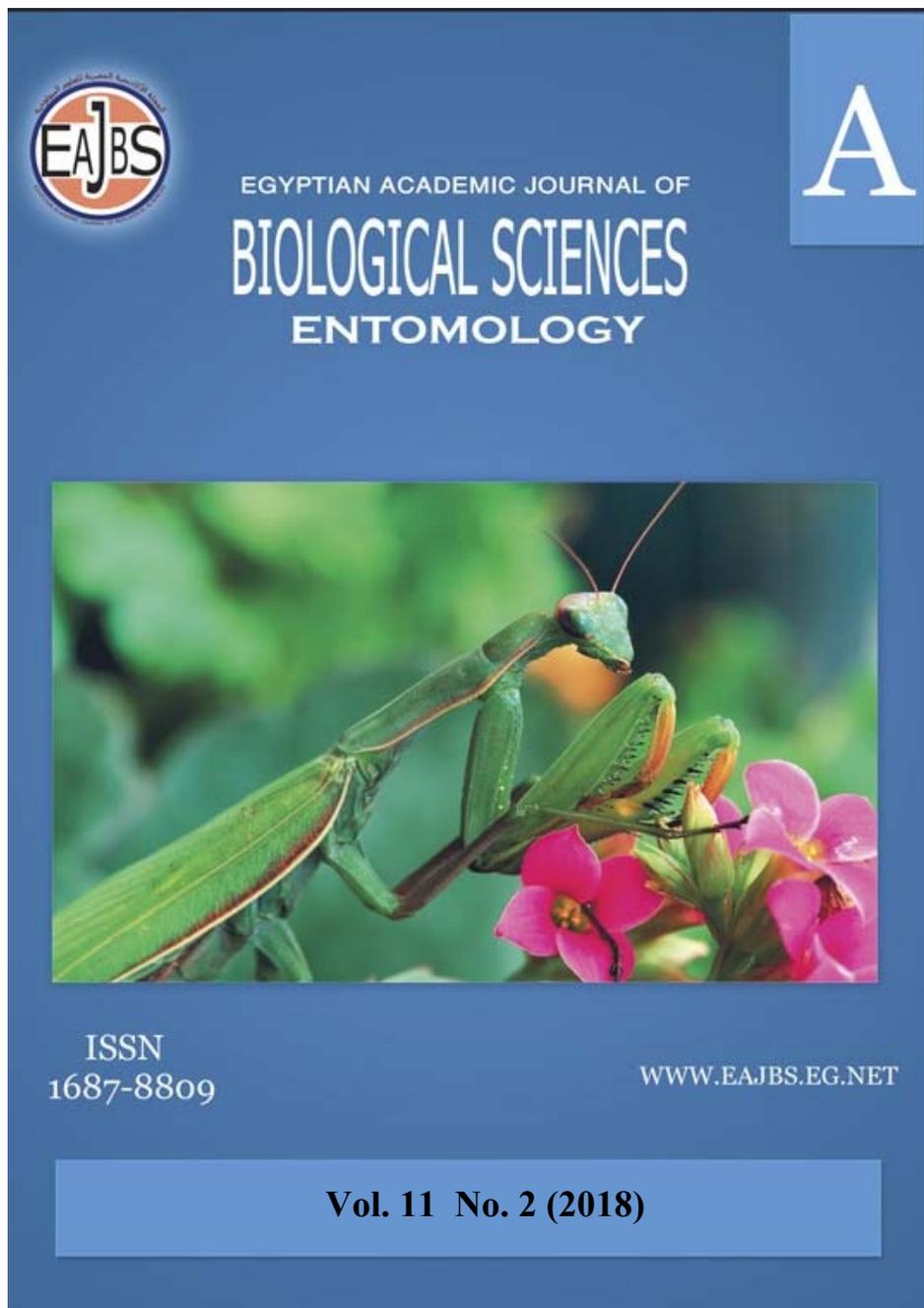


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Changes in Vitellogenic Proteins During The Reproductive Cycle of The Female Tick, *Argas persicus*

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ARTICLE INFO

Article History

Received: 20/2/2018

Accepted: 25/3/2018

Keywords:

Argas persicus,
vitellogenic protein,
female reproductive
cycle

ABSTRACT

Proteins in the freshly deposited eggs of mated fed female *Argas persicus* were electrophoretically separated into twelve fractions or egg vitellins (EVts) with Mwt of 24.72-131.45kD. One fraction (131.45kD) existed only in the egg and eleven were exogenous EVts with extraovarian counterparts (vitellogenins) in the hemolymph (HL). Six of them were common EVts (24.72-65.53kD) with similar counterparts in the HL of both male and female and five were female-specific EVts (78.65-112.92 and 48.67kD) with similar counterparts only in the female HL.

Eleven vitellogenins (Vgs) and ten exogenous vitellins (Vts) with similar electrophoretic mobilities to EVts were identified in the HL and ovaries, respectively, of mated females. The number and percent amount of the detected vitellogenic protein fractions varied in the different physiological states (10) studied (unfed, 0-7 and 20 day after feeding, daf). Generally, there was an increase of the two parameters in ovary vitellin fractions (with appearance of new Vts) and a decrease in HL vitellogenin fractions during vitellogenesis at the late period of the female reproductive cycle (4-7 and 20 daf).

The obtained results support the concept of the uptake of hemolymph Vgs by the ovaries and deposition as Vts in the oocytes during vitellogenesis which may extend till the end of the oviposition period (20 daf) in the female *A. persicus*.

INTRODUCTION

The fowl tick *A. persicus* infects poultry and wild birds in Egypt and several countries around the world (Hoogstraal et al., 1975). This species is of a considerable veterinary importance, acts as an efficient reservoir and a vector of viral, rickettsial and spirochaetal infectious agents to birds (Khalil, 1979) and probably to human (Darwish et al., 1977).

