Gross and Microscopic Structures of the Female Reproductive System in the Whip-tail Stingray *(Dasyatis bleekeri)*

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Abstract

The female reproductive organs of the whip-tail stingray *(Dasyatis bleekeri)* were studied under light microscope. The ovary was found only on the left side. It was embedded behind the epigonal organ. It contained developing follicles at various stages along with atretic follicles and copora lutea. The granulosa of the ovarian follicle had 2 types of cell: columnar follicular cells and large cells. The female reproductive ducts were found on both sides, each was divided into an oviduct, nidamentary gland, uterus and vagina. The wall of the oviduct consisted of a mucosa, muscular layer and serosa. the nidamentary gland wall had a large number of transverse folds. Its mucosal layer had numerous glands which contained PAS positive granules. The mucosal wall of the uterus was thrown into many longitudinal folds and secreted a fluid that gave a PAS positive. The vaginal wall was formed of a mucosa, muscularis and adventitia. The lower ends of the left and right vagina joined and became a common vagina which opened into the cloaca.

1.Introduction

The whip-tail stingray *(Dasyatis bleekeri)* is a cartilaginous fish in the order Rajiformes, subclass Elasmobranchii, class Chondrichthyes [1]. It is found in the Thai marine environment and nearby. It is of economic importance because the flesh is edible and its skin is now used in leather goods. The cartilaginous fish are of particular interest to reproductive biologists in many aspects such as internal fertilization, pattern of sex differentiation, gonadogenesis and reproductive tract development, all closely resembling those of amniotes. Information on the reproductive structures of the cartilaginous fish is rare, much of it originating from sharks and dogfish. The structures of the male reproductive system have been reported [2] but not of the female.

2.Materials and Methods

Five female whip-tail stingrays were obtained from the gulf of Thailand close to Ta-prig and Laem-hin district in Trard province, 400 kms south-east of Bangkok. The fishes were captured in October, 1992.

The reproductive organs were dissected out immediately after catching because they disintegrate within 2 hours. The tissues were cut into 0.5 cm. sizes and fixed in Bouin's solution for 24 hours and processed for paraffin technique. The paraffin sections were cut at 5-6 μm and were divided into 3 groups. The first group was stained with Harris hematoxylin and eosin. The second group was stained with Masson's trichrome and the last group was stained with Periodic acid Schiff (PAS). The sections were examined under a light microscope.

The gross structure of the ovary, oviduct, uterus and vagina were examined *in situ*. The sizes were measured and photographed.

3.Results

Gross anatomy of whip-tail stingray

The mean body weight was 3.0 kg. The body length and width were 40 cm. and 36 cm. respectively. The mean tail length was 75 cm.

Sex determination can be noticed easily because the male has one pair of claspers which lie on the medial edge of the pelvic fins but these are absent in the female.

Gross structure of ovary

The ovary was pink in color with a smooth surface. The mean size was 0.8 cm. in length and 3 cm. in width. The proximal part was broader than the distal part. It was found only on the left side. It was suspended from the dorsal body wall by a broad mesovarium which carried blood vessels and nerves and was embedded behind the epigonal organ. On opening the abdominal wall the epigonal organ covered the ovary. The epigonal organ was carefully removed so as to see the ovary clearly.

Gross structure of reproductive ducts

The female reproductive ducts were found on both sides divided into 4 parts which were the oviduct, oviducal or nidamentary gland, uterus and vagina [1].

The mean length of the oviducts were 15 cm. long and were lined parallel to the spinal column. The shape of the anterior part close to the falciform ligament was like a funnel and called osteum of the oviduct. The posterior part was enlarged into a bulb-like structure of 0.7 cm. in diameter called the oviducal or nidamentary glands. The outer surface of the nidamentary gland was smooth but the inner surface had a large number of transverse lamellae.

The nidamentary gland connected to the uterus is a large bulb-like structure 2.5 cm. in diameter. The last part of the duct was the vagina which was 2 cm. long. The inner surface was lined with disperse papillae. The lower ends of the left and the right vagina merged and became a common vagina which opened into the cloaca (Figure 1).

