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REVIEW

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Diversity and dynamics of fish ovaries: Insights into reproductive strategies, hormonal regulation, and ovarian development

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Summary. Fish ovaries exhibit a remarkable diversity in shape, size, and organization, reflecting the myriad reproductive strategies employed by different species. This review delves into the intricate biology of fish ovaries, highlighting their structural diversity and the hormonal regulation that governs ovarian development and oocyte maturation. Key hormones include pituitary gonadotropins (GTHs) and maturation-inducing hormones (MIHs), which initiate oocyte growth and maturation. GTHs stimulate ovarian production of estradiol-17β and 17α,20β-DP, which induce oocyte maturation via MPF formation. Sex steroids like estrogens and progestogens, synthesized from cholesterol, play crucial roles. Other hormones, including growth hormone, prolactin, thyroid hormones, IGFs, ACTH, and melatonin, influence ovarian activity. The review also explores the varied reproductive strategies among fish, including oviparity and viviparity, and discusses how environmental factors like water temperature and photoperiod influence ovarian histology. Understanding the complex interplay between these factors is essential for advancing fisheries management, conservation, and aquaculture practices. Additionally, the evolutionary trajectory of fish ovaries underscores their adaptation to diverse ecological niches, contributing to the survival and reproductive success of fish species. The ovarian stroma provides structural support and houses various cell types, including dendritic cells (DCs), endocrine cells, and telocytes, contributing to follicle growth and hormone production, essential for reproductive success in fish. Fish ovaries are a crucial aspect of fish biology, with their structure and function intricately regulated by hormonal, environmental, and seasonal factors.

Key words: Biology, Hormones, Oogenesis, Reproduction, Stroma, Teleosts, Viviparity

Introduction

In female fish, the ovaries are reproductive organs that can vary in shape and size depending on the species and reproductive strategy. The enormous variety of fish species and their reproductive methods are reflected in the fascinating and varied field of fish ovary biology (Jalabert, 2005).

The ovaries in most teleosts have the female gonads fully within the visceral cavity, however, in Pleuronectiformes, like B. robinsi, the ovaries normally develop as far out of the visceral cavity as possible, between the muscles that move the anal fin rays, the hemal spines of the caudal vertebrae, and the pterygiophores of the anal fin. The anus, genital, and urine openings are situated extremely anteriorly in the Pleuronectiformes because the anal fin begins noticeably frontally and the first anal pterygiophore is bent. This suggests that the gonoduct runs forward and the ovaries are positioned posteriorly (Kobelkowsky, 2012).

Most salmonid and eel species reproduce once or several times per year, however, some only spawn once before dying. Some species may breed every two or three years. Some teleost species and some oviparous skate species seem to reproduce all year long (Burton and Margaret, 2017). Fish that reproduce once and then perish have synchronous ovaries, meaning that every oocyte is in the same developmental stage. Group-synchronous ovaries, which contain at least two populations of oocytes at various maturation stages, are characteristic of species that spawn only once annually during a brief breeding season, which is frequent in teleosts. Over the course of the breeding season, group-synchronous ovaries may ovulate intermittently,

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releasing oocytes in batches. Oocytes at all stages of development are present in an asynchronous ovary (Mokhtar, 2021).

Ovarian development may be impacted by variations in water temperature, photoperiod, and food availability. In species with seasonal reproductive cycles, seasonal alterations in the histology of fish ovaries are frequently seen (Arockiaraj et al., 2004).

The ovarian stroma, which is made up of vascular collagenous connective tissue and a few strands of smooth muscle fibers, holds the developing ova together. Along with the atretic follicles, the finger-like ovarian (ovigerous) lamellae also house ovarian follicles in various stages of oogenesis. The ovarian wall protrudes ovarian-like lamellae into the ovocoel, and these lamellae are where oogenesis takes place. The stroma is severely compressed during spawning because the eggs get larger (Milton et al., 2018).

This review aims to provide a comprehensive overview of the diversity and dynamics of fish ovaries, exploring their structure, function, hormonal regulation, and the evolutionary adaptations that enable fish to thrive in various ecological niches. By understanding these aspects, we can better appreciate the complexity of fish reproduction and its implications for conservation and aquaculture.

Structural diversity

The enormous anatomical variety among different species of fish is one of their ovaries' most noticeable characteristics. The shape, size, and organization of fish ovaries can vary widely, reflecting the unique reproductive strategies of each species. Some have a single lobe, while others have several, and their appearance can vary from being small and compact to big and diffuse. This variety demonstrates how easily fish may adapt to their habitats and reproductive niches (Porcu et al., 2022).

Kobelkowsky (2012) found that most teleostean species have two ovaries, except the eel S. marmoratus and the needlefish S. marina, which possess just an elongated right ovary. There are symmetric and asymmetric ovaries. The majority of teleost species belong to the first group, where the differences in size between the two ovaries are negligible. There are noticeable variations in the size and form of certain species. For example, in the anchovy *A. mitchilli*, the left ovary is larger and grows below the gut, whereas the right ovary is smaller. On the other hand, the ovaries of *O. gomesii* and *A. anale* are separated, long, and parallel, lacking a distinct gonoduct. Certain Poeciliidae species, like the guayacón *G. regain* and the guapote of the Lerma *P. infans*, do not retain an interovarian septum; instead, both ovaries fuse together. The embryos are contained in this saccular gonad, which develops asymmetrically on the right side of the liver and digestive tract.