Studies of changes in the hemolymph (HL) and ovary proteins are essential not only for understanding the different processes associated with reproduction in ticks (Diehl et al., 1982) but also with the ticks interrelationships to pathogens they transmit (Boldbaatar et al., 2008). Furthermore, proteins in the arthropod vectors of

disease may provide specific target molecules toward which new control approaches could be directed (Mayer et al., 1999).

Changes in HL and ovarian total protein and fractions have been reported during oogenesis and oviposition of female ticks (Tatchell, 1971; Diehl et al., 1982; Shanbaky et al., 1990a, b; Yousry, 2011; Radwan et al., 2015). In argasid and ixodid ticks, egg yolk proteins (vitellins) were found to be synthesized endogenously inside the ovary (Araman, 1979) or exogenously outside the ovary as vitellogenins with subsequent transfer to oocytes via the HL (Taylor and Chinzei, 2001). Variations in the concentration of vitellogenins have been suggested as indicators of different stages of oogenesis and may be used in studies involving the regulation of vitellogenesis and reproduction (James and Oliver, 1996).

The present work is intended to provide some basic knowledge about changes of vitellogenic protein fractions in the HL and ovaries of *Argas persicus* during the reproductive cycle of the female. Native protein fractions in the HL, ovaries and freshly deposited eggs are electrophoretically separated, compared and vitellogenic protein fractions are identified. Male HL protein fractions are also examined for comparison.

MATERIALS AND METHODS

Ticks:

The fowl tick *Argas persicus* was collected from domestic chicken houses in Banisweif governorate. Tick colony was maintained in laboratory at $27\pm 1^{\circ}\text{C}$, 75% RH and 16 hr light and ticks were fed on domestic pigeon (*Columbia livia*) according to Khalil (1979).

Sample Preparation:

Mated unfed and fed ticks 1 hr and on different days after feeding (0, 1-7 and 20 daf) were used to provide HL, ovaries and freshly deposited eggs. Samples were obtained and prepared as described by Radwan et al. (2015).

Total protein:

The total protein concentration of the samples was measured photometrically at 595 nm and compared to a standard of bovine gamma globulin according to Lowery et al. (1951).

Gel Electrophoresis:

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) was used to separate HL, ovaries and egg protein fractions (Smith, 1976). Before electrophoresis, the total protein of each sample was adjusted to 1mg protein/ml. Molecular weight (Mwt) standards of 10-120kD were prepared in the solubilization buffer to assist in the Mwt determination of the sample protein fractions. Electrophoresis in SDS was done using 1 mm-thick slab gel that consisted of 5 % polyacrylamide stacking gel followed by 10 % gel. Bromophenol blue was used as tracking dye. Twenty μl of each sample were added to each well of the gel. Two wells in each slab were used for the Mwt standards and the blank. Electrophoresis was done at $27\pm 1^{\circ}\text{C}$ using 20 mA/plate for approximately 5 hr. The gels were stained with Coomassie blue for proteins. The gels were photographed and scanned using a DCD-16 Gelman densitometer to determine the relative concentration (% amount) of the resolved protein bands. Vitellogenic protein fractions with similar electrophoretic mobilities to egg protein bands (Mwt in egg \pm 1kD) were identified in the HL of mated female (and male) ticks and in the ovaries of the different physiological states examined (unfed and fed on 0-7 & 20 daf).

RESULTS AND DISCUSSION

I- Native Protein Fractions:

I.1. Hemolymph (HL) Protein Fractions In Female And Male *A.Persicus*:

The HL protein of mated female *A.persicus* unfed and fed 0-3 daf were electrophoretically separated into 27 protein fractions banding patterns (rows) with molecular weight of 10.85-125.98kD and a number of protein fractions (or bands) of 23-24 fractions in the different physiological states (lanes) with a total of 116 fractions during this period (Fig.1a). On 4-7 and 20 daf, the banding patterns slightly decreased to 25 banding patterns with Mwt of 12.19-110.98kD and a number of 24 protein fractions in each of the physiological states examined, with a total of 120 fractions during this period (Fig.1b). The slight increase in the total number of protein bands in the latter period may point to a slight increase of protein synthesis and/or release in the HL which exceeded utilization (Engelmann, 1979).

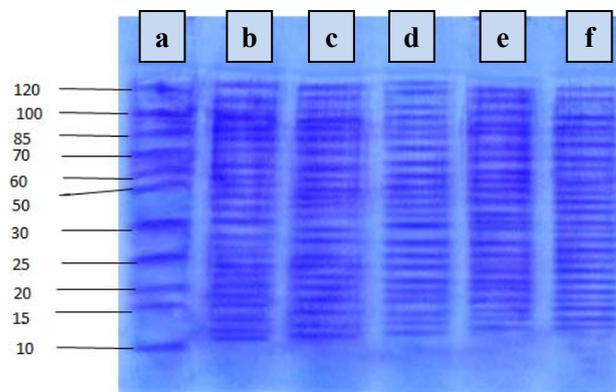


Fig. (1a): Electrophoretic protein fractions in the hemolymph of mated unfed and fed female *Argas persicus* on different days after feeding (daf) a. Standards molecular weight, b, c, d, e & f : unfed, immediately, one, two & three daf.

