NOTES ON THE OÖGENESIS, EGG MEMBRANE, AND MICROPYLE OF RED SHINER (NOTROPIS LUTRENSIS)

By

YEN LU

Bachelor of Science

Taiwan Normal University

Taipei, Taiwan

Republic of China

1967

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
August, 1969

NOTES ON THE OÖGENESIS, EGG MEMBRANE, AND MICROPYLE OF RED SHINER (NOTROPIS LUTRENSIS)

Thesis Approved:

Roy W. Jones
Thesis Adviser

Bryan P. Glass

L. Herbert Bureau

D. D. Durham
Dean of the Graduate College

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENT

The author wishes to express her deepest appreciation to her major adviser, Dr. Roy W. Jones, the Head of the Zoology Department, for his guidance, patience and criticism during this study and the preparation of this thesis.

Dr. George A. Moore, Dr. Milton R. Curd and Dr. Rudolph J. Miller identified the fish for this study and were a great help in development of techniques and in the selection of literature. Dr. L. H. Bruneau and Dr. Bryan P. Glass were very gracious in giving advice and criticism throughout the study.

CHAPTER I

INTRODUCTION

This thesis deals with a histological study of the micropyle and the egg membranes of the red shiner (Notropis lutrensis). Very little work has been done on either the micropyle or the egg membranes of fishes. Eigenmann in 1890 found that "in teleosts the micropyle is formed by some follicular cells lying at the animal pole of the egg, sending a long protoplasmic projection through the zona radiata into the egg". Mark (1890) in a study of micropyles stated that the micropylar plug (a mass of granulosa cells found in the micropylar funnel) "may mechanically determine the presence and form of a funnel".

Moore in 1944 observed a 'star' shaped structure with many rays in the chorion of Notropis girardi Hubbs and Ortenburger, which he assumed was a micropyle. A large number of teleosts and many species of Notropis have similar prominent micropyles (Jones, personal communication). It has been found that the micropyle in the egg of insects plays a role in the entrance of the sperm into the egg (Raven 1961). Mark (1890) suggested that these micropyles function as a

passageway for the sperm in fishes.

Because the prominent micropyle in Notropis lutrensis was representative of those observed in other species of this genus and fishes of other genera, it was felt that a histological study of its origin in the ovary was justified. No previous studies except that of Moore in 1944 concerning the micropyle or egg membranes of any Notropis were found in the literature.

CHAPTER II

MATERIALS AND METHODS

Notropis lutrensis (Baird and Girard) has two common names, red shiner and redhorse shiner. This species belongs to the family Cyprinidae and was first taken in 1852 from Otter Creek, its type locality, a tributary of the North Fork of the Red River (Jordan, Everman, and Clark 1930), probably in the present Tillman County, Oklahoma. The species ranges mainly west of the Mississippi River from Minnesota and Wyoming south to Mexico (Moore 1968).

The main diet of this species includes algae, insects, and crustaceans. Spawning takes place in spring and early summer. The maximum length of the adult is about 4 inches. The black blotch between the halves of the lower jaw, the absence of a bold, black caudal spot, and nine anal rays serve to distinguish the red shiner from other members of the subgenus Cyprinella. The lateral line has 30 to 40 scales. The back and sides of the body are usually silvery or steely olive and fade to white on the belly. During the breeding season, the male's back and sides become a dark, steely blue, its fins, belly and behind the shoulders are

tinged with red or orange (Koster 1957).

The specimens studied were collected from Salt Creek, in Osage county near Fairfax, Oklahoma. This creek is a tributary of the Arkansas River. The fish were caught either by net or electrical shock. The first specimens were collected on June 1, 1968. Most of the ovaries had reached full size. Individual eggs were distinguishable with the unaided eye in freshly removed ovaries. Some of the ovaries were reduced in size because some of the eggs had been laid. More specimens were collected on February 12, 20; April 6, 14, 30; and May 15, 1969. The ovaries in February were small. Ovaries obtained in February were not very different in size and appearance from testes obtained at the same time. Since it was too early to show the breeding color on the male fish, the anatomy of all the fishes was studied and dissection of both kinds of gonads was done. During April the ovaries were larger and could easily be distinguished from testes. The males began to show the beautiful orange breeding color in mid-May, and some ova in the ovaries had reached full maturity at this time. One interesting fact is that in this species the males are usually larger than the females. Each fish studied was from 2 to 3 inches long.

