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EXPOSURE UNDER CHOLINE CHLORIDE EXHIBITS SUCCESSFUL GONADAL MATURATION OF INDIAN MAJOR CARPS AND AIR-BREATHING TELEOSTS IN A SEMI-INTENSIVE PISCICULTURE SYSTEM: A HISTOTECHNOLOGICAL INTROSPECTION

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Abstract

Surveillance under direct field-pond application of choline chloride in addition to farm-made-aqua-feed under semi-intensive culture system was investigated on the gonadal maturity in two Indian Major Carps *Catla catla* (Catla) and *Labeo rohita* (Rahu) and in two air-breathing teleosts, e.g., *Clarias batrachus* (Magur) and *Anabas testudineus* (Koi) reared in a ratio of 2:5:1:1:: Catla:Rahu:Magur:Koi for a period of 90-d both during dry [November to January as control-dry (CD) and treatment-dry (TD)] and in breeding seasons [June to August as control-breeding (CB) and treatment-breeding (TB)]. Results were compared with control [C: pond (C) fed only with farm-made-aqua-feed] and treatment [T: ponds (P1 and P2) fed with farm-made-aqua-feed plus feed-grade choline chloride]. The histological observations of ovary under control condition in both the seasons (CD and CB) depicted the follicular layer separation, follicular atresia, resulting into non-fertile oocytes, and ovarian tissue necrosis, declination of yolk granules, while under choline supplementation in both the seasons (TD and TB), the fish species showed ripe and developed oocytes resulting into excellent reproduction performance and steroidogenesis as well as ovulation especially in breeding season. Besides, the exposure of choline (TB) has improved manifolds in the seminiferous tubules of testis of the experimental fish species with the development of increased sertoli cells, development of mature