In viviparous fish, the gonoduct plays a vital role as the offspring passes through it to reach the outside world during delivery. As a result, the gonoduct creates a barrier between the ovary's germinal zone and the exterior. The gonoducts of *Poecilia reticulata* and *Poeciliopsis gracilis* were examined by Campuzano-Caballero and Uribe (2014, 2017), who described it as a muscular tube with several longitudinal folds of the mucosa extended into the gonoductal lumen. Single cuboidal or columnar epithelium with ciliated and non-ciliated cells, as well as a stroma of connective tissue, smooth muscle, and serosa, line the internal wall of the gonoduct.

Melano-macrophage centers are amorphous or spherical aggregates of melanocytes, lymphocytes, and macrophages that are sporadically seen in the connective tissue, next to the epithelium. The existence of these centers implies that they play a role in the immune system's defense of the ovary and developing embryos. The movement of spermatozoa during insemination and embryos during delivery may be facilitated by the presence of both ciliated and non-ciliated epithelial cells (Uribe et al., 2019).

Gymnovarian and cystovarian fish ovaries are the two major forms. Some ancient fish species have ovaries known as gymnovarian, which are distinguished by the oocytes' (egg cells') dispersion throughout the ovarian tissue. Contrarily, most teleosts have cystovarian ovaries, which are made up of a large number of individual ovarian follicles, each of which has a single egg (Ando and Makioka, 1998).

Reproductive strategies

The diverse reproductive methods used by fish species are evident in the ovaries of fish. Fish that are oviparous lay their eggs outside of their body. Their ovaries manufacture eggs that are released into the water, where fertilization occurs. Some are viviparous, which means that the eggs grow inside the ovary and are born young. To these various tactics, the ovary's shape, size, and egg output have been modified (Quintana et al., 2004).

Oviparity

The mature eggs (called oocytes) are produced by the ovaries and discharged into the aquatic environment. Many teleosts frequently use this reproductive method (Adebiyi et al., 2011). In cartilaginous fish, all batoids of the six Rajidae families and more than 100 species of sharks in the Orectolobiformes, Carcharhiniformes, and Heterodontiformes groups exhibit oviparity (Compagno, 1990). There are two forms of oviparity: maintained and expanded. Large egg cases are fertilized, encased in an egg case, deposited, and hatch after a period of up to 15 months in almost all oviparous cartilaginous species with extended oviparity. The egg case acts as a shell that generally has tendrils and sticky filaments that aid in attaching the egg to some sort of structure or substrate where the eggs incubate. The egg case also hardens after being deposited to protect the embryos from predation. Almost all the embryonic development in this reproductive strategy takes place inside the egg case, away from the mother's body (Musa et al., 2018). Retained oviparity is a rarer phenomenon that describes species where the development of the cased eggs continues longer in the oviduct before the eggs are released into the sea. When several egg cases are held in the oviduct before being discharged, it is known as retained oviparity in certain scyliorhinid catsharks (Compagno, 1990).

Viviparity

Some fish species give birth to live offspring. In viviparous species, the ovaries form embryos within the ovarian follicles, and these embryos take maternal nutrients before birth. Due to this adaption, the embryos can grow inside the mother's body, where she can shelter and feed them until they are strong enough to survive outside the body (Macías-Garcia and Saborío, 2004). Compared with other reproductive techniques like oviparity, viviparity is less frequent in fish. There are 54 families of fish, including 40 families of chondrichthyans and 14 families of teleosts, which have species that give birth to living offspring even though the majority of fishes are oviparous. Many fish families, including the Poeciliidae (guppies and mollies), Anablepidae (four-eyed fish), Goodeidae (splitfins), and some sharks, exhibit viviparity (Xu et al., 2022). Viviparity can take many different forms depending on the species. As a result, in viviparous teleosts, the ovary serves as a structure for oogenesis as well as a storage location for the spermatozoa received during insemination. It also facilitates the fertilization of oocytes and the growth of offspring up until birth. Therefore, viviparous fish have unique adaptations and mechanisms to support live birth. These could involve changes to the female reproductive system, such as the presence of brood pouches in some species (Helmstetter et al., 2016).

Types of viviparity according to maternal nutrition (Lode, 2012)

1-Full viviparity (Hemotrophic viviparity): In full viviparity, the mother provides the embryos with all of their nutrition through a placenta-like structure. The developing embryos receive oxygen and nutrition directly from the mother, and the offspring are born fully grown and ready to live on their own. Characteristic representatives include surfperches, seahorses, lemon sharks, splitfins, and pipefish.

2-Histotrophic viviparity: In histotrophic viviparity, the mother develops specialized glandular tissues or secretions that serve as the embryo’s main source of nutrition. The embryos absorb the nutrients but they may not form a true placental link. This type is observed mainly among sharks but is also reported in a few bony fish.

The endothelium of maternal capillaries, the follicular epithelium, the embryonic surface epithelium, and the endothelium of embryonic capillaries make up the follicular placenta. Therefore, a placenta enables the mother to provide nutrition to the embryos during gestation rather than depositing large amounts of nutrients into the oocyte during oogenesis (Gunn and Thresher, 1991). It was possible to identify specialized features of the follicular cells that increased the active transfer of nutrients between the mother and embryos by analyzing the placenta structure in several species of poeciliids, such as an increase in the number and length of microvilli. A straightforward variety of pseudo-placenta is found in the genus Poeciliidae. In this instance, the ovarian follicle walls generate a complex network of capillaries that project outward as villi and form a close association with the growing embryos' exterior surfaces (Uribe et al., 2019).