After being fixed in Tellyesniczky's or Smith's fixative, the ovaries were washed with tap water; upgraded

through 30, 50, 70, 90, 100 percent isopropyl alcohol, absolute isopropyl alcohol and xylene; cleared in xylene and embedded in paraffin. Sections were cut at 6 to 10 micra and mounted on slides with Sass's adhesive. Some sections were stained with Harris Hematoxylin and Eosin, and others were studied using the phase microscope without staining. Stained and unstained sections were mounted in picolyte or euparal. Various stages and sizes of the eggs were observed from the smallest ova in the ovary to mature eggs ready to be laid at a later time of the year. Photographs were taken with a Kodak Pony IV 35mm camera mounted on a Spencer trioc ular microscope. Measurements of the micropyles and egg membranes were made with the aid of an ocular micrometer.

There is not much difference in results obtained using Tellyesniczky's or Smith's fixative. Shorter time in storage yields a better result. As the oöcytes ripen, the sectioning procedure was found to be more difficult due to less cytoplasm present among the yolk granules. The yolk thus separated from the section more easily. Ten micra is suggested to be the best thickness for sectioning. The writer would like to call attention to the use of the Euparal technique. This is suggested for unstained sections, especially for the ripe eggs. The chance of these sections being ruined is due very largely to going through so many hydrating, staining, dehydrating and clearing solutions. There are two things that

may happen; the oil elements in the yolk granules may be dissolved during the procedures and the yolk granules may fall out from the sections. Humason (1967) suggested sections should be mounted after 95% ethanol. The writer found the concentration of isopropyl alcohol between 90 and 95%, after the sections went through xylene, would get better results. An appropriate time has to be carefully estimated before applying the euparal. Exposure in the air too long will cause the tissue to shrink and dry out; too short, alcohol dissolves in euparal and will trap a lot of air bubbles. Generally speaking, comparing the mounting media euparal and picolyte, air bubbles are more easily trapped in the former, but they will disappear by placing the slides on a warming plate overnight.

CHAPTER III

LITERATURE REVIEW

Development of the gonads of teleosts is under hormonal control of the pituitary gland (Hoar 1955). Gametogenesis in teleosts starts in the embryo and is continuous throughout the active sexual life. Primordial germ cells originating in mesoderm are destined to give rise to the gametes. These cells migrate to the epithelium of the germinal ridge through the blood stream or by amoeboid movement. After repeated mitotic division in the germinal epithelium, primary oöcytes are formed and enter into the initial stages of oögenesis (Balinsky 1961).

According to Wallace (1903) the oögonia of Zoarces originate by delamination from the germinal epithelium or by repeated division of primordial germ cells, which he named the "cell nests". There are large numbers of oögonia contained in each cell nest but only one develops into an oöcyte, the rest of the cells degenerate.

Balfour (1879) studied the ovary development of an Elasmobranch fish and found "nests" of primitive ova of Scyllium confined to the outer part of the germinal epithe-

lium. Either all the primitive ova fuse or one primitive ovum is transformed into a permanent ovum. The cells of the germinal epithelium arrange themselves almost immediately around each ovum and form a single-layered follicular epithelium. Dildine (1933) stated that in Lebistes the oögonia are derived from primordial germ cells.

Egg membranes are named for their origins: primary membranes, secondary membranes and tertiary membranes. The primary membranes are the membranes which develop from the oöcytes. The secondary membranes are formed in the ovary by the follicular epithelium. The tertiary membranes are formed after ovulation in the genital ducts. Mark (1890) recognized four kinds of egg membranes in fishes: 1) Vitelline membrane, 2) Zona radiata, 3) Villous layer which is found in Lepidosteus, 4) Müller's capsular membrane found in the pike and perch. The vitelline membrane represents the cell membrane of the egg. Raven (1961) considered it as the hardening of the outer layer of the cytoplasm of the oöcyte. This membrane may not exist in all telesotean eggs; some appear to be formed after fertilization at the time the egg makes contact with the water (Richards 1931). Mark thought that the zona radiata and villous layers are produced by the oöcyte and the Müller's capsular membrane is the product of the follicular epithelium. Eigenmann (1890) classified the

egg membranes as follows: 1) Eggs with single membrane, that is zona radiata. This membrane either appears as a single layer of uniform structure or is differentiated into an outer and inner layer. 2) Eggs with a zona radiata and an outer membrane which bears appendages. 3) Eggs with a zona and thick outer layer produced by the secretion of the follicular cells.