Microscopic structure of the female reproductive organs

Ovary The outermost surface of the ovary was covered with the germinal epithelium which was composed of simple squamous or simple cuboidal epithelial cells (Figure 2). The inner layer was connective tissue stroma with ovarian follicles in different stages including atretic follicles and corpora lutea. The ovarian follicles could be divided into four types which were primordial, primary,previtellogenic and vitellogenic follicles [3],[4].

1.Primordial follicle. Each consisted of an ovum enveloped by a single layer of squamous follicular cells. The mean follicular size was 100.67 μm (S.D = 9.95 ,n = 7). Most of the primodal follicles were found near the tunica albuginea (Figure 3).

2.Primary follicle. The follicle had only one layer of cuboidal or columnar follicular cells enclosed in an ovum. There were some large round cells interposed among these follicular cells. (Figure 4) The mean size of the follicle was 128.25 μm (S.D = 18.63, n = 10) Some ova showed abnormal mitosis called aberrant follicle (Figure 5).

3.Pre-vitellogenic follicle (non-yolky follicle). The follicle was about 341.53 μm in diameter (S.D = 98.90, n = 13). Each consisted of a yolk-free oocyte surrounded by a multilayer of granulosa or follicular cells. There were two types of granulosa cells, some large round cells interposed among columnar cells. (Figure 6) The large round cells had round concentric nuclei and clear cytoplasm. The zona radiata surrounded the oocyte plasma membrane. This layer was stained magenta with PAS. Beneath the granulosa layer was the basement membrane and then the theca layer.

4.Vitellogenic follicle (yolky follicle). The follicle was composed of a yolky oocyte surrounded by a multilayer of granulosa cells. There were a lot of yolk platelets in the cytoplasm of the oocyte. The mean diameter of the vitellogenic follicle was 1443.37 μm (S.D = 527.18, n = 10) The theca can be distinguished into theca interna and the theca externa (Figure 7).

Corpus luteum (postovulatory follicle). After ovulation, the follicle transformed into a corpus luteum. The histological changes in three stages have been described. Stage 1 was charaterized by an indented granulosa layer and later became a low granulosa fold (Figure 8). The granulosa had 2 cell types, columnar cells and large round cells the same as those of the vitellogenic follicles. The follicular cavity contained a lot of yolk platelets dispersed evenly and densest at the area close to the granulosa layer. Stage 2 : The granulosa fold protruded deeper into the follicular cavity. The core of the fold had more connective tissue. There was a single layer of cuboidal cells surrounding the theca interna. The rest were similar to those in stage 1 (Figure 9). Stage 3 : The granulosa fold completely filled the center of the structure causing a reduction in the follicular space. The large cells became smaller and began to degenerate. Many small blood vessels and a large amount of connective tissue invaded the granulosa fold. The theca layer was reduced in thickness. The whole structure was reduced in size with degeneration of the granulosa cells. (Figure 10).

Atretic follicle. Follcles that can not develop to ovulatory follicles are called atretic follicles. They can occur at any stage of follicular development. The degeneration of follicles is known as atresia or regression. Atresia of the primordial and primary follicles was characterized by shrinkage and degeneration of the oocyte. The follicular cells degenerated later and the follicle became distorted (Figure 11). During the degeneration of previtellogenic follicles there was no granulosa fold. The degeneration of vitellogenic follicles could be divided into 4 stages.

Stage 1: The granulosa layer folded like villi with a small amount of connective tissue at the core of the fold. The granulosa had no large cells, the follicular cells lengthened. At this stage phagocytosis of yolk had begun. The theca interna had a thin band of densely packed collagen fibers (Figure 12).

Stage 2 : The granulosa fold protruded into the follicle and began to anastomose. More connective tissue was found in the fold. The degeneration of yolk increased. The follicular cells were hypertrophy and commenced degeneration. The theca interna had a thin layer of collagen fibers. In the adjacent layer there were cells forming a tube-like structure. The thin layer of connective tissue surrounded the outermost layer (Figure 13).

Stage 3 : Follicles were getting smaller with a thin wall. The granulosa layer was unorganized and degenerated. More connective tissue infiltrated into the follicles, resulting in a smaller follicular space. The yolk was completely degenerated with follicular cells and nuclear debris scattered in the follicular space (Figure 14).

