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Review Article

The Essential, Complex and Multiple Functions of the Ovary of the Viviparous Teleosts *Xenotoca eiseni* (Goodeidae) during Non-Gestation and Gestation Stages

Mari Carmen Uribe*, Gabino De la Rosa Cruz, Adriana García Alarcón and Juan Carlos Campuzano Caballero

Laboratorio de Biología de la Reproducción Animal Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, Insurgentes, Ciudad de México, Mexico

***Corresponding Author**: Mari Carmen Uribe, Laboratorio de Biología de la Reproducción Animal Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, Insurgentes, Ciudad de México, Mexico.

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Abstract

Teleosts show unique features of the female reproductive system compared with the rest of vertebrates which define their viviparity. Teleosts do not have oviducts, therefore, the ovary of viviparous teleosts is the site, not only for the development of oocytes, but also it allows the reception of spermatozoa for the internal fertilization of the oocytes and the intraovarian gestation of offspring until birth; that is intraovarian gestation, unique in vertebrates. The intraluminal gestation proper of *X. eiseni* as a goodeid, permits that the nutrition of the embryos is complemented by absorption of nutrients obtained from the maternal tissues. The nutrition of *X. eiseni* during the initial part of gestation by the consumption of yolk is lecithotrophic, but, for the low amount of yolk in the mature oocytes, when the yolk ends, the nutrition is complemented by taking nutrients from the maternal tissues, that is, matrotrophic nutrition. Specifically, the species of Goodeidae have trophotaeniae, extension of the embryonic gut to the lumen of the ovary, used for matrotrophic nutrition.

Keywords: *Viviparity; Oogenesis; Yolk; Intraluminal Gestation; Trophotaeniae*

Introduction

Viviparity in teleosts has an exceptional morpho-physiological condition among vertebrates. Condition that produce a great interest in the analysis of this type of gestation. That is, teleosts lack Müllerian ducts during the embryogenesis, then, teleosts do not have oviducts. Consequently, the female reproductive system is integrated only by the ovary which posterior region communicates the ovary to the exterior. The posterior region of the ovary lacks germinal cells and it is call gonoduct [1-4]. As a consequence of the absence of oviducts, in viviparous species, gestation occurs into the ovary, as intraovarian gestation, unique among vertebrates [1,2,5-7].

An essential process of the viviparity is associated with the internal fertilization of the oocytes, therefore the occurrence of insemination, when the spermatozoa enter to the ovary. Thus, there are specific aspects related to the establishment of physiological relationships between the ovarian tissue and the intraovarian presence of spermatozoa.

The viviparous species of teleosts of the family Goodeidae are endemic to the central plateau of Mexico. As all goodeids, *Xenotoca eiseni* has a single ovary with saccular structure with a central lumen [7-9]. The lumen contains complex substance secreted by the ovarian tissues. This substance is called histotrophe or embryotrophe. Similarly to that described in all goodeids, the histology of the ovarian wall consists of four tissue layers, which, from the interior (the ovarian lumen) to the exterior (the coelom), they are: a) germinal epithelium; b) stroma, formed by loose vascularized connective tissue, containing follicles in different stages of development; c) smooth muscle layers; and d) serosa, formed by scarce connective tissue and mesothelium [6,10,11]. The ovarian tissue is lined by the germinal epithelium, adjacent to the lumen. The germinal epithelium contains oogonia, situated among somatic epithelial cells [10,11]. The ovarian mucosa forms folds that extend into the ovarian lumen, called lamellae, where follicles in diverse stages of oogenesis may be located [2,8,12].

In the intraovarian gestation of viviparous teleosts, the development of embryos may occur according to two types: a) as intrafollicular gestation where the embryos remain in the follicle during their development and move into the ovarian lumen immediately before birth, as occurs in poeciliids; or b) as intraluminal gestation, in which the early embryo moves from the follicle to the ovarian lumen where gestation continues, as occurs in goodeids [1,2,6,9,12-14]. Thus, the intraluminal gestation is the case of *X. eiseni*.

The nutrition of the embryos during gestation establish essential changes related with the change in the site of gestation to the maternal ovary. This condition involves new relationships during embryonic development, as the possible acquisition of nutrients. Consequently, the nutrition in *X. eiseni* revealed the possible disposition of nutrients, not only stored in the egg during the oogenesis, but also obtaining furthermore from the maternal tissues during gestation [9].