Early investigators showed much interest in the origin and the nature of the zona radiata in the vertebrates. Their conclusions have been discussed in the works of Mark (1890) and Eigenmann (1890). Some of them believe that egg membranes are the product of the oöcyte while the others attribute them to the follicular epithelium.

As a result of his studies on the ovaries of Trichiurus savala and Triacanthus brevirostris, Chaudhry (1955) suggested that the zona pellucida is secreted by the follicular epithelial cells. Thing (1918) described the follicular origin of the zona pellucida of turtles as composed of two different layers: the outer, denser and thicker and the inner, narrower, clear and striated.

A few flat cells scattered over the surface of the oöcyte formed a layer of follicular epithelium after proliferation. Shelton (1964) stated that in Dorosoma petenense the follicular epithelium appears before the zona radiata is found. In Scyllium (Balfour 1878) the follicular cells

become columnar, highly vacuolated and granular. Mark (1890) suggested that the follicular epithelium is then transformed into a membrane in some species. The function of follicular layers have been determined by many workers as yolk deposition, the nutrients passing through the canals in the zona radiata to the cytoplasm (Eigenmann 1890; Thing 1918; Hoar 1955; Balinsky 1961).

Both primary and secondary egg membranes are often present before fertilization takes place and usually they are impenetrable to the sperm. Sometimes one or more micropyles are situated at or near one of the poles of the egg and perforate through these membranes (Richards 1931).

Several important papers concerning the micropyle and egg membranes of the teleost egg appeared before 1900. Mark (1890) described the formation of micropyle in the gar, Lepidosteus and gave a critical review of the literature concerning the egg membranes and micropyle. Brunch (1855, in Mark) found that the orifice of the micropyle is of exactly the same size as the spermatozoan. He also distinguished three regions -- an approach, a fundus, and a neck or cylindrical canal. His (1873, in Mark) stated that only one spermatozoan can pass the micropylar canal at a time. Hoffmann (1881, in Mark) thought that there exists a distinct plug of granulosa cells occupying the depression in the egg membrane at the micropylar region in Leuciscus rutilus. Owsjannikow

(1885, in Mark) thought that the inner micropyle can be regarded as an enlarged canal of the zona, and it plays a role in the nutrition and growth of the egg.

Mark thought the function of the cell nearest to the bottom of the funnel -- micropylar cell -- to be a kind of mould for the formation of the micropyle, i. e., a mechanical device for producing a micropylar funnel, also to absorb the already formed membrane as necessary to allow the passage of spermatozoan. Eigenmann (1890) observed seasonal changes in the egg membranes and formation of the micropyle of nine teleost species. Moore (1944) found the micropyle in Notropis girardi and made a brief description of its appearance. Shelton (1964) gave a detailed description of the oögenesis and egg membranes of Dorosoma petenense but he did not make any observation or description of the micropyle. Bottrell (1962) described the circular micropyle and the egg membranes of Hybopsis aestivalis tetranemus (Gilbert). Sliger (1967) described the structure of the micropyle and egg membrane of Hybognathus placitus (Girard). The micropyle of H. placitus is funnel shaped with a number of folds radiating outward from the funnel rim and is very similar to that of Notropis. The micropylar funnel is filled with granulosa cells with a single large cell located at the tip of the funnel. No indication was observed of micropyle or folds when the zona radiata had a thickness of less than 0.008mm in H. plac-

itus. Little has been reported subsequently concerning either the micropyle or the egg membranes of fishes.

In some teleosts some mature eggs are not laid, but are resorbed by the follicular epithelium (Litt 1952). The fate of the follicular cells around the oöcyte is variable. They may either form 'post-ovulation' corpora lutea through hypertrophy of cells of the ruptured follicle at ovulation (Wallace 1903), or form the 'pre-ovulation' corpora lutea ovarian follicles, which serves as the main endocrine tissue of the teleost ovary (Hoar 1955).

CHAPTER IV

OBSERVATIONS

Morphology of the Ovary

The bilobed hollow ovaries of Notropis lutrensis are located in the dorsal portion of the body cavity in close membranous union with the swim bladder. The two lobes join posteriorly to form a single oviduct which empties into a urogenital sinus behind the anus. The ovary is covered with the pigmented peritoneum characteristic of the minnow. Beneath the peritoneum is the thin tunica albuginea of connective tissue.

Internally the ovary consists of ovigerous lamellae, which project from the wall into the central lumen. Each lamella contains a large number of eggs. The lumen can be recognized during the early stage of development but becomes obscure later as oöcytes gradually mature and pass into it filling it.

