

Photoperiod-Mediated Synchronization of Reproductive Hormones, Follicular Development and Calcium Homeostasis in Common Carp (*Cyprinus carpio*)

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Abstract

Photoperiod is a major environmental regulator of biological rhythms and reproductive processes in teleost fishes. The present study evaluates the effects of altered light–dark regimes on neuroendocrine activity, ovarian development, and calcium homeostasis in the Indian major carp Cyprinus carpio. Adult fish were maintained for 120 days under five photoperiodic conditions: control (L12:D12), long photoperiod (L16:D8), short photoperiod (L6:D18), constant light (LL), and constant darkness (DD). Circulating levels of estradiol, progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and growth hormone (GH) were assessed in relation to gonadosomatic index (GSI), ovarian follicular dynamics, and plasma calcium levels.

Fish exposed to the long photoperiod (L16:D8) exhibited significantly elevated estradiol and progesterone levels, increased GSI, and a higher proportion of vitellogenic follicles, indicating enhanced ovarian maturation and reproductive activity. In contrast, short photoperiod and continuous darkness resulted in suppressed gonadotropin secretion, reduced GSI, and increased follicular atresia, reflecting inhibition of the reproductive axis. Constant light and constant darkness disrupted endocrine balance and reproductive homeostasis, suggesting circadian desynchronization under extreme photic conditions. Variations in plasma calcium levels across photoperiod treatments further highlight the association between light regulation, endocrine function, and mineral metabolism.

These findings demonstrate that appropriate photoperiod manipulation, particularly long-day regimes, can effectively enhance reproductive performance in C. carpio. The study provides practical insights for the application of chronobiological principles in aquaculture to improve broodstock management and breeding efficiency.

Keywords: Photoperiod; Reproductive endocrinology; Ovarian development; Gonadosomatic index; Follicular dynamics; Calcium homeostasis; Aquaculture management

1. Introduction

Environmental light is a primary synchronizer of biological rhythms and is crucial for coordinating endocrine and metabolic processes in vertebrates (Refinetti, 2016). In teleost fishes, photoperiod-driven entrainment of circadian and seasonal timing systems regulates the hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–interrenal (HPI) axes, thereby integrating reproduction, energy allocation, and stress physiology (Bromage *et al*., 2001; Falcon *et al*., 2010).

In most species, periodic events such as gonadal growth and spawning are aligned with predictable daily and annual environmental changes, particularly photoperiod and temperature, allowing anticipation and optimisation of reproductive timing (Falcon and Munoz-Cueto, 2024). Accordingly, manipulation of light regimes in captivity can advance, delay, or suppress maturation depending on the species and the photic schedule applied.

Photic information is detected primarily by the retina and the pineal gland and is translated into endocrine signals via rhythmic melatonin secretion (Zahangir *et al*., 2014). Melatonin, in turn, modulates downstream neuroendocrine pathways that govern gonadotropin-releasing hormone (GnRH) and pituitary gonadotropins, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thereby influencing gametogenesis, vitellogenesis, and ovarian maturation (Migaud *et al.,* 2010).

Calcium also contributes to reproductive function by acting as an intracellular second messenger in hormone synthesis, receptor activation, and follicular growth, linking rhythmic signalling with metabolism and gonadal activity (Faulkner and Holt, 2009). However, compared with endocrine endpoints, the coupling between photoperiod-driven hormonal rhythms and calcium regulation has received limited attention in cultured teleosts, despite its relevance to oocyte development and overall physiological homeostasis.

With increasing demand for fish protein, hatcheries and farms require practical approaches to improve broodstock performance, spawning efficiency, and resilience to stress-induced reproductive failure. Photoperiod manipulation is a non-invasive and environmentally sustainable management tool that can be incorporated into routine aquaculture operations. Therefore, the present study evaluates the effects of altered photoperiods on hormonal profiles, gonadosomatic index (GSI), ovarian follicular dynamics, and plasma calcium in *Cyprinus carpio,* to generate applied insights for light-based broodstock management.

