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Effect of juvenile hormone analogue (Admiral) on embryogenesis of the soft tick *Argas persicus* (Oken)

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ABSTRACT

Embryonic development of the fowl tick *A. persicus* was investigated during cleavage stage, blastoderm formation, gastrulation and organogenesis. Cleavage started 1 h post-oviposition (POP) and is indicated by presence of vitellophages of different sizes. Blastoderm is formed at 48 h POP. Secondary vitellophages are observed by 24 h POP. By 72 h POP, the germ band is formed. At 96 h POP, while the embryonic envelops are formed, gastrulation of the germ band takes place. Segmentation and differentiation of germ band, as well as, blastokinesis occur at 120 h POP. The stomodaeal and proctodaeal invagination started to differentiate at 120 h POP. The anterior and posterior midgut rudiments can be observed at 144 h POP. By 168 h POP, the nervous system, as well as, rectal sac and malpighian tubules are formed.

Histological study revealed that application of JHA (Admiral) to newly laid eggs of *A. persicus* blocked the embryonic development of affected eggs at cleavage stage and before blastoderm and pole cells formation. In 24 h-old treated eggs, few cleavage nuclei appeared with absence of secondary vitellophages and pole cells. In 48 h-old treated eggs, the blastoderm was rudimentary and irregularly arranged. In 72 h-old treated eggs, disintegration of the rudimentary blastoderm and cracking of the cytoplasm could be seen. Starting from 96-old eggs until hatching at 168 h, complete destruction of the eggs was clear.

Key words: *Argas persicus*, Juvenile hormone analogue, embryogenesis

INTRODUCTION

Several histological studies of the embryogenesis of insects especially those belonging to order Diptera (West *et al.*, 1968; Sandescu ahd Tacu, 1970; Raminani and Cupp, 1978 and Radwan *et al.*, 1993). Hymenoptera (Tawfic, 1975) and Hemiptera (Shaarawi *et al.*, 1982 and Ajidagba *et al.*, 1983) has been reported. However, little information on the embryogenesis of ticks, especially the soft ticks was available. Preliminary studies have been indicated for the soft tick *Aragas persicus* (El-Rammah, 1981). Most investigations of tick embryogenesis were focused on hard ticks such as the camel tick *Hyalomma dromedarii* (El-Kammah *et al.*, 1982); the cattle tick *Boophilus annulatus* (El-Kammah *et al.*, 1987).

Embryonic development is subject to inhibition by the same hormonally active materials which are effective in blocking the metamorphosis of postembryonic insects (Slama and Williams, 1966). The embryonic development can be blocked as early as the blastoderm stage by exposing unfertilized eggs to juvenoids (Riddiford and Williams, 1967). Application of JHA on newly emerged females, as well as, newly deposited eggs may have the same effect on embryonic development (Matolin, 1970). Blocking of embryonic development during or after blastokinesis was observed in the

bed bug *Cimex lectularius* by application of JHA to either freshly laid eggs or parent females (Shaarawy *et al.*, 1982).

Bassal (1974) reported that acyclic terpene (ACT) completely blocked embryonic development of eggs that had not reached the vitellogenesis stage or were in this stage at the time of ACT application to female. However, embryological development of the northern deer tick *Ixodes dammini* is not disrupted by different concentrations of insect growth regulator "fenoxycarb" (Slusser and Sonenshine, 1992).

Inhibition of embryonic development at different stages was observed in the tick *Boophilus annulatus* (El-Kammah *et al.*, 1987) after treatment with different pesticides.

In a previous work (Radwan *et al.*, 2009) the JHA (Admiral) proved to be effective in reducing egg viability of the Fowl tick *Argas persicus* both by topical application and immersion of eggs in solution of different concentrations.

The present study aimed at detailed histological study of embryogenesis of normal and JHA-treated eggs of the fowl tick *Argas persicus* using the light microscope.