The oocytes are fertilized in the ovarian follicle to begin intraovarian gestation. All embryonic development, up until birth, takes place inside the follicle. Each oocyte has a particular structure at its periphery where the spermatozoa make contact with the oocyte, making intrafollicular fertilization feasible (Iida et al., 2019). A duct connecting the ovarian lumen to the oocyte membrane is created by this structure, which enters each oocyte as a funnel-shaped invagination of the ovarian lining to the follicular epithelium. This invasion is known as a delle. As a result, the delle is the only route by which the spermatozoa can reach the oocyte. Intrafollicular embryonic development begins when spermatozoa fertilize mature oocytes inside the follicle (Mokhtar, 2021).

Ovivivparity

Another method of reproduction used by the majority of shark, ray, and rockfish species is ovoviviparity. Fish that are ovoviviparous have their eggs fertilized inside of them. The eggs stay inside the mother while they grow, providing a higher level of defense against predators and severe environmental circumstances compared with fish that are oviparous. Another benefit of ovoviviparity is that the young are more likely to survive on their own in the wild since they have developed to a more advanced stage when they are born alive (Kawaguchi et al., 2015).

Teleosts use a variety of reproductive techniques, such as intricate kinds of parental care and external fertilization. The structure and function of their ovaries can vary greatly, indicating how they have adapted to fit into various ecological niches (Jalabert, 2005).

Among the noteworthy adaptations are:

Batch Spawning: During the spawning season, a number of teleosts can generate and release their eggs in batches, increasing the likelihood of successful fertilization and the survival of the progeny.

Livebearing: A few teleosts, such as mollies and guppies, can fertilize internally and give birth to live young, which is an adaptation that can increase the survival of the juveniles.

Hermaphroditism: Individuals of certain species can switch their sex at any moment in their lives, having separate testicles and ovaries. This method optimizes the success of reproduction in a variety of social contexts (Goodwin et al., 2002).

Evolution of fish ovaries

The history of fish ovary evolution is a fascinating journey through time that reflects the complex diversifications and adaptations that fish have undergone (Adolfi et al., 2023).

The primitive gonadal organs that were not yet well specialized into ovaries and testes were probably present in the earliest vertebrates, such as the jawless ostracoderms (Koya and Muñoz, 2007). Although the methods by which these rudimentary fish reproduced are poorly known, the development into more specialized reproductive organs would have conferred evolutionary advantages.

Fish with Jaws (Gnathostomes): The emergence of jawed fish led to notable developments in reproductive architecture. Sharks and rays are examples of early cartilaginous fish (Chondrichthyes) that display a range of reproductive methods, including viviparity (live birth) and oviparity (egg-laying) (Penfold and Wyffels, 2019). Their paired ovaries have the capacity to yield large, yolk-rich eggs, a characteristic that aids in the development of embryos inside eggs or, in the event of a live birth, inside the body.

Osteichthyes, or bony fish: The great majority of contemporary fish species are bony fish, which exhibit much more variation in ovarian development and reproductive tactics. The lobe-finned fish (Sarcopterygii) and the ray-finned fish (Actinopterygii) are the two main clades of this category. The ancestors of tetrapods, Sarcopterygii Lobe-finned fish, often had paired ovaries. Certain species, such as lungfish, have special reproductive adaptations. For example, they can burrow into the mud to survive during droughts, which affects their reproductive cycles (Clement, 2019).

Actinopterygii Ray-finned fish have the most varied ovarian anatomy and physiology. The most evolved and diverse creatures in this group are the teleosts. To produce a large number of little eggs, teleost fish have evolved extremely specialized ovaries (Henderson et al., 2023). This is useful for species with high fertility rates and those that live in habitats where there is great pressure from predators that eat the eggs and larvae.

Numerous factors, such as environmental conditions, predation pressures, and reproductive techniques that increase offspring survival, have influenced the evolution of fish ovaries (Torsabo et al., 2022). Fish have been able to establish themselves in a variety of environments, including freshwater rivers and lakes, and deep seas, thanks to the development of sophisticated ovarian structures and varied reproductive strategies.

A unique characteristic of almost all viviparous teleosts occurs when, during the early stages of embryonic development, the right and left ovaries fuse, forming a single ovary (Wourms, 1981). This fusion establishes a single and saccular ovary, with the germinal epithelium lining the internal lumen. Another distinctive feature of teleosts among vertebrates is that teleosts do not develop Müllerian ducts during embryogenesis, as occurs in the rest of vertebrates; consequently, teleosts do not have oviducts. The ovary's caudal zone, also known as the gonoduct, is where the germinal zone of the ovary communicates with the exterior. This ovarian zone is devoid of germinal cells (Wourms, 1981). As a result, the lumen of the gonoduct and the ovarian germinal zone are continuous. At the boundary between the gonoduct and the germinal region of the ovary, several mucosal folds grow to produce a limit that resembles a cervix (Campuzano-Caballero and Uribe, 2014).

Unlike other viviparous vertebrates, where gestation occurs in the uterus, viviparous teleosts undergo gestation in the ovary due to the absence of oviducts. Therefore, in viviparous teleosts, the ovary serves as both the site of oogenesis and the organ that receives and stores spermatozoa during insemination, allowing for the fertilization of oocytes and the growth of progeny until birth (Uribe et al., 2019).