Stage 4 : The follicles reduced in size and nearly all follicular cells were degenerated. At the end of this stage, the follicular cells disappeared. A large number of blood spaces were in connective tissue inside the follicle (Figure 15).

Microscopic structure of the female reproductive duct

Oviduct. The wall of the oviduct consisted of the mucosa, the muscularis and the serosa (Figure 16).

The mucosa was thrown into longitudinal folds which branched into primary and secondary folds. Most of the epithelium lining the fold was simple columnar with small number of pseudostratified columnar. Some epithelial cells had cilia. Underlying the epithelial layer was a loose connective tissue layer. The secretion found in the lumen was carbohydrate substance that could be stained with PAS.

The muscularis was composed of an inner circular and outer longitudinal layer of smooth muscle. The serosa consisted of loose connective tissue and mesothelium.

Nidamentary gland. The wall had a large number of transverse folds. The epithelium lining was ciliated pseudostratified columnar (Figure17). The mucosa had a large number of tubular glands separated by connective tissue. The glandular cells contained numerous PAS positive granules (Figure 18). Underlying the mucosa was a circular smooth muscle layer and the outermost was dense collagen fibers.

Uterus. The uterus is the largest part of the reproductive duct. The mucosal wall was thrown into many longitudinal folds similar to villi and were called trophonemata (Figure 19). These folds contained blood vessels and connective tissue. The epithelial lining was simple columnar epithelium. Underneath were collagen fibers mixed with smooth muscle cells, lymph nodules and small blood vessels. The secretion in the lumen was stained magenta with PAS.

Vagina. The wall of the vagina was composed of 3 layers : a mucosa, a muscularis and an adventitia. The epithelium of the mucosal layer was transitional epithelium of multilayers of round or oval shaped cells (Figure 20). Underlying connective tissue was mainly collagen fibers which penetrated into the epithelial layer (Figure 21). The muscularis consisted of smooth muscle oriented randomly, rich in collagen fibers and blood vessels. The adventitia contained a layer of loose connective tissue.

4.Discussion

The female whip-tail stingray has only one ovary on the left side as have some genera of elasmobranch, *Urolophus* and *Dasyatis.* Most other elasmobranch have paired ovaries which are symmetrical. In some elasmobranch such as *Scyliorhinus, Carcharhinus and Mustelus*, only the right ovary becomes functional [1]. The ovary is embedded in the epigonal organ which is the hemopoietic organ, testes are also embedded in this tissue [2], [5].

The ovarian follicles are the same as those found in the ovary of other elasmobranch [6], [7], [8]. The granulosa layer of the ovarian follicle consisted of stratified columnar epithelium. There were two types of granulosa cells : The small columnar cells and large round cells, which is a unique characteristic of cartilaginous fish in the order Rajiformes [1]. Large cells play an important role in the deposition of yolk [7]. In cartilaginous fish in the order Squaliformes e.g. sharks and dogfish, the granulosa layer has only one cell type [9], [10].

Corpus luteum had folded mucosa similar to those found in ovoviviparous elasmobranch, *Squalus acanthias* [10]. Inside the corpus luteum there are yolk platelets dispersed in the follicular cavity between the granulosa folds the same as those reported by Hisaw and Hisaw [6]. But the viviparous elasmobranch, *Mustelus canis* had no yolk platelets inside the corpus luteum [11].

The atretic follicles found in the stingray are the same as those reported in other elasmobranchs including oviparous, ovoviviparous and viviparous [4],[8],[10],[12], [13],[14],[15]. The atresia had 4 stages similar to the reports of Te Winkle [11] and Saidapur [8]. There were no large cells in the granulosa of atretic follicles from the begining of stage 1 because they were expelled from the granulosa layer [8].

The female stingray had two reproductive ducts and both function in the same way as those found in most elasmobranchs. In *Centrophorus squamosus* only the right side is functional and in *Dasyatis* and *Myliobatis* only the left side is developed (1). The female reproductive ducts were divided into 4 parts, based on Dodd [1].

