



Structural Dynamics of Hydrated Oocytes in Captive Black Seabream, *Acanthopagrus berda*: Insights from Oocyte Morphology

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Abstract

This study investigates the morphology, ultrastructure, and degeneration patterns of hydrated oocytes in captive broodstock of *Acanthopagrus berda* during the breeding seasons from 2022 to 2024. Ovarian biopsy samples consistently revealed the presence of hydrated oocytes, identified as large, transparent, spherical cells that progressively degenerated when not spawned. Light microscopy and scanning electron microscopy (SEM) provided detailed insights into oocyte developmental stages, from early vitellogenic oocytes to hydrated oocytes (300-700 µm), with an emphasis on the degeneration of hydrated oocytes in female broodstock and hormone-induced females that failed to spawn. Notable surface changes included pore canal opening patterns and zona radiata folding, indicative of hydration-related structural modifications. Hydrated oocytes exhibited a smoother zona radiata with widely spaced pore openings compared to the densely pitted surface of mature oocytes. Progressive degeneration was characterised by membrane folding, shrinkage, rupture of the zona radiata, and eventual disintegration into irregular cellular debris. Similar patterns were confirmed in hormone-induced females at an inshore hatchery, where hydrated oocytes retained within the ovarian lumen failed to ovulate and underwent similar degeneration. The degeneration process was marked by cytoplasmic collapse, persistent nuclei in early

stages, and complete structural breakdown in advanced stages. The study underscores the importance of optimising hormonal treatment protocols and environmental conditions to prevent oocyte retention and to improve reproductive outcomes in marine fish broodstock management.

Keywords: Broodstock, ovarian biopsy, picnic seabream, scanning electron microscopy, zona radiata

Introduction

The coordinated processes of oocyte growth, maturation, hydration, and ovulation intricately govern reproductive success in teleost fishes. Among these, oocyte hydration marks the final stage of oocyte maturation and plays a critical role in preparing the egg for fertilisation and ensuring its buoyancy and viability in the marine environment (Lubzens, Young, Bobe, & Cerdà, 2010). In marine teleosts, hydration is characterised by rapid water influx, swelling of the oocyte, and structural reorganisation of the zona radiata to accommodate volume expansion and prepare the oocyte for spawning. The successful completion of final oocyte maturation, hydration, and ovulation is thus essential for reproductive success in both wild and captive populations.

In aquaculture systems, however, reproductive dysfunctions such as incomplete oocyte maturation, failed ovulation, and oocyte retention are frequently encountered in broodstock management. These disruptions are often linked to suboptimal environmental conditions or insufficient endocrine stimulation, leading to the degeneration of hydrated oocytes and reduced spawning efficiency (Zohar &

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Mylonas, 2001; Zupa et al., 2014). Despite the widespread application of hormonal therapies to induce spawning, the failure of hydrated oocytes to ovulate remains a significant bottleneck, particularly in species with complex reproductive physiology.

The use of ovarian biopsy has become a valuable, minimally invasive technique for monitoring gonadal development and assessing the progress of oogenesis in various teleost species (Farrell, Hüsey, Coad, Clausen, & Clarke, 2012; Papadaki et al., 2018). When coupled with high-resolution imaging tools such as scanning electron microscopy (SEM), biopsy-based analyses enable detailed characterisation of oocyte ultrastructure, including the surface morphology of the zona radiata and cytoplasmic organisation—features that are critical for understanding the functional state of oocytes during maturation and degeneration.

Acanthopagrus berda (Forsskal, 1775), commonly known as the black seabream, is a commercially important sparid species found in estuarine and coastal waters across the Indo-West Pacific. Recent advances in broodstock management and the optimisation of breeding techniques in the Indian subcontinent have demonstrated the species' strong potential for commercial-scale aquaculture (Abbas et al. 2019; Suresh Babu et al. 2022). Investigations into the reproductive biology of wild populations have shown that *A. berda* is a digynous protandrous hermaphrodite with asynchronous oocyte development (Shilta et al., 2019). Egg quality and production vary considerably in asynchronous batch spawners. Shilta et al. (2019) reported active males with a total length ranging from 14 to 25 cm and active females with total lengths ranging from 25 to 45 cm in *A. berda*. Although considerable progress has been made in understanding its reproductive biology under natural conditions, limited information is available on oocyte maturation dynamics, particularly in captive broodstock conditions. Understanding the morphological and ultrastructural changes associated with hydrated oocytes and their degeneration when retained, is essential for improving broodstock management and spawning induction protocols in fish hatcheries.

