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Oocyte degeneration and post-ovulatory developments in the ovary of captive broodstock of black seabream, *Acanthopagrus berda*

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Original Article

Abstract

The study used ovarian biopsy and histological analysis to investigate oocyte hydration, follicular atresia, and post-ovulatory follicles (POFs) in captive broodstock of black seabream. *Acanthopagrus berda.* Mature females were collected from the Kali Estuary, Karwar, India, and reared in floating net cages. Ovarian development was assessed using routine biopsy and histological techniques. Biopsy samples revealed the presence of hydrated oocytes, confirming ovarian maturity, while histological analysis identified distinct stages of oocyte hydration, including germinal vesicle breakdown and membrane fragmentation. In hormonally induced females, histological sections demonstrated the uneven distribution of hydrated oocytes across ovarian lobes, with a higher abundance in the posterior region. Follicular atresia was observed in both biopsy and histological samples, with alpha and beta atretic oocytes indicating progressive ovarian degeneration. The presence of numerous atretic oocytes in hormone-induced females suggested a possible link to spawning failure. Additionally, POFs were identified in regressing and regenerating ovaries, providing evidence of previous spawning events. The study highlights the value of ovarian biopsy as a cost-effective, real-time tool for assessing broodstock suitability while emphasizing the importance of histological analysis in understanding reproductive dynamics. These findings contribute to the improvement of broodstock management strategies in aquaculture.

Keywords: *Sparidae, oocyte atresia, hydration, post-ovulatory follicles, biopsy*

Introduction

Captive broodstock development and maturation studies are fundamental for ensuring continuous seed production in any aquaculture candidate species. The maturation and reproductive cycles of various commercially significant fish species have been extensively studied in captivity to establish standardized breeding protocols. The captive maturation and reproductive dynamics of sparids also have been systematically evaluated by several authors (Abu-Hakima *et al.,* 1984; Chang and Yueh, 1990; Abou-Seedo *et al.,* 2003; Black and Black, 2013; Shilta *et al.,* 2019; Suresh Babu *et al.,* 2022 a, b) and found species specificity in maturation and development (Mylonas *et al.,* 2011).

The picnic seabream or black seabream, *Acanthopagrus berda*, is a commercially important species with both recreational and aquaculture potential for which captive broodstock development, breeding and seed production technology has been standardised (Abbas *et al.,* 2019; Suresh Babu *et al.,* 2022a). Several aspects of its biology have been documented, including species confirmation (Shilta *et al.,* 2021), reproductive biology (Shilta *et al.,* 2018a), annual reproductive cycle (Shilta *et al.,* 2019), and feeding behaviour (Shilta *et al.,* 2018b). However, detailed studies on cellular changes during reproductive development for these species need to be evaluated further.

Oocyte degeneration and post-ovulatory developments are important mechanisms that influence the oocyte maturation process in fishes. Follicular atresia, the degeneration of oocytes and their surrounding follicular cells is a normal part of oogenesis. Hunter and Macewicz (1985) classified the atretic oocytes into several classes based on the degenerative

process. Post-ovulatory follicles (POFs) in fish are the remnants of the follicular complex remaining in the ovary after eggs are released, serving as indicators of previous spawning. Ferreri *et al.* (2021) described the presence of various types of POFs based on the degree of degeneration and lumen formation. Studies on oocyte atresia and POFs will give significant insights related to the nature of spawning, reproductive developments and quality of broodstock of the species.

This work aimed to investigate the process of oocyte atresia and the development of POFs in *A. berda* using the traditional histological analysis and simple ovarian biopsy technique in this commercially important protandrous hermaphrodite species for insight into better broodstock management for the species.

