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Research Article

A COMPARATIVE STUDY ON THE HISTOLOGY OF REPRODUCTIVE ORGANS IN *ARTEMIA* URMIANA AND *ARTEMIA PARTHENOGENETICA* IN URMIA LAKE REGION

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ABSTRACT
The present research is conducted to investigate the probability of a Histologic difference between the genital system of (bisexual) *Artemia* urmiana and local *Artemia Parthenogenetica* in the Urmia lake region. The species of bisexual A. urmiana were being cultivated in an Incubator for hatching and breeding. The samples of bisexual and A. parthenogenetica were harvested in separate Incubators and were placed in a 10% Formalin and Bowen after reaching maturity for further studies. The prepared samples were pigmented by Hematoxilin and Eosin method, and were examined under LM (Light Microscopy) and then some photomicrographs were prepared. The findings of a histology study on the genital system resulted in a difference between brown theme maker glands, smooth and skeletal muscles, uterus cell walls, somatic cells, and oogonia in between the two groups under study. No significant difference was observed in the histology structure of the genital system of the noted groups. However, in local A. parthenogenetica, the uterus cells wall were long and thin with an oblong nucleus, and the cysts included a heterochromatin nucleus, intense basophilic and less granules. The diagonal of the cyst was recorded and observed smaller in the parthenogenetic specie rather than the A. urmiana species.

Keywords*: Histology, Genital System, Artemia urmania, Artemia Parthenogenetica, Urmia Lake*

INTRODUCTION
The Aquaculture industry has been noticed by many expertise because agriculture and animal husbandry sources have been reduced. The Aquatic products are rich source of vitamin, protein, minerals, and unsaturated fatty acids. A significant point in Aquaculture is the Aquatic's nurture that includes 40-50% of the cost of nourishment. The Aquatics feed of two kinds of sources: living and non-living. Among different kinds of animal living sources *Artemia* is very important because it includes of 52±8/8% protein, fatty, favorable fatty acids, and Amino acids.

The reproductive organ of female *Artemia* consists of a pair of ovaries, a pair of oviduct, and a uterine bag. The ovaries are tubular and geminate that start from the eleventh strap of the chest and end to abdominal straps. After ovulation, the zygotes enter the oviduct and are saved there. They are broken when the oviduct secretions begin (Hafezieh, 2003). The zygotes become spheral immediately after exiting from the ovary and are lead toward the uterus through two oviducts. The fertilized zygotes turn into nauplius with free swimming in a favorable condition (reproduction by hatchery-vivitary method) and they leave the mother ovary (Lawns and Sorjelos, 2003).

Most studies conducted in the world on *Artemia* are done frequently in morphology, reproduction, and breeding fields. Along with this, in histology field, we can point to Wolfe's study in 1971 on the genital system of male Artemia. In 1980, Criel conducted a research on the morphology of the genital organ of female Artemia. After observing with an electron microscope, findings showed that the formation of yolk membrane starts immediately after the zygotes are placed in the uterus, even without female's intercross.

In 1980, Criel studied the Fallopian tube of *Artemia* and stated that the ovums are temporarily saved in the oviduct. Along with it, the oviduct creates the side cysts. The oviduct affects the first meiotic divisions. Finally, Browne *et al.*, studied the biology of *Artemia* in 1990.

Most conducted researches in Iran which are related to this field are based on biometry-morphology. For instance, we can refer to a research conducted by Asem and Rastegar-Pouyani in 2008 that studied the A.

urmiana morphology in different geographical stations of the Urmia Lake. Their research in 2009 on the biometric difference of two *A. parthenogonetica* populations of the Urmia lake region, pointed to a research on Artemia's Bio-assay characters in different distinct of Iran by Peykaran Mana *et al.*, in 2010. Men have reached significant success in reproduction and breeding of the Aquatic based on their knowledge on Artemia's bio-behaviors. Certainly, scientific knowledge of biologic behaviors of a creature enables human to protect it and at same time reach a favorable utilization of that living creature.
The aim of this research was to compare histological characters of genital organs between A. urmiana and A.parthenogenetica.