MATERIALS AND METHODS

Ticks:

The soft tick, *Argas persicus* (Oken) was collected from a domestic chicken house at Banisweif Governorate, Egypt. The ticks were colonized into the laboratory at 27^{o}C$\pm$1 & 75% R.H. and 16 hrs. daylight (as described by Kaiser, 1966).

Treatment:

The juvenile hormone analogue (Admiral, from Sumitomo Company) was provided by Prof. Reda Fadeel, Ain-Shams University, Faculty of Science.

Newly laid eggs (0-1) h postoviposition (POP) were treated topically by 1.5, 15, 150 and 1500 µg of the hormonal material in 15 µl acetome / 30 eggs respectively. The hormonal material was applied topically by micropipette directly on eggs.

In dipping technique, newly laid eggs (0-1) h postoviposition were immersed in 100-200 µl acetone solution containing one of four different doses (100, 150, 170, 200 µg / 30 eggs) of Admiral. The experiment was repeated 5 times (30 X 5) and eggs were dipped for 1 minute in each solution. Eggs used as control were treated with appropriate amount of pure acetone. Selected stages from normal (0-1 h, 48 h and 96 h after oviposition) and treated eggs (0-1 h, 48 h and 96 h after treatment) were processed for histological study.

Histological Study:

The F1 egg batches of ten females were selected to study the embryonic development. Some eggs from each batch were allowed to hatch to assure that batch was viable. To determine the stages of embryonic development, samples were taken at intervals of 24 h for 168 h (the hatching period). Eggs were prepared for microscopic examination as follows:

Eggs were dechorionated in 6% sodium hypochlorite for 5 min and then washed 3 times for 20 min with distilled water. Eggs were then placed in aqueous bouin's solution for 24-48 hrs depending on the stage of development and dehydrated in consecutive baths of 30, 50, 70, 85, 95 and 100% ethanol. These steps were followed by clearing in xylene, embedding in paraffin and sectioning 5-6 µm. Thick sections using a rotator microtome were placed on slides and stained using modified method of Harris, hematoxylin stain.

RESULTS AND DISCUSSION

Histological study of normal embryogenesis:

Saggital section of softened eggs were examined as early as (0-1 h) "newly laid eggs" (Fig. 1) and 12 h (POP) to describe structure and cleavage of the zygote. The successive stages were examined at intervals of 24 hours for 168 hours (hatching period).

At the present study, no mitotic and meiotic stages can be observed, even as early as 0 h postoviposition. This agrees with finding of Ajidagba *et al.* (1983), where no meiotic figures were observed in *Stomoxys calcitrans* at 15 min after deposition. This is also the case in *Apanteles glomeratus* (Tawfik, 1975) and in *Musca domestica* (Radwan *et al.*, 1993).

Cleavage and blastoderm formation:

The onset of cleavage in *Argas persicus* is 1 hour post-oviposition (POP) (about 1% development), 3 h POP (about 1.4% development) in each of *Ornithodorous moubata* (Aeschliman, 1958) and the hard tick *Hyalomma dromedarri* (El-Kammah *et al.* 1982). The onset of cleavage in *Boophilus annulatus* is 1-2 h POP (about 3% development).

In *A. persicus* cleavage nuclei appeared during the 12 h POP. The protoplasm of the egg differentiated into a densely staining peripheral layer, the periplasm, and an inner cytoplasmic network containing the yolk granules (Fig. 2).

At the posterior end of the egg, just beneath the vitelline envelope, lies the germ line determinant as a distinct irregular line of darkly stained granules. After 24 h (about 14% development), cleavage nuclei have increased in number and migrated to the periphery of the egg; but some migrate back into the yolk as secondary vitellophages (Fig. 3). At the same time, dark granules begin to appear in the periplasm at the posterior pole of the egg (pole cells) (Fig. 3). Primary vitellophages observed in the centre and delimiting cell furrows are clear.