The present study investigates the morphological features, ultrastructural characteristics, and degeneration patterns of hydrated oocytes in captive *A. berda* broodstock using light microscopy and SEM. In addition, comparative analysis was performed on

hormone-induced females to assess the consistency of degenerative processes, to enhance our understanding of reproductive physiology in *A. berda*, and contribute to the refinement of spawning strategies in marine aquaculture.

Materials and Methods

The study investigated the occurrence and degeneration of hydrated oocytes in captive broodstock of *A. berda* maintained in cages. A graphical representation of the research work carried out is presented in Fig. 1. Regular ovarian biopsies and microscopic observations were conducted to identify hydrated oocytes and examine their surface morphology using scanning electron microscopy (SEM). Under hatchery conditions, hormone-induced breeding trials were performed, and daily ovarian biopsies were conducted to assess hydrated oocytes in the ovaries of unspent females.

Live broodstock of *A. berda* were collected from the Kali estuary, Karwar, and maintained in 6 m diameter floating cages at ICAR-CMFRI, Karwar. Year-wise batches included 230 (2019–2020), 223 (2020–2021), and 136 (2021–2022) individuals, weighing 205–968 g, with sex ratios ranging from 1:1.07 to 1:1.24 (male : female). Fish were initially acclimated to a diet of chopped low-value fish and subsequently transitioned to a commercial pellet feed (Growel India Pvt. Ltd) containing 40% crude protein and 10% crude lipid. Feeding was carried out at 5% of body weight per day. The diet was supplemented biweekly with a vitamin-mineral mix (1 g kg⁻¹) and cod liver oil (10 mL kg⁻¹), whereas raw squid and oyster meat were provided weekly at 3% of body weight to promote gonadal development.

Ovarian biopsies were performed using a minimally invasive catheterisation technique. Female fish (20 fish for each sampling) were anaesthetised with 0.01% clove oil to minimise stress during handling. Sterile plastic catheters (1.6–2.0 mm diameter; Ramsons Scientific and Surgical Industries Pvt. Ltd., New Delhi) were carefully introduced through the genital opening to extract ovarian tissue samples, following the method described by Suresh Babu et al. (2022). The retrieved ovarian samples (n=20) were immediately fixed in 10% neutral buffered formalin. Fixed samples were then examined under a compound light microscope (Zeiss Axio Scope A1) equipped with a Jenoptik ProgRes C3 digital camera to determine the stages of oocyte development.

For induced breeding trials, four pairs of fish (1 male and 1 female in each pair) were successfully induced to breed using the GnRH analogue injection, as per Abbas et al. (2019). Both males and females were given an initial dose of 0.25 mL kg⁻¹ fish, and the female was given the same second dose after 6 h as intramuscular injections. After a 42-h latency period, ovarian biopsy was carried out at 24-h intervals for the next 5 days.

For examining oocyte ultrastructure, selected biopsy samples were processed for SEM. Tissues were fixed in 2.5% glutaraldehyde (0.1 M sodium cacodylate buffer, pH 7.5) for 3 h, rinsed, and post-fixed in 1% osmium tetroxide for 1 h. After buffer washes, samples were dehydrated in graded acetone, dried using a Hitachi HCP2 critical point dryer, and sputter-coated with gold using a Quorum SC76220 coater. SEM imaging was performed using a Tescan VEGA3 microscope at various magnifications to analyse surface morphology as described by Suresh Babu et al. (2022).

Results and Discussion

Hydrated oocytes were consistently observed in ovarian biopsy samples (approximately 10 to 30% of the samples) collected from captive broodstock throughout the breeding season (August to February) during the assessment period of 2022–2024. The percentage of hydrated oocytes ranged from 10–70% of the total oocytes depending on the maturation stage and season. Representative photomicrographs of these biopsy samples are presented in Fig. 2a. Hydrated oocytes were identified as large, transparent, spherical oocytes, initially exhibiting a distinct nucleus. As degeneration progressed, these oocytes

displayed characteristic signs of atresia, including the breakdown of the cell membrane and disintegration of the cytoplasm. The variation in the diameter of oocytes and fertilised eggs is shown in Fig. 2b. The oocyte and egg diameter increased considerably during maturation. Late vitellogenic oocytes showed the smallest diameter (~450 µm), while hydrated oocytes expanded to ~690 µm due to yolk hydration. The fertilised eggs exhibited the largest diameter (~760 µm), indicating successful water uptake and embryonic development after fertilisation. This progressive increase in diameter reflects normal oocyte maturation, hydration, and spawning readiness in the species.