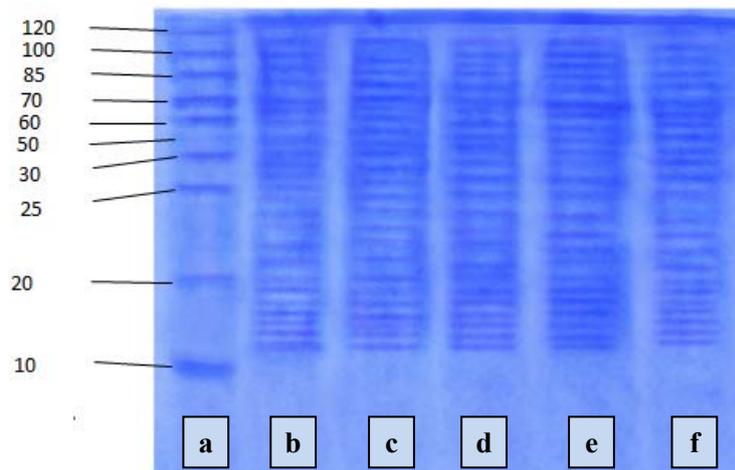


Fig. (1b): Electrophoretic protein fractions in the hemolymph of mated fed female *Argas persicus* on different days after feeding (daf) a. Standards molecular weight, b, c, d, e & f: Four, five, six, seven & twenty daf.

The HL proteins of mated male *A.persicus* unfed and fed 0-7 and 20 daf were electrophoretically separated into 14 protein fractions banding patterns with Mwt of 22.72-166.65kD and a number of protein fractions of 11-12 fractions in the different physiological states studied with a total of 116 fractions (Fig.2a,b). Fifty eight protein fractions were electrophoretically separated in each of the early (unfed & 0-3 daf) and late (4-7 & 20 daf) period of the reproductive cycle as represented by the physiological states studied. Almost all protein fractions in the male HL had more or less corresponding bands in the female HL except eight fractions with high Mwt of 129.10-166.61kD appeared on 4-7 and 20 daf in male HL and were not represented in the female. These male specific HL proteins might be related to needs of the male accessory glands for production of proteinaceous components and substances in the spermatophore (Oliver, 1982). However, the number of protein fractions in the female HL of the different physiological states examined and their total sum were nearly two times higher in the female (23-24 and 236 fractions) than in male HL (11-12 and 116 fractions) during the whole period of study (unfed, 0-7 and 20 daf). The present results and the higher concentrations of the total protein in the HL of the female *A.persicus* than in male on 3-7 daf (Radwan *et al.*,2015) are in agreement with findings in previously studied argasids such as *A.hermannii* (Shanbaky *et al.*,1990 a) and *Ornithodoros erraticus* (Yousery,2011) and reflect the increasing demands of female argasids for proteins during vitellogenesis.

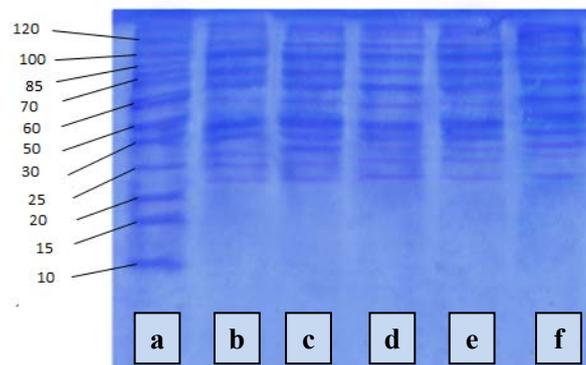


Fig. (2a): Electrophoretic protein fractions in the hemolymph of mated unfed and fed male *Argas persicus* on different days after feeding (daf). a. Standards molecular weight, b; unfed, c, d, e & f: immediately, one, two & three daf.

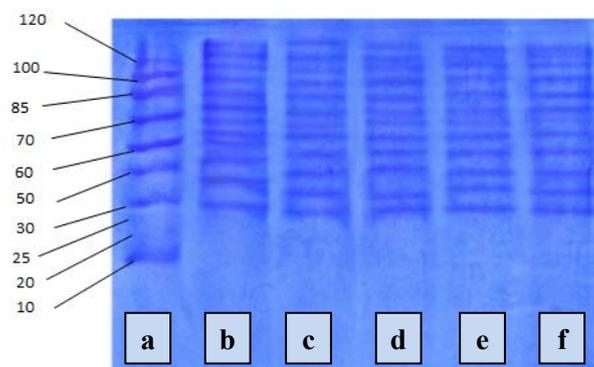


Fig. (2b): Electrophoretic protein fractions in the hemolymph of mated fed male *Argas persicus* on different days after feeding (daf). a. Standards molecular weight, b, c, d, e & f: Four, five, six, seven & twenty daf.

I.2. Ovary Protein Fractions in Female *A.persicus*:

The ovary proteins of mated female *A.persicus* unfed and fed 0-3 daf were electrophoretically separated into 22 banding patterns (rows) with Mwt of 10.98-122.79kD and a number of protein fractions of 17-20 fractions in the different physiological states with a total of 91 fractions during this period (Fig.3a). On 4-7 and 20 daf, the banding patterns increased to 27 patterns with Mwt of 9.51-140.76kD and a number of protein fractions of 20-23 fractions in the different physiological states with a total of 107 fractions during this period (Fig.3b). The evident increase in the numbers of ovary protein fractions on 4-7 and 20 daf conforms to results of the total proteins in *A.persicus* (Radwan et al., 2015) where their concentration in the ovaries was noticeably increased on the 4th daf to reach maximal levels on 5-7 daf which followed the maximal levels of the total proteins in the female HL on 4-7 daf. This suggested an uptake and/or increased synthesis of proteins in the ovaries of *A.persicus* on 4-7 and 20 daf. In the present work, it was found that the increase in number of the ovary native protein fractions in *A.persicus* was associated with an increase in number (5-8 fractions) and percent amount (23-38.83 %) of the ovary vitellins (Vts). Also, there was 2.9 fold increase in number of small native protein fractions with low Mwt of less than 30kD on 4-7 and 20 daf (49 fractions) more than in the unfed and fed 0-3 daf (17 fractions). This might be attributed to a breakdown of larger protein probably to be incorporated into other vitellogenic and non vitellogenic proteins. In the present work, five native protein fractions with high Mwt disappeared from the ovaries (122.79 and 108.93-109.87kD) on 4-7 and 20 daf. Chinzei (1986) reported that during the uptake of vitellogenins (Vgs) into the ovary, they are processed to Vts by proteolysis and then incorporated into the oocytes.