The nidamentary gland of the stingray had a uniform structure, but the nidamentary gland of oviparous elasmobranch, *Scyliorhinus Caniculus* can be divided into three distinct zones, an anterior albumen secreting zone, a narrow mucus secreting zone and a large area of shell-secreting zone.The names are descriptive of the function of various zones. The shell-secreting zone acts as a storage of sperm from insemination [1]. The function of the nidamentary gland in the stingray may be the same.

The whip-tail stingray is ovoviviparous because the young that were found in some pregnant females had yolk attached but there was no placenta connecting the young with the uterus. During pregnancy only one side of the paired uterus was functioning. Therefore, the young were found in only the left or in the right side (Chatchavalvanich and Visuttipat, unpublished observations). The innermost layer of the uterus has villi-like structure. Saidapur [8] named these villi trophonemata. The trophonemata secretes fluid called embryotroph or uterine milk which is an important nutreint source for the young [16]. Embryotroph secreted from the uterus gives a PAS positive, this is due to the glycoprotein content [17]. The distal part of the left and right vagina joined and became a common vagina. The inner layer of the vagina was full of knots (nodules). These

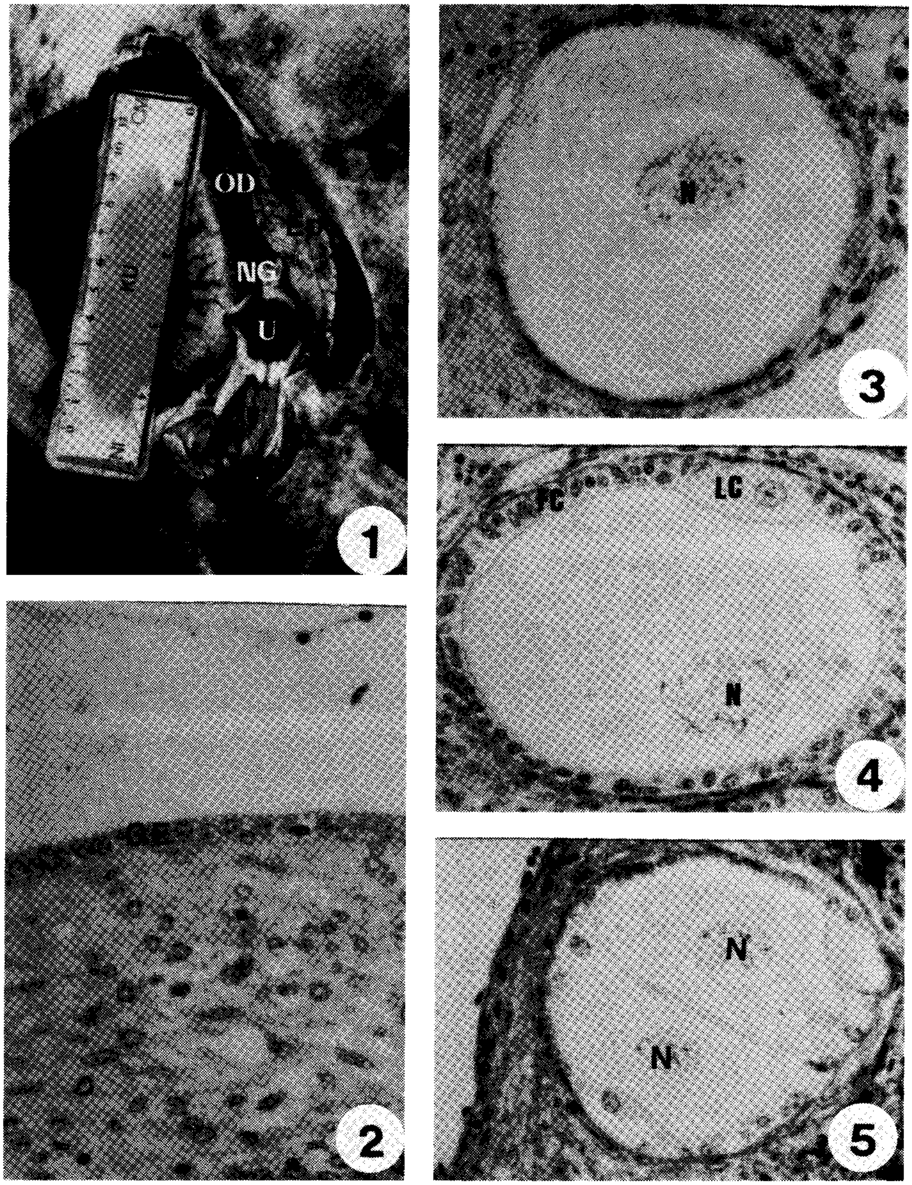


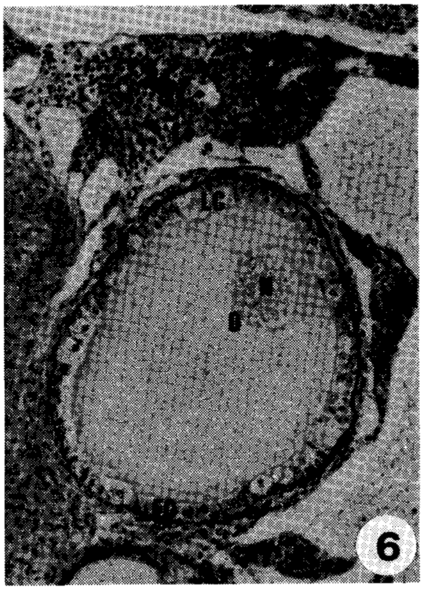
Figure 1 Gross structure of the female reproductive system in stingray. OD = oviduct, NG = nidamentary gland (dissected), U = uterus (dissected), V = vagina (dissected), EP = epigonal organ which covered the ovary.

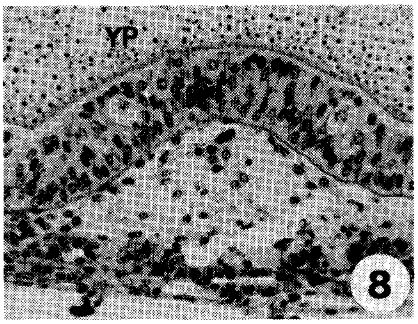
Figure 2 The surface of the ovary was covered by germinal epithelium (GE). (H&E; x500).

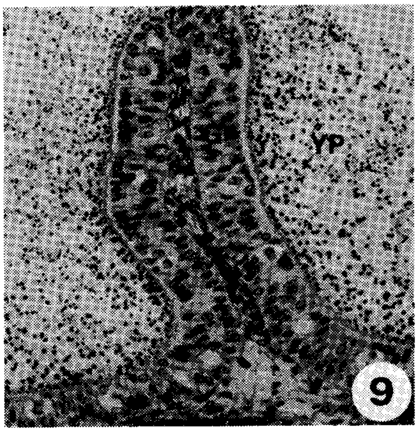
Figure 3 Cross section of the ovary showing primordial follicle. FC = follicular cell, N= nucleus of the ovum. (H&E; x500).

Figure 4 Cross section of the ovary showing primary follicle. FC = follicular cell, LC = large cell, N = nucleus, (H&E; x500).

Figure 5 Abnormal mitosis of the ovum. N = nucleus. (H&E; x500).







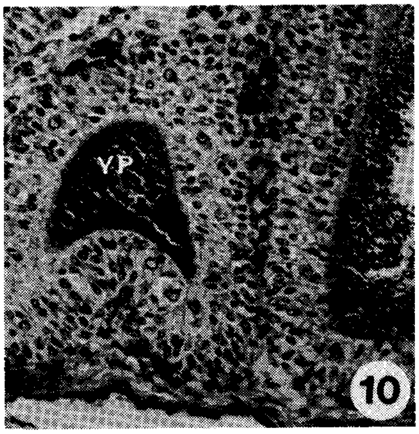


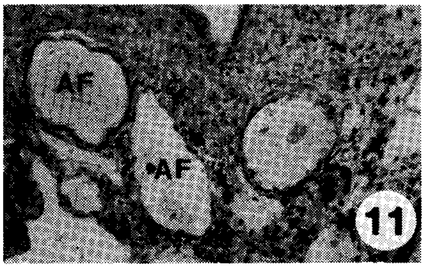
Figure 6 Pre-vitellogenic or non-yolky follicle consisted of multilayer of follicular cells enclosed an ovum. N = nucleus of the ovum, LC = large cell, FC = columnar follicular cell. Th = theca. (H&E; x200).