Hormonal control and molecular mechanisms of oocyte maturation

A number of hormones play a role in the intricately regulated and extremely advanced hormonal management of the fish ovary. The coordination of ovarian growth, oocyte maturation, ovulation, and reproductive timing is critically dependent on these hormones. These hormones are secreted and controlled by the endocrine system, which is composed of the pituitary gland, gonads, and other endocrine organs (Ramezani-Fard et al., 2013). Several important hormones involved in the hormonal regulation of the fish ovary are listed below:

GTHs, or gonadotropin hormones

Oocyte growth and maturation are primarily initiated by pituitary gonadotropins, or GTHs. The ovarian production of steroidal mediators of oocyte growth (estradiol-17β) and maturation (MIH; 17α,20β-dihydroxy-4-pregnen-3-one, 17α,20β-DP in salmonid fishes; 17α,20β,21-trihydroxy-4-pregnen-3-one, 20β-S in sciaenid fishes) is what mediated the actions of GTHs. It is known that the interaction between the granulosa and thecal cell layers results in the generation of estradiol-17β and 17α,20β-DP by salmonid ovarian follicles. Just before oocyte maturation, there is a noticeable change in salmonid steroidogenesis in the ovarian follicle layer from estradiol-17β to 17α,20β-DP. This shift may be a consequence of dramatic changes in the expression of genes encoding various steroidogenic enzymes (Melamed et al., 1998) It has also been demonstrated that the liver synthesizes egg membrane proteins in response to estradiol-17β. The way that 17α, 20β-DP, and 20β-S induce maturation is via binding to the plasma membrane of the oocyte. The maturation of vertebrate oocytes is regulated by MIH, which is progesterone in all vertebrates except in fish, where it is 17α,20β-DP. The oocytes stop at the prophase of meiosis I once they have reached their maximum growth. Oocytes are released from this arrest by MIH. MIH stimulates the production of a dimeric protein kinase complex called maturation promoting factor (MPF), of which cell division cycle (Cdc2) kinase is the catalytic and cyclin B is the regulatory component. (Bhattacharya et al., 2007). MPF has been isolated from mature carp oocytes. Luteinizing hormone (LH) acts on the ovarian follicle layer to produce MIH (17α, 20β-dihydroxy-4-pregnen-3-one, 17α, 20β-DP, in most fishes) (Nagahama et al., 1993).

Estrogens and progestogens

These are sex steroids synthesized from cholesterol. The cleavage of the cholesterol side chain by cytochrome P450 cholesterol side chain cleavage (P450scc) is the initial step in the production of steroids. This steroidogenic enzyme is found in the mitochondrial inner membrane. Since cholesterol is lipophilic and the gap between the two mitochondrial membranes is aqueous, a particular transport protein known as steroidogenic acute regulatory protein (StAR) mediates the passage. StAR is located in steroidogenic tissues and is produced in response to cell stimulation to generate steroids (Arakane et al., 1997). According to Govoroun et al. (2001), in addition to P450scc, other significant steroidogenic enzymes include 17-hydroxylase/lyase (P450c17), 3β-hydroxysteroid-dehydrogenase (3βHSD), 11β-hydroxylase (P45011β), and P450 aromatase (cyp19). Cyp19 is the enzyme responsible for catalyzing the permanent transformation of androgens into estrogens. Cyp19 plays a vital role in sex differentiation since it regulates the balance between the two types of steroids, as all estrogens are derived from androgens, and Cyp19 is located at the end of the steroidogenic pathway. In tetrapods, Cyp19 is found as a single copy. Teleost fish, on the other hand, have two isoforms of cyp19, which result from distinct genes that were duplicated from an ancestral gene. Referred to as gonadal aromatase, P450aromA or cyp19a, one isoform is largely localized in the gonad; the other is primarily localized in the brain and is known as brain or neural aromatase, P450aromB or cyp19b (Chang et al., 2005).

Pituitary peptides

Growth hormone (GH) and prolactin are two additional pituitary peptides that may have an impact on ovarian activity (Le Gac et al., 1993). Whittington and Wilson (2013) discussed the role of prolactin in fish reproduction, which includes reproductive development and cycling, brood care behavior, pregnancy, and nutrient provisioning to the young.

Thyroxine-T4 (thyroid hormones)

Fish metabolism and development are regulated by thyroid hormones, and this has an indirect impact on ovarian growth and function (Deal and Volkoff, 2020).

IGFs, or insulin-like growth factors

The pituitary and ovaries both produce IGFs, which promote ovarian growth and development (Yamamoto et al., 2011). IGF1 has been discovered in the developing ovaries of some fish species, and specific binding sites for both IGF1 and IGF2 have been established in ovarian and testicular tissues of various fish species, including zebrafish and tilapia (Yuan and Hong, 2017). In diverse fish species, both IGF1 and IGF2 were found to activate thymidine incorporation in goldfish vitellogenic follicles and increase oocyte maturation (Yuan et al., 2018).

Adrenocorticotropic hormone (ACTH)

The pituitary gland produces ACTH that can indirectly affect ovarian function by influencing steroidogenesis in inter-renal tissues (Sousa et al., 2015).

Melatonin

Melatonin, a hormone that controls circadian rhythms, can modify the timing and seasonality of reproduction in some fish, which can have an impact on ovarian activity (Maitra and Hasa, 2016). A study by Chattoraj et al. (2005) showed that prior incubation with melatonin accelerates the action of MIH on carp oocyte maturation.

Endocrine-disrupting chemicals (EDCs) can alter fish ovarian function in modern environments by interfering with hormonal signaling, which could result in aberrant reproduction (Bhattacharya, 1992).

Environmental elements like temperature and photoperiod frequently have an impact on the precise regulation of these hormones and how they interact, which varies according to the species. Fish may use seasonal or continuous reproduction as one of their reproductive techniques (Baroiller et al., 2009).

The time and rate of gonadal development are influenced by temperature. Cooler temperatures can postpone spawning, although warmer conditions frequently quicken the maturation process.