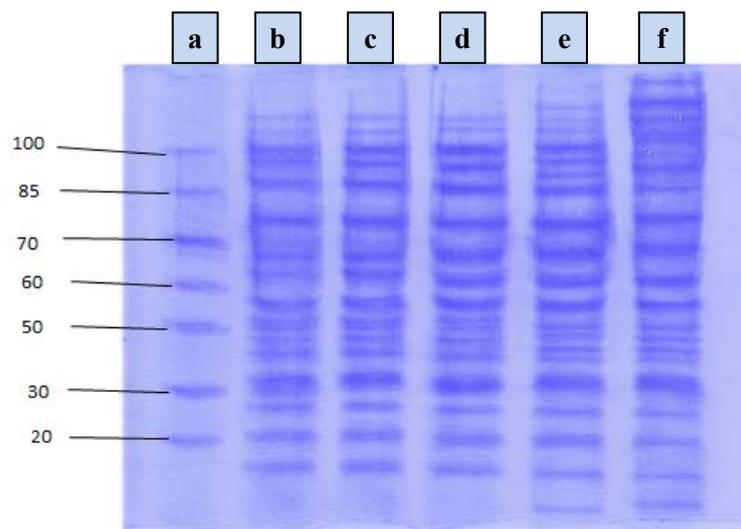


Fig. (3a): Electrophoretic protein fractions in the ovaries of mated unfed and fed female *Argas persicus* on different days after feeding (daf). a. Standards molecular weight, b; unfed, c, d, e & f: immediately, one, two & three daf.

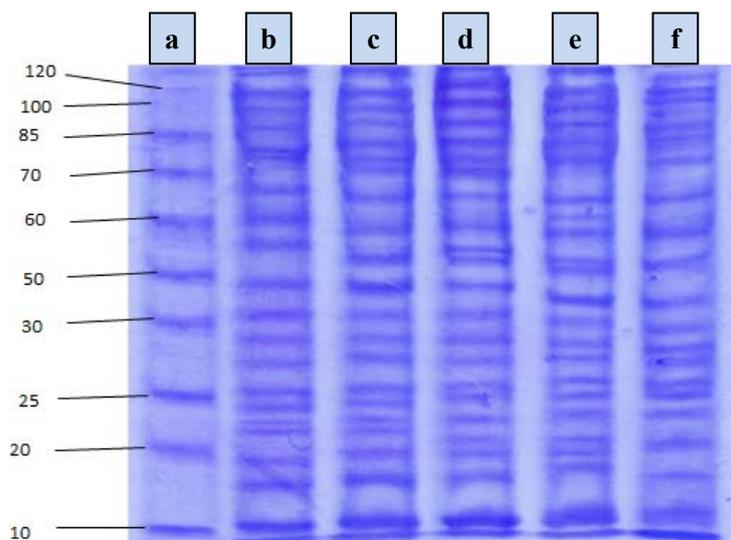


Fig. (3b): Electrophoretic protein fractions in the ovaries of mated fed female *Argas persicus* on different days after feeding (daf). a. Standards molecular weight, b, c, d, e & f: Four, five, six, seven & twenty daf.

In comparison with female HL, the total number of protein fractions in the HL of female *A.persicus* was much greater than in the ovaries throughout the whole period of study (236 in HL:198 in Ov.), in the unfed and 0-3 daf (116 in HL:91 in Ov.) and on 4-7 and 20 daf (120 in HL:107 in Ov.). This is attributed to the fact that protein fractions in the HL represent the outcome of biosynthesis, release, uptake and utilization of protein of the different physiological processes and activities in the whole body of the tick, while protein fractions in the ovaries represent an outcome of protein synthesis, uptake and utilization in the ovaries during egg development and oviposition.

II Egg And Vitellogenic Protein Fractions of Adult *A.persicus*:

II.1. Egg Protein Fractions (Vitellins):

Proteins of the freshly deposited egg of mated fed female *A.persicus* were electrophoretically separated into twelve protein fractions or egg vitellins (EVts) with Mwt of 24.72-131.45kD (Table 1) and percent relative amounts of 2.61-13.57 % where band of 24.72kD had the highest percent amount of the egg proteins. One fraction with the highest Mwt (131.45kD) was detected only in the deposited eggs and eleven EVts had corresponding protein bands with similar electrophoretic mobilities in the HL or HL and ovaries and were considered as exogenous EVts with Mwt of 24.72-112.92kD. These were presumed to originate outside the ovaries as vitellogenins (Vgs) with subsequent transfer to the ovaries via the HL (Taylor and Chinzei,2001) to be selectively taken up through receptor mediated endocytosis or binding protein in the ovaries (Friesen and Kaufman,2002 & 2004; Boldbaatar et al.,2008). In *A.persicus*, five female specific EVts (78.65-112.92 and 48.67kD) had corresponding Vgs in the HL of the female but not in the male and six were common EVts (24.72-65.53kD) with similar counterparts (Vgs) in the HL of both male and female ticks.