Development of the oöcyte

Six stages of developing oöcytes have been found and

classified by their physical appearances in addition to the 0.0017mm spherical oögonia (Figure 1) and the ruptured, contracted, convoluted and slightly swollen follicles (Figure 10).

Stage 1:

Ova in this stage has irregular outlines, sizes range from 0.019 to 0.085mm. The cell has a single, large spherical nucleus which occupies 75% of the cell volume. The cytoplasm is homogeneous. The slightly acidophilic nucleoplasm contains a single, basophilic, spherical nucleous (Figure 1).

Stage 2:

Sizes range from 0.085 to 0.2mm. The cytoplasm in most cells is highly basophilic although some cells are becoming granular and less basophilic. Several nucleoli form close to the periphery of the nucleus which occupies 50% of the cell volume. The villous layer appears in traces. The basophilic, squamous, follicular cells enclose the egg cell (Figure 4).

Stage 3:

Size is approximately 0.3mm. Cytoplasm is less basophilic, ranges from scattered to densely vacuolated. The

nuclear membrane is irregular in shape, yet the multiple, basophilic nucleoli can easily be recognized arranged around the periphery of the nucleus. The basophilic, early stage of the membrane appears as a thin, non-cellular structure about 0.002mm in thickness. The radial, darkly stained striation of the villous layer is apparent (Figure 5).

Stage 4:

Some cells reach 0.4mm in diameter. The yolk begins to accumulate in vacuoles in the acidophilic cytoplasm. The nucleus is rather small and its membrane becomes obscure. The egg membrane (villous layer and early stage of zona radiata) increases in thickness to 0.0026mm. A multiple, cuboidal, basophilic follicular layer of cells has formed by proliferation. The vitelline membrane begins to differentiate into a very thin, transparent, non-cellular structure distinctly separated from the surface of the oöplasm (Figure 5).

Stage 5:

The largest eggs reach 0.5mm in cross section. The cytoplasm is filled with yolk spherules. The nuclear membrane and nucleoli are no longer evident. Radial striation is apparent all through the egg membrane (villous layer and late stage of zona radiata) which ranges from 0.0068 to

0.01mm. The striation of the vitelline membrane is very apparent (Figure 6).

Stage 6:

Size approximately 0.6mm. Yolk spherules form large globules in the acidophilic cytoplasm. The villous layer and zona radiata are 0.012mm in thickness. Oöcytes are expelled into the central lumen, the irregular shape is due to the loss of the support of stroma. The multiple follicular epithelium has degenerated to two or three layers which surround the oöcyte. The vitelline membrane has increased slightly in thickness and adheres to the oöplasm (Figure 2, 3).

Although various stages of oöcytes are distributed in the ovary at all times, the seasonal changes cause a large number of certain oöcytes to appear at any given time. The highly basophilic cytoplasm, a large nucleus and nucleoli are characteristic of the oöcytes of February fish.

Abundant oöcytes of stages 1, 2, and few of stage 3 are present. In April, 80% of the volume of the ovary is filled with oöcytes of stage 4. About 80% of the volume of the ovary is filled with oöcytes of stage 5 and 6 in May. Several ruptured follicles are found toward the end of this month. About one third ruptured follicles, one third of stages 5, 6 oöcytes and one third of oögonia and stages 1,

2, 3, 4 oöcytes are in the ovary in June.

The villous layer is most distinguishable in stage 6, where it appeared as 0.0017mm thicker than the zona radiata. Each villus looks like the extension of one pore-canal of the zona radiata. There is a thin dark shadow between the villous layer and the zona radiata (Figure 2, 3).

The follicular epithelium first appears as a flat, single layer of polygonal cells. Repeated duplication results in many layers of cells being formed. The follicular cells become more columnar when the oöcytes mature, and then degenerate, but the nucleus remains spherical (Figure 10, 12). No mature oöcyte without follicular epithelium was found either in the lumen of the ovary or in the oviduct. Since no effort was made to observe the fish in spawning, it is not certain whether the ova are shed with part of the follicular epithelium attached or with a naked egg membrane.

Description of the Micropyle

In the slides studied there was no apparent evidence or sign of the micropyle till quite late in the maturation of the ovum, when the egg membrane (villous layer and zona radiata) was not less than 0.007mm in thickness. The mouth of the micropyle is oval with seven or eight folds radially toward the center. The zona radiata is thicker while the follicular epithelium is thinner in this area. There is a sin-

gle micropylar cell at the tip of the funnel. The funnel taper is approximately 0.043 to 0.119mm at the surface to 0.026 to 0.043mm at the bottom. The micropylar canal is continuous with the funnel and passes through the zona radiata. The length of the funnel ranges from 0.077 to 0.094mm and is filled with layers of follicular cells (Figure 7, 8, 9, 11).