Being the intraovarian gestation of goodeids a so complex process, it is important contribute in this analysis, as in *X. eiseni*, in the study of changes of the ovary during the different conditions of the reproductive cycle, throughout non-gestation and gestation.

Aim of the Study

The goal of this study is, taking, as a model the species *X. eiseni* (Goodeidae), providing a histological analysis of the ovarian structures involved in non-gestation and gestation processes. During non-gestation ovaries previtellogenesis and vitellogenesis where selected; and, during gestation ovaries in early gestation and advanced gestation where selected.

Materials and Methods

The management of the specimens used in this study followed the Guidelines for the Use of Fishes in Research of the American Fisheries Society. Eighteen female adults *X. eiseni* (281 ± 0.8 mm total length) were processed from April to September 2014, from aquaria maintained by Harry J. Grier. Four stages of reproduction were considered according to the classification of Haynes [15]: 2 stages in non-gestation: previtellogenesis and vitellogenesis, and 2 stages in gestation: early embryos in cleavage-neurulation, and late embryos in late eye development. Three females with embryos during each stage of reproduction were selected (total = 12 females). The specimens were sedated by adding six drops of Tropical fish, Tranquilizer/Calmer, Sedate STK#3110 to a liter of water. After sedation, the abdominal cavity was opened by a lateral incision, and the ovary was quickly excised and fixed in glutaraldehyde and formaldehyde fixative. After fixation, the ovaries were dehydrated in a series of graded ethyl alcohols (30, 50, 70, and 95%). The dehydrated ovaries were embedded in glycol methacrylate plastic resin (JB-4 embedding kit). The embedded ovaries were sectioned at 5 μm thickness. Sections were stained with hematoxylin and eosin (H and E).

Results

The ovary of *X. eiseni* is a saccular structure, with a central lumen (Figure 1A). The ovarian mucosa forms lamellae expanded to the lumen, composed of columnar germinal epithelium and stroma, containing follicles in development (Figure 1A). Follicles containing early previtellogenic oocytes are observed in the ovarian mucosa near the epithelium (Figure 1B). The oocytes grow by increasing the ooplasm, organelles and deposition of lipid droplets (Figures 1A-1E). During vitellogenesis (Figures 2A-2E), the oocytes have a progressive accumulation of homogeneous fluid yolk, intensely acidophilic (Figures 2B-2D); the germinal vesicle (oocyte nucleus) is irregular in shape and during maturation moves to the animal pole (Figure 2E). During late oogenesis, the oocytes attain a diameter of 0.63 mm.

During the advanced development of oocytes, occurs the insemination, then, the spermatozoa enter from the gonoduct to the lumen of the germinal region of the ovary. Consequently, at this time there are groups of spermatozoa situated in the ovarian lumen near the germinal epithelium (Figure 2E). Some of the spermatozoa are located adjacent to the apical end of the epithelial cells (Figure 3D).

After fertilization, the embryonic development is initiated. The early embryos, during cleavage stage, in blastula, are seen in the ovarian lumen, already in the intraluminal position (Figures 3A), this situation will be continued throughout all gestation. The blastodisc has the same position as the animal pole, it is dorsally situated. The yolk is in the ventral position, some yolk particles are seen into the blastomeres indicating the initial utilization of yolk by the embryos in their nutrition. At the end of neurulation (Figures 3B and 3C), when the embryo up till now has yolk continues the nutrition utilizing the yolk, but also occurs the development of a complementary type of nutrition, a vascularized extension of the hindgut call trophotaeniae, expands to the exterior of the embryo to the ovarian lumen, as an absorptive tissue of histotrophe dissolved in the ovarian fluid. Trophotaeniae progress in great capacity of vascularization and absorption by the columnar epithelium which surrounds this structure; then this epithelium may take the histotrophe contained in the lumen, and the blood vessels of the trophotaeniae transport the contains of the histotrophe to the embryo. Progressively, the reduction of yolk is completed, since then the embryo is nourished by the trophotaeniae, taking the nutrients from the maternal tissue, by absorbing the histotrophe.

