Anatomical and Histological studies on the Female Reproductive System of the Red Swamp Crayfish *Procambarus Clarkii.*

Abd El-Halim A. Saad and Montaser M. S. Hassan
Zoology Department, Faculty of Science, Ain Shams University, Cairo. Egypt.

ABSTRACT

The present study revealed that the female reproductive system consists of a trilobed ovaries (Y-shaped) with an oviduct on each side, which opens with the female gonopore on the coxopodite of the third cheliped. The ovary passes through three stages, immature, premature and mature stages. In the first stage, the ovary is white in colour and the ova do not contain yolk. The ovary in the second stage acquires the yellow colour due to deposition of yolk granules. Finally it became brown due to the formation of the cortical granules at the periphery of the egg. Histologically, the ovary is surrounded by a transparent membrane composed of connective tissue. Each oocyte is surrounded by a layer of cuboidal follicular cells. The oocytes are more or less spherical in shape. The larger and mature oocytes are arranged at the periphery while smaller and younger oocytes are found at the center of the ovary. The oviduct has a thin wall and a wide lumen. The inner most layer is simple cilliated columnar cells. The internal lining of the oviduct forms numerous narrow finger-like folds. It is followed by a thin layer of connective tissue and circular muscle layer.

Key words: anatomy, histology, female reproductive system, *Procambarus clarkii.*

INTRODUCTION

The freshwater crayfish, *Procambarus clarkii* is known to receive a great environmental and economic importance. It is considered as one of the largest crustacean animal in the most freshwater systems. It was introduced into the Egyptian freshwater bodies in early 1980s. During the last decade, this invader has become a substantial member of the Egyptian aquatic fauna in many areas of Egypt. *P. clarkii* is native to southern U.S.A., northern Mexico and Mississippi valley. It had been successfully introduced to west India, Europe, the Middle East, eastern Asia and Africa i.e. it invaded various habitats (Huner, 1992).

In Egypt, *P. clarkii* had invaded most of the governorates of upper and lower Egypt. Its distribution has extended from Northern Delta to Assuit (Ibrahim *et al.* 1995; Saad & Emam, 1998; Shakir & Ibrahim 1998; and Mubarak 2001). This invader has an amphibious mode of life, enabling it to live out of water for long times because its gills are moistened with water and can utilize the atmospheric oxygen. This animal can withstand the life in shallow water and it is highly adaptable and resistant for severe unfavorable conditions

due to its hard exoskeleton. Moreover, it burrows deep tunnels in the banks of irrigation canals to survive and overcome drastic seasonal fluctuations (Ibrahim *et al.*, 1995). Moreover, Ibrahim *et al.* (1997) and Soliman *et al.* (1998b) investigated the habitat and behavior of the red swamp crayfish while Soliman *et al.* (1998a) examined its reproductive biology. Aly (2000) examined the pathological effects of Jojoba seed oil (plant extract) and fenthion (organophosphorus) insecticide on the ovary, digestive gland and muscles of the *P. clarkii*. Moreover, Sharshar and Geasa (1998) investigated the effect of copper on the hemocytes of *P. clarkii* which caused destruction and change of their ratio. Tolba (1999) used this crayfish as a biological indicator for water contamination with copper and cadmium. He found that the respiration rate increases as the pollutants increase. Habashy (2004a&b) explained the effect of natural and artificial diets, temperature and salinity on *P. clarkii*. Ibahim *et al.* (2006) studied its reproductive behavior. Ramalho *et al,* (2008) explained the effect of salinity and temperature on survival and growth of juvenile *P. clarkii.*

Most of the previous studies on the crayfish focused on the ecology, physiology and aquaculture. However, the anatomical and histological studies were not so integrated to give complete information about this animal. Although the crayfish is widely distributed in the Egyptian freshwater ecosystems, there is little information about this spices (IAA, 1995). Therefore, the present work was designed to study the anatomical and histological structure of the reproductive systems of this invader which considered one of the strongest factors influencing his survival and resistance to the environmental conditions.

MATERIALS AND METHODS

Specimens of *P. clarkii* were collected from the irrigation canals from EL-Kanater El-Khayreyia 20 Km North of Cairo using gobia baited with fishes. These animals were taken alive in wide container to the laboratory. They were placed in glass aquaria 20 x 25 x 30 cm that were filled with dechlorinated tap water which was aerated by using air pumps. The water was continuously changed every two days. They were fed on lettuce leaves and formulated diets. Only fully grown animals of 10-12 cm total length were used.