MATERIALS AND METHODS

The bisexual and A. parthenogenetica samples were placed in separate incubators for hatching and breeding after being confirmed by expertise. After reaching sexual maturity, they were placed in a 10% Formalin and Bowen stabilizer solutions and were sent to the laboratory. The urmiana material was wrapped in a cleaning separately with the parthenogenetica specie (due to the sample's small size) and were then placed in the Autotechnicon device for histological preparation, and after that they were cut by the Microtome device to a 0/7 thickness. The samples were then pigmented through Hematoxilin and Eosin staining (Pousty & Adibmoradi, 1382).

RESULTS

The female *Artemia* can be easily recognized by the ovary that include zygotes and are exactly placed behind the branchiopoda's eleventh placenta above the intestine of the Gastro Intestinal (GI). The zygotes were observed as two ovaries in the abdominal. The ovary was wider in the hind and thinner to the head and was observed generally pear-shaped (Figure 1.)
Oviduct includes of one thin part and a wide part that the oogonia pass through it to the uterus. The histology study on A.urmiana, showed that the uterus had a one-layer covering with oblong cells and clear nucleus that were bigger toward the outlet. After that, the basal lamina layer, the loose connective tissue, and finally the smooth muscle exist one after another. It is covered by the body's chitin from outside, knowing that there is a delicate connective tissue between the muscles and the chitin layer (Figure 2).

Figure 1: The female *Artemia* uterus

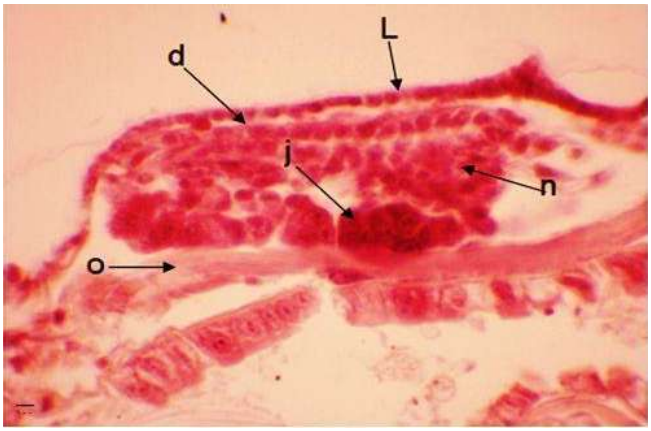

Figure 2: Longitudinal section of uterus in bisexual A.urmiana, H&E, 600x
L- The chitin layer, d- The uterus covering, n- The somatic cells; j-The oogonia cells, o- The smooth muscle

Constructions of large blood vessels were observed around the uterus and ovary that were very close to the uterine lining from about. The muscles are plain in the female genital organs that turn into synergistic muscles when it gets close to the outlet. The uterine lining cells include of cubic cell muscles with a clear

and spherical nucleus and a specific nucleolar above the muscles. The cysts consist of granules that can confirm the vitellusacinuses inside and have a clear nucleus (uchromatin) and specific nucleolar, that indicate activity and hatchery (Figure 3).

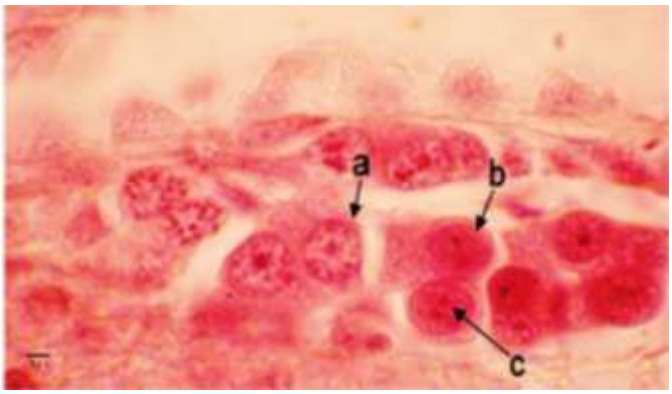

Figure 3: Cysts of bisexual A.urmiana that include a nucleus and a specific nucleolar. H&E, 1500x
a- Cysts, b- Nucleus, c- Nucleolar

It should be noticed that the younger cysts are bigger and have a chromatic nucleus and a greater number of granules. There exists an Eosinophili uniform fluid caused by the cysts accumulation around the uterus (Figure 4)