Formation of secondary vitellophages is also described in *Stomoxys calcitrans* (Ajidagba *et al.*, 1983) and *Phlebotomus papastasi* (Abbassy *et al.*, 1995a) . The vitellophages have a variety of functions. They are concerned with breakdown of yolk, and later when the yolk is enclosed in the mid gut, they may form part of the mid gut epithelium. They are also involved in the formation of new cytoplasm and are responsible for contraction of the yolk (Giorgi and Nordin, 1994).

Pole cells can be seen arranged at posterior pole of the egg. In *A. persicus* they have no definite number. This is also true in *Musca domestica* (Radwan *et al.*, 1993), while in sand fly (Abbassy *et al.*, 1995a) there is a variable number of pole cells (6-10). Mitotic activity during this period of development in very high.

The blastoderm formation in *A. persicus* eggs occur 2 days POP (about 28% development), while in *Hyalomma dromedarri* (El-Kammah *et al.*, 1982) 8 days POP (about 40% development), 5-8 days POP (about 42% development) in *Boophilus annulatus* (El-Kammah *et al.*, 1987). This variation is probably due to difference in prehatching periods.

Gastrulation:

In the present study, at 72 h POP (about 43% development), the blastoderm thickens along the midventral line, forming the germ band (Fig. 4). The cells on the dorsal and lateral sides of the blastoderm become flattened to form the serosa. This is also the case in *Musca domestica* (Radwan, *et al.*, 1993), *Phlebotomus papatasi*

(Abbassy *et al.*, 1995a). In *Manduca sexta*, serosa is formed 12 h POP (about 10% development) (Lamber and Dorn, 2001).

On the dorsal side a small group of cells invaginate slightly to constitute the dorsal organ (Fig. 5). By 120 h POP (about 71% development) the dorsal organ is absorbed in the yolk.

While the embryonic envelopes are formed at 96 POP (57% development) gastrulation of the germ band in *A. periscus* takes place where it first appears in the middle region of the egg and then spread anteriorly and posteriorly. This also occurs in *Boophilus annulatus* (El-Kammah *et al.*, 1987). However, in *Drosophila melanogaster* (Turner and Mahowald, 1977). The invagination of the germ band first appeared at anterior pole of the egg and then spread ventrolaterlly as paired mesodermal bands.

The germ band is formed of multilayer strips of cells that are differentiated into two layers of ectoderm and mesoderm (Fig.5).

Segmentation:

The germ band extends the anterior length of the egg on the ventral side and is differentiated by 120 h POP. The ectoderm cells proliferated in the anteroventral area to form a U-shape symmetrical band (Fig. 6). The opithosome and four ambulatory segments were formed on one side of the germ band consisting of 3-4 layers of cells. The precheliceral and pedipalp lobes are formed on the other side consisting of 2-3 layers of cells. The portion of the germ band extending on the dorsal side of the egg become contracted and shorten (Fig. 6). As a result of this contraction, the mouth parts and the bases of the ambulatory segments are shifted in position simultaneously to a more ventral position (Fig. 7).

In the present study, the germ band differentiation and segmentation of the external features are observed within 24 h of the germ band formation. This is also true in *O. moubata* (Aeschliman, 1958) and in *Hyalomma dromedarii* (El-Kammah *et al.*, 1982).

Blastokinesis:

Initially (0-96 h) the ventral side of the embryo faces the ventral side of the egg (Fig. 7). At 120 h POP the embryo undergoes and opposite longitudinal rotation (180^{o}), causing the ventral side of the embryo to face the dorsal side of the egg (Fig. 8). This phenomenon has been observed also in *Aedes aegypti* (Raminani and Cupp., 1978), *Manduca sexta* (Dorn *et al.*, 1987a), *Phlebotomus papatasi* (Abbassy *et al.*, 1955b) and *Antheraea yamami* (Baba *et al.*, 1997).