For the anatomical studies, live animals were dissected in the laboratory without using any anesthetic drugs. The female reproductive system was removed away from the specimens using dissecting microscope and placed in 0.6 % saline solution.

Other specimens were dissected for the histological and histochemical studies. The reproductive system were removed and placed directly into the appropriate fixative. The fixatives were Bouin's fluid and 10% formalin solution for 24 hours. After fixation the tissues were dehydrated using ascending series of ethyl alcohol, cleared in terpineol and embedded in paraplast. Serial sections were cut at 5 um thickness. The sections were deparaffinized, hydrated and stained by various stains.

Staining solutions were Mayer's hematoxylin countered with eosin (HX&E), periodic acid Schiff (PAS) for general carbohydrates and mercury bromophenole blue for general protein. The slides were cleared in xylen and mounted in DPX.

RESULTS

Anatomy of the Female Reproductive System:-

The crayfish *P. clarkii* is dioesious crustacean animal. The female gonopods (Gp.) are located on the first abdominal segment (Fig. 1). This pair of appendages is small and minute. The pleopods of females are larger in size than those of males of the same age or size carrying numerous setae. They are classified into two types, oosetae and stiff setae for eggs and young attachment.

The female reproductive system consists of two ovaries. They unite together forming a trilobed (Y-shaped) gonad (Fig. 2). The ovaries are found in the thoracic cavity above the digestive gland and just beneath the heart. It is formed of two anterior lobes and another posterior one. One oviduct arises from each side at the junction between the anterior and posterior lobes. The oviducts extend to the coxopodite of the third walking legs where they open to outside with the female genital opening. The genital opening (Go.) is circular and covered with a thin transparent flap (Fig. 1).

The ovaries include three stages according to yolk deposition, colour and size of both ova and ovaries. The first stage has white colour, second or vitellogenic is yellow to orange in colour and the third or mature stages is brown in colour (Fig. 2). The white ovary is the smallest stage; very delicate and measuring 5-10 mm in length and 1-2 mm in diameter with very small ova "oogonial stage". The oviduct is thread like and transparent. The yellow ovary or the vitellogenic stage is larger in size measuring 12-15 mm in length and 3-4 mm in diameter. The oviducts are transparent. The yolk granules accumulate in the cytoplasm in this stage.

The brown ovary or mature stage is bright to dark brown and have large size measuring 20-40 mm in length and 5-7 mm in diameter. The oocytes attained their largest size and the oviducts are wide.

Histology of the Female Reproductive System:

1- The Ovary:

Histologically, the ovary of *P. clarkii* is compact without lumen (Fig. 3). It is surrounded by a transparent membrane composed of connective tissue. Each oocyte is surrounded by a layer of follicular cells which are cuboidal. The oocytes are more or less spherical in shape. The larger and mature oocytes are arranged at the periphery while smaller and younger oocytes are found at the center of the ovary.

Stage I (oogonial stage):-

The oocytes are small in size measuring about 120-200 um in diameter. The cytoplasm is weakly acidophilic, finely granulated and lacking the yolk

granules (Fig. 4). The nucleus is large, spherical and containing network of chromatin granules. There are no nucleoli inside the nucleus and the nuclear membrane is sharply distinct. The oocyte is surrounded by a single layer of flattened follicular cells (fl.) (Fig. 4). The oocytes gave a week reaction with bromophenol blue (Fig. 5).

Stage II (vitellogenic stage):-

The ovaries in this stage are yellowish in colour. It represents the active stage of yolk formation in which the yolk begin to accumulate in the oocytes (Fig. 6). Firstly, the yolk granules appear at the periphery of the cytoplasm and spread towards the center leaving a clear zone around the nucleus, the perinuclear zone (p.z.) (Fig. 7). The oocytes increase in size measuring about 200-400 um in diameter. The nucleus is more or less centrally located with several prominent nucleoli which adhere to the nuclear membrane (Fig. 7). Each oocyte is surrounded with a single layer of follicular cells (Fig. 8). The yolk granules of the oocytes react positively with bromophenol blue (Fig. 9).