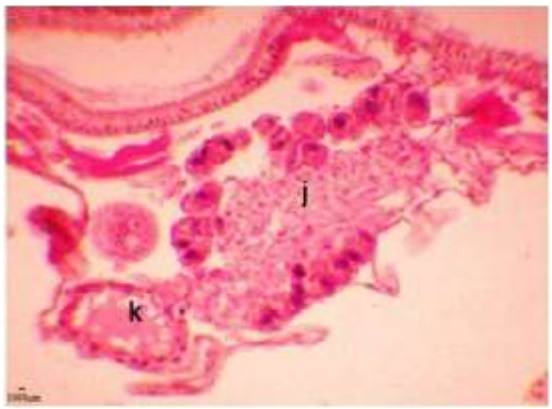

Figure 4: The ovary of bisexual A.urmiana, H&E, 150x
j-The oogony cells can be observed in the center, k- A transverse of the uterus

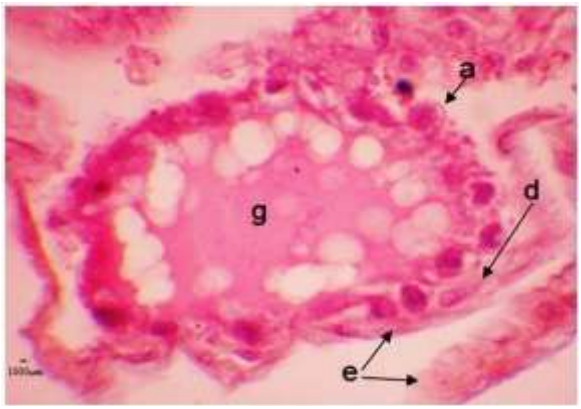

Figure 5: A transverse of the uterus that indicates that there is no cysts of bisexual A. urmiana, H&E, 600x
g- The uterine fluid, a-The cysts, d-The uterine lining cells, e-The connective tissue

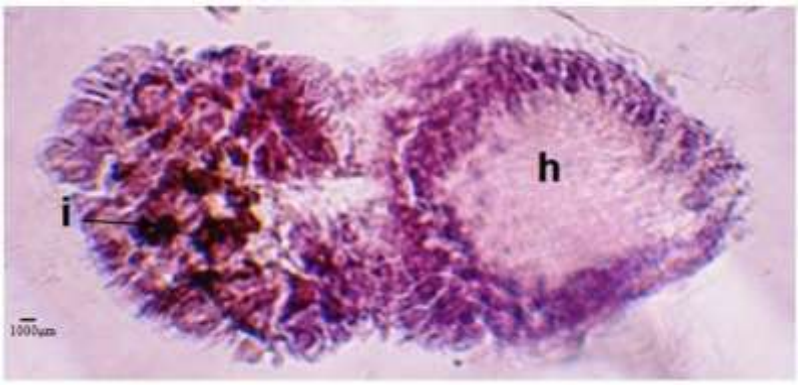

Figure 6: the theme builder pigment glands next to the ducts of bisexual A.urmiana cysts. H&E, 1500x
h-The duct with laminated covering tissue, i-Theme builder pigment glands

Some facts were pointed out about bisexual A.urmiana which are the same in the parthenogenetica specie, considering the uterine lining cells are oblong, thin, and have a clearer and fusiform nucleus.

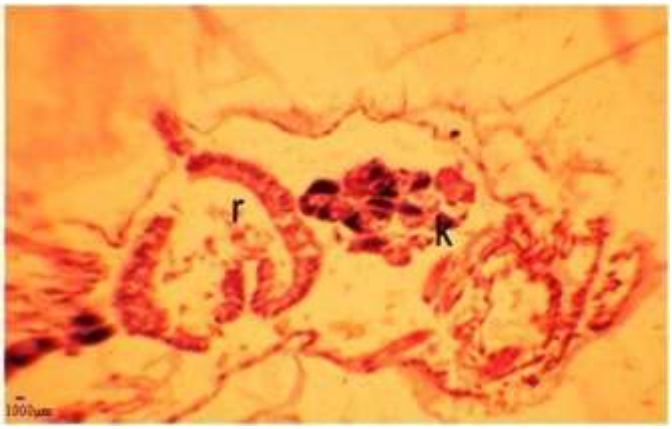

Figure 7: A cross-section of the uterine of A. parthenogenetica, H&E, 150x
r-The Intestine in center, k-The uterus