2. Materials and methods:

Adult Indian common carp (*Cyprinus carpio*) were collected on 17 January 2024 from Shyagale, Davangere, Karnataka, India, and transported to the laboratory for acclimatization. Fish were maintained for 20 days in glass aquaria (50 L) containing non-chlorinated water and were prophylactically treated with chloramphenicol. During acclimatization, fish were fed a balanced diet ad libidum.

After acclimatization, fish with an average total length of 14 ± 1 cm and body weight of 41 ± 1 g were selected for the experiment. Fish were randomly assigned to five photoperiod treatments (n = 8 fish/group): control (L:D 12:12), short photoperiod (L:D 06:18), long photoperiod (L:D 16:08), constant light (LL; L:D 24:00), and constant darkness (DD; L:D 00:24). Each group was maintained in a separate aerated chamber equipped with an automated light on/off system controlled by a chronometer. Water was changed daily, and continuous aeration was provided to maintain adequate dissolved oxygen.

At the end of the experimental period, fish were sacrificed and processed separately for hormone assay

and ovarian histology. For hormone analysis, four fish per group with comparable body weight were sacrificed; the whole body was homogenized in methanol, and the homogenate was centrifuged at 3000 rpm for 12 min. The resulting supernatant was collected for hormone estimation. For histological analysis, ovaries were dissected, blotted, weighed using an electronic balance, and fixed immediately in Bouin's fixative. Tissues were subsequently processed and embedded in paraffin wax.

Paraffin sections (5 µm) were prepared and stained with haematoxylin and eosin. Follicular kinetics were assessed from 40 slides per fish (10 from the anterior, 20 from the middle, and 10 from the posterior region of the ovary) in four fish per group. Follicles were classified as previtellogenic follicles (PVF), vitellogenic follicles (VF), and atretic follicles (AF).

Data were analyzed using one-way analysis of variance (ANOVA). The gonadosomatic index (GSI) was calculated for each fish using the formula: GSI = (ovarian weight/body weight) × 100, and mean values were compared among photoperiod treatments.

3. Results:

Hormone assays showed that the short photoperiod (L:D 06:18) increased growth hormone (GH) to 0.05 ng/mL, whereas continuous darkness (DD; L:D 00:24) inhibited GH. In contrast, the long photoperiod (L:D 16:08) and continuous light (LL; L:D 24:00) did not markedly alter GH levels. Follicle-stimulating hormone (FSH) decreased significantly under DD ($P < 0.05$; 1.23 mIU/mL) and showed a slight reduction under the short photoperiod, while L:D 16:08 and LL did not appreciably change FSH. Luteinizing hormone (LH) was significantly reduced in DD ($P < 0.05$; 0.77 mIU/mL) and under the short photoperiod; LH was also lower under L:D 16:08 and LL, but the reduction was less pronounced than in DD. Estradiol (E2) declined sharply under the short photoperiod (64.5 pg/mL) compared with all other groups. Relative to the control (L:D 12:12), E2 was reduced in both LL and DD but increased markedly under L:D 16:08 ($P < 0.05$; 1336 pg/mL). Progesterone increased under both the short and long photoperiods ($P < 0.05$; 0.49 ng/mL and 0.40 ng/mL, respectively), whereas DD caused a significant decline (0.26 ng/mL). Continuous light also increased progesterone relative to the control, but to a lesser extent than L:D 16:08 or L:D 06:18.

Gonadosomatic index (GSI) values indicated that DD and the short photoperiod exerted an inhibitory effect on the reproductive axis in *C. carpio* ($P < 0.05$; 9.22 ± 0.03 and 4.56 ± 0.01, respectively). By contrast, extending the light phase by 4 h (L:D 16:08) had a stimulatory effect and produced the highest GSI (11.90 ± 0.01), whereas continuous light did not markedly alter GSI compared with the control.

Follicular kinetics showed that fish maintained under the long photoperiod exhibited a significant increase in both previtellogenic follicles ($P < 0.05$; PVF; 2084 ± 5) and vitellogenic follicles ($P < 0.05$; VF; 1972 ± 3), whereas atretic follicles (AF) were almost absent in this group. In contrast, the short photoperiod produced a reversed pattern, with a significant increase in AF ($P < 0.05$; 38 ± 1) and reductions in VF (916 ± 3) and PVF (1430 ± 2). Complete absence of light (DD) caused a marked reduction in PVF (804 ± 3) and VF (1120 ± 2), while AF increased significantly ($P < 0.05$; 48 ± 5), indicating that light availability is critical for maintaining ovarian follicular development in this species.