In the scanning electron microscopy (SEM) analysis (Fig. 3A–C), the vitellogenic oocytes measured between 300 and 400 µm and contained a solid spherical structure filled with granular material, indicating the presence of yolk granules. The oocytes were surrounded by connective tissues and blood vessels for nourishment. The advanced vitellogenic oocytes were dispersed, spherical structures with more liquefied contents, measuring 400–450 µm. The hydrated oocytes were large, spherical, measuring 600–700 µm, with more surface folding. Surface morphology at higher magnification was performed focusing on the vegetative pole of the oocyte (Fig. 3a-c). Early vitellogenic oocytes (Fig. 3a) displayed early pore canal formation on the zona radiata. In advanced vitellogenic oocytes (Fig. 3b), the zona radiata exhibited densely packed pore canal openings and a rough surface texture. In contrast, the zona radiata of hydrated oocytes (Fig. 3c) appeared smoother, with widely spaced pore canals and prominent surface folds, indicative of structural changes associated with hydration and imminent ovulation.

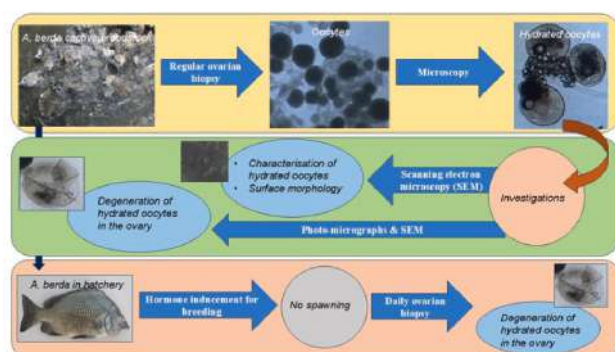


Fig. 1. A graphical representation of the research work carried out related to the morphology and degeneration of hydrated oocytes in *A. berda*

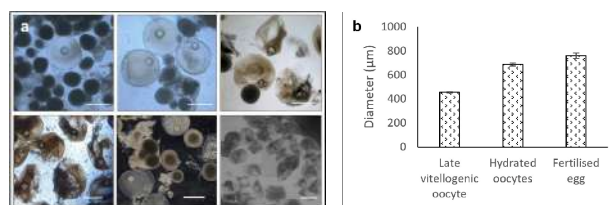


Fig. 2. a) Representative photomicrographs of the biopsy samples indicating the presence of hydrated oocytes (large transparent oocytes and their degenerating stages) collected during the assessment period 2022–24. (Scale bar = 500 µm). b) Variation in the diameter of oocytes and fertilised eggs

During broodstock management, the water quality parameters such as salinity (27–32 ppt), temperature (27–32 °C), pH (7.6–8.2), dissolved oxygen (4.0–4.8 mg L⁻¹) and total ammonia nitrogen (0.01–0.05 mg L⁻¹) remained within ranges conducive to maintaining the broodstock.

Progressive stages of hydrated oocyte degeneration were evaluated through both light microscopy and SEM. Photomicrographs of advanced vitellogenic oocytes (Fig. 4a), along with various stages of hydrated oocyte degeneration (Fig. 4b–e), were compared with corresponding SEM images of advanced vitellogenic oocytes (Fig. 4f) and degenerating oocytes (Fig. 4g–j). SEM analysis corroborated the morphological changes observed under light microscopy, clearly delineating the sequence of degeneration. Following hydration, spherical oocytes exhibited initial shrinkage and folding of the zona radiata. As degeneration progressed, oocytes transformed into cup-like structures with pronounced aberrations on the zona radiata. Ultrastructural analysis revealed rupture of the oocyte membrane (Fig. 4i), likely facilitating the release of cytoplasmic contents into the surrounding ovarian lumen—an observation consistent with the light micrographs (Fig. 4d). The mean thickness of the zona radiata in aberrant hydrated oocytes was measured at $1.95 \pm 0.062 \mu\text{m}$. In advanced stages, hydrated oocytes were reduced to irregular, folded debris, indicating complete degradation.