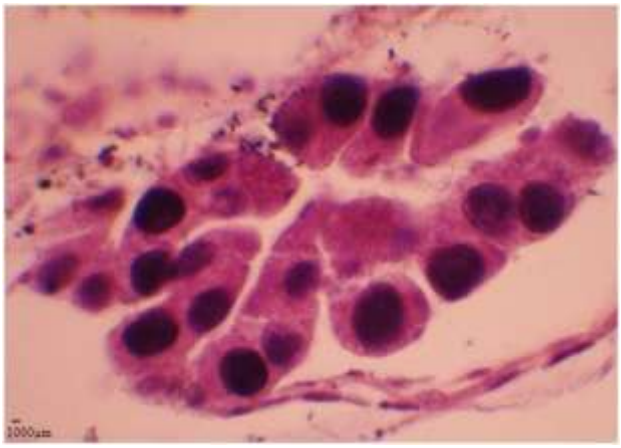

Figure 8: The ovary of A. parthenogenetic, heterochromatinecysts, H&E, 600x

The same muscles of the bisexual *Artemia* are plain and they change to skeletal muscles when they get close to the outlet (Figure 9.)

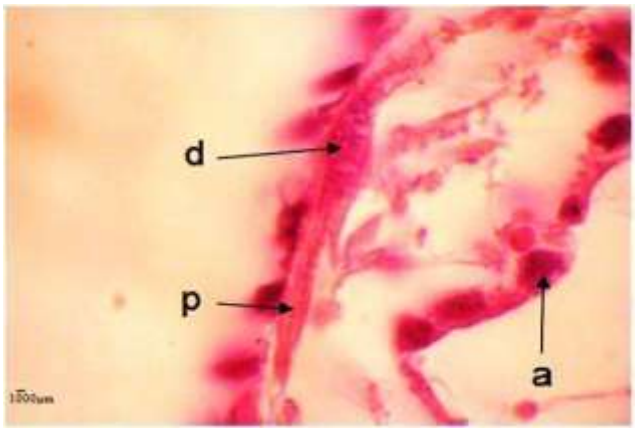

Figure 9: The uterus of A.parthenogenetica, H&E, 1500x
p- Skeletal muscles, a-cysts, d-Uterine lining cells

The cysts are smaller and darker in the parthenogenetica specie and have less granules and are placed one after another in the outlet.

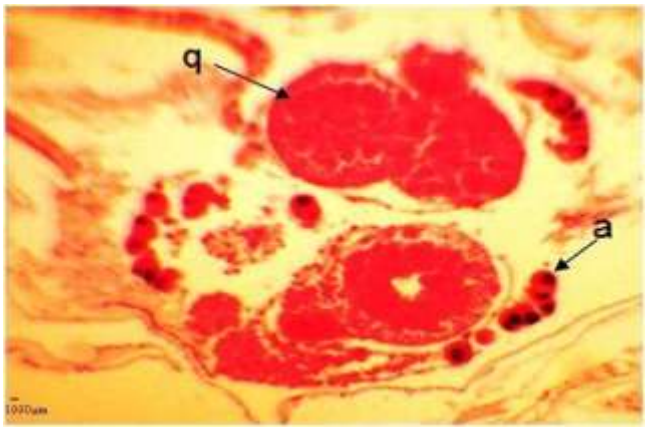

Figure 10: A part of the A. parthenogenetica uterus that is divided into two parts by bloody vessels, H&E, 150x
a-Cysts, q-Bloody Vessels

DISCUSSION

The growth and development of the reproductive organs starts from the difference of the first seminal cells and are happened when reproduction takes place along the first part of seminal ducts. Oviduct origins from the ovaries placed in the abdominal. During ovulation and reproduction, the zygotes are saved in small accessory bags and are away from the uterus (Browne *et al.*, 1990, 141-146).

There are two kinds of reproduction in all species of Artemia: hatchery and hatchery-vivitary. The embryo evolves to the emboly step in specific conditions (for instance, high salinity and low oxygenated water). At this level the cysts are covered by a thick membrane (which is secreted by brown constituent glands placed in the uterus). After that they enter the inactive level of metabolism stop (sleep mode). The cysts are then released by the female *Artemia* (hatchery reproduction) (Lawns and Sorjelos, 2003).

The Artemia's uterus is single and cod-like. The posterior part of the uterus is linked to a short outlet and is opened to the body from that part (Figure 4).

The fluid inside the uterus (Figure6-4) facilitates the cysts' movement from the uterus toward the inside part of the ducts and then outside of the body. Criel (1980) observed the secretory cells in the uterine ducts of Artemia. The existence of fluid in the recent research approves this.

