McNATY, 1980). The transaminases catalyze theformation of pyruvate which produce the active acetate molecules. (HARPER et al., 1979). Our results show that the follicular fluid contains high levels of transaminases which increase with follicular development. So it is suggested that transaminases may affect ovarian steriodogensis. This suggestion is supported by the finding that total cholesterol was significantly higher in the serum of cows with medium or large follicules (PUROHIT and KOHLL, 1977), ewes (IBRAHIM et al., 1984) and in She-camels (IBRAHIM, 1989).

It is obvious from data presented in Table (2) that acid and alkaline phosphatases are found at considerable levels in the follicular fluid of different groups. These results come in accordance with previous reports (CERLETTI & ZICHELLA, 1961; CAUSIG et al., 1971; WISE, 1987 and YOUIS et al., 1988). It can be noticed that level of acid phosphatase (ACP) in the follicular fluid decreased significantly with the increase in follicular size from small to medium size, but was not influenced in mature follicles. These findings agree partially with previous studies (WISE, 1987 and YOUNIS et al., 1988).

Meanwhile, the activity of alkaline phosphatase (ALP) in the follicular fluid was nearly the same and did not correlate with the follicular size. This result is not in agreement with those obtained by WISE (1987) and YOUNIS (1988). The species difference may be the factor responsible for such disagreement. It is of interest to note that, the activities of both ACP and ALP in the follicular fluid of the different groups are significantly higher than the corresponding levels in the serum. The role of granulosa cells in the contribution of these enzymes may be acceptable.

During follicular development the ovaries are metabolically active, so it needs more energy for this increased metabolism, and as ACP and LAP catalyze the hydrolysis of phosphate esters in acidic and alkaline pH respectively (Harper et al., 1979), so it is suggested that the energy required for metabolic activity of the ovaries may be partially obtained from hydrolysis of phosphate esters. The activity of phosphatases depends on the pH, and as the ACP level in follicular fluid is higher in small follicles, so its pH is acidic, and so energy needed during early development may be taken through ACP. While as the follicle grows, the ALP levels in the follicular fluid increases and the pH changes from acidic side to alkaline side therefore, energy required may be taken through ALP.

The concentration of sodium in the follicular fluid was significantly reduced in the mature follicles only, Table (3). This finding disagrees with the results of previous studies in buffaloes (YOUNIS et al., 1988). Similarly, the present results revealed that potassium concentration decreased significantly in the follicular fluid of mature follicles (Table 3). This finding disagrees with the result recorded in cattle (WISE, 1987) but comes in some accordance with the results obtained in buffloes (YOUNIS et al., 1988). The species difference may be the factor creating this disagreement. In general, the present data showed that sodium and potassium concentrations in the follicular fluid was nearly close to their levels in the serum. EDWARDS (1974), reported similar conclusion. Bearing in mind that the monovalent cations, sodium and potassium, are in the main elements responsible for the creation of osmotic pressure, it can be considered that the osmotic pressure of the follicular fluid is nearly similar to that of the serum. This consideration receives some support from earlier reports (EDWARDS, 1974 and SHALGI et al., 1972b). Moreover, sodium and potassium regulate cell membrane permeability and the acid - base balance (HARPER et al., 1979). So it also concluded that the pH of follicular fluid is similar to the serum pH. This conclusion receives some support from earlier reports (ZACHARIAE and JENSEN, 1958). It is suggested that the similarity osmotic pressure and pH of follicular fluid to that of serum may provide a suitable environment for occyte maturation. The results of the current work showed that the concentrations of both calcium and inorganic phosphorus in the follicular fluid were, in general, similar to their levels in serum, Table (4), so they pass freely through membranes of thefollicle. These results receives confirmation from earlier studies by PETKOV, ANTANOV and DZHUROVA (1969). The presence of calcium in the follicular fluid may have a role inregulating permeability of the membranes of the follicle, HARPER et al. (1979). The presence of phosphate in the follicular fluid is also important, as esters has a great importance in energy transfer (HARPER et al., 1979), and its presence in the follicular fluid provide a source of energy for metabolic activities of the follicle.

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