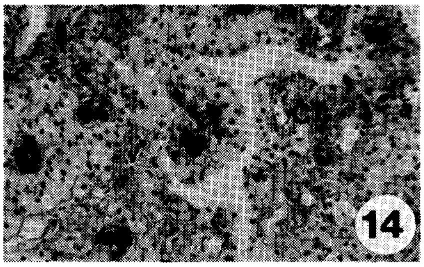
Figure 7 Vitellogenic or yolky follicle showing yolk platelets (YP), large cells (LC), columnar follicular cells (FC), zona radiata (ZR), basement membrane (BM), theca interna (TI), theca externa (TE) and blood vessel (BV). (PAS; x300).

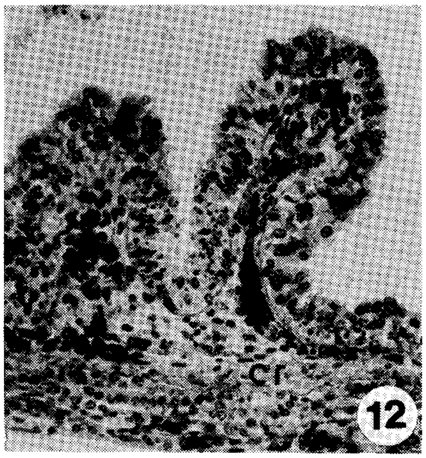
Figure 8 Corpus luteum stage 1 showing indented granulosa layer (Gr), YP = yolk platelets.(H&E; x200).

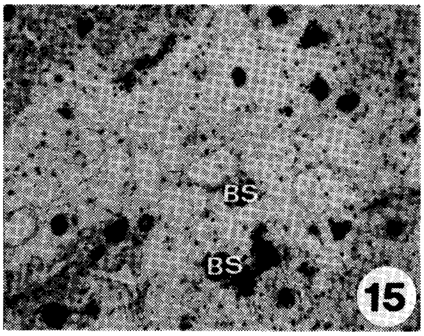
Figure 9 Corpus luteum stage 2 showing granulosa fold (Gr). (YP) = yolk platelets. (H&E; x200).

Figure 10 Corpus luteum stage 3, the granulosa fold filled the center of the structure causing a reduction of the follicular space. YP = yolk platelets. (H&E; x200).









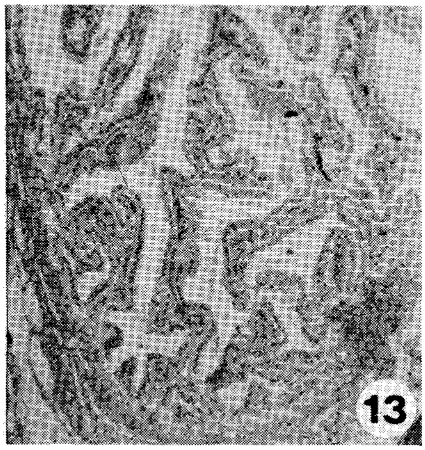


Figure 11 Cross section of the ovary showing atretic follicle (AF). (H&E; x80).

Figure 12 Atresia of vitellogenic follicle stage 1, showing folded granulosa (Gr) had no large cells. CT = connective tissue. (Trichrome; x270).

Figure 13 Atresia of vitellogenic follicle stage 2 showing granulosa fold protruded into the follicle and began to anastomose. (H&E; x75).

Figure14 Atresia of vitellogenic follicle stage 3 showing the granulosa layer was unorganized and degenerated. CT = connective tissue. (H&E; x175).

Figure 15 Atresia of vitellogenic follicle stage 4 showing nearly all follicular cells were degenerated. BS = blood space. (H&E; x175).

Figure 16 Cross section of the oviduct showing the mucosal folds (M), the muscular layer (Mu) and the serosa (Se). (H&E; x40).