Plasma calcium varies across photoperiod treatments. Long photoperiod (L16:D8) and continuous light (LL; L24:D0) showed lower calcium levels ($P < 0.05$; 1.2 mg/dL and 1.5 mg/dL,

respectively) compared with the control (2.3 mg/dL), suggesting that extended light exposure may suppress calcium homeostasis. In contrast, the short photoperiod (L6:D18) and continuous darkness (DD; L0:D24) showed higher calcium levels ($P < 0.05$; 2.9 mg/dL and 2.9 mg/dL), indicating that reduced light exposure may enhance calcium retention and/or alter calcium metabolism.

4. Discussion

The endocrine responses observed in this study support photoperiod as a primary environmental cue regulating reproductive physiology in *Cyprinus carpio*. The marked elevation of estradiol and progesterone under the long photoperiod (L:D 16:08) suggests activation of the hypothalamic–pituitary–gonadal (HPG) axis, as widely reported for teleost fishes (Bromage *et al*., 2001; Falcon *et al*., 2010). Prolonged light exposure may reduce the duration and/or amplitude of nocturnal melatonin secretion, thereby modifying hypothalamic signaling to gonadotropin-releasing hormone (GnRH) neurons and pituitary gonadotropin release. In turn, changes in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) support enhanced steroidogenesis and ovarian maturation, consistent with the hormone profile recorded in the present study.

Similar photoperiodic effects have been documented in salmonids such as *Oncorhynchus mykiss* and *Salmo salar*, where day length extended advanced gonadal development through melatonin-mediated modulation of gonadotropin secretion (Migaud *et a*l., 2010). The comparable response in *C. carpio* indicates that the brood stock of this species can be effectively managed using controlled light regimes to promote maturation under captive conditions.

In contrast, extreme lighting conditions (constant light, LL; and constant darkness, DD) are expected to disturb normal circadian organization and melatonin rhythmicity, which can impair downstream endocrine signaling (Falcon *et al*., 2010). In the present study, suppression of gonadotropins and sex steroids under LL and especially DD coincided with poorer ovarian condition, suggesting disruption of reproductive homeostasis under these regimens. Such effects may be accompanied by activation of the stress axis (HPI) and oxidative challenges that favour follicular degeneration; however, targeted measurements (e.g., cortisol and oxidative stress markers) would be required to confirm these mechanisms.

The adverse endocrine and reproductive patterns observed under LL and DD agree with earlier reports in *Danio rerio* and *Oreochromis niloticus*, where non-natural lighting schedules altered hormonal profiles and reduced reproductive output (Zahangir *et al.*, 2014). Together, these findings emphasize that maintaining an appropriate light–dark cycle is important for reproductive stability in cultured fishes.

Histological observations and follicular kinetics corroborated the endocrine data and confirmed that ovarian development in *C. carpio* is photoperiod dependent. The predominance of vitellogenic follicles under L:D 16:08 indicates active yolk deposition and follicular recruitment, consistent with elevated estradiol and higher GSI. Conversely, the increase in atretic follicles under short photoperiod and particularly under DD reflects impaired follicular progression and increased degeneration when photic cues are reduced or absent.

The concordance between follicular composition (PVF, VF and AF) and circulating hormone levels indicates that photoperiod-mediated endocrine shifts are closely linked to ovarian outcomes. Similar relationships between photoperiod, steroid profiles, and ovarian dynamics have been reported in other teleosts (Renuka and Joshi, 2012), supporting the use of follicular kinetics as a sensitive indicator of reproductive status under captive lighting regimes.

Plasma calcium also varied with photoperiod, indicating an association between light regime and mineral homeostasis. In the present study, calcium levels were lower under extended illumination (L:D 16:08 and LL) and higher under reduced illumination (L:D 06:18 and DD). Calcium is a key regulator of cell signalling and contributes to steroidogenic activity and oocyte maturation through calcium-dependent pathways (Faulkner and Holt, 2009). Although the direction of change suggests that altered lighting can modify calcium balance, additional analyses (e.g., dietary intake, osmoregulatory status, and calcium fluxes) are required to clarify whether these shifts reflect changes in uptake, retention, or

metabolic utilization during reproduction.

