

INVESTIGATING THE HISTOLOGY OF FEMALE *ARTEMIA URMIANA*'S REPRODUCTIVE TRACT

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ABSTRACT

The present study aimed to investigate the histology of female *Artemia urmiana*'s reproductive tract. The samples of female *A. urmiana*'s reproductive tract were confirmed by the experts in *Artemia* Research Center in Iran and were put in hatching incubator for growth. After reaching maturity, they were put in Formalin of 10% for further investigation. Furthermore, after separating the female *Artemia* and carrying out tissue processing through the method of light microscopic examination; hence, the photo-micrographics were extracted out of them. Also, through studying the histology of female *A. urmiana*'s reproductive tract, the brown skin-construction glands, smooth and skeletal muscle, uterus wall cells, as well as somatic and Oogonium cells were separated and distinctly observed.

KEY WORDS: Histology, female reproductive tract, *A. urmiana*.

INTRODUCTION

Urmia Lake is considered as one of the largest reservoirs in Western Asia and as one of the few permanent lakes with super-saturated salt. This lake includes organisms such as green algae, different types of bacteria, and a kind of hard skin creature with a special feature entitled as *A. urmiana* which is regarded as the only bisexual species in Iran. Not only *Artemia* can be found in this lake but also it can be detected in the reservoirs near Urmia Lake (Hafeziyeh, 2002). *Artemia*'s body surface is covered with an infinitely thin flexible exo-skeleton called 'Chitin' which is linked to its muscles inside (Drewes 2002). Chitin is continuously molted so that the time intervals and patterns required for different stages of molting are distinct in the larval and adult stages (Hafeziyeh, 2002). The size of female *Artemia* is 10-12 mm and of male *Artemia* is 8-10 mm. The size difference between the sexes can be seen as an advantage for mating and fertilization since regarding the female's reproductive system, the female carries the male in the fertilization process. Also, *Artemia* lacks sexual behavior and complex mating system; therefore, the large size of the female is necessary for reproductive in the mating process. This phenomenon indicates an evolutionary relationship between *Artemia*'s reproductive system and sexual dimorphism of body size (Asem, 2007). The reproductive tract of a female *Artemia* consists of a pair of ovaries (i.e., Oviduct) and the uterine sac (Hafeziyeh 2002). Ovaries begin with the eleventh chest cord and end

with the sixth abdominal cord. After ovulation, the eggs enter into Oviduct and remain there from several hours to several days. Afterwards, the eggs are influenced by uterus tube secretion. This uterus tube is extended to build the sacs nearby. The cells within the sacs walls are so long that they cannot be identified as cells of the uterus tube. These sac-like components are separated from the uterus with a shutter. Approximately, forty to sixty minutes after mating, the shutter is opened and eggs in the uterus, where they are fertilized, are released. Between the two mating time intervals and the opening of the shutter, the front and middle parts of the uterus serves as a gathering place of sperm. Hence, the spermatozoons are matured in the female uterus (Criel, 1980). Among a large number of studies which have been carried out on *Artemia*, Pilla and Beardmore's (1994) research is outstanding in that they investigated the genetic and morpho-metric distinction among the bisexual *Artemia* species which inhabit the old areas' water reservoirs. In addition, Asem et al. (2007) scrutinized the synchronic as well as synthetic species of parthenogenetica and bisexual *Artemia* obtained from the Urmia Lake and its adjacent swamps by Agh et al. (2009). Furthermore, Asem (2009) compered the biometric characteristics of two groups of *Artemia parthenogenetica* inhabiting in the beach side and within the lake of Urmia. Moreover, Peykarn-Mana et al. (2010) investigated the biometrics characteristics of *Artemia* in four regions of Iran. Nevertheless, little research has been carried out on the histology of female *A.urmiana's* reproductive tract. A study which is of significance regarding such histology is the one done by Criel (1980) titled as 'The Morphology of female *Artemia's* reproductive Tract and Its Fallopian Tube'. Also, another study pertained to this line of research is the one carried out by Najaf Asaadi et al. (2014) titles as 'The Comparison Study of female *A.urmiana's* reproductive tract histology with *A.parthenogenetica* inhabiting Urmia Lake'. The present study aims to investigate the basis of biological studies on *Artemia*; hence, it tries to scrutinize the histological characteristics of the female *A.urmiana's* reproductive tract since the biological behaviors of organisms helps us to protect our environment and exploit from their presence efficiently.