Ovarian biopsy samples collected at 24-hour intervals from four hormone-induced females during the spawning period are depicted in Fig. 5. Observations between 46 and 120 h post-induction revealed

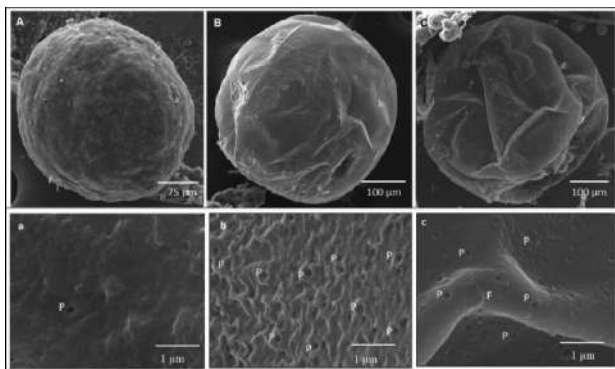


Fig. 3. Ultrastructure of the (A) early vitellogenic, (B) advanced vitellogenic, and (C) hydrated oocytes and their corresponding surface morphology (a, b and c respectively)

progressive degeneration of hydrated oocytes, consistent with the degeneration patterns recorded in biopsies from captive broodstock. These results confirm that hydrated oocytes retained in the ovary, if not ovulated or spawned, undergo a distinct degenerative process that leads to structural disintegration and resorption. Water quality during the hormone-induced breeding trials remained within suitable ranges for spawning, with salinity of 31–33 ppt, temperature of 28–30 °C, pH of 7.8–8.2, dissolved oxygen of 4.5–5.2 mg L⁻¹, and ammonia of 0.003–0.01 mg L⁻¹.

In marine pelagophilic fishes, oocyte maturation is characterised by a substantial increase in egg volume (approximately 67–75%) primarily due to osmotic water uptake (Cerdà, Fabra, & Raldúa, 2007). This hydration process enhances egg buoyancy and provides an internal water reserve, facilitating embryo survival and dispersal in the hyperosmotic marine environment, with water content comprising up to 90–95% of the egg's total weight (Lubzens et al., 2010). This process is termed oocyte hydration. Aquaporins play a vital role in oocyte hydration in fish by facilitating rapid water transport across the oocyte membrane during final maturation (Cerdà, 2009). Increased aquaporin expression enhances water uptake by the oocyte, causing swelling and the formation of hydrated eggs needed for buoyancy and successful fertilisation. This hydration process is regulated by hormones and osmotic changes associated with yolk protein breakdown, ensuring proper egg quality and embryonic development in teleosts (Fabra et al., 2006).

Ovarian biopsy has been established as a reliable, minimally invasive method for monitoring repro-

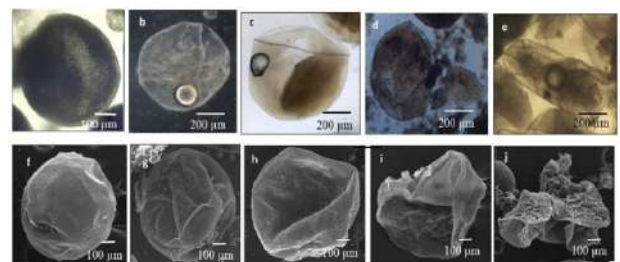


Fig. 4. Photomicrographs of (a) advanced vitellogenic oocyte and various stages of degenerated hydrated oocytes (b – e) and the corresponding representative scanning electron micrographs for advanced vitellogenic oocyte (f) and degenerated hydrated oocytes (g – j) observed in the biopsy samples

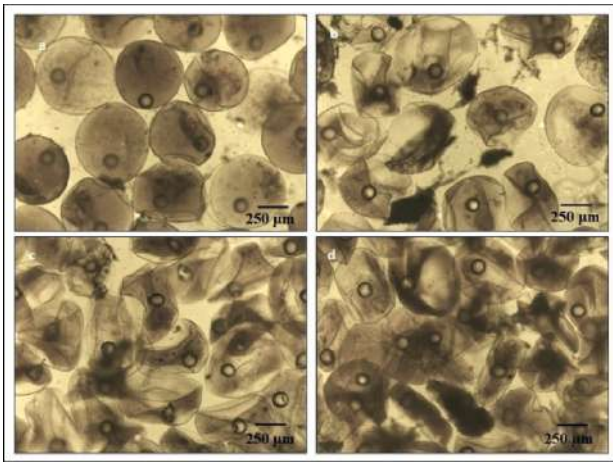


Fig. 5. Photomicrographs of biopsy samples taken at 24 h intervals from an unspent female: (a) hydrated oocytes with initiation of degeneration 48 h after induction; gradual degradation of the hydrated oocytes at (b) 72 h, (c) 96 h and (d) 120 h

ductive status and assessing the presence of hydrated oocytes in marine teleosts (Finn, Østby, Norberg, & Fyhn, 2002; Farrell et al., 2012; Papadaki et al., 2018). The current findings in *A. berda* align with these earlier reports, validating biopsy as an effective tool for reproductive assessment.