Overall, the results highlight practical opportunities for brood stock management in aquaculture. Under captive conditions, long-day photoperiods (L:D 16:08) promoted endocrine activation, higher GSI, and vitellogenic follicle development, whereas short photoperiod and continuous darkness were associated with endocrine suppression and increased follicular atresia. Therefore, optimization of photoperiod (and, where applicable, spectral composition) may provide a non-invasive approach to improve maturation and spawning preparedness while minimizing the reproductive disruption associated with extreme or inappropriate lighting schedules.

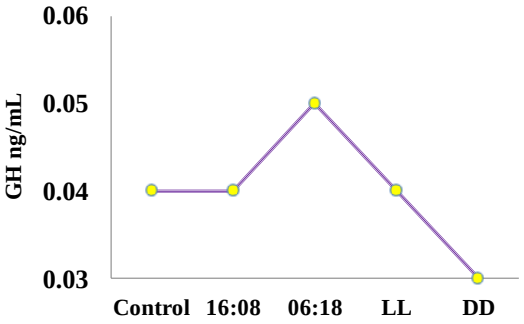
In conclusion, photoperiod manipulation markedly influenced reproductive endocrinology, ovarian maturation, follicular dynamics, and calcium homeostasis in *Cyprinus carpio*. The long-day regimen (L:D 16:08) was the most favorable condition, showing enhanced estradiol and progesterone levels, higher GSI, and predominance of vitellogenic follicles, indicating accelerated ovarian development and reproductive readiness. In contrast, short photoperiod and especially continuous darkness suppressed gonadotropic and steroidogenic activity and were associated with increased follicular atresia, demonstrating that reduced or absent photic cues disrupt reproductive homeostasis. Photoperiod-dependent shifts in plasma calcium further suggest a close coupling between light-driven endocrine regulation and mineral metabolism during ovarian development. Collectively, these findings provide an applied basis for using optimized photoperiod schedules—particularly L:D 16:08—as a practical, non-invasive tool to improve brood stock performance and spawning preparedness in carp hatcheries. Future studies integrating melatonin, cortisol, and oxidative stress markers, together with fertilization and hatchability outcomes, will strengthen the translation of chronobiological light management into reliable aquaculture breeding protocols.

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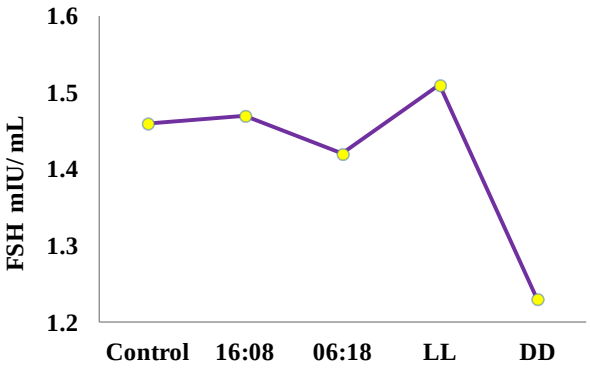
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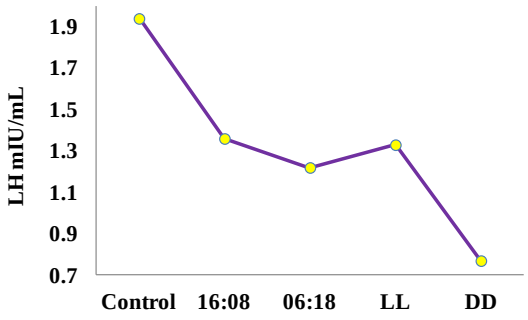
Graph-I: Hormone profile in fish exposed to varied photoperiod (1) GH, (2) FSH, (3) LH, (4) Estrogen, (5) Progesterone, (6) calcium, (7) GSI, (8) vitellogenic and pre-vitellogenic follicles, (9) atretic follicles.