Stage III (late vitellogenic or mature stage):-

The oocytes in this stage are large in size measuring from 400-600 um in diameter and the colour of the ovaries ranging from bright brown to dark brown. Oocytes become harden immediately prior to spawning and measure from 800-1500 um in diameter (Fig. 10). The cytoplasm is restricted to a very narrow zone around the nucleus. The nucleus is more or less centrally located with several nucleoli. In this stage the follicular cells are flattened.

2- The oviduct:-

It is a short straight tube that transports the mature oocytes to outside measuring about 9mm in length and 2-3 mm in diameter.

Histologically, it has a thin wall and a wide lumen (Fig. 11). The inner most layer is simple cilliated columnar cells. Each cell has finely granulated cytoplasm and central spherical nucleus with centrally located nucleolus (Fig. 12). The internal lining of the oviduct gives numerous narrow finger-like folds. It is followed by a thin layer of connective tissue and circular muscle layer.

DISCUSSION

The ovaries of the crayfish *P. clarkii* are one pair, trilobed and Y-shaped. These observations are in agreement with those reported by Boolootina and Heyneman (1962) Marshall and Williams (1975) working on the crayfish *Astacus.* On the other hand, El-Sherief (1987) described the ovaries of the crab, *Portunus pelagicus* and the squat lobster *Thenus orientalis* respectively as they are H-shaped.

This study explained that, the colour of the ovary of crayfish *P. clarkii* changes during the maturation; the white or immature, the orange or vitellogenic and dark brown or mature. The colour of ovary of the lobster *Homarus americanus* ranges from chartreuse to black. Burton (1995) found that these variations in colour are due to the differences in the amount of ovoverdin

deposited in the oocytes. Furthermore, Kulkarni *et al.* (1991) reported that the colour of the ovary of *P. clarkii* changes during the maturation; it is transparent, translucent white and finally attaining a dark-greenish dark-brown colour when fully mature. Meanwhile, Hussein and Obuid-allah (1992) reported that the ovaries of the fresh water prawn *Cardiane nilotica* are orange in colour. McRae and Mitchell (1995) found that the ovaries of the crayfish, *Cherax albidus* have seven maturation stages according to the colour and yolk deposition. They are white, cream, yellow, orange, green, dark green and gray. The ovaries of the red swamp crayfish *P. clarkii* have three stages according to the colour; the white or immature, the yellow or mature and brown or fully mature (Mubarak, 2001).

The ovary is compact organ and the oocytes are more or less spherical in shape. The mature oocytes are arranged at the periphery while younger oocytes are found at the center of the ovary. Unlike this description, Borradail *et al.* (1963) found that the gonads of both sexes of the crayfish *Astacus* are hollow. Moreover, Hashem *et al.* (1991) observed that the ovary of the female prawn *Penaeus kerathurus* is compact and has an internal core of oogonial cells and oocytes in various stages. Furthermore, the ovary of the crayfish *P. clarkii* has a central core of oogonial cells while the mature oocytes appear at the periphery. Similar results were obtained by Heiba (1998).

In the present study the young oocytes are lacking yolk granules. The yolk appears at the periphery of the oocytes and extends towards the center of the fully mature oocytes. These results were supported by Stephens (1952) who reported that ovarian maturation is considered as yolk deposition. Beams and Kessel (1963) declared that the cortical region in large oocytes become loaded with yolk granules. However, Marshall and Williams (1975) indicated that the eggs of crayfish *Astacus* are centrolecithal. Moreover, Kulkarini *et al.* (1991) noted that in late vitellogenic stages oocytes appeared pale. On the other hand, Mubarak (2001) indicated that the yolk globules aggregate in the periphery and then extend towards the center of the mature oocyte. Medina *et al.* (1996) observed a conspicuous cortical rods at the periphery of the mature oocytes of the ovary of the *Penaeus kerathurus*. The cortical rods are invaginations of the plasma lemma of the oocyts. These rods on the oocyte surface are synthesized shortly before spawning. There are no cortical rods were observed in *P. clarkii*. Hilmy *et al.* (1986) found that the premature oocytes appeared without or with little amount of yolk granules where yolk is formed as granules, globules and spheres. Moreover, Sagia *et al.* (1997) studied the eggs of the crayfish *Cherax quadricarinatus*. He indicated that vitellogenesis is characterized by a rapid deposition of yolk and other proteins in the oocyte, which results in fast increase in the oocyte diameter. Therefore, the same author stated that yolk is the main component of the crustacean eggs and it is consisted of proteins, lipids and carbohydrates.