Variations in temperature can affect the ovary's cellular composition. For instance, ideal temperatures encourage the proper development of follicles but excessive heat might result in atresia and anomalies in follicular growth (Arula et al., 2019). Spawning in many fish species is triggered by particular temperature ranges. Changes in spawning cycles or decreased reproductive success may arise from departures from these ideal parameters (Liu et al., 2022).

For seasonal breeders, photoperiod is a crucial environmental cue. It aids in coordinating reproductive processes with ideal environmental circumstances to ensure the survival of progeny. By controlling the amounts of hormones such as GTHs, variations in photoperiod can have an impact on the ovarian cycle. Longer daylight hours usually promote gonadal maturation and development (Akhoundian et al., 2020). The beginning of the breeding season is marked by a number of fish species by an increase in daylight. This synchronization makes sure that spawning takes place when the environment is most conducive to the survival of the eggs and larvae (García et al., 2022).

Immunohistochemistry

To identify and map the distribution of different proteins, hormones, or biological components inside the ovarian tissue, immunohistochemistry (IHC) is used on fish ovaries. The specific antigens or indicators being studied and the specific research goals influence the use of antibodies. The following common antibodies are utilized in IHC analyses of fish ovaries:

The SRY-box transcription factor 9 (gene: SOX9) is an essential transcription factor in the development of several tissues and organs, including chondrogenesis and sex determination. One of the *SOX* gene family members is *SOX9*. During gonad differentiation, SOX9 is increased in the testes and downregulated in the ovaries. In zebrafish, two *SOX9* genes have been identified: *SOX9a* and *SOX9b*. In chondrogenic tissues, both are expressed. Whereas *SOX9a* is expressed in the zebrafish gonad testis, *SOX9b* is localized in the ovary. These results suggest that whereas *SOX9* expression in fish gonads appears to be modestly variable, it is conserved in vertebrate chondrogenic organs (Chiang et al., 2001).

The protein Myostatin, also known as growth differentiation factor-8 (GDF-8) is a member of the Transforming growth factor (TGF)-β superfamily and was initially shown to be a negative regulator of the formation of skeletal muscle (Østbye et al., 2001). Notably, recent research has investigated myostatin expression and potential roles in a variety of reproductive organs, including the zebrafish ovary (Mohamedien et al., 2023).

Proteins called aquaporins control how much water is transported inside cells (Chauvigné et al., 2011). They may play a role in osmoregulation during egg formation in fish ovaries. Cell proliferation markers, such as CD117 and PCNA can be used to gauge the ovaries' rate of cell division and reveal details about tissue expansion and development. Positively stained cells were detected in the follicle epithelium of all stages of growing oocytes and degenerating oocytes. Furthermore, PCNA was found to be present in nuclei of oogonia and oocytes in meiotic prophase (Korfsmeier, 2002). A precursor protein called vitellogenin assists in producing the yolk in growing oocytes. In fish ovaries, oocyte maturation and yolk deposition are investigated using antibodies against vitellogenin (Ruan et al., 2020). The apoptotic marker cleaved caspase-3 has a role in the control of ovarian follicular atresia. The apoptotic processes in the ovaries can be studied with these antibodies. Vascular Endothelial Growth Factor (VEGF): antibodies against growth factors can be used to study ovary angiogenesis, tissue growth, and development (Santos et al., 2015).

Follicle development

The astonishing variety in egg formation is also visible in fish ovaries. Many microscopic eggs are produced by the ovaries of some species, while fewer, relatively large eggs are produced by the ovaries of other species. Various factors, including parental care, egg preservation, and environmental conditions, are frequently connected to this variation in egg size (Patiño and Sullivan, 2002). A critical step in the ovaries is oogenesis, the formation of oocytes. Oocyte development, maturation, and growth inside ovarian follicles are all part of this process. Before they are prepared for fertilization, oocytes go through several phases of development and maturation starting as undifferentiated oogonia (Chmilevskii and Kameneva, 2003).

Ovulation and ovarian follicle development follow cyclical patterns in many teleosts, with specific hormonal regulation. The length of these cycles can vary, and they are frequently influenced by elements including temperature, photoperiod, and seasonal fluctuations. The effectiveness of their reproductive activities depends on the intricate and tightly controlled process of ovarian follicle growth in teleosts (Mohamedien et al., 2023). The most diverse group of fish are teleosts, and the development of ovarian follicles varies between species as a result of the diversity of their reproductive techniques and ecological niches (Viana et al., 2018). The basic phases and distinguishing characteristics of ovarian follicle development in teleosts (Fig. 1) include:

1. Oogonia

The process starts early in the life of the fish, when undifferentiated germ cells, or oogonia, multiply and transform into primary oocytes inside the ovaries. By meiosis I, these initial oocytes are arrested.

2. Primary growth phase

In this stage, the primary oocytes enlarge and the somatic cells around them expand to create previtellogenic follicles. The developing oocyte is supported and fed by the somatic cells. During primary growth, abundant ribosomes, the rough endoplasmic reticulum, and mitochondria are grouped in the cytoplasm. At the end of this stage, the chorion is developed, while microvilli start to be evident (Cárdenas et al., 2008).

3. Vitellogenesis

Oocytes accumulate yolk material, primarily lipids and proteins, during this critical period. Oocyte size increases noticeably at this stage, and vitellin, a protein that forms the yolk, is incorporated into the oocyte. A precursor protein called vitellogenin is ingested by growing oocytes and is crucial for the formation of eggs. At this time, the yolk vesicles enlarge. Large yolk globules are formed from the protein-rich yolk granules. Near the oocyte's center, several spherical yolk globules (platelets) emerge and spread centrifugally until just a thin cytoplasmic shell is left on the outer edge. The ooplasm is filled with many fat vacuoles (Hara et al., 2016).