Organogenesis:

The mouth parts development:

At 144 h POP (about 85% development), the palps, hypostome and basis of the chelicera are beginning to differentiate. The mouth parts opened to the anteroventral line of the embryo (Fig. 8). The buccal apparatus was visible. By 168 h POP, the ambulatory segments 1, 2, 3 increased in length while the fourth segment remained undeveloped. The mouth parts also enlarged in size. The basis of chelicerae came together in front of the mouth while the median lobes of the pedipalps unite together to develop the hypostome. The remainder of each pedipalp form a functional palp (Fig. 9). This also occurs in *Hyalomma dromedarri* (El-Kammah *et al.*, 1982) and in *Phlebotomus papatasi* (Abbassy *et al.*, 1995 b) with respect to different in number of embryonic unit.

The alimentary canal:

At 120 h POP (about 71% development) a pouch is first formed from elongation and invagination of the ectodermal cells anterior and posterior to

ambulatory segments (Fig. 8). At 144 h POP, the stomodaeal and proctodaeal invagination are well developed (Fig. 10). It is difficult to distinguish between the differentiated mesodermal band and the anterior and posterior mid gut rudiment. This is also true in *Phlebotomus papatasi* (Abbassy *et al.*, 1995 b). In the present study, the stomodaeum did not invaginate before the presumptive anterior mid gut rudiment cells, but they invaginate nearly at the same time. The invaginating posterior mid gut rudiment and proctodaeal cells are not histologically distinct as are those of the anterior mid gut rudiment and stomodaeum. This is also true in *Culex fatigans* (Davis, 1966).

In *A. persicus*, the rectal sac and malpighian tubules (Fig. 11) are formed at 168 h POP (about 100% development). In *Hyalomma dromedarri* (El-Kammah *et al.*, 1982) the proctodaeum is formed on 11 days POP (about 52% development) and the rectal sac, anus and malpighian tubules is formed 17-18 days POP (about 80% development) and in *Anthreaea yamami* (Baba *et al.*, 1997) the protodaeum formation occurs at 36 h POP (about 15% development).

The nervous system:

At 120 h POP, the germ band became short and broad by the multiplication of the ventral region of the ectoderm cells and the movement of these cells towards the ventral invagination forming ganglionic tissue (Fig. 12). At 168 h POP, the brain appeared as two ganglionic mass dorsal to the prechelicerae lobe. These two ganglionic mass are separated by the oesophagus. The dorsal ganglion is the supra-oesophageal ganglion, and the ventral one is the suboesophageal ganglion, which is formed from neural cell aggregates of the gnathal region (Fig. 12). Generally, the nervous system in *A. persicus* appeared 144 h POP (about 85% development) developing from the ectoderm. The basic development of the nervous system shows that it is alike throughout the Argasidae and Ixodids (Eichenberger, 1970 and Anderson, 1973). The outline of nervous system appeared later in *H. dromedarri* (El-Kammah *et al.*, 1982) 15 days POP (about 80% development), while in the *O. moubata* (Aeschliman, 1958) 72 h POP (about 30% development). In *Phlebotomus papatasi* (Abbassy *et al.*, 1995c) brain formation started at 108 h POP (about 50% development) from ectodermal cells. By 156 h POP (about 72% development), the brain is completely developed.

The embryo of *A. persicus* became enclosed by a cuticular membrane (Fig. 11). The yolk mass remained dorsally in a yolk sac surrounded by a membrane. Hatching started 7 days POP in *A. persicus*, 9-10 and 20-21 days POP in the *O. moubata* (Aeschliman, 1958), *H. dromedarri* (El-Kammah *et al.*, 1982) respectively, and in *Thermobia domestica* (Rost *et al.*, 2004) 14 days POP. Until prehatching stage the alimentary canal was not clearly divided into branches. This may explain the fact that the larval stages of *A. persicus* and *H. dromedarri* cannot feed before four days (El-Kammah and Abdel Wahab, 1979).