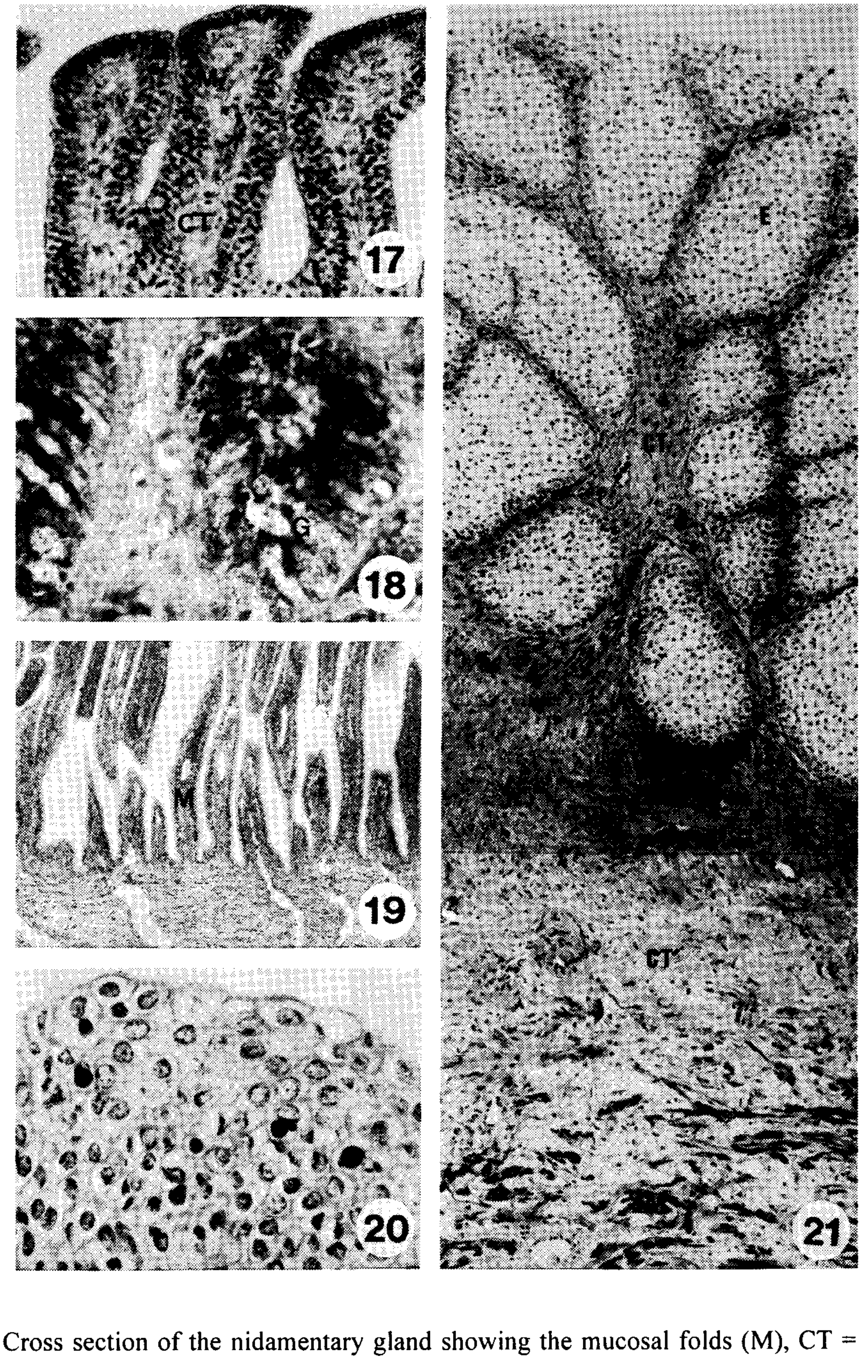


Figure 17 Cross section of the nidamentary gland showing the mucosal folds (M), CT = connective tissue. (Trichrome; x125).

Figure 18 Cross section of the nidamentary gland showing the glandular cells contained numerous PAS positive granules (G). (PAS; x800).

Figure 19 Cross section of the uterus showing the mucosal fold (M). (Trichrome; x125).

Figure 20 Cross section of the vagina showing transitional epithelium. (Trichrome; x800).

Figure 21 Cross section of the vagina showing the epithelium (E), connective tissue (CT) and smooth muscle (SM). (Trichrome; x125).

knots may be important in providing an anchor for the clasper spine copulation [12].

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6.References

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