Material and methods

Fish collection

For the development of broodstock, *A. berda* was collected from the Kali Estuary, Karwar, Karnataka, India. The details are provided in Table 1. The collected fish were stocked in 6-m diameter galvanized iron (GI)-framed circular floating net cages at the marine cage farm of ICAR-Central Marine Fisheries Research Institute, Karwar, Karnataka, India and reared for one year. The fish collected from the estuary, brought to the marine cage farm in oxygenated packs, after acclimatisation was released to the cages following prophylactic treatment by $KMNO_4$ bath for 10 min. The fish were slowly weaned from low-value fish feeding to commercial pellet feed in a few days. The fish were reared on a formulated pellet diet (40% crude protein, Growel India Pvt. Ltd) at a feeding rate of 5% of body weight. Additionally, a vitamin-mineral mixture (1 g kg^{-1} feed) and cod liver oil (10 ml kg^{-1} feed) were supplemented twice weekly. To enhance dietary diversity, raw squid and oyster meat were provided at 3% of body weight once per week.

Biopsy

Ovarian development in captive broodstock reared in cages was studied using samples obtained through cannulation fortnightly with catheters (1.6 mm to 2 mm diameter; Ramsons Scientific and Surgical Industries Pvt. Ltd., New Delhi, India). Tissue samples for biopsy observation were fixed in 1% formalin prepared in a normal saline solution. Biopsy samples were analyzed under a compound microscope (Zeiss Axio Scope A1, equipped with a Jenoptik ProgRes C3 digital camera) to assess ovarian development and confirm maturity stages.

Induced breeding trials

As a part of the routine breeding program, a pair of brooders (1 male and 1 female) was induced with maturation hormone. A female weighing 490 g and a running male weighing 380 g were induced using Gonadotropin Releasing Hormone analogue (GnRH analogue) (OvaFish, Bhoomi Aqua International. Pvt. ltd) as per Suresh Babu *et al.* (2022 a). Ovarian tissue samples from the anterior, mid and posterior parts of the induced and unspawned female were taken to study the distribution of matured and hydrated oocytes.

Histology

To assess ovarian development and confirm maturity stages, ovaries were carefully dissected from fish samples. Tissue samples collected from the mid-region of the ovary were fixed in neutral buffered formalin, dehydrated through a graded ethanol series, and embedded in paraffin wax. Longitudinally embedded ovaries were sectioned to a thickness of 5 µm, dewaxed, and rehydrated through an ethanol series. The sections were stained with hematoxylin and eosin, and permanent slides were prepared following the method of Gabe (1976). Photomicrographs were captured using a compound microscope (Zeiss Axio Scope A1, equipped with a Jenoptik ProgRes C3 digital camera) to confirm maturity stages and

Table 1. Details of *A. berda* broodstock collection, maturation studies and breeding trials

Attribute	details
Location	Kali estuary, Uttarknnada district, Karnataka, India (14°51′0″N, 74°8′0″E)
Gear used	cast nets
Period of broodstock collection	August 2022 – December 2022
Number of fishes collected	126
Average length of fish	23.78 ± 0.23
Weight range of fishes	234 g to 960 g
Sampling period for ovarian biopsy	August 2023 to April 2024 once in every month 10 fishes were canulated
Sampling period for ovarian histology	August 2023 to December 2023 (Occasionally) fishes from the broodstock were dissected for ovarian histology
Induced breeding trial	December 2023

analyse developmental details. Ovarian tissue samples from the unspent female (380 g) were taken to study the distribution of hydrated oocytes and POFS.

Results

Oocyte hydration and ovulation

To assess the distribution of matured oocytes within the ovary, a histological examination was performed on a female that exhibited spawning failure following GnRH analogue induction. During biopsy (Fig. 1), mature oocytes (Fig. 1a) exhibited an increase in diameter from 450 to 650 µm due to hydration. The hydrated oocytes (Fig. 1b) appeared as large, transparent, spherical structures with a distinct nucleus. Histological analysis of the germinal vesicle breakdown stage (Fig. 1c) demonstrated the disintegration of the migrated nucleus into the cytoplasm, accompanied by the coalescence of oil globules into larger oil droplets. In histological sections, hydrated oocytes (Fig. 1d) appeared as large vacuolated structures, partially delineated by fragmented oocyte membranes. Macroscopically the ovary was fully mature, occupying a significant portion of the body cavity (Fig. 2a). Hydrated oocytes were observed in an oozing state from the oviduct (Fig. 2b).