Histological study of treated eggs:

The development of eggs treated with Admiral appeared to be blocked at cleavage division and before blastoderm and pole cells formation. In 0 h-old treated eggs, vacuolation of cytoplasm could be seen (Fig. 13). During the following 24 h of treatment, only few cleavage nuclei appeared with absence of the pole cells and secondary vitellophages (Fig. 14). In some affected eggs only few cleavage nuclei migrated towards the periphery and some of them entered the periplasm. Generally the yolk is irregularly deposited, and the destruction of eggs continues by disintegration of the yolk mass into tiny droplets (Fig. 15). At 48 h-old eggs treated

with Admiral the blastoderm was often rudimentary and irregularly arranged (Fig. 16). At 72 h-old eggs treated with Admiral, we can observe the disintegration of the rudimentary blastoderm, the cracking of the cytoplasm (Fig. 17).Treated eggs at 96 h until hatching at 168 h, show complete destruction of eggs (Fig. 18). Blockage of embryogenesis during cleavage by application of different juvenile hormone analogues was also reported in *Musca domestica* (Shanbaky *et al.* 1993) and in cat flea *Ctenocephalides felis* (Meola *et al*., 1993b & Palma *et al*. 1993). While in *Cimex lectularius* (Shaarawi *et al*., 1982) blockage of embryogenesis by juvenile hormone analogues occur during or after blastokinesis. Ultrastructure studies of *A. persicus* eggs treated with JHA (Admiral) (Radwan *et al.*, 2008) demonstrated that ovicidal activity of Admiral resulted from an inhibition of the embryonic development at cleavage stage and before blastoderm and pole cell formation. Gadallah *et al.* (1989) showed that treatment of *A. arbaeus* with juvenile hormone III lowers the total protein only during the first two days of embryogenesis.

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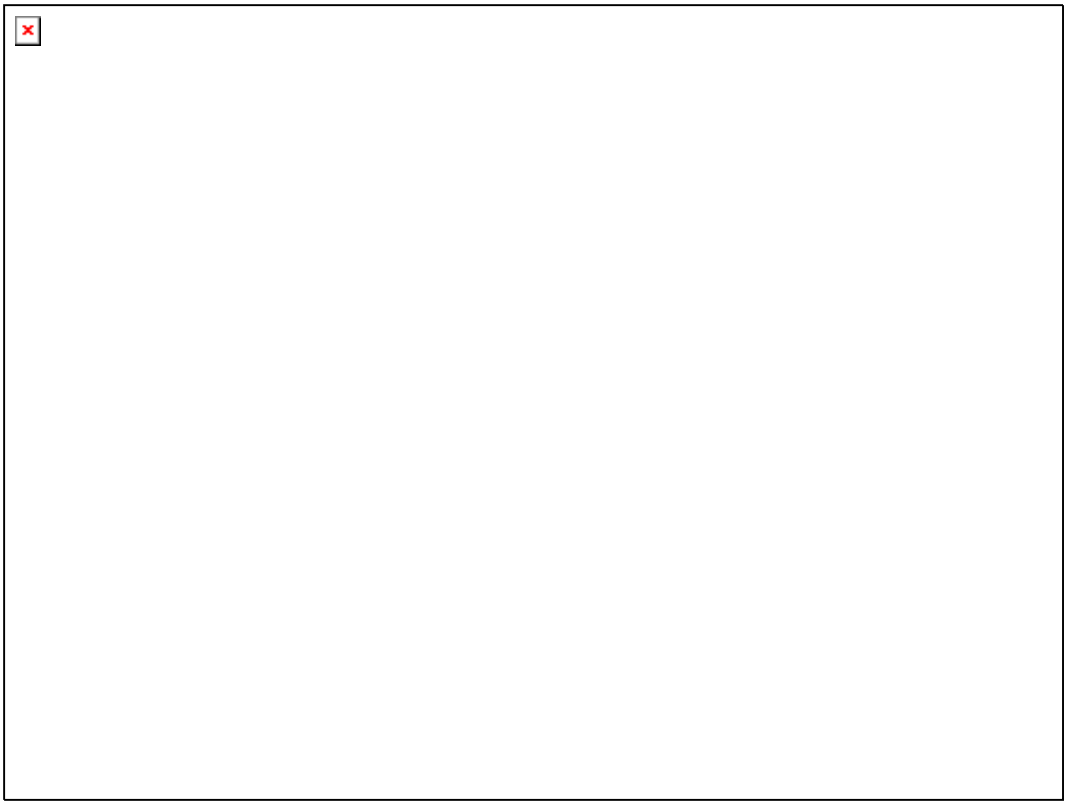