In the advanced stage of gestation, during late eye development (Figures 4A and 4B), the trophotaeniae are larger and displaced in the ovarian lumen; some regions of the trophotaeniae are in apposition to the maternal epithelium (Figure 4A), consequently, it will be near the maternal blood vessels, forming an emerging placental connection, increasing the possibility of interchanges with the maternal tissue. The maternal tissue associated with the embryonic trophotaeniae is absolutely essential in the nutrition of the embryos until birth. At this time, there are some late vitellogenic oocytes suggesting that the late oogenesis is activated at the end of gestation.

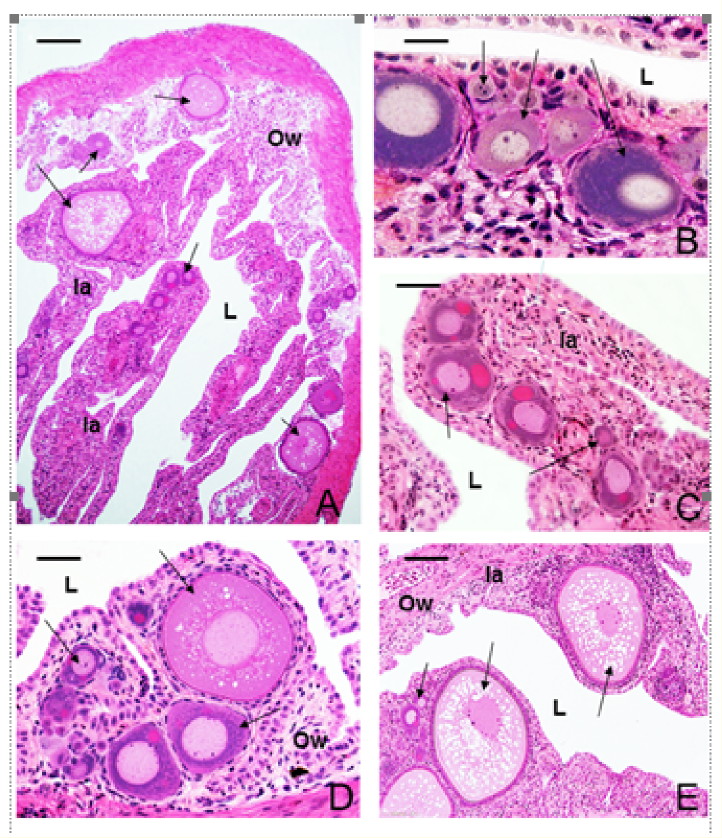

Figure 1: *Ovary of the goodeid Xenotoca eiseni containing oocytes in follicles in different stages of previtellogenesis. A: Portion of the ovarian wall containing previtellogenic oocytes. B: Germinal epithelium with oocytes in early previtellogenesis. C: Oocytes with the formation of early cortical alveoli with eosinophilic affinity. D, E: The increase in size of the oocyte by the deposition of lipid droplets, seen as clear spaces. Ovarian wall (Ow), folds of the ovarian wall, lamellae (la) expanded to the ovarian lumen (L), follicles with previtellogenic oocytes included in the ovarian wall (→). Hematoxylin-Eosin. Bars = A: 100 µm, B: 20 µm, C: 40 µm, D: 20 µm, E: 50 µm.*