CHAPTER V

DISCUSSION AND SUMMARY

Six stages of developing oöcytes were described and compared with Shelton's (1964) classification of the oögenesis of the threadfin shad and Sliger's (1967) histological study of the egg of the plains minnow. The oögenesis of all teleosts is similar; the only difference is in the occurence and appearance of the egg membranes. The egg membrane in the red shiner contains three parts, the vitelline membrane which adheres to the oöplasm, the outer thicker villous layer which is composed of a large number of villi, and the inner thinner part, the zona radiata, which is passed through by the pore-canals. Mark has shown the villi of the villous layer are rooted in these canals. No membrane is formed before the follicular cells appear around the oöcyte. The first apparent membrane is acidophilic and structureless. As this membrane thickens, the peripheral radial striation becomes apparent. Shortly the radial striation spreads to the inner portion. This means that the zona radiata forms after a large part of the villous layer is already developed. Mark has demonstrated that both layers are the product

of the oöcyte.

The irregularity of cell membrane shape in the young oöcyte is caused by the pressure of crowding in the lamellae of the ovary, ripe oöcytes which have been expelled into the central lumen of the ovary and lack support of any stroma are also irregular in shape due to crowding.

The folding of the wall of the micropyle may function as a device which prevents premature entry of water. After the ova are spawned, water may penetrate the egg by the way of the micropyle (Jones, personal communication), and a perivitelline space is then formed between the vitelline membrane and the zona radiata. These folds, by restricting the aperture of the micropyle and increasing frictional surface of the funnel, may slow down the water speed and retard entry into the perivetelline space. This would prevent rupture by too rapid passage of water into the interior of the ovum.

There is always a mass of granulosa cells in the micropylar funnel and a single micropylar cell at the tip which Eigenmann(1890) suggested was differentiated from the granulosa cells and served as a sort of mould for the formation of the micropyle. The question is: What factors cause these cells to aggregate there and induce such a structure? Since the ruptured follicular epithelium later transforms and differentiates into the main endocrine tissue of the

teleosts ovary (Hoar 1955), it may be that the follicular cells secrete some kind of substance which causes the formation of the micropyle. The change in the following epithelium from basophilic in early stages to an acidophilic later stage suggests some change in chemical control during maturation. The follicular cells in the later stages of development of the oöcyte are unevenly distributed (Mark 1890 some follicular cells may grow larger and higher and as a result overlap the neighboring ones). This multiplication, overlapping each other cannot be avoided (Figure 12).

The multiplication results in masses as well as layers of follicular cells. Among them the largest mass of follicular cells might secrete enough of a chemical substance (which might have existed in the cells as a sort of 'repressor') and thus differentiate one of the cells (micropylar cell) to induce the surface of the oöcyte to form the micropyle.

LITERATURE CITED

Austin, C. P. 1968. Ultrastructure of fertilization. Holt, Rinehart & Winston. New York. 91-108.

Balfour, F. M. 1878. On the structure and development of the vertebrate ovary. Royal Micros. Soc. London. 383-438.

Bottrell, C. E. 1962. Notes on the embryology and egg membranes of Hybopsis aestivalis tetranemus (Gilbert). Unpublished Oklahoma State University Master's thesis.

Bottrell, C. E., Ingersol, R. H., Jones, R. W. 1964. Notes on the embryology, early development, and behavior of Hybopsis aestivalis tetranemus (Gilbert). Trans. Amer. Soc. 83 (4): 391-399.

Chaudhry, H. S. 1956. The origin and structure of the zona pellucide in the ovarian eggs of teleosts. Zeitschrift für Zellforschung und Mikroskopische Anatomie. 43, S: 478-485.

Dildine, G. C. 1933. Germ cell origin and gonad differentiation in the viviparous top minnow, Lebistes reticrelatus. Anat. Rec. 575: 88.

Eddy, Samuel. 1957. How to know the freshwater fishes. Sm. C. Brown Co. Dubeque, Iowa. 253 pp.

Eigenmann, E. H. 1890. On the egg membranes and micropyle of some osseous fishes. Bull. Harvard Mus. Comp. Zool. 19 (2): 129-154.