The ovary of the crayfish *P. clarkii* has younger oocytes among the mature ones even in the fully mature ovary. Each oocyte is surrounded by a layer

of follicular cells which are cuboidal in shape, the same results were obtained by Heiba (1998) and Mubarak (2001). Stephens (1952) suggested that not all the oocytes in the ovary of the *Cambarus* pass through the same stage of development. Moreover, Talbot (1981) and Kularini *et al.* (1991) studied the ovaries of the *Homarus americaus* and *P. clarkii*. They postulated that most of the oocytes of a given ovary are of approximately the same size but there are always immature oocytes present among the mature ova in the same gonad.

The ovary of the of the present crayfish passes through three stages, the oogonial (measuring about 120-200 um in diameter), vitellogenic (measuring about 200-400 um in diameter) and mature stage, (measuring from 400-600 um in diameter) Kulkarini *et al.* (1991) classified the ovarian development in the red swamp crayfish *P. clarkii* into seven stages according to the size: oogonial (< 10 um), immature (10-65 um), a vitellogenic (66-160 um), early vitellogenic (161-245 um), midvitellogenic (246-455 um), late vitallogenic (456-980 um), postvitellogenic and resorptive. On the other hand, Hashem (1991) classified the oocyte development in the prawn *Penaeus japonicus* into four stages: early immature (spent stage), immature (advanced stage), mature stage, Ripe stage. Medina *et al.* (1996) reported that there are five stages of oocyte maturation of the shrimp, *Penaeus kerathurus*: previtellogenic, early vitellogenic, late vitellogenic, mature and spent or degenerating.

Aly (2001) reported that the ovarian oocytes of the red swamp crayfish *P. clarkii* are classified into three stages according to their size, density of the yolk globules and colour of the ovary into: immature oocytes (< 0.5 mm), mature oocytes (> .05 mm) and fully mature oocytes (< 1.3 mm). Moreover, McRea and Mitchell (1995) discussed that prior to spawning, the ova are about 1200-2000 um and the ovary is very large.

All these studies agree with the results found in this study where they were sorted according to the size, yolk deposition and the colour of the ovary. But there are some differences where there is no resorptive, atretic or degenerating stages were observed and previtellogenic, oogonial avitallogenic and immature stages are compatible here with oogonial stage. Furthermore, the early mid and late vitellogenic stages are the stages of yolk deposition which in the present study were named as vitellogenic stage.

Heiba (1998), Aly (2000) and Mubarak (2001) reported that there are four cell types in the ovary of the *P. clarkii*: follicle cells, germinal cells, nutritive cells and oocytes. These results do not agree with the present observations.

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EXPLANATION OF FIGURES

Fig. 1:-Photograph of ventral view of female crayfish showing gonopods (gp) and genital opening (go) on the 6th. thoracic appendages.

Fig. 2:-Photograph of the female reproductive system showing the three stages (A) oogonial (B) vitellogenic and (C) fully mature.

Fig. 3:-Photomicrograph of T.S. of white ovary showing immature oocytes (i.o) surrounded by follicular cells ((fl). [Bouin, HX&E. (160 X)].

Fig. 4:-Photomicrograph of enlarged part of T.S. of white ovary showing immature oocytes (i.o.) surrounded by single layer of flattened follicular (fl.) cells and the vesicular nucleus arrows. [Bouin, HX&E.(422X)].

Fig. 5:-Photomicrograph of T.S. of white ovary showing the weak reaction with protein stain and the sharply distinct nuclear membrane arrows. [10% Formalin, Bromophenol blue.(422X)].

Fig. 6:-Photomicrograph of T.S. of yellow ovary showing vitellogenic oocytes (v.o.) yolk granules (y.g.). [Bouin, HX&E.(43X)].

Fig. 7:-Photomicrograph of part of T.S. of vitellogenic ovary showing yolk granules (y.g.) perinuclear zone (p.z.) and several nucleoli (ni.). [Bouin, HX&E.(160X)].