Generally, EVts constitute the major proteins (about 90-95% of all proteins) in the egg yolk (Diehl, 1970) and serve as main nutrients for developing embryo.(Kamel et al.1982). Several authors studied yolk protein in egg extracts and separated protein fractions by electrophoretical and immunological techniques (Diehl, 1970; Araman, 1979). Using SDS-PAGE, tick EVts have been resolved into

6-9 polypeptides in *Dermacentor andersoni* (Boctor and kamel, 1976), *Ornithodoros moubata* (Chinzei et al.,1983), *D.variabilis* (Rosell and Coons, 1991) and *Ixodes scapularis* (James and Oliver, 1996).Tatchell (1971) identified two hemoglycoproteins in the HL and egg of female *Boophilus microplus* but was unable to demonstrate their immunological identity despite their similar electrophoretic mobility. Shanbaky et al. (1990 b) used 22 EVts of *Argas hermanni* to make antisera for immunoelectrophoretic analysis of Vgs and Vts in the HL and ovaries, respectively. They found that almost all exogenous Vts and their corresponding Vgs were hemoglycolipoproteins with a relatively low molecular mobilities of 66.2 to > 200kD. In female argasid and ixodid ticks, EVts were found to be synthesized endogenously inside the oocytes as Vts (Araman, 1979) or exogenously mainly in the fat body as Vgs (Chinzei and Yano, 1985), released into the HL to be transported and selectively incorporated into oocytes as Vts (Araman, 1979; Taylor and Chinzei,2001). In the present study, no endogenous Vts were identified in the freshly deposited eggs of female *A.persicus* as in the case of *A.hermanni* where 7 endogenous EVts were restricted to the ovaries and the deposited egg without corresponding fractions in the HL (Shanbaky et al., 1990 b).

Table (1): Egg proteins (vitellins) and vitellogenic proteins in the hemolymph (vitellogenins) of male and female and the ovaries (vitellins) of the female *Argas persicus*

Egg vitellins Mwt (kD)	Ovary vitellins	Hemolymph vitellogenins		Egg and vitellogenic proteins
		Female	Male	
131.45	-	-	-	Egg Vt
112.92	-	+	-	Exo. ♀ Spec. Vt*
100	+	+	-	Exo. ♀ Spec. Vt
89.732	+	+	-	Exo. ♀ Spec. Vt
78.649	+	+	-	Exo. ♀ Spec. Vt
65.525	+	+	+	Exo. com. Vt**
60	+	+	+	Exo. com. Vt
55.490	+	+	+	Exo. com. Vt
48.674	+	+	-	Exo. ♀ Spec. Vt
38.213	+	+	+	Exo. com. Vt
29.638	+	+	+	Exo. com. Vt
24.723	+	+	+	Exo. com. Vt

*Exo. ♀ Spec. Vt., exogenous female specific vitellins.

** Exo. Com. Vt., exogenous common vitellins.

II.2.Vitellogenic Protein Fractions in The Hemolymph and Ovaries of *A.persicus*:

Vitellogenic protein fractions with similar electrophoretic mobilities to EVts were identified in the HL or HL and ovaries of mated female *A.persicus*. Male HL proteins were also examined for comparison (Table1). Eleven vitellogenins (Vgs) were identified (24.72-112.92kD) in the HL of female, six of them were common Vgs which were identified in the HL of both male and female and five were female-specific Vgs identified in the HL of only female. Also, ten exogenous vitellins (24.72-100kD) were identified in the ovaries of females in the different physiological states examined.

Vitellogenins in the HL of mated female *A.persicus* (Table 2 and 3) were detected in the unfed female (5 fractions and 18.28% amount) and increased in number (8 fractions) and percent amount (28.48%) as early as 1 hr after feeding (0 daf) and up to the 2nd daf (6-7 fractions and 21.89-29.50% amount) with appearance of 7 new Vgs (112.92, 101.22, 88.85, 64.36, 58.8, 47.73 and 38.73kD) in the HL during this period (Table 3). On the 3rd and next days after feeding, the number and percent amount of Vg fractions relatively decreased (5-6 fractions and 15.79-19.89% amount) to reach minimal levels (3 fractions and 10.50% amount) on 20 daf (at the end of oviposition). Similarly, vitellins (Vts) in the ovaries of mated female *A.persicus* (Table 2 and 3) were detected in the unfed mated female (4 fractions and 19.10% amount) but their number and percent amount remained more or less constant after feeding (4 fractions and 16.81-19.16% amount) almost in all early physiological states (Table 2) with appearance (Table 3) of three new Vts (60.85, 38.12 and 25.59kD). However, the number and percent amount of Vt fractions in the ovaries noticeably increased (5-8 fractions and 23-38.83% amount) on 4-7 and 20 daf with the highest levels and appearance of two new exogenous Vt fractions (78.33 and 28.81kD) on the 4th daf in addition to a third new Vt (89.73kD) which appeared in the ovaries on 6, 7 and 20 daf (Table 3). Appearance and disappearance of the vitellogenic protein fractions occurred in the different physiological states examined in the present study. Generally, appearance of protein fractions might be attributed to their production at higher rates than their utilization while disappearance might be due to their consumption, breakdown or incorporation in other vitellogenic or nonvitellogenic proteins (Engelmann, 1979). In the present work, the total number of Vg & Vt fractions detected in the HL and ovaries were 31 & 22 and 25 & 33 fractions during the early (unfed, 0-3 daf) and late (4-7 and 20 daf) period, respectively, of the reproductive cycle of the female *A.persicus*. The increase in number and percent amount of Vt fractions in the ovaries in the different physiological states examined (Table 2) during the latter period in comparison with the lower levels and relative stability of the two parameters during the early period suggested an increase of yolk deposition in the ovaries and that vitellogenesis occurred on 4-7 and 20 daf. Furthermore, the aforementioned results support the concept of the uptake of Vgs by ovaries during vitellogenesis (Taylor and Chinzei, 2001; Friesen and Kaufman, 2004) which may extend during the oviposition period of ticks where ovaries contain oocytes in different stages of development and maturity (Balashov, 1972). This leads to more consumption of Vgs and has been manifested as a decrease in the number (3 fractions) and percent amount (10.5% amount) of Vg fractions at the end of oviposition (20 daf) in *A.persicus* (Table 2). Also, all ovary Vts of this tick species (10 Vts) have been identified as being exogenous with Vg counterparts in the HL (Table 1 and 3). The decrease in number and percent amount of Vg fractions in the different physiological states and their total number during vitellogenesis and at the end of oviposition (4-7 and 20 daf) than during previtellogenesis (unfed and 0-3 daf) suggested Vgs uptake during vitellogenesis and oviposition. In addition, appearance of most Vt fractions in the ovaries of female *A.persicus*, followed or accompanied the detection of their Vg counterparts in the HL (Table 3). Three exogenous ovary Vts (89.73, 78.33 and 28.81kD) detected on one or more day(s) during 4-7 and 20 daf were preceded by the early appearance of their corresponding Vg counterparts in the HL of the unfed (79.67 and 30.70kD) and fed female immediately (0 daf) after feeding (88.85kD). These three Vgs remained in the HL solely during the previtellogenesis prior the detection of their corresponding Vts in the ovaries during vitellogenesis. The early