Since the size, shape, and distribution of the tiny vesicles around the oocyte periphery are remarkably similar to those of the alveoli at the yolk vesicle stage, they may be cortical alveoli. The zona radiata is thick and the follicular epithelium (granulosa) is composed of cuboidal cells. The theca folliculi is divided into an outer vascular thecal layer and an inner cellular theca layer. The oocytes increase in size and the number and diameter of yolk globule oocytes vary significantly between the breeding and non-breeding seasons (Mokhtar, 2017).

The oocyte yolk globule stage is the most crucial since it is during this stage that vitellogenesis takes place, which causes the oocyte to rapidly incorporate enormous amounts of exogenous vitellogenin from the liver, leading to substantial oocyte expansion. Some authors claimed that Estradiol is produced by the expanding ovarian follicles. Through blood arteries that supply the theca cell layer, this steroid exits the follicle and travels to the liver, where it causes the creation of vitellogenin. Vitellogenin travels through the bloodstream to the ovary, where it is absorbed by the egg and deposited as yolk protein, which is used as a building block and an energy source after fertilization (Hiramatsu et al., 2015).

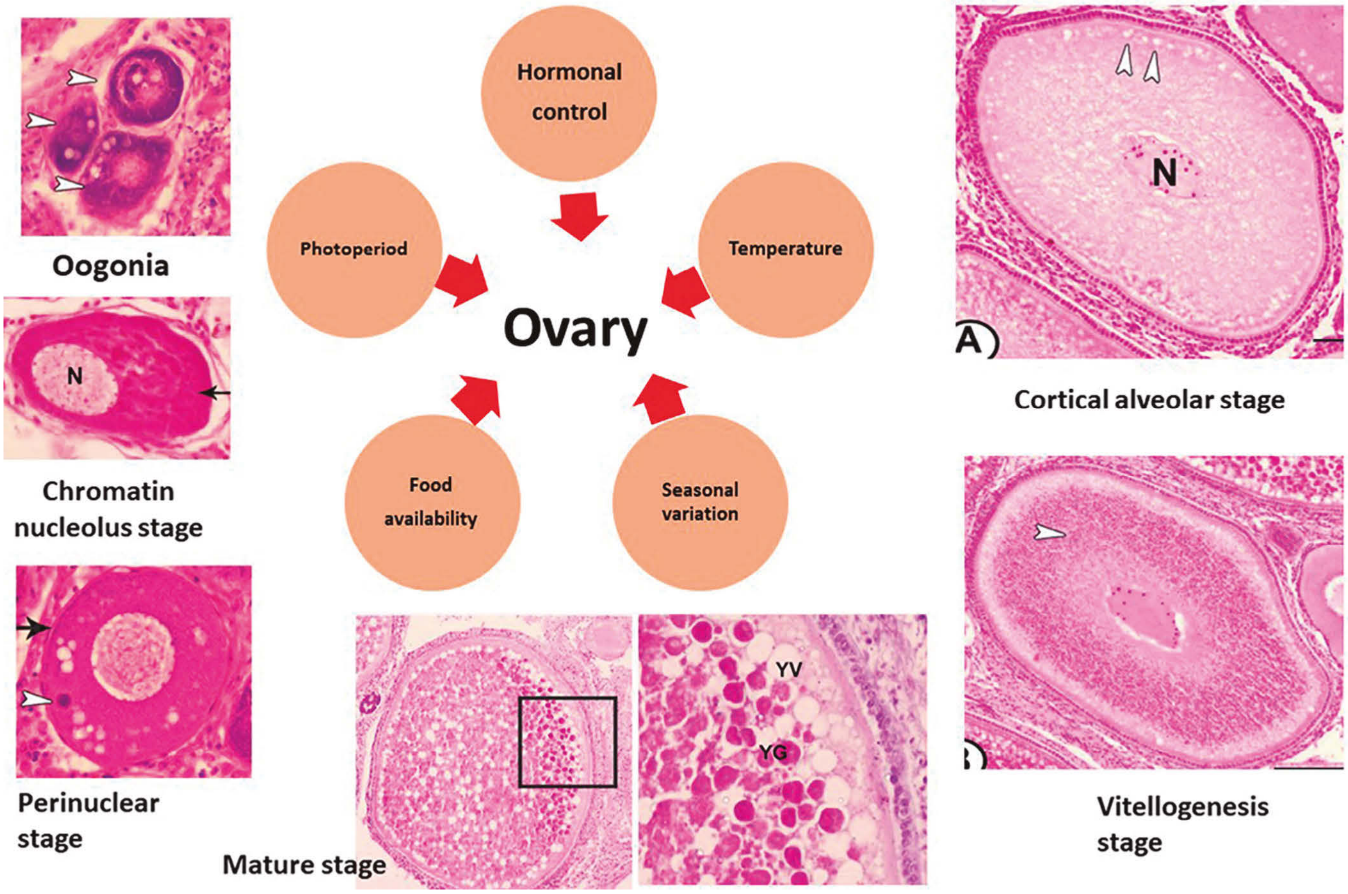

Fig. 1. Diagram showing the stages of oogenesis and factors affecting the ovary.

4. Secondary growth phase

As the oocytes mature, meiosis resumes and the follicles continue to expand.