The oviduct is surrounded by a layer of muscles that are circular smooth and longitudinal (Figure 5-4). The synergistic muscle in the outlet of the uterus and ducts can be effective in the exit of the cysts (Figure 9-4). Browne *et al.,* (1990) have observed the circular smooth and longitudinal muscles that are in accordance with the results of the research.

When the cysts reach maturity, their cytoplasm gets smaller and a dark, spheral nucleus is appeared. According to Peykaran Mana *et al.,* in 2010, the cysts have a larger diameter and longer naupliusin A. urmania compared to other cysts. This finding is in accordance with the findings of present research.

The existence of skeletal muscles in the uterus outlet and duct can be effective in the exit of the body (Figure 9). In the parthenogenetica field, the uterus lining cells are longer and thinner and consist of a thinner outlet. The parthenogenetica cysts were smaller and had fewer granules (Figure 11). In general, high potential for hatchery is due to the small size of the zygote. Therefore, the small size of the zygote represents its high quality (Asem, 2007). The findings of the present research are in accordance with the findings of Pilla and Beardmore in 1994 and with PeykaranMana *et al.,* in 2010. But there are in contrast with the findings of Asem's research, conducted in 2007.

No significant difference was observed in the reproductive organs of A.urmiana and A.parthenogenetica, except that the cysts were smaller, intense basophil, and had a decrease in their granules in the parthenogenetica specie.

The current research is conducted to examine the probability of a histological difference between the reproduction organs of (bisexual) female A.urmiana with A.parthenogeneticain the Urmia Lake.

REFERENCES

Asem A and Rastegar-Pouyani N (2008). Morphological Differentiation of *Artemia*urmiana Gunther, 1899 (Crustacea: Anostraca) in Different Geographical Stations from the Urmia Lake, Iran. *Medwell Journal* **3**(2) 222-228.

Asem A *et al.,* (2009). Biometric comparison of two parthenogenetic populations of *Artemia* Leach, 1819 from the Urmia Lake basin, Iran (Anostraca: Artemiidae). *Zoology in the Middle East* 47.

Asem A, Rastegar-Pouyani N and Agh N (2007). Biometrical Study of Artemiaurmiana (Anostraca: Artemiidae) Cysts Harvested from Lake Urmia (West Azerbaiijan,Iran). *Turkish Journal of Zoology* **31** 171-180.

Browne Robert A, Sorgeloos Patrick and Trotman Clive NA (1990). *Artemia Biology* (CRC Press) 141-6.

Criel G (1980). Morphology of the female genital apparatus of Artemia: a review 75-87. In: *The Brine Shrimp Artemia. Morphology, Genetics, Radiobiology, Toxicology* edited by Persoone G, Roels O, Jaspers E (Universa Press, Wetteren, Belgium) **1** 345.

Criel G (1980). Ultrastructural observations on the oviduct of Artemia: A review. In: *The Brine Shrimp Artemia. Morphology, Genetics, Radiobiology, Toxicology* edited by Persoone G, Roels O, Jaspers E (Universa Press, Wetteren, Belgium) **1** 345.

Hafezie M (2003). *Artemia* (Salty water shrimp). Iran ShilatResearch Organization-Managing Scientific Information, (Aslani Cultural Institute Publications) 66-68.

Lawns P and Sarjelus P (2003). *Artemia: The Function of Artemia in Reproduction and Breeding of the Aquatics* Translated by Hasani Sh and Jafari M (Daryasar Publications) 21.

PeykaranMana N *et al.,* (2010). Biometrical characters of *Artemia* from four Iranian regions. *Iranian Journal of Fisheries Sciences* **10**(2) 294-303. Available: http://www.jifro.ir/browse.php?a_id=177&slc_lang=en&sid=1&printcase=1&hbnr=1&hmb=1

Pilla EJS and Beardmore JA (1994). Genetic and morphometric differentiation in old World bisexual species of *Artemia* (the brine shrimp) **73** 47-56.

Pousty I and Adibmoradi M (1382). *Comparative Histology and Histotechnique* (Tehran University, Tehran) 540-556.

Wolfe AF (1971). A histological and histochemical study of the male reproductive system of *Artemia* (Crustacea, Branchiopoda). *Journal of Morphology* **135** 51-70.