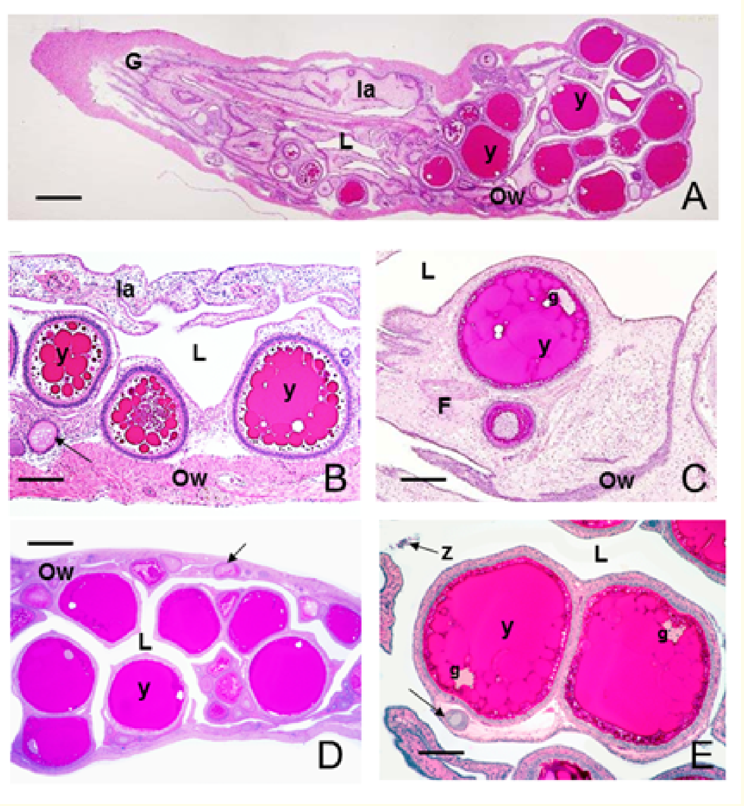

Figure 2: *Ovary of the goodeid Xenotoca eiseni containing oocytes in follicles in different stages of vitellogenesis. A: Ovary in longitudinal section, the posterior zone of the ovary forms the gonoduct (G), Ovarian wall (Ow) and lamellae (la) of the ovarian wall expanded to the lumen (L), follicles with vitellogenic oocytes with progressive deposition of fluid yolk, included in the ovarian wall, some of them indicated with (y). B: oocytes with progressive deposition of yolk during vitellogenesis. C, D, E: Full grown oocytes containing homogeneous yolk, and the germinal vesicle (g) displaced to the periphery of the animal pole. Also, follicles with previtellogenic oocytes may be seen, some of them indicated (→). Groups of spermatozoa (Z) may be seen in the lumen. Hematoxylin-Eosin. Bars = A: 1 mm, B: 200 μm, C: 100 μm, D: 300 μm, E: 70 μm.*

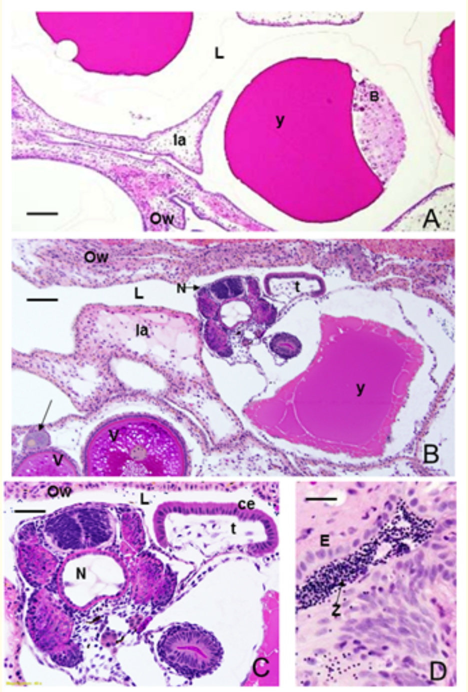

Figure 3: *Ovary of the goodeid Xenotoca eiseni containing embryos in early gestation. The embryos are in the ovarian lumen (L), in intraluminal gestation. A: Embryos during cleavage stage, forming the blastula with the blastomeres in the animal pole, and the yolk (y) ventrally situated; lamellae (la). B, C: Embryo at the end of neurulation (N); the embryo has abundant yolk, and, at the same time is developing an extension of the gut call trophotaenia (t). The embryo in neurula is seen with more magnification in C. The extension of the trophotaeniae lined with columnar epithelium (ce) is seen. The ovarian wall (Ow) contains follicles with oocytes during previtellogenesis (→), and vitellogenesis (V), blastula (B), neurula (N), ovarian lumen (L), lamellae (la). D: Spermatozoa (Z) in the ovarian lumen border the ovarian epithelium (E). Hematoxylin- Eosin. Bars = A: 100 μm, B: 200 μm, C: 100 μm, D: 50 μm.*