appearance of new Vgs in the HL of female *A.persicus* after feeding and the relative increase in number and percent amount of Vg fractions during previtellogenesis conform to findings in previous studies on ticks where early appearance and increase in number or concentration of Vgs occurred during oogenesis (Diehl, 1970; Chinzei, 1983; Shanbaky et al., 1990 b; Schriefer, 1991; Rosell and Coons, 1991; James and Oliver, 1996; Friesen and kaufman, 2002). In agreement with the present results Schriefer (1991) found that the increase in Vg level in *Hyalomma dromedarii* during oogenesis was followed by a decrease after oviposition.

Table (2): Number and percent amount of vitellogenic protein fractions in the ovary and hemolymph (HL) of mated adult *Argas persicus* on different days after feeding (daf) during the reproductive cycle of the female.

Days after feeding	Ovary vitellins		Female HL vitellogenins		Male HL vitellogenins	
	Number	% amount	Number	% amount	Number	% amount
unfed	4	19.10	5	18.28	3	14.42
0 daf	4	16.81	8	28.48	3	13.54
1 daf	6	-	7	29.50	1	6.23
2 daf	4	19.16	6	21.89	1	6.44
3 daf	4	17.47	5	15.79	3	13.09
4 daf	8	38.83	6	19.76	1	4.997
5 daf	5	23	5	18.07	1	6.45
6 daf	7	28.81	5	18.91	0	0
7 daf	7	29.01	6	19.89	0	0
20 daf	6	25.14	3	10.50	0	0

In *A.persicus*, five Vgs were identified in the HL of the female but not in male HL and were considered as female-specific Vgs (Table 1). Four of these Vgs had a relatively high Mwt of 79.67, 88.85, 101.22 and 112.92kD and one Vg fraction with Mwt of 47.73kD. Most of these female-specific Vgs appeared after feeding in the HL (Table 3) during previtellogenesis but their Vt counterparts appeared in the ovaries during vitellogenesis (fractions of 78.33 and 89.73kD) or in the freshly deposited egg (fraction of 112.92kD) which suggested an uptake by the ovaries. In *A.hermannii*, eight hemoglycolipoproteins were restricted to the HL of mated female but not detected in male HL and were considered as female-specific Vgs (Shanbaky et al., 1990 b). In *Amblyomma hebraeum*, Friesen and Kaufman (2002) identified two polypeptides (Vg 211 and 148kD) in HL from vitellogenic female but not in HL of male and nonvitellogenic females. The two Vgs immunologically corresponded to minor polypeptides (6) of similar Mwts in the ovary.

As was mentioned before, six Vgs with similar electrophoretic molecular mobilities to EVts (24.72-65.53kD) appeared in the HL of both male and female *A.persicus* and were considered as common Vgs (Table1). The occurrence of Vgs common to both sexes has been shown immunologically in the argasid ticks *Ornithodoros moubata* (Diehl, 1970; Chinzei et al., 1983) and *A.hermannii* (Shanbaky et al., 1990 b). The electrophoretic patterns of the HL proteins of *Hyalomma* and *Argas* sp. (Dolp and Hamdy, 1971) and the extracts of the ixodid *Amblyomma maculatus* (McGowan et al., 1982) were similar but not identical in males and females of each species. In *A.persicus*, 6 Vgs were identified and 0-3 Vg fraction (s) were detected in the HL of the different physiological states examined

in male with total number (sum) of 13 Vg fractions and Mwt of 25.27-65.44kD . These numbers were much less than in the female HL (Tables 1 & 2) which indicates the increased needs of the female for vitellogenins.

Table (3): Egg and vitellogenic protein fractions in hemolymph (HL) and ovary (OV) of female *Argas persicus* on different days after feeding (daf).

Egg	Molecular weight (kD)																			
	unfed		0daf		1daf		2daf		3daf		4daf		5daf		6daf		7daf		20daf	
	HL	OV	HL	OV	HL	OV	HL	OV	HL	OV	HL	OV	HL	OV	HL	OV	HL	OV	HL	OV
131.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
112.92	-	-	112.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	100	-	100.86	-	100.86	101.22	-	-	-	100	98.92	-	-	-	-	-	98.92	-	-
89.732	-	-	88.85	-	88.85	-	-	-	88.85	-	-	-	-	-	-	90.71	-	89.73	-	89.73
78.649	79.67	-	-	-	-	-	-	-	-	-	-	78.33	79.03	-	-	-	-	-	-	-
65.525	-	66.18	-	66.18	64.36	66.18	-	-	-	-	64.81	66.22	-	65.01	-	65.01	66.49	64.61	-	65.01
60	-	-	-	-	-	60.85	58.8	60.42	60.85	61.28	-	60.75	60	-	60.21	59.195	-	-	-	-
55.49	56.46	55.57	54.22	55.04	56.46	55.04	-	55.04	-	55.04	-	55.33	-	55.33	56.46	54.59	54.77	-	56.46	-
48.674	-	49.21	47.73	49.21	-	48.43	47.73	-	-	-	-	-	-	-	-	-	-	-	-	-
38.213	-	-	38.73	-	37.84	-	-29.48	38.12	30	38.12	-	-	-	-	-	-	30	38.73	-	37.12
29.638	30.70	-	28.98	-	29.74	-	30.71	-	28.98	-	28.55	28.81	30	28.96	28.55	28.66	28.55	30.65	29.02	29.55
24.723	25.44	-	25.22	-	25.44	25.59	25.44	24.71	24.69	24.71	25.42	25.13	24.43	25.38	24.62	25.64	24.62	25.90	24.62	25.90
	23.79	-	23.79	-	23.5	-	-	-	-	-	24.43 23.51	23.79	23.69	24.39	23.51	25	23.51	25	-	25