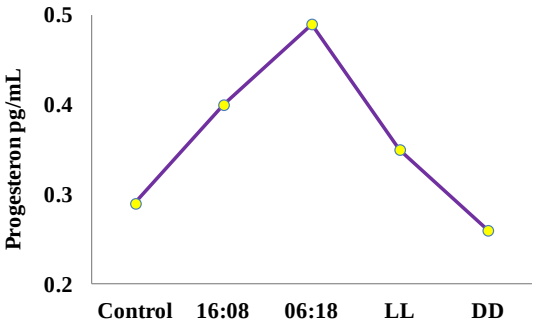
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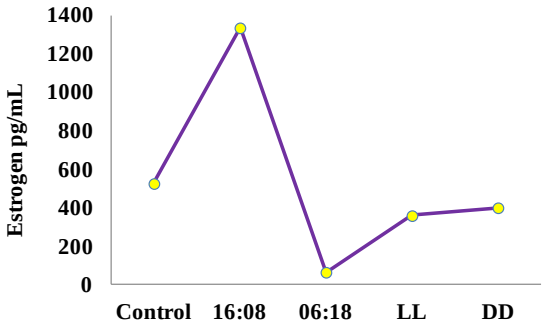
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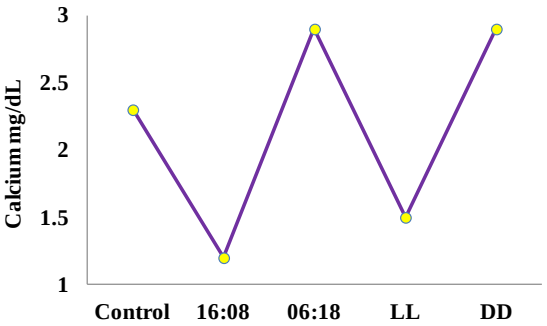
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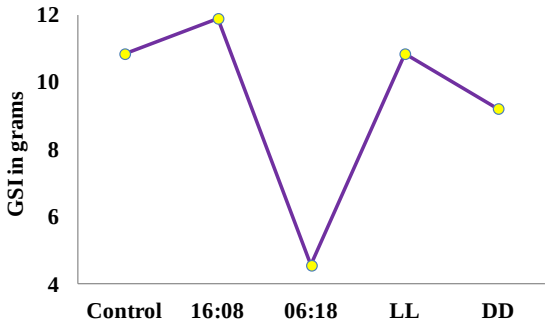
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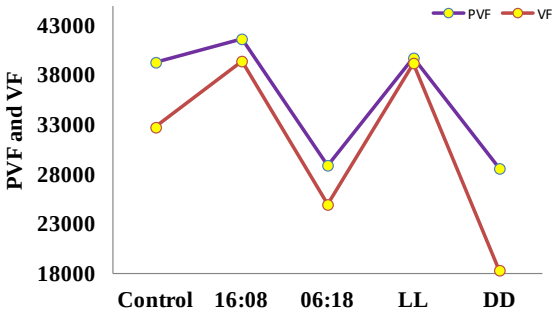
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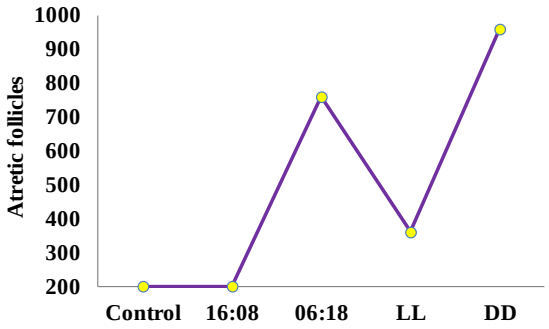
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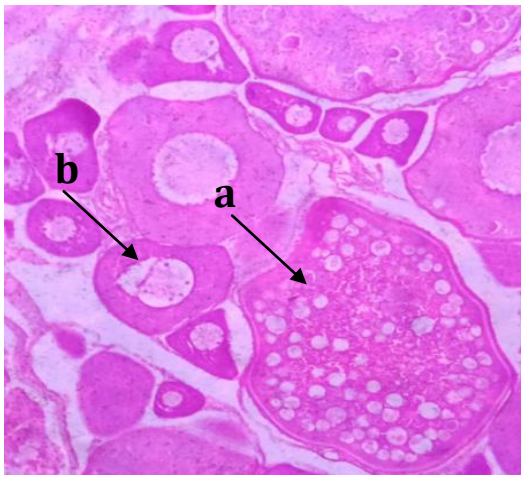
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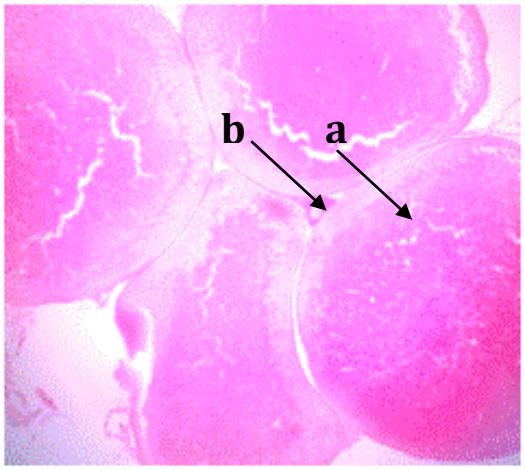
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Legend to graph 1: Hormone profile, serum calcium and follicular dynamics in fish exposed to varied photoperiod (1) GH, (2) FSH, (3) LH, (4) Estrogen, (5) Progesterone, (6) calcium, (7) GSI, (8) vitellogenic and pre-vitellogenic follicles, (9) atretic follicles.