Gray, Peter. 1958. Handbook of basic microtechnique. McGraw-Hill Book Co. New York. 252 pp.

Hickling, C. F. and E. Rutenberg. 1936. The ovary as an indicator of the spawning period in fishes. Marine Biol. Assoc. of the United Kingdom. Journal (Plymouth) NS. 21: 311-317.

Hoar. W. S. 1955. Reproduction in teleost fishes. Soc. for Endocrinology. (Great Britain) Memoirs.: 4: 5-24.

Hubbs, C. L. 1943. Terminology of the early stages of fishes. Copeia 43 (4): 260.

Humason, G. L. 1962. Animal tissue technique. W. H. Freeman and Co. San Francisco. 569pp.

Jordan, D. S., B. W. Everman, H. W. Clark. Check list of the fishes and fishlike vertebrates of north and middle America. U. S. Government Printing Office. Washington. 670pp.

Koster, W. J. 1957. Guide to the fishes of New Mexico. University of New Mexico Press. 116pp.

Lee, Bolles. 1937. The microtomist's vade-mecum. P. Blakiston's Son & Co. Inc. Philadelphia. 784pp.

Litt, B. D. 1952. Histological changes in the ovary of the white crappie, Pomoxis annularis Rafinesque, correlated with age and season. Unpublished Master's thesis. University of Missouri.

Mark, E. L. 1890. Studies of Lepidosteus. Bull. Harvard Mus. Comp. Zool. 19 (1): 1-127.

Moore, G. A. 1944. Notes on the early life history of Notropis girardi. Copeia (4): 209-214.

Moore, G. A. 1968. Fishes. Part two in vertebrates of the United States. By Blair, Blair, Brodkorb, Cagle and Moore. McGraw-Hill Book Co., Inc. New York 33-210.

Raven, C. P. 1961. Oögenesis: The storage of developmental information. Pergamon Press. New York 274pp.

Raven, C. P. 1959. An outline of developmental physiology. Pergamon Press. New York. 230pp.

Richard, K. K. 1965. Development and ecological significance of Nuptial Tubercles of the Red Shiner, Notropis lutrensis. Copeia (4): 462-7.

Richard, K. K. and W. L. Minckley. 1965. Changes with growth in selected body proportions of the cyprinid fish Notropis lutrensis. The Southwestern Naturalist 10 (3): 151-155.

Richards, Aute. 1931. The outline of camparative embryology. John Wiley & Sons, Inc. New York 444pp.

Shelton, W. L. 1964. The threadfin shad, Dorosoma petenense (Günter): Oögenesis, seasonal ovarian changes and observations on life history. Unpublished Oklahoma State University Master's Thesis.

Sliger, W. A. 1967. The embryology, egg structure, micropyle and egg membranes of the plains minnow. Hybognathus placitus (Girard). Unpublished Oklahoma State University Master's Thesis.

Thing, A. 1918. The formation and structure of the zona pellucida in the ovarian eggs of turtles. Amer. J. Anat. 23: 337-352.

Wallace, W. 1903. Observation on ovarian ova and follicles in certain teleostean and elasmobranch fishes. Royal Micros. Soc. London. 47: 161-213.

Wilson, E. B. 1926. The cell in development and heredity. MacMillan. New York. 1232pp.

Zuckerman, Solly. 1962. The ovary (I). Academic Press. New York. 575pp.