Plate I: Embryogenesis of soft tick, *Argas persicus* (Oken), cleavage, blatoderm and germ band formation:

Fig. 1: Sagittal section through 0 h old egg, showing the chorion (ch), vitelline membrane (V), periplasm (P), yolk (Y), cytoplasm (CY), and germ line determinant (GLD) in the posterior pole of the egg. (X = 400).

Fig. 2: Sagittal section through 12 h old egg, showing periplasm (P), cleavage nuclei (CN), cytoplasm (CY) and yolk (Y). (X = 400).

Fig. 3: Sagittal section through 24 h old egg, showing arrangement of cleavage nuclei at the periphery, pole cells (PC), secondary vitellophages (SV), cleavage nuclei (CN), and delimiting cell furrows (CF). (X = 1000).

Fig. 4: Sagittal section through 72 h old egg, showing blastoderm thickening to form germ band (GB), formation of the serosa (Ser). (X = 1000).

Fig. 5: Sagittal section through 96 h old egg, showing the dorsal organ (DO), ectoderm (Ect), mesoderm (Mes). (X = 1000).

Fig. 6: Saggital section through 96 h old egg, showing germ band lobes (GBL), amnion (Am), germ band (GB), serosa (Ser). (X = 1000).

Plate II

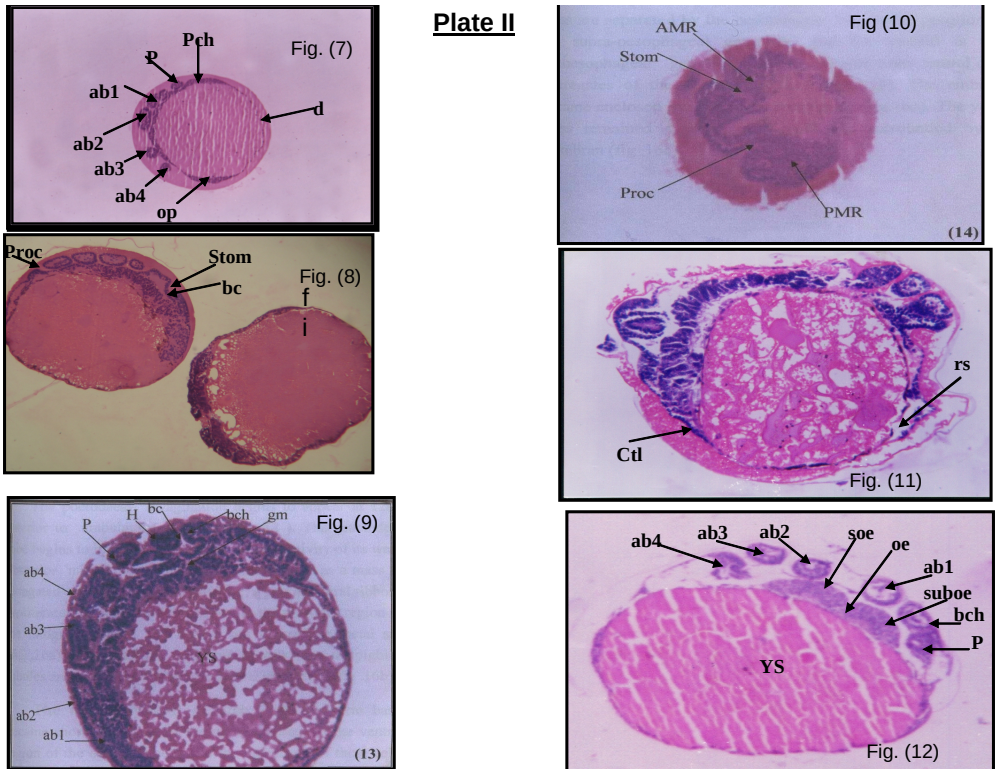


Plate II: Embryogenesis of soft tick, *Argas periscus* (Oken), segmentation, blastokinesis, and organogenesis:

Fig. 7: Sagittal section through 120 h old egg, showing movement of the ambulatory segments (ab1–ab4) and mouth parts (opsithosome (OP), precheliceral lobe (Pch), pedipalp (P) and dorsal surface (d). (X = 600).

Fig. 8: Sagittal section through 120 h old egg, showing blastokinesis, proctodaeum (Proc), stomodaeum (Stom), buccal cavity (bc), and ganglionic mass (gm). (X = 400).

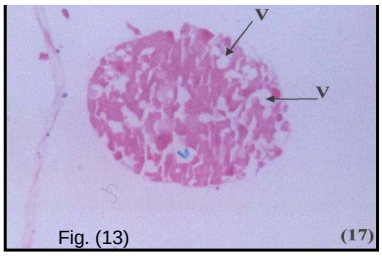
Fig. 9: Sagittal section through 144 h old egg, showing the buccal cavity (bc), ambulatory segment (ab1 – ab4), hypostome (H), basis of chelicerae (bch), ganglionic mass (gm), pedipalp (P), and yolk sac (Ys). (X = 600).

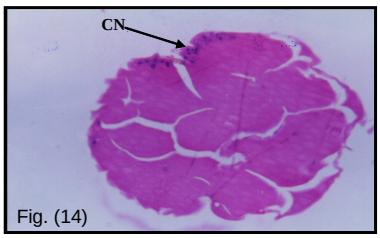
Fig. 10: Sagittal section through 144 h old egg, showing the stomodeael invagination (Stom), proctodeael invaginatin (Proc), anterior mid gut rudiment (AMR), and posterior mid gut rudiment (PMR). (X = 400).

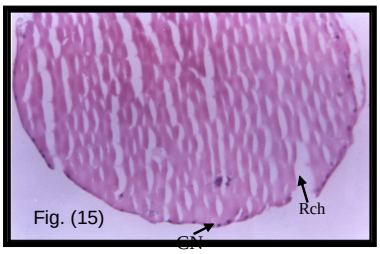
Fig.11:Sagittal section through fully formed embryo, showing rectal sac (rs), cuticular membrane (ctl) and yolk sac (Ys). (X = 600).

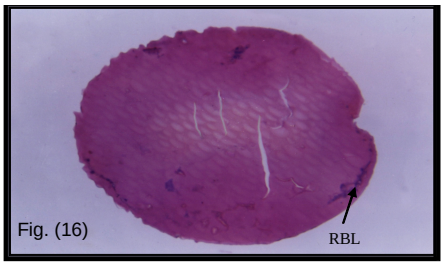
Fig.12:Sagittal section through fully formed embryo, showing oesophagus (Oe), supraoesophageal ganglion (Soe), suboesephageal ganglion (Suboe) and yolk sac (Ys). (X = 600).