The present study elucidates the structural and morphological dynamics of hydrated oocytes in *A. berda* using light and scanning electron microscopy (SEM), with particular emphasis on their development, hydration, and subsequent degeneration. The consistent detection of hydrated oocytes in ovarian biopsy samples from captive broodstock over successive breeding seasons (August–February) highlights prolonged reproductive readiness under controlled conditions. However, the failure of these oocytes to ovulate or spawn, followed by their degeneration, underscores potential physiological and endocrine constraints in captivity that require further study.

Photomicrographs of routine biopsy samples clearly revealed the hydration of advanced vitellogenic oocytes and their retention in the ovary in *A. berda*. SEM analysis clearly distinguished the surface morphology of the oocyte membrane among early vitellogenic, advanced vitellogenic, and hydrated oocytes. Early vitellogenic oocytes showed a granular cytoplasm and solid structure, while advanced vitellogenic oocytes exhibited liquefaction and increased diameter. Hydrated oocytes displayed

further enlargement with prominent surface folding, indicating increased turgor pressure and readiness for spawning. Notably, the surface morphology of the zona radiata changed progressively from dense pore canal openings in advanced vitellogenic oocytes to smoother, sparsely pitted surfaces in hydrated oocytes. Such modifications are attributed to mechanical stretching during hydration, leading to an increase in oocyte volume, as previously reported in other teleosts (Cerdà et al., 2007; Lubzens et al., 2010). These pore canal opening arrangements are known to be species-specific and play a functional role in oocyte-environment interaction and fertilisation. Kaviani, Shabanipour, and Mirnategh (2013) reported pit-like remnants on the surface of advanced vitellogenic Zebrafish oocytes. Kim, Lee, Ju, Kim, and Lee (2011) reported pits as concentric circles around the micropyle in *Epinephelus fasciatus*. Weber, Sato, Santos, Rizzo, and Bazzoli (2012) reported oocyte surfaces with pore canals, covered by a thick jelly coat. Perini, Sato, Rizzo, and Bazzoli (2013) described distinct variations in pit patterns on the oocyte surface between the two fish species *Curimatella lepidura* and *Steindachnerina elegans* in the future vegetative pole region. The distinct morphology of hydrated oocytes may serve as an identifying characteristic for recognising the oocytes of this species in the wild.

Degeneration of hydrated oocytes was characterised by distinct morphological transitions (initial shrinkage, membrane folding, zona radiata collapse, and eventual disintegration into irregular debris). This degeneration was observed not only in broodstock but also in hormone-induced females, suggesting that retained hydrated oocytes undergo a conserved pattern of deterioration when ovulation is unsuccessful. Light microscopy and SEM collectively revealed the gradual breakdown of cytoplasmic integrity, collapse of oocyte structure, and eventual resorption, consistent with the “egg-retaining condition” described in other marine species (Zupa et al., 2014).

Natural spawning in captive enclosures such as marine cages has been reported in several commercial fish species, allowing fertilised eggs to escape and disperse into the natural environment. This phenomenon has been termed “escape through spawning”. Farming fish to sexual maturation and spawning can therefore lead to the escape of fertilised eggs, or “escape through spawning” (Uglen et al., 2012). Escape through spawning has

been associated with environmental implications that affect the genetic variability of wild stocks, predation, and spread of diseases (Thorstad et al., 2008; Jensen, Dempster, Thorstad, Uglem, & Fredheim, 2010). This phenomenon has been reported in commercial fishes such as Atlantic cod (Uglem et al., 2012), bluefin tuna (Medina et al., 2016), salmon and trout (Jensen et al., 2010). Recently, the escape of fertilised eggs from captivity has been reported in gilthead sea bream *Sparus aurata* (Somarakis, Pavlidis, Saapoglou, Tsigenopoulos, & Dempster, 2013). The presence of hydrated oocytes in ovarian biopsy samples of *A. berda* therefore raises the possibility of the occurrence of “escape through spawning” in this species also.