APPENDIX

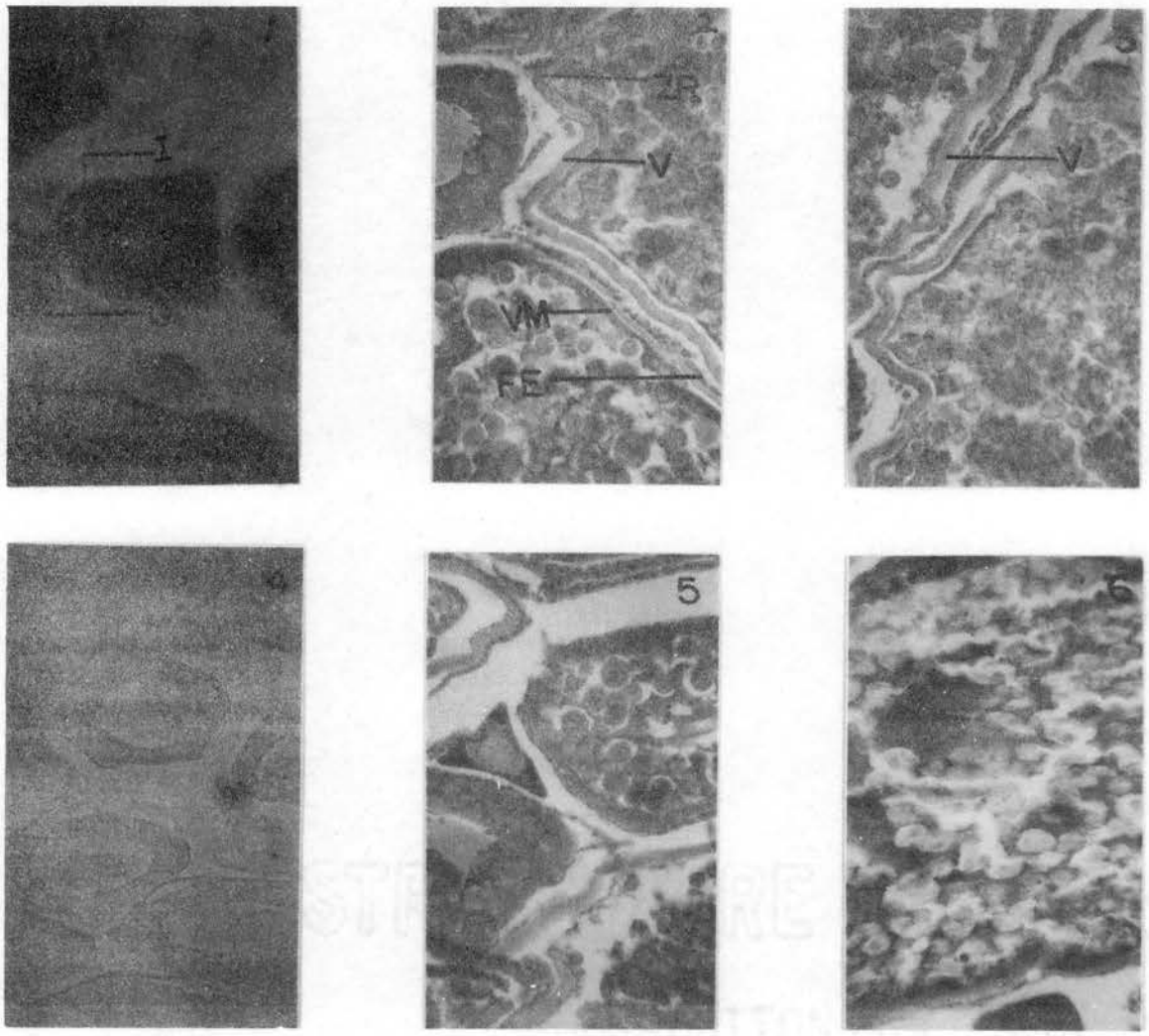

Figure 1. The Small Oogonium (0) is Shown Beside a Stage 1 (I) (1940X).

Figure 2. Villous Layer (V), Zona Radiata (ZR) and Vitelline Membrane (VM) (860X).

Figure 3. Follicular Epithelium (FE) and the Villi of the Villous Layer (V) (860X).

Figure 4. Stage 2 Oöcyte (860X).

Figure 5. Lower Oöcyte is in Stage 3 (860X). Upper One is in Stage 4 (860X).

Figure 6. Stage 5 Oöcyte (in part). Notice the Yolk Granules Accumulated in the Vacuoles (860X).