REFERENCES

- Araman, S.F. (1979): Protein digestion and synthesis in ixodid females, PP 385-395. In I.J.G. Rodriguez [ed.], Recent advances in acarology. Academic, New York.
- Balashov, Y.S. (1972): Blood sucking ticks (Ixodoidea) – vectors of diseases of man and animals. Misc. Publ. Entomol. Soc. Am. 8: 273-300.
- Boctor, F.N. and Kamel, M.Y. (1976): Purification and characterization of two lipovitellins from eggs of the tick *Dermacentor andersoni*. Insect Biochem.; 6, 233-240.
- Boldbaatar, D.; Battsetseg, B.; Matsuo, T.; Hatta, T.; Umemiya-Shirafuji, R.; Xuan, X. and Fujisaki, K. (2008): Tick vitellogenin receptor reveals critical role in oocyte development and transovarial transmission of *Babesia* parasite. Biochem. cell Biol.; 86(4): 331-44.
- Chinzei, Y. (1983): Quantitative changes of vitellogenin and vitellin in adult female tick *Ornithodoros moubata*, during vitellogenesis. Mie. Med. J.; 32: 117-127.
- Chinzei, Y. (1986): Vitellogenin biosynthesis and processing in a soft tick *Ornithodoros moubata*, PP. 18-25. In: D. Borovsky & A. Spielman [eds.], Host regulated developmental mechanisms in vector arthropods. Proceedings of the Vero Beach symposium, University of Florida-IFAS, Florida Med. Entomol. Lab., Vero Beach.
- Chinzei, Y. and Yano, I. (1985): Fat body is the site of vitellogenin synthesis in the soft tick, *Ornithodoros moubato*. J. compara. Physiol.; 155B, 671-678.
- Chinzei, Y.; Chino, H. and Takahashi, K. (1983): Purification and properties of vitellogenin and vitellin from a tick, *Ornithodoros moubata*. J. compara. Physiol.; 152B, 13-21.

- Darwish, M. A.; Imam, I. Z. E.; Omar, F. M. and Hoogstraal, H. (1977): A seroepidemiological survey for Crimean-Congo hemorrhagic fever virus in humans and domestic animals in Egypt. J. Egypt. Public Health Assoc.; 52:156-63.
- Diehl, P.A. (1970): Zur oogenesis bei *Ornithodoros moubata* Murray (Ixodidea: Argasidae). Unter besonderer Berücksichtigung der vitellogenese. Acta. Tropica.; 27, 302-255..
- Diehl, P.Q.; Aeschlimann, A. and Obenchain, F.D. (1982): Tick reproduction: oogenesis and oviposition. In: Obenchain F.D., Galun R. (Eds.), physiology of ticks. Pergamon press, Oxford, VK, PP 277-350.
- Dolp, R.M. and Hamdy, H. (1971): Biochemical and physiological studies of certain ticks (Ixodoidea). Protein electrophoretic studies of certain biological fluids of *Argas* (Argasidae) and *Hyalomma* (Ixodidae). J. Med. Entomol. 8: 636-642.
- Engelmann, F. (1979): Insect vitellogenin: identification, biosynthesis and role in vitellogenesis. Adv. Insect physiol.; 14: 49-108.
- Friesen, K.J. and Kaufman, W.R. (2002): Quantification of vitellogenesis and its control by 20-hydroxyecdysone in the ixodid tick, *Amblyomma hebraeum*. J. Insect physiol.; 48(8): 773-782.
- Friesen, K.J. and Kaufman, W.R. (2004): Effects of 20 – hydroxyecdysone and other hormones on egg development and identification of a vitellin – binding protein in the ovary of the tick, *Amblyomma hebraeum*. J. Insect physiol.; 50:6, 519-527.
- Hoogstraal, H.; Guirgis, S.S.; Khalil, G.M. and Kaiser, M.N. (1975): The subgenus *persicargas* (Ixodoidea: Argasida: *Argas*).27. The life cycle of *A. (p.) robertsi* population samples from Taiwan, Thailand, Indonesia, Australia and Srilanka-South Asian. J. Trop. Med. Pub. Hlth.; 6:532-539.
- James, A.M. and Oliver, J.H. (1996): Vitellogenin concentrations in the hemolymph and ovaries of *Ixodes scapularis* ticks during vitellogenesis. Exp. Appl. Acarol.; 20: 639-647.
- Kamel, M.Y.; Shalaby, F.Y. and Ghazy A.M. (1982): Biochemical studies of tick embryogenesis DNA, RNA, Hemoprotein, Guanosine and Guanine in developing eggs of *Hyalomma dromedarii*. Insect Biochem.; Vol 12 No 1: 15-28.
- Khalil, G.M. (1979): The subgenus *persicargas* (Ixodoidea: Argasidae: *Argas*). The life cycle of *A. (P.) persicus* in the laboratory. J. Med. Entomol.; Vol. 16(3): 200-206.
- Lowery, O.H.; Rosebrough, A.A. and Randall, R.J. (1951): Protein measurement with the folin – phenol reagent J. Boil. Chem.; 193:265-275.
- Mayer, R. T.; Cunningham, G. and Gupton, J.(1990): Insecticides based on differences in metabolic pathways. Safer insecticides Ernest Hodgson Ronald J. Kuhr 1990 Dekker, INC.New York.
- McGowan, M.J.; Homer, J.T. and Barker, R.W. (1982): Electrophoretic and antigenic comparisons of the ixodid ticks *Amblyomma maculatum* and *A. americanum* (Acari: Ixodidae). Florida Entomol.; 65(4): 447-452.
- Oliver, J.H. (1982): Tick reproduction: Sperm development and cytogenesis p 245-275. In physiology of ticks by Obenchain, F.D. and Galun R.(Eds) Pergamon Press, New York, London.
- Radwan, W.A.; Helmy, N.; Shanbaky, N.M. ; Bakr, R.F.A; Salem, D.A.M. and Abd El-Hamid (2015): Effect of Lufox on haemolymph and ovarian protein in soft tick, *Argas persicus*. Egypt. Acad. J. Biol. Sci.:(A-Entomology)Vol. 8(2): 35-48.