5. Mature follicle

The mature follicle is distinguished by the accumulation of several empty, big vacuoles at the oocyte's periphery and the enlargement of the yolk globules. The nucleus (germinal vesicle), which typically happens at the end of vitellogenesis, gradually disappears, migrates to the animal pole beneath the oocyte surface, and finally breaks down. The oocyte has a large diameter in the mature follicle. The ovarian lamellae are thick and fully obliterate the ovaries during the breeding season (Francolini et al., 2003).

The protein yolk globules begin to form as the germinal vesicle breaks down, the oocyte's volume expands quickly, it ovulates into the ovarian lumen and develops into a mature ovum. Due to the breakdown of the nuclear membrane and the dispersion of its contents in the cytoplasm at the end of this stage, the nucleus is not visible during the maturation stage. The follicular layers and the zona radiata reach their maximum thickness and are encircled by cuboidal granulosa and theca cells. Between the follicular epithelium and the theca layer is a thin, Periodic acid-Schiff (PAS)-positive fibrous layer known as the basal lamina. The yolk vesicles, on the other hand, contain glycoprotein and demonstrate a high positive reaction to PAS stain. Lipoprotein makes up the majority of the yolk globules, with a small amount of carbohydrates. In addition to being the main food supply, yolk globules have a role in embryogenesis. The proteins in the yolk are also helpful for monitoring the environment (Reading et al., 2018).

A positive immunoreaction to MMP9 (matrix metalloproteinase 9) is expressed on the wall of mature ovarian follicles. A particular class of proteolytic enzymes, called matrix metalloproteinases (MMPs), are essential for the growth and luteinization of follicles. MMPs are the enzymes that degrade connective tissues and extracellular matrix to aid in tissue remodeling (Mokhtar and Hussein, 2020).

During the spawning season, the EM results indicated that the zona radiata increased in thickness and was crossed perpendicularly by pore canals that contained processes from both oocytes and zona granulosa. The granulosa cells contained many mitochondria, rER, sER, and vesicles. Meanwhile, the thecal layer consisted of fibroblast-like cells.

Ovulation is the process by which the oocytes are expelled from the ovary once they have matured. This includes the rupture of the ovarian wall and subsequent release of eggs from the ovarian follicles, and it is frequently triggered by hormonal changes or environmental cues, such as changes in temperature or photoperiod (Goetz and Garczynski, 1997).

6. Fertilization

After ovulation, the mature oocytes are discharged into the watery environment to undergo fertilization. External fertilization takes place when male fish sperm fertilize the eggs that the female has released. The term "embryo" now refers to the fertilized eggs (Iwamatsu, 2000).

Atretic follicles, or corpora atretica, are the outcome of oocyte degeneration and phagocytosis-induced resorption. The teleost ovary frequently develops follicular atresia, which affects both the oocytes and the follicular wall. The connective tissue surrounding the follicle thickens and becomes more vascular, while the atretic follicular cells grow and phagocytize the oocytes that were not spawned The elastic characteristics of the theca externa are likely to be responsible for the follicles' contraction and folding. The sizes of the atretic follicles are too varied to measure since they are amorphous structures that appear in oocytes at any stage of maturation but are always visible during the post-spawning period. Because of this, the presence of damaged yolky oocytes indicated recent spawning (Corriero et al., 2021).

The process of follicular regression began with the oocyte nuclear membrane shrinking and disintegrating, which caused chromatin to disperse within the ooplasm. This was followed by a notable hyperplasia and hypertrophy of the granulosa and follicular cells, which showed a strong phagocytic activity to absorb the liquefied yolk particles (Thomé et al., 2009). Granulocytes and rodlet cells penetrated the regressed follicles and were observed on the follicular wall

Neutrophils exhibited a great reactivity to Myeloperoxidase-3 (MPO), whereas rodlet cells showed robust responsiveness to MMP-9 and α-smooth muscle actin (Mokhtar and Hussein, 2020). The yolk was nearly phagocytized and resorbed in the advanced stage of follicular atresia, and the regressed follicle lost its integrity and seemed to be made up of a cellular mass of phagocytic cells (Besseau and Faliex, 1994). Dendritic, eosinophil, and neutrophil cells were found between these phagocytic cells within the atretic follicle using transmission electron microscopy (TEM). In addition, a large number of phagosomes, granules, and lysosomes were seen in the cytoplasm of granulocytes and phagocytic cells. Caspase-3 IHC labeling demonstrated that apoptosis was involved in the later phases of follicular regression (Mokhtar and Hussein, 2020).

The ovarian stroma, which is mostly made up of connective tissue, blood arteries, and nerves, is thought of as the ovary's structural support system. Sex-reversal species exhibit more pronounced alterations in this stroma during both the spawning and non-spawning seasons. Blood enters the mesovarium on the dorsal side of the ovary and travels to the ovary through multiple arteries that branch off the dorsal aorta in teleosts. Arterioles, which supply each follicle in the ovaries, conduct it by passing via a stalk of stromal components. Various types of cells were detected in the stroma (Fig. 2) including telocytes, rodlet cells, mast cells, eosinophils, neutrophils, lymphocytes, fibroblasts, macrophages, melanocytes, adipocytes, DCS, and endocrine cells (steroidogenic, interstitial) (Mokhtar, 2019).

DCs are tiny cells with delicate processes that are present around theca cells and blood arteries in conjunction with macrophages (Sayed et al., 2022). They express c-kit and the S-100 protein. According to TEM images, DCs are distinguished by their processes, large, indented heterochromatic nuclei, and rER. Lysosomes and occasionally phagocytized items are visible in the cytoplasm. They also exhibit mitochondria, phagosomes, vacuoles of varied sizes, and macropinocytotic vesicles (Mokhtar et al., 2023a-c).