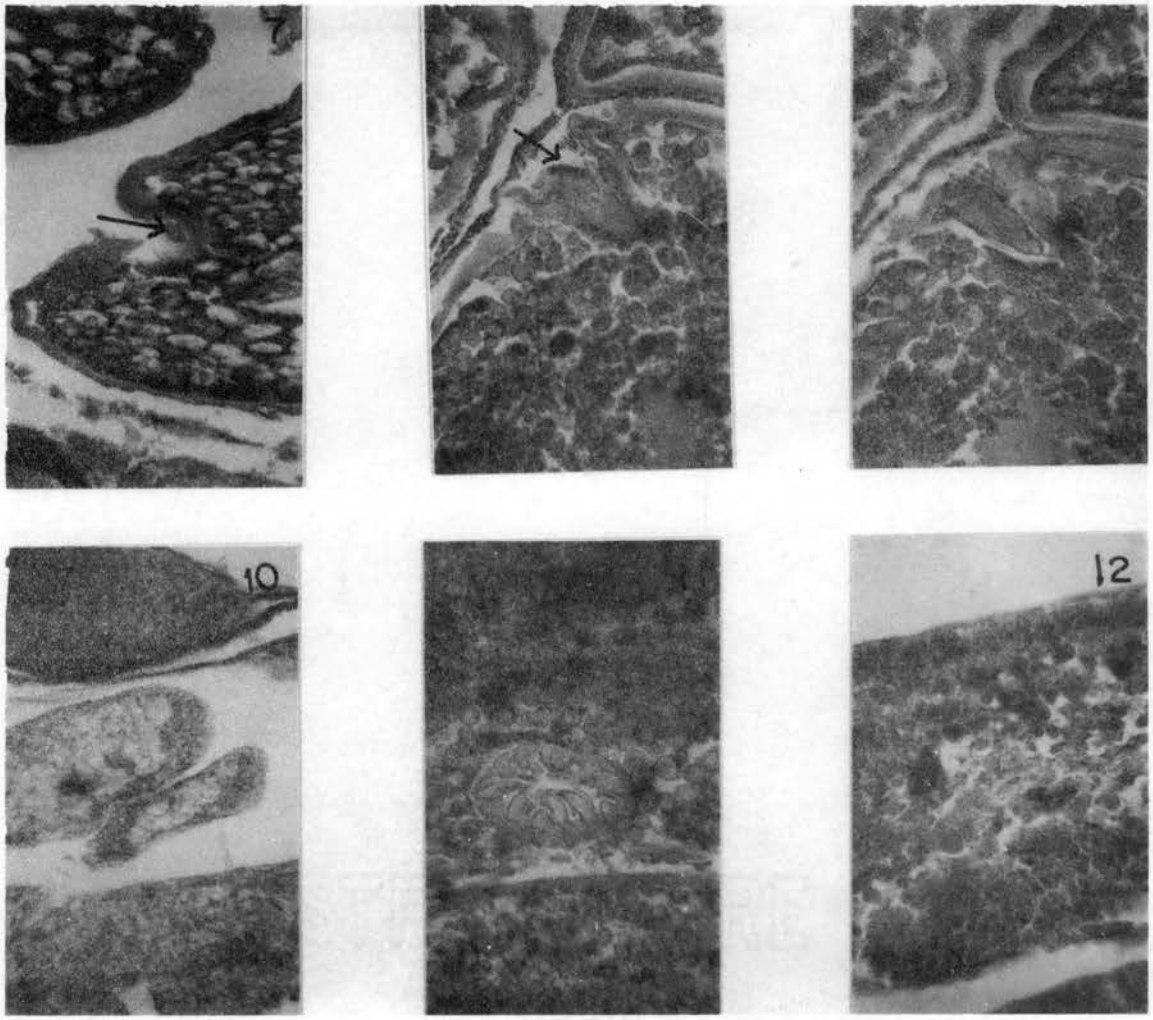

Figure 7. Micropyle with Micropylar Cell (860X).

Figure 8. Micropyle in a Stage 6 Oöcyte (860X).

Figure 9. Next Section of Figure 8. Notice the Micropylar Funnel and Canal (860X).

Figure 10. Four Ruptured Follicles (860X). The Top One had Changed to Lutein Cells. The Other Three have Begun Transformation.

Figure 11. Cross Section of Micropylar Funnel Obtained by Cutting Tangential Sections of the Egg (860X).

Figure 12. The Follicular Epithelium of the Oöcyte (860X). Notice the Columnar Arrangement of the Follicular Cells Along the Edge. The Center has Many Different Sizes of Follicular Cells.

VITA

/

Yen Lu

Candidate for the Degree of

Master of Science

Thesis: NOTES ON THE OÖGENESIS, EGG MEMBRANE, AND MICROPYLE OF RED SHINER (NOTROPIS LUTRENSIS)

Major Field: Zoology

Biographical:

Personal Data: Born in Chung-Ching, China, October 9, 1944, daughter of Chan-Yin and Moo-Yun Lu.

Education: Attended primary school, high school and college in Taipei, Taiwan, Republic of China; graduated from Taiwan First Girls' High School 1962; B. A. Taiwan Normal University; completed requirements for the Master of Science, Oklahoma State University, Stillwater, Oklahoma, August, 1969.

Professional Experience: Biology Teacher in Pei-Tou High School, Pei-Tou, Taiwan, Republic of China 1966-1967; Graduate Teaching Assistant, Zoology Department, Oklahoma State University 1968-1969.