- Rosell, R. and Coons, L.B. (1991): Purification and partial characterization of vitellin from the eggs of the hard tick, *Dermacentor variabilis*. Insect Biochem.; 21: 871-885.
- Schriefer, M.E. (1991): Vitellogenesis in *Hyalomma dromedarii* (Acari: Ixodidae): a model for analysis of endocrine regulation in ixodid ticks. PhD dissertation, Old Dominion University, Norfolk, VA.
- Shanbaky, N.M.; Mansour, M.M.; Main, A.J. and Helmy, N. (1990a): Changes in total hemolymph and ovary proteins during oogenesis in *Argas (Argas) hermanni* (Acari: Argasidae). J. Med. Entomol.; 27:992-985.
- Shanbaky, N.M.; Mansour, M.M.; Main, A.J. and Helmy, N. (1990b): Vitellogenic and non vitellogenic proteins in hemolymph, ovaries, and eggs of *Argas (Argas) hermanni* (Acari: Argasidae). J. Med. Entomol.; 27: 986-992.
- Smith, I. (1976): Chromatographic and electrophoretic techniques. Zone Electrophoresis, Vol. II, 4th ed. William Heinemann Medical Book, London.
- Tatchell, R.J. (1971): Electrophoretic studies on the proteins of the hemolymph, saliva, and eggs of the cattle tick, *Boophilus microplus*. Insect Biochem.; 1: 47-55.
- Taylor, D. and Chinzei, Y. (2001): Vitellogenin and its synthesis in the soft ticks. In: Holliday, R.B., Walter D.E., Proctor H.C., Norton R.A., Colloff M.J. (Eds.), Acarology: proceeding of the 10th International Congress, Conterra, Australia, pp. 622-627.
- Yousery, A. (2011): Digestion of blood proteins in an ornithodorine tick vector of borreliosis (*Ornithodoros erraticus*). M.Sc., Faculty of Science, Ain Shams University.

ARABIC SUMMARY

تغيرات البروتينات المحية و المولدة للمح أثناء دورة التكاثر في إناث القراد أرجاس بيرسيكس

نادية احمد حلمي- نوال محمود شنبكى¹- وفاء احمد رضوان¹ -¹ رضا فضيل على بكر^{2&1} - داليا عبد البديع سالم¹ - اميرة السيد عبد الحميد¹

1 - قسم علم الحشرات - كلية العلوم - جامعة عين شمس

2 - قسم الأحياء - كلية العلوم - جامعة بيشه - بيشه - المملكة العربية السعودية

تم استخدام التفريد الكهربائي لفصل بروتينات البيض المنتج حديثاً من إناث القراد أرجاس بيرسيكس إلى اثني عشر شريط بروتيني محي بيضي بوزن جزئي من 24,72 إلى 131,45 كيلو دالتون منهم شريط واحد تم تعيينه في البيض فقط (131,45 كيلو دالتون) و إحدى عشر شريط بروتينات محية بيضية خارجية ستة منهم شائعة تم تعيين نظراء لهم (بروتينات مولدة للمح) في هيموليمف كلا من الإناث و الذكور (24,72 - 65,53 كيلو دالتون) و خمسة شرائط بروتينات محية خاصة بالإناث وجدت شبيهاتها في هيموليمف الإناث فقط بوزن جزئي 78,65 - 112,92 و 48,67 كيلو دالتون .

تم تعيين عدد عشرة شرائط من البروتينات المحية الخارجية و إحدى عشر شريط من البروتينات المولدة للمح في كل من المبيض و الهيموليمف على التوالي و ذلك باختيار شرائط البروتينات الأصلية ذات الوزن الجزيئي و الحركي المشابه لبروتينات البيضة. و اختلفت أعداد و نسب تركيزات شرائط البروتينات المحية و المولدة للمح في مبيض و هيموليمف إناث القراد المتزاوج في المراحل الفسيولوجية المختلفة (غير المغذى و المغذى من صفر إلى سبعة يوم و أيضا عشرون يوماً بعد التغذية) التي تم فحصها (10 مراحل) في الدراسة الحالية. و عامة زادت أعداد و تركيزات شرائط البروتينات المحية و ظهر الجديد منها داخل المبيض و صاحب ذلك نقصان في أعداد و تركيزات شرائط البروتينات المولدة للمح في هيموليمف القراد في الفترة الأخيرة من دورة التكاثر (4-7 و 20 يوم بعد التغذية) مما يشير إليها كفترة يتم فيها ترسيب معظم البروتين المحي داخل المبيض لنضوج البويضات كما تشير الدراسة إلى إمتصاص البروتينات المولدة للمح من الهيموليمف إلى المبيض و إمتداده حتى نهاية وضع البيض (20 يوم بعد التغذية) في أنثى القراد اللين من نوع أرجاس بيرسيكس .