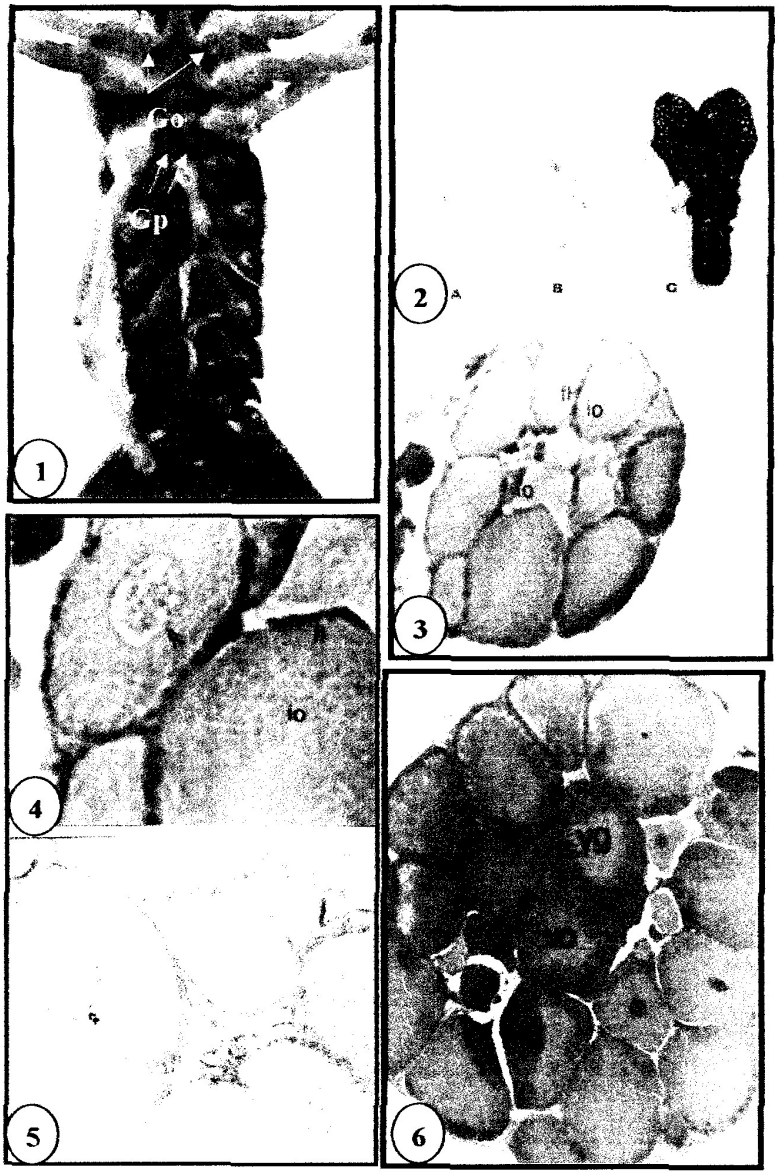
Fig. 8:-Photomicrograph of T.S. of yellow ovary showing vitellogenic oocytes (v.o.) surrounded by single layer of follicular cells (fl.). [Bouin, HX&E.(422X)].

Fig. 9:-Photomicrograph of T.S. of yellow ovary showing strong reaction of vitellogenic oocytes for the protein contents (y.g.). [10% Formalin, Bromophenol blue.(160X)].

Fig. 10:-Photomicrograph of T.S. of mature ovary showing mature oocytes (m.o.) surrounded by single layer of flattened follicular cells (fl.). [Bouin, HX&E. (160X)].

Fig. 11:-Photomicrograph of T.S. of the oviduct showing the finger-like folds (fo.) and the wide lumen (lu.). [Bouin, HX&E.(160X)].