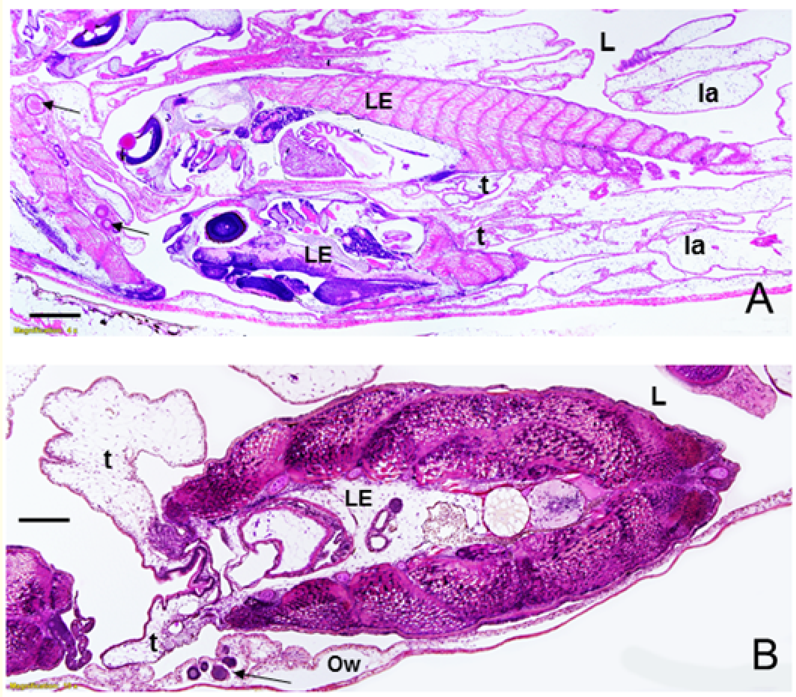

Figure 4: *Ovary of the goodeid Xenotoca eiseni containing embryos in late gestation. A: Embryos during Late Eye Development (LE) in longitudinal section. The embryos do not have any more yolk, but it is observed the apposition of the embryonic trophotaeniae (t) to the maternal tissue. The ovarian tissue contains follicles in previtellogenesis (→); lamellae (la) and ovarian lumen (L). B: Embryo during Late Eye Development (LE) in transversal section, the hindgut has trophotaeniae to the ovarian lumen, some portion border the maternal tissue. Hematoxylin-Eosin. Bars = A: 500 µm, B: 100 µm.*

Discussion

The viviparity in teleosts, 14 families in which viviparity occurs [2,5], is unique among viviparous vertebrates because the intraovarian gestation [5,13]. This character develops several adaptations for the development of embryos into the ovary. Thus, the structure of the ovary of *X. eiseni*, where besides the oogenesis also occurs insemination and gestation [8], implies great changes during the reproductive cycle, where all these processes occur.

In the ovary of *X. eiseni* there are follicles during all stages of reproduction. The morphology of the oocytes during oogenesis present similar characteristics as described in the oogenesis of other teleosts [16]. There are previtellogenic follicles throughout non-gestation and gestation, but vitellogenic follicles are not seen during the most part of gestation, except at the end of gestation, suggesting that at this time it is advanced the development of mature oocytes, prepared for the next fertilization and gestation after birth of the embryos.

The intraluminal gestation, taking place in the ovarian lumen, has been described in several families of viviparous teleosts as: Embiotocidae, Parabrotulidae, Zoarcidae and Goodeidae [2,13]. In the analyzed species, fertilization occurs into the follicle and soon the embryo goes to the ovarian lumen where the development continues [2,13]. In *X. eiseni* during very early embryogenesis, in blastula stage, we observed clearly the embryos already situated in the ovarian lumen.

The intraluminal gestation proper of *X. eiseni* as a goodeid, permits that the embryos, surrounded by the complex histotrophe, may take the substances from there [5]. The histotrophe has been detailed in other goodeids as *Xenoophorus captivus* [17]. *Skiffia bilineata* and *Ameca splendens*, and also in *X. eiseni* [6], considering the vital importance for the development of the embryos.