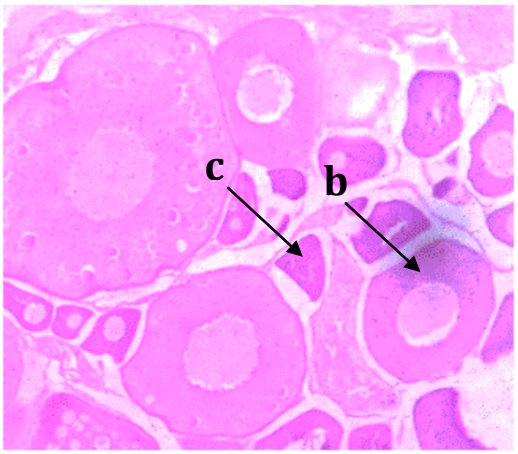
Figure-I: Follicles observed in fishes exposed to varied photoperiodic conditions and photic alterations



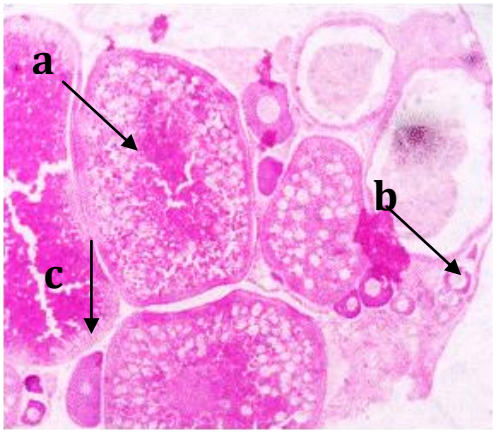
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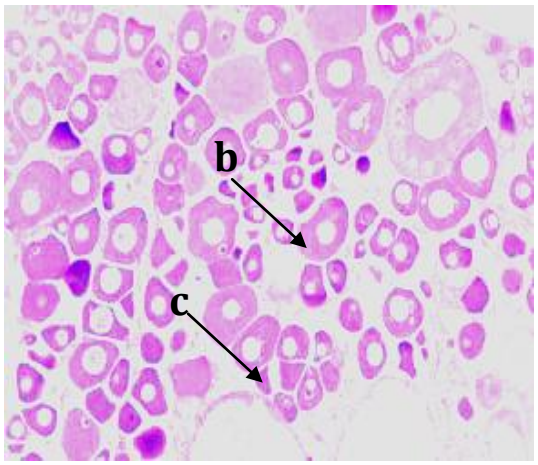
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Legend to figure-1: Follicles observed in fishes exposed to varied photoperiodic conditions and photic alterations (1) L:D 12:12, (2) L:D 16:08, (3) L:D 06:18, (4) LL, (5) DD

Abbreviations: (a) Vitellogenic follicles, (b) Pre-Vitellogenic follicles, (c) Atretic follicles.