Fig. 12:-Photomicrograph of part of the wall of the oviduct showing the ciliated short columnar cells (c.cl.c.) and circular muscles (c.m.) [Bouin, HX&E. (422X)].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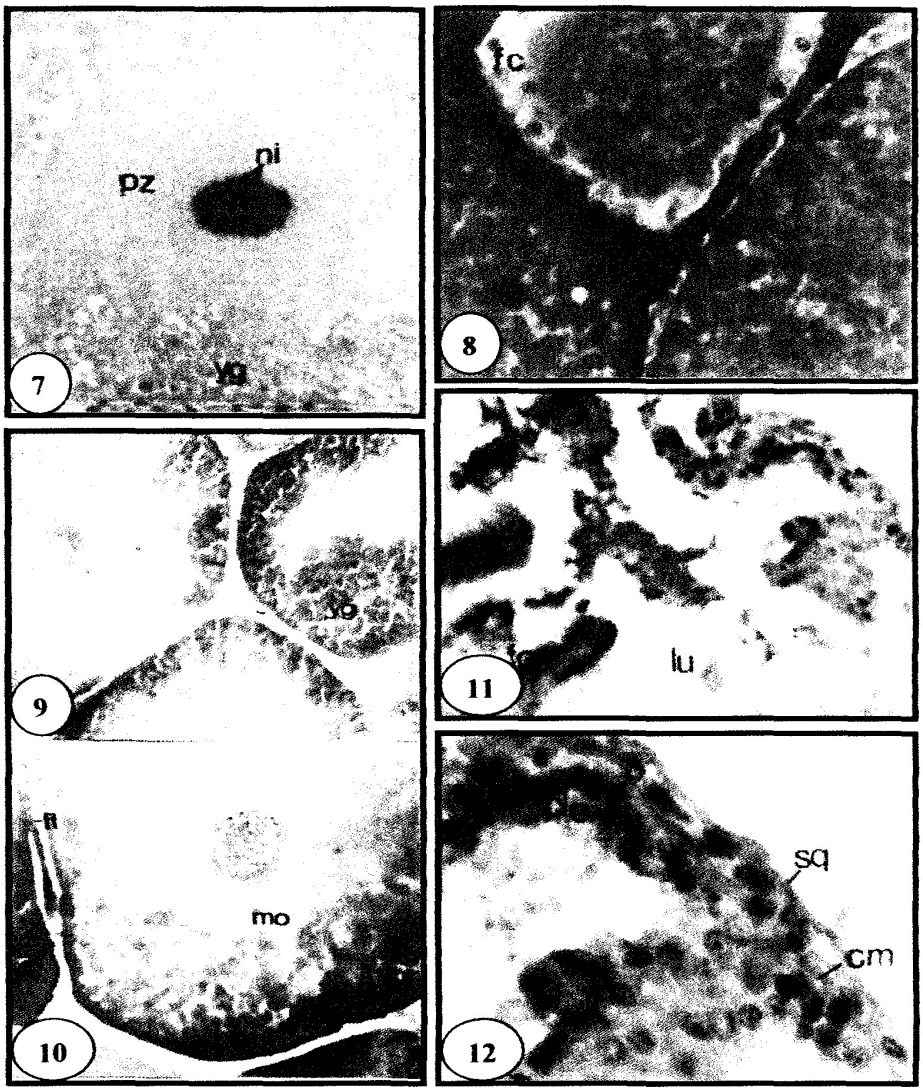
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دراسات تشريحية وهستولوجية علي الجهاز التناسلي الأنثوي لاستاكوزا المياه العذبة بروكامبارس كلاركي.

عبد الحليم عبده سعد - منتصر محمد سيد
قسم علم الحيوان- كلية العلوم- جامعة عين شمس - القاهرة - مصر.

أوضحت الدراسة التشريحية أن الجهاز التناسلي الأنثوى لاستاكوزا المياه العذبة بروكامبارس كلاركي. يتكون من مبيضين ملتحمين من الخلف مكونان منسل ثلاثي الفصوص على شكل حرف Y حيث تمتد قناة البيض من نقطة الإلتحام على كلا الجانبين إلى اسفل لتفتح بالفتحة التناسلية علي الزائدة الكلابية الثالثة. ويمر المبيض بثلاث مراحل للنضج؛ المرحلة الغير ناضجة ويكون لونه أبيض ثم مرحلة تكوين المح ولونه أصفر ثم مرحلة النضج ويكون فيها بني اللون. ومن الناحية الهستولوجية يحاط المبيض من الخارج بنسيج ضام رقيق شفاف وتوجد خلايا جرابية مكعبة تحيط بكل بويضة. و البويضات كروية الشكل؛ وتظهر البويضات الناضجة على حواف المبيض بينما توجد البويضات الغير ناضجه فى الداخل. وقناة البيض ذات جدار رقيق يتكون من طبقة خلايا حرشفية تليها طبقة دائرية من العضلات الملساء وتجويفها واسع، به ثنايا ومبطن بطبقة من الخلايا العمودية المهدبة .