Method and Instruments

After obtaining the permission of experts in Artemia Research Center in Urmia, the *Artemia* samples were put in hatching incubator for growth. After reaching maturity, they were put in the stabilizing solutions of 10% Formalin for further investigation in laboratory. Furthermore, after separating the female *Artemia*, due to the microscopic size of sample, Atoutechnicon machine was utilized for carrying out the histological process (i.e., dehydrating, clearing, and so forth). Samples were painted with molding Paraffin and were cut with microtome machine into a thickness of 7 μm through the method of Hematoxiline and Eosin (Pousty and Adib Moradi, 2002, pp. 556-540).

CONCLUSION

The female *Artemia* can be identified through the ovaries containing egg which is located behind the eleventh pair of Trachopods in upper side of intestines of the digestive tract. The ovary is wider in the rear part and is thinner in the front section; also, it is formed as a pear (picture 1).



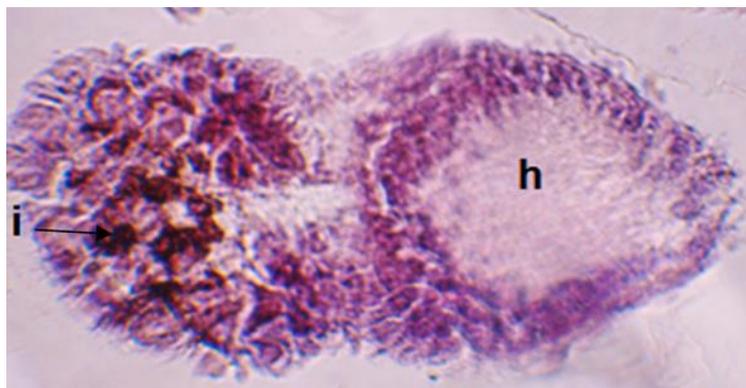
Picture 1: *A. urmiana's* ovary, H and E, 150x, j: the Oogonia cells in center, k: the width incision of uterus

The histological investigation of *A. urmiana's* uterus shows that the layer of covering histology is magnified with epithelial cells and clear nucleus toward the outlet; then, the layer of basal lamina has weak tissue and smooth muscles. Although there exists a delicate connective tissue between the muscles and Chitin layer, this layer which covers the outer part of *A. urmiana's* body is visible. Around the ovary and the uterus, large blood vessels are observed which are very close to the uterus wall. The muscles around the connective tissue are located after basal lamina as smooth muscles; however, toward the outlet the muscles become skeletal (Picture 2).



Picture 2: the length incision of *A. urmiana's* uterus, H and E, 600x, L: Chitin layer, d: uterus covering, n: somatic cells, j: Oogonia cells, o: smooth muscle

Above the muscles, the cube-like cells with spherical bright and clear nucleus create the uterus wall. The oogoniums turn into cyst in certain conditions and the posterior part of the uterus connect to a short outlet conduit which is opened toward the outside part of its body (picture 3).



Picture 3: *A. urmiana*'s pigmented shell glands near the excretory Cyst tube, H and E, 1500x, h: tube with laminated covering tissue, i: pigmented shell glands

urmiana's cysts possess certain nuclei which are active for hatching. Moreover, Granules can be found in these cysts. The younger and larger cysts possess euchromatin (bright) nucleus with more granules. The granules within these cysts indicate the existence of vitelous seeds. The cytoplasm of mature cysts are smaller, and the nucleus is darker and more spherical (picture 4).



Picture 4: Cysts with certain core and nuclei in *A. urmiana*, H and E, 1500 x, a: cyst, b: core, c: nucleus

With the uterus, the Eosinophilia homogeneous fluid can be observed due to the density of cysts (picture 5).



Picture 5: Width incision of the uterus without cysts in *A. urmiana*, H and E, 600x, g: fluid within the uterus, a: cyst, d: cell within the uterus wall, e: connective tissue

DISCUSSION

Two types of reproductive (egg laying and egg laying - parity) can be found in all strains of *Artemia*. In special circumstances (for example, high salinity and low oxygen in water), Female *A. urmiana* are able to change their conditions between these two types of reproductive so that the embryos be grown into the embryo stage. At this stage, the cysts are covered by a thick crust. Then, they enter a stopping or inactive metabolism (i.e., sleep mode) stage,; therefore, the cysts are released by the female *Artemia* (i.e., reproductive through egg-laying) (Lavenz and Sarjelus, 2002). Shell-shaped glands (located inside the uterus) which have yellow to brown pigments are thick around the cysts (Figure 3). Najaf Asaadi et al. (2014) investigated the existence of these shell glands which is in line with the results of the current study. Fluid in the uterus (as shown in picture 5) causes the cyst to move from the uterus toward the tube and out of the body. Consistent with the results of the present study, Criel (1980) found that *Artemia's* tubal secretory cells indicate the presence of fluid. Likewise, the egg sac is surrounded by a smooth layer of circular and linear muscles (Figure 2) as demonstrated by Najaf Asaadi et al. (2014).

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