The nutrition of the embryos of *X. eiseni* during gestation by the consumption of yolk, called lecithotrophy is similar in oviparous species [18], ends approximately during the first third of gestation [9]. This is defined for the low amount of yolk in the mature oocytes of *X. eiseni* [9], this character implies that because the yolk contained in the mature oocytes is not enough for all the gestation, when the yolk is completely consumed, the nutrition is complemented by taking nutrients from the maternal tissues, that is, matrotrophy. Similar sequence is described in other viviparous vertebrates [18], as an evolutionary process, discussed by Blackburn [19].

The intraluminal gestation allows that, even when lecithotrophy is occurring in early gestation, the matrotrophy is initiated with the development of trophotaeniae, extensions of the hindgut to the ovarian lumen with great capacity of absorption. Then, the nutrition is complemented by absorption of nutrients obtained from the maternal tissues, at that time. The matrotrophy surge as an essential process, initially coexisting, at the same time, both lecithotrophy and matrotrophy.

During the middle gestation of *X. eiseni*, when the reduction of the yolk is completed, the trophotaeniae have been enlarged. Some extensions of the trophotaeniae are lining the maternal ovarian tissue developing an emerging placental connection, increasing the possibility of interchanges with the maternal tissue. The characteristic structure in *X. eiseni* that permits this great level of interchanges with the maternal tissues is a brush-border at the apical end of the epithelium of the trophotaeniae, proper of an absorptive epithelium [20]. Schindler and de Vries [21], described similar cellular component of the trophotaeniae in embryos of the goodeid *X. captivus* considering the clear signs of endocytotic activity at their apical surfaces. The trophotaeniae is not more useful after birth when the offspring is going out of the maternal ovary. Then, this structure has to disappear. This important process of regression has been studied in *X. eiseni* [22] observing that the trophotaeniae show preliminary regression at birth and then disappear within few days.

Then, the nutrition in *X. eiseni* by the yolk during the initial part of the development is characterized by the lecithotrophy; meanwhile the development of the trophotaeniae by the nutrition from the maternal tissue, characterized the matrotrophy. Several analyses [9,13,18,19,23] considered that the evolution of the viviparity involves the transition from lecithotrophy to matrotrophy. In that sense, the occurrence of both type of nutrition in goodeids define the species of this family very valuable for the analysis of this evolutionary process in viviparous vertebrates.

Matrotrophy may be considered in advantage for viviparity, allowing the possibility to provide a source of necessary nutrients to the developing embryo from the maternal tissues [5]. In *X. eiseni* this change of nutrition has been described indicating this adaptation only possible in the condition of viviparity. Integrating these elements of the embryonic nutrition of *X. eiseni*, we consider that the most advanced type of viviparity occurs in goodeids in which the yolk has become greatly reduced, and the embryos in an intraluminal condition develop the widest diversity of maternal–embryonic adaptations, as trophotaeniae, for obtaining nutrients from the maternal tissues [9].

As it is mentioned by Blackburn [19], studies on functional and evolutionary morphology continue to play a central role in our attempts to understand viviparity and mechanisms of fetal nutrition.

Then, in *X. eiseni*, as occurs in other goodeids, and in other viviparous teleosts, the maternal ovary offers to the embryos during their viviparous development such essential, complex and multiple functions. The ovary is not only the structure where oogenesis occurs, with the oocyte development with the nutrients stored in the yolk, but also it allows the insemination with the reception of spermatozoa and their proper maintenance in the ovarian lumen, the internal fertilization of the oocytes, and the intraovarian gestation of offspring until birth. The intraluminal development of the embryos in gestation arises fundamental processes as it is the secretion to the ovarian lumen of the histotrophe, the nutrient transfer and gas exchange through the development of a placenta by the apposition of maternal and embryonic tissues by the trophotaeniae, allowing a great level of interchanges with the ovarian tissues in this viviparous process.

Conclusion

The ovary of *X. eiseni* develops multiple functions during the reproductive cycle, similar to other goodeids, being the site of oogenesis and intraluminal gestation. In these aspects, this analysis defined features in this species as: the embryos are discharged to the ovarian lumen at early cleavage; occurs simultaneous lecithotrophic and matrotrophic nutrition during the first third of gestation, with the development of trophotaeniae since neurulation; formation of a placental relationship with the apposition of portions of the trophotaeniae to the ovarian epithelium; the presence of late vitellogenic oocytes at the end of gestation.

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