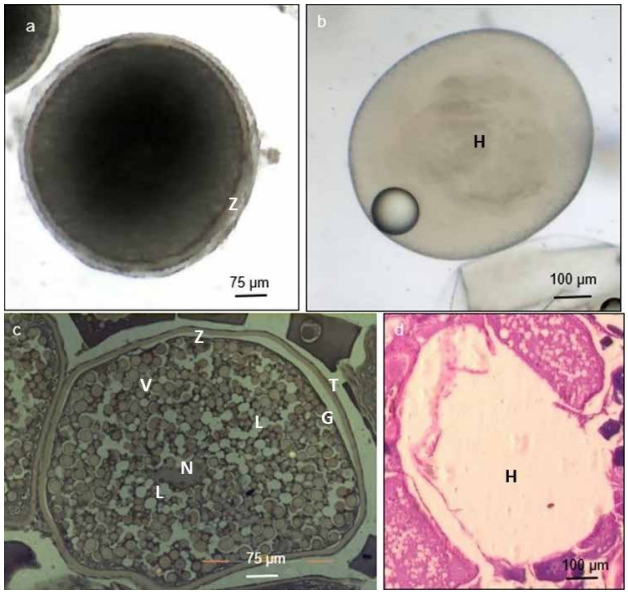

Fig. 1. Photomicrograph of various oocytes: (a) fully matured oocyte ready for hydration in biopsy; (b) hydrated oocytes in biopsy; (c) matured oocytes in histology; and hydrated oocytes in histology (d). Notations as N- nucleus, L- lipid vesicle; V – vitellogenic granules, T – thecal layer, G – granulosa layer, Z – zona radiata, H – hydrated oocyte. (Magnification 20 x)

Follicular atresia

Both biopsy and histological analyses of captive broodstock (Fig. 3) revealed the presence of atretic oocytes. In biopsy samples, normal spherical oocytes (Fig. 3a) exhibited yolk granules uniformly distributed around a well-defined zona radiata. In contrast, atretic oocytes (Fig. 3b) appeared smaller than mature oocytes and were characterized by darkened cytoplasm, a distorted or indistinct zona radiata, and a reduction in yolk granules. Atretic oocytes were sporadically observed in biopsy samples collected between August and April.

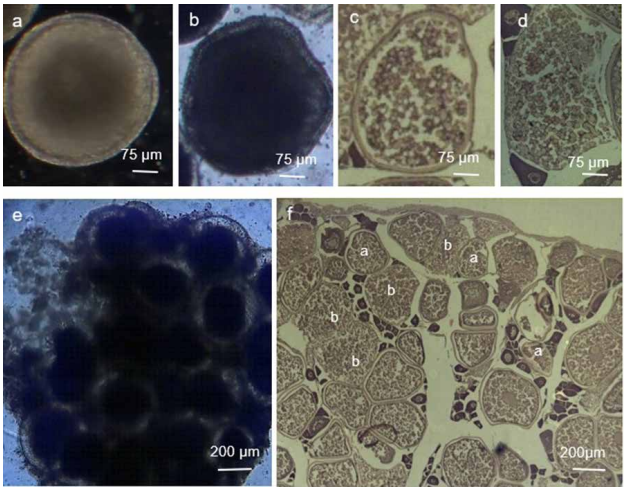

Fig. 3. Difference in the oocyte morphology: (a) Normal oocyte and (b) atretic oocyte observed in biopsy; (c) Alpha and (d) beta atretic oocytes observed in histology; (e) bunch of advanced atretic oocytes observed in biopsy; (f) presence of oocyte atresia in a histological section of ovary. Notations as a- alpha atresia and b -beta atresia

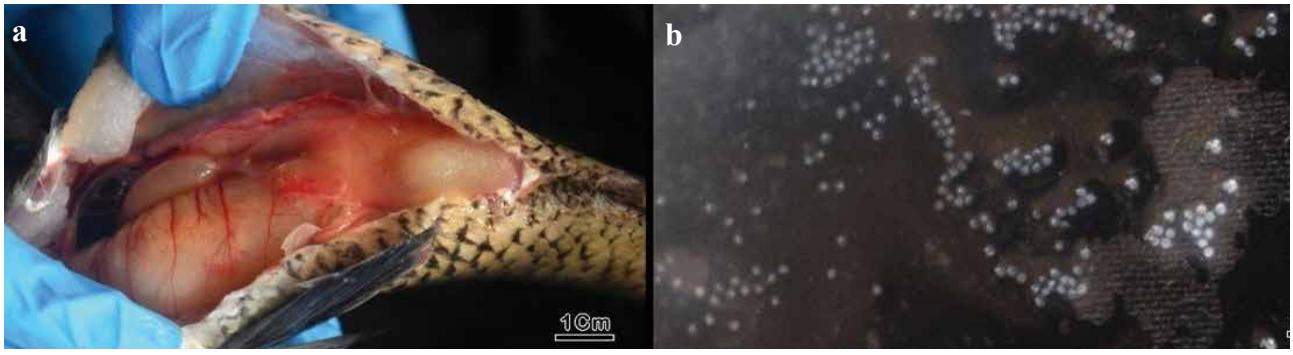

Fig. 2. (a) Ovary of a hormone-induced female with hydrated oocytes and (b) the stripped eggs

Histological examination identified two primary types of atresia. In alpha atretic oocytes (Fig. 3c), the nucleus and its surrounding membrane underwent degradation, resulting in the dispersal of nuclear contents into the cytoplasm. Concurrently, yolk granules and lipid droplets began to coalesce, leading to morphological alterations. Additionally, the zona radiata exhibited fragmentation and progressive degeneration. Beta atretic oocytes (Fig. 3d) were distinguished by the complete resorption of the zona radiata and the formation of large vacuoles in the oocytes. During biopsy sampling, advanced atretic oocytes (Fig. 3e) were observed in clusters, with distinct peripheral degeneration of yolk granules, appearing as clear zones. The morphological characteristics of both alpha and beta atretic oocytes facilitated their clear identification in histological sections (Fig. 3f).

Histological analysis of ovarian tissue from hormone-induced females (Fig. 4) demonstrated the presence of numerous atretic oocytes, indicating the initiation of ovarian degeneration. The simultaneous occurrence of both alpha and beta atretic oocytes suggests a progressive and severe degenerative process, which might contribute to spawning failure.

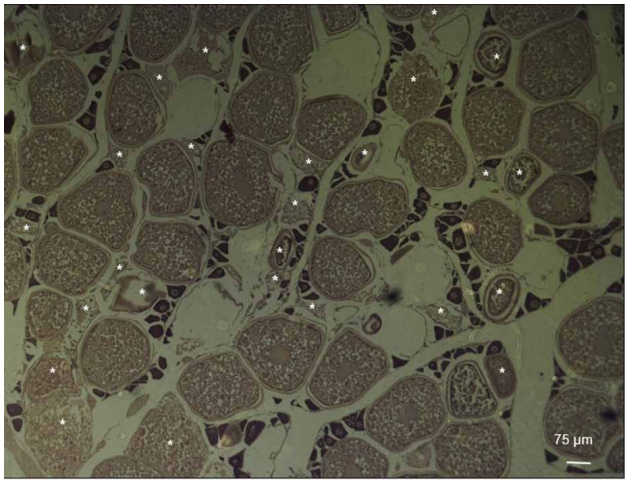

Fig. 4. Histology of the ovary of the hormone-induced female with spawning failure showing numerous atretic oocytes in the ovary. Notations as *– atretic oocytes

Post ovulatory follicles

The characterization of post-ovulatory follicles (POFs) in histological sections of the ovary of *A. berda* is presented in Fig. 5. POFs (Fig. 5a) were identified by the presence of ovarian follicles with canaliculated lumens, degenerated thecal and granulosa cells, a lightly stained basement membrane, and the absence of large vacuoles. Histological analysis revealed that POFs were prominently observed in regenerating (Fig. 5b) and regressing ovaries (Fig. 5c), indicating prior ovulation events and batch spawning activity.

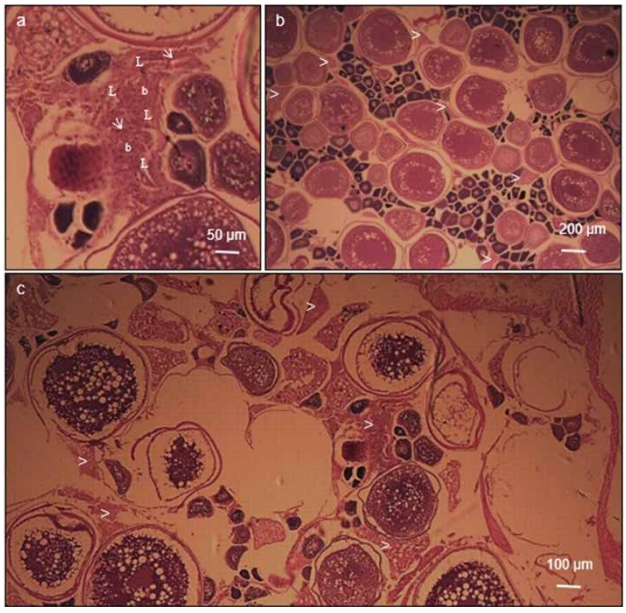

Fig. 5. (a) Post ovulatory follicles (POFs) identified in the histological section of the ovary of the captive broodstock of *A. berda.* (b) Presence of POFs in ovarian histological section of a regenerating ovary of *A. berda.* (c) Presence of POFs in ovarian histological section of regressing ovaries of *A. berda.* Notations as >–POFs, L – Lumen, b – basement membrane, arrowhead – theca and granulosa layers

Discussion

Reproductive success in seabreams (*Sparidae*) is closely linked to key physiological processes such as oocyte hydration, atresia, and post-ovulatory follicle (POF) resorption. Understanding these processes is essential for effective broodstock management, particularly in aquaculture settings. Only limited works have been taken up to unveil the reproductive development in the black seabream *A. berda.*

Hydration of oocytes

Oocyte hydration is a critical event in the final stages of oocyte maturation, leading to an increase in oocyte volume and the acquisition of buoyancy, which facilitates successful fertilization and embryonic development (Finn *et al.,* 2002).

The present study demonstrates that both ovarian biopsy and histological analysis effectively confirm the presence of hydrated oocytes in the ovary of the mature captive *A. berda*. Farrell *et al.* (2012) investigated oocyte maturation, hydration, and the characteristics of spent ovaries in boarfish (*Capros aper*). Similarly, oocyte hydration and hardening have been assessed in Atlantic halibut (*Hippoglossus hippoglossus*) using various techniques, including biopsy (Finn *et al.,* 2002). The presence of hydrated oocytes is a key characteristic of ripe or running ovaries. In biopsy samples, running ovaries were identified by the presence of hydrated oocytes, consistent

with the findings of Suresh Babu *et al.* (2022a). Histological sections revealed vacuolated structures devoid of cellular details, similar to observations reported by Pajuelo *et al.* (2008) in the sharp snout seabream (*Diplodus puntazzo*) and by Kalamarz-Kubiak and Guellard (2019) in the round goby (*Neogobius melanostomus*).

In females induced for breeding using a commercial GnRH-inducing agent, the ovaries were observed to be fully mature, occupying a substantial portion of the body cavity. The presence of hydrated oocytes signals readiness for spawning, making it a critical marker for broodstock selection. Variations in oocyte hydration across ovarian lobes can impact fertilization success and indicate spawning irregularities (Coward and Bromage, 2001). In certain species, oocyte development is uniform throughout the ovarian lobes (Coward and Bromage, 2001). However, in other species, oocyte diameter gradually increases from the anterior to the posterior region (Beumer, 1979; Alvarez-Lajonchère *et al.,* 2013). These studies were based on histological observations. However, since biopsy also yielded hydrated oocytes, a simple and cost-effective analysis of ovarian development can be designed using the technique. Alvarez-Lajonchère *et al.* (2013) employed biopsy to examine oocyte development across different ovarian lobes along the entire ovarian length.

Follicular atresia

Follicular atresia is a physiological process in which oocytes and their surrounding follicular cells undergo degeneration, ultimately leading to their resorption in the ovary. This process is a normal component of fish oogenesis and occurs throughout the ovarian cycle, with a higher prevalence in regressing ovaries during the post-spawning period. An increased incidence of follicular atresia can negatively impact fecundity and, in severe cases, lead to reproductive failure. Atresia may occur at any stage of oocyte development (Corriero *et al.,* 2021) and progresses rapidly, with complete resorption occurring within a short timeframe (Kjesbu *et al.,* 1991; Murua and Motos, 2006).

Hunter and Macewicz (1985) classified follicular atresia into four stages; Alpha (α) Stage as the initial phase marked by early signs of degeneration in the oocyte and follicular cells, Beta (β) Stage characterized by increased degeneration, detachment of some follicular cells, and structural deterioration, Gamma (γ) Stage with further degeneration of the oocyte and follicular cells, accompanied by follicular collapse and Delta (δ) Stage, the final stage, where the follicle is almost completely collapsed, with extensive cellular degradation. In the present study characteristics of only two types of atretic oocytes only could be adopted according to these characteristics. Alternatively, some studies categorize atresia into three stages-early, advanced, and late–based on morphological characteristics such as yolk degradation and hypertrophy of follicular cells.

In the present study, atretic oocytes were identified in both biopsy and histological samples, predominantly in the mature ovarian phases of *A. berda*. In the biopsy, these atretic oocytes appeared smaller than mature oocytes and were distinguished by their darker cytoplasm, disrupted or indistinct zona radiata, and reduced yolk granule content. Histological analysis identified two primary types of atresia: Alpha atretic oocytes exhibited nuclear membrane breakdown, with nuclear contents dispersing into the cytoplasm. Yolk granules and lipid droplets began to coalesce, leading to alterations in oocyte morphology. The zona radiata underwent fragmentation and degradation. Beta atretic oocytes were characterized by the complete resorption of the zona radiata and the formation of large vacuoles.

Several studies have employed biopsy and histology to investigate oocyte atresia in various fish species. Corriero *et al.* (2021) identified atresia in vitellogenic oocytes of greater amberjack (*Seriola dumerili*) using ovarian biopsy. Kurita *et al.* (2003) utilized histological techniques to highlight the significance of oocyte atresia in fecundity regulation in herring (*Clupea harengus*). Skjaeraasen *et al.* (2012) investigated the factors influencing fecundity, atresia, and skipped spawning in Northeast Arctic cod (*Gadus morhua*).

In females induced for breeding using a commercial GnRH-inducing agent, the ovary exhibited a high prevalence of atretic oocytes, indicating that it was in a regressing phase. This ovarian condition likely contributed to spawning failure despite hormonal inducement. An excessive occurrence of follicular atresia beyond normal physiological levels can significantly reduce fish fecundity and may lead to reproductive failure in both wild and captive-reared fish populations. (Corriero *et al.* 2021). Studies on fishes have shown that environmental stressors, nutrition, and hormonal treatments influence atresia rates (Murua and Motos, 2006; Skjaeraasen *et al.,* 2012).