Spawning failure in marine fish under captive conditions arises from a complex interplay of physiological, environmental, and management-related factors. A primary cause is disruption of the hypothalamic–pituitary–gonadal (HPG) axis, leading to incomplete oocyte maturation or failed ovulation due to insufficient luteinizing hormone or maturation-inducing hormone release (Zohar & Mylonas, 2001; Mylonas, Fostier, & Zanuy, 2010). Environmental misalignment, such as suboptimal temperature, photoperiod, or salinity, further disrupts reproductive timing (Pankhurst & Munday, 2011). Nutritional deficiencies, particularly in essential fatty acids and vitamins, impair gamete quality and steroidogenesis (Izquierdo, Fernández-Palacios, & Tacon, 2001). Stress from handling or poor water quality elevates cortisol, thereby suppressing reproductive hormones (Pankhurst, 2011). Additionally, improper hormonal induction protocols and poor broodstock conditioning contribute to egg retention and degeneration (Zupa et al., 2014). Behavioural and social factors, such as skewed sex ratios or the absence of spawning stimuli, also affect successful spawning (Mylonas, Zohar, Pankhurst, & Kagawa, 2011). Addressing these interconnected factors is essential for improving reproductive success in marine aquaculture.

Ovarian contraction plays a crucial role in facilitating oocyte transit from the ovary to the oviduct and subsequently to the external environment. Spontaneous contractions of the ovarian wall have been documented across multiple teleost families and are thought to be driven by smooth muscle layers within the tunica albuginea or theca cells (Bills, Callaway, Kessler, & Taylor, 2008; Piccinno et al.,

2014). These contractions are not limited to ovulation but may serve to mix ovarian fluid, reposition oocytes, or assist in post-ovulatory follicular regression (Bartley, Kippax, & Taylor, 1992; Zupa et al., 2014). In species such as the mummichog (*Fundulus heteroclitus*), contractions have been shown to move ovulated oocytes posteriorly (Bartley et al., 1992). Dysfunction of this mechanism may result in the retention of hydrated oocytes in the ovary and oviduct, leading to spawning failure.

The persistence of hydrated but unspawned oocytes in captive broodstock highlights a key reproductive bottleneck. In wild settings, oocyte hydration is tightly coupled with ovulation, ensuring timely release and external fertilisation. However, in captive systems, disjunctions between hormonal signalling and mechanical ovulatory responses, such as ovarian contraction, may impair this process. Factors contributing to this impairment may include inadequate environmental cues (e.g., photoperiod, salinity, substrate), suboptimal broodstock conditioning, or inefficient hormone therapy that fails to fully stimulate the hypothalamic–pituitary–gonadal (HPG) axis.

The observed degeneration of oocytes, particularly the formation of cup-like structures and the breakdown of the zona radiata, underscores the structural vulnerability of hydrated oocytes when retained post-hydration. Furthermore, the prolonged presence of degenerating oocytes within the ovary can negatively affect subsequent reproductive cycles by inducing local inflammation or interfering with follicular recruitment (Corriero, Zupa, Mylonas, & Passantino, 2021).

The present study provides critical insights into the structural and morphological dynamics of hydrated oocytes in *A. berda* under captive conditions. The consistent presence, followed by the ultimate degeneration, of unspawned hydrated oocytes indicates a significant reproductive bottleneck associated with impaired ovulatory mechanisms. Scanning electron microscopy revealed progressive ultrastructural changes during oocyte development, hydration, and degeneration, highlighting the vulnerability of retained oocytes. These findings suggest that although oocyte hydration is achieved, spawning failure may result from disruptions in hormonal signalling, environmental cues, or broodstock conditioning that prevents the initiation of ovulation. The occurrence of post-hydrated

oocytes (PHOs) also raises the possibility of escape through spawning in this species. Degenerating oocytes may compromise ovarian health and affect future spawning cycles. Therefore, optimising environmental parameters and refining induced breeding protocols are essential for improving spawning success and reproductive efficiency in captive seabream broodstock management.

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Ethical Statement

All procedures involving the handling and experimentation of live animals in this study adhered to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for fish experimentation, issued by the Department of Animal Husbandry and Dairying, Government of India. The experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee of ICAR-Central Marine Fisheries Research Institute (CMFRI), Kochi, under Project code MDN/HYD/18.

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