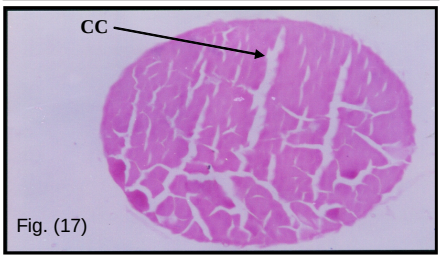
Plate III











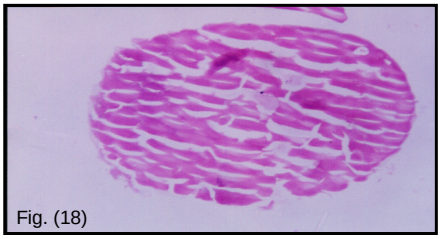


Plate III: Embryogenesis of *A. persicus* eggs treated with JHA (Admiral):

Fig. 13: Sagittal section of 0 h old treated egg, showing vacuolation of the cytoplasm (V). (X = 400).

Fig. 14: Sagittal section through 12 h- old treated egg, showing decrease in number of cleavage nuclei (CN) at periphery and absence of the pole cells. (X = 600).

Fig. 15: Sagittal section through 24 h-old treated egg, showing few cleavage nuclei migrating to the periphery, and ruptured chorion (Rch). (X = 1000).

Fig. 16: Sagittal section through 48 h-old treated egg, showing rudimentary and irregularly arranged cells of blastoderm (RBL). (X = 1000).

Fig. 17: Sagittal section through 72 h-old treated egg, showing disintegration of the rudimentary blastoderm, and cracking of the cytoplasm (CC). (X = 1000).

Fig. 18: Sagittal section through 96 h-old treated egg, showing complete lysis and degeration of the egg content. (X = 1000).

ARABIC SUMMARY

تأثير شبيه هرمون الشباب (أدميرال) على التطور الجنينى للقراد اللين أرجاس برسيكس (أوكن)

نادية حلمى أحمد – وفاء أحمد رضوان – نهى عونى جنيدى – شيماء صلاح الدين محمود
قسم علم الحشرات – بكلية العلوم – جامعة عين شمس

تمت دراسة التطور الجينينى لقراد الدواجن، أرجس برسيكس، خلال مرحلة التفلج ، تكوين البلاستودرم ، التبطين ، والتعضى. وقد تبين أن مرحلة الأنقسام (التفلج) تبدأ بعد ساعة من وضع البيض حيث تتميز بوجود أحجام مختلفة من خلايا المح. وتتكون طبقة البلاستودرم وتظهر خلايا المح الثانوية عند عمر 24 ساعة للبيض. وتتكون الطبقة الجرثومية عند عمر 72ساعة. وتبدأ مرحلة التبطين أثناء تكوين الأغلفة الجنينية عند عمر 96ساعة. تبدأ حركات الجنين بعد 120 ساعة من وضع البيض وذلك بعد ظهور حلقات الجسم وتميز الطبقة الجرثومية. عند 144 ساعة من عمر البيض أمكن تمييز البدايات المستقبلة للجزء الأمامى والخلفى للمعى المتوسط. ويتكون الجهاز العصبى وكيس المستقيم وأنابيب ملبيجى عند عمر 168ساعة.

أثبتت الدراسات الهستولوجية أن تطبيق المادة الهرمونية (أدميرال) على البيض حديث الوضع قد أدى الى توقف النمو الجنينى عند المرحلة الاتقسامية (التفلج) وقبل تكوين البلاستودرم والخلايا القطبية. وعند عمر 24 ساعة لوحظ ظهور عدد قليل من الأنوية الأنقسامية مع غياب خلايا المج الثانوية والخلايا القطبية. وبعد 48 ساعة من المعاملة لوحظ وجود بعض خلايا البلاستودرم الضامرة مع توزيعها العشوائى. لوحظ اختفاء طبقة البلاستودرم الضامرة وحدوث تشقق فى الستيوبلازم عند 72 ساعة من المعاملة. ابتداء من 96 ساعة من المعاملة حتى النفس عند 168 ساعة ظهر تدمير كامل لمحتوى البيض المعامل.