Although atresia can be extensively studied, histological analysis is not a practical tool for real-time field identification and selection of broodstock for breeding programs. In this context, the application of ovarian biopsy for the detection of atretic oocytes could facilitate informed decision-making regarding the suitability of female fish for breeding. Additionally, detailed histological characterization of atretic oocytes could aid in developing a standardized protocol for identifying ovaries in the degenerative phase, thereby improving broodstock selection strategies.

Post ovulatory follicles

Post-ovulatory Follicles (POFs) serve as indicators of recent ovulation and can provide insights into spawning frequency and reproductive cycles (Ferreri *et al.,* 2021). The resorption rate of POFs varies among species, affecting the assessment of spawning intervals and ovarian recovery (Yoda and Yoneda, 2009). Their presence in histological sections confirms prior spawning events and helps differentiate actively spawning females from those in regression (Klibansky and Scharf, 2013).

Post-ovulatory follicles (POFs) were identified by the presence of ovarian follicles with canaliculated lumens, degenerated thecal and granulosa cells, a lightly stained basement membrane, and the absence of large vacuoles. The identification and analysis of POF ageing are critical for determining spawning intervals, post-ovulation timing (Ferreri *et al.,* 2021), and spawning frequency (Yoda and Yoneda, 2009). The rate of POF resorption varies among species and is particularly rapid in multiple spawning species, as the ovary requires space for the maturation of successive oocyte batches (Korta *et al.,* 2010).

The degeneration of POFs has been classified into three stages in seabream (*Dentex hypselosomus*) (Yoda and Yoneda, 2009) and six stages in European anchovy (*Engraulis encrasicolus*) (Ferreri *et al.,* 2021). In *A. berda*, POFs were categorized into three distinct phases: an initial phase with minimal signs of degeneration, a secondary phase characterized by a reduction in follicular size, and a final phase in which the follicles appeared shrunken and irregularly shaped.

In the present study, POFs were prominently visible in histological sections of regenerating and regressing ovaries, providing clear evidence of previous ovulation events and batch spawning. The presence of POFs in histological samples was consistent with observations in *Pagrus pagrus* (Klibansky and Scharf, 2013) and *Neogobius melanostomus* (Kalamarz-Kubiak and Guellard, 2019).

According to Nancy *et al.* (2011) and Lowerre-Barbieri *et al.* (2011), ovarian development follows a distinct sequence of reproductive phases: among these phases, the developing phase (preparatory phase) occurs before spawning and is characterized by vitellogenesis, with no POFs present. The spawning-capable phase is marked by the presence of advanced maturation stages, such as the tertiary yolk granule stage. The regressing or spent phase represents the final stage of the reproductive cycle and is characterized by a high prevalence of atretic oocytes, POFs, and a limited number of mature oocytes; this phase is typically of short duration. Following regression, the regenerating phase begins, marked by the presence of oogonia, primary germ cells, atretic oocytes, and POFs, signalling the initiation of a new reproductive cycle.

Conclusion

Ovarian biopsy and histological analysis are effective in assessing oocyte hydration, follicular atresia, and post-ovulatory follicles (POFs) in the captive mature *A. berda*. The identification of hydrated oocytes confirmed ovarian maturity, while the presence of atretic oocytes in regressing ovaries indicates potential reproductive challenges, particularly in hormonally induced females. The characterisation of POFs further provides insights into spawning history and ovarian cycling. Given its simplicity and cost-effectiveness, ovarian biopsy emerges as a valuable tool for real-time broodstock selection, while histological analysis remains essential for detailed reproductive assessments. These findings contribute to improving broodstock management and reproductive success in aquaculture.

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Author contributions

Conceptualization: PPSB; Methodology: MTS, MP, AA, PND; Writing Original Draft: PPSB, TH, Data Analysis: NR, PPSB Supervision: BI Data Collection: PND, TH, AG.

Data availability

The data are available and can be requested from the corresponding author.

Conflict of interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of sensitive samples/ protected environments.

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