The endocrine (steroidogenic, interstitial) cells are polyhedral cell cords or clusters that can be seen around tiny blood arteries in the stroma or around mature vitellogenic follicles and exhibit significant activity towards S-100 protein, silver stain, and positive reactivity to bromophenol blue. According to TEM results, polymorphic secretory granules, tubular mitochondrial cristae, and sER dominate the endocrine cells. It has been proposed that stromal components constitute the source of the steroid-producing cells. Additionally, ovarian lumen development and germ cell differentiation are both influenced by the steroids that steroid-producing cells release. There is a possibility that these cells manufacture estrogens (Nakamura et al., 1996).

Small spindle-shaped cells known as TCs have two or more lengthy cell processes known as telopodes. They are either dispersed throughout the stroma in between the other connective tissue cells or surrounding blood capillaries and atretic follicles. They displayed positive silver and dark toluidine blue staining. Desmin immunoreactivity was strongly expressed in the bodies and telopodes of TCs. Additionally, c-kit and s-100 protein immunoreactivity were seen in the telopodes (Hussein et al., 2023a). Moreover, TCs expressed SOX9 and TGF-β (Mokhtar et al., 2023a). TCs are unique interstitial cells that are widely distributed in many organs of fish, including the skin of the upper lip of silver carp (Sayed et al., 2021), the stomach of catfish (Mokhtar, 2022), around the bile ducts of grass carp (Mokhtar, 2018), heart and pancreas of molly fish (Hussein et al., 2023b ; Zaccone et al., 2024), as well as gills and intestinal bulb of molly fish (Mokhtar et al., 2022, 2023b).

Macrophages were frequently observed in the ovarian stroma, which play a central role in immune reactions (Mokhtar and Abdelhafez, 2021). A protein responsible for bacterial recognition found on the surface of macrophages is CD14. These cells possess a repertoire of antimicrobial molecules stored in their granules and lysosomes. Polarization states observed in mammalian macrophages, termed M1 and M2, also likely exist in fish macrophages (Zaccone et al., 2023). Sayed et al. (2024) found that the macrophages in the ovarian stroma of molly fish expressed APG5, IL-1β, TGF-β, S100, NF-κB, CD68, Iba-1, and Ach. Rodlet cells were encountered in the ovaries of tilapia (Mokhtar, 2019). A recent study supposed that the origin of these cells is the bulbous arteriosus (Mokhtar et al., 2024).

Mast cells (MCs) are ovoid or rounded immune cells that exhibit amoeboid activity. They can be found in the skin, blood vessels, digestive tract, gills, and ovaries (Galli et al., 2005). These cells can control inflammation and are engaged in a number of the body's defense systems (Abraham and John, 2010). Histamine and serotonin are two examples of bioactive mediators stored in their unique cytoplasmic granules (Shim et al., 2019). Numerous studies have shown that the functional features of MCs in teleosts are quite similar to those of mammals. Furthermore, the MCs of fish include granules that are similar to those of mammals, including lysozyme, serotonin, and particular antimicrobial peptides (AMPs) known as piscidins (Alesci et al., 2022).

Recent trends in fish ovary biology

Advances in environmental monitoring, aquaculture techniques, and molecular biology have been the driving forces behind recent changes in fish ovary biology.

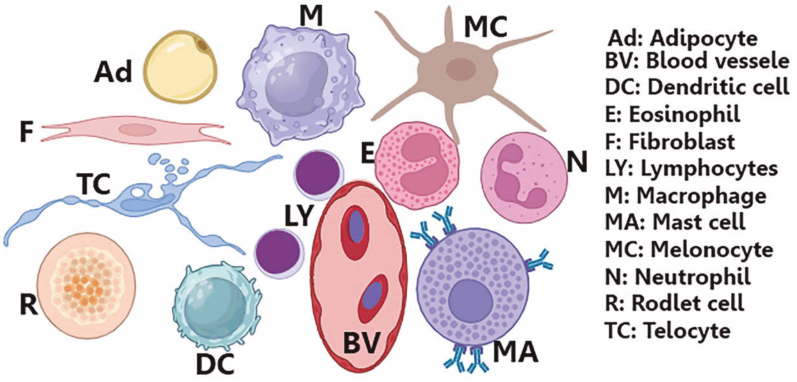

Fig. 2. Diagram showing various cells in the ovarian stroma.

These patterns show an increasing knowledge of fish reproduction, which is important for sustainable fishing, conservation initiatives, and raising aquaculture productivity. Important patterns consist of:

Genomic sequencing

Detailed transcriptomic and genomic investigations of fish ovaries are now possible through developments in next-generation sequencing technologies. These investigations aid in the identification of genes and regulatory networks implicated in hormone control, oogenesis (the formation of eggs), and responses to environmental stresses (Lu and Luo, 2020).

Epigenetics

The study of how epigenetic changes, like DNA methylation and histone modification, affect gene expression during fish oogenesis has drawn more attention in recent years. Through epigenetic modifications, these investigations are revealing how environmental factors impact reproductive health and development (Sumon et al., 2022).

Selective breeding and genetic manipulation

To increase reproductive efficiency and improve egg quality, aquaculture procedures are increasingly using genetic manipulation and selective breeding techniques. For instance, CRISPR-Cas9 technology is being investigated to modify genes linked to stress tolerance and fertility (Strømsnes et al., 2022).

Cryopreservation

Methods for preserving ovarian tissue and fish eggs are being created and enhanced. Maintaining genetic variety and assisting with the restocking of endangered species depend on this (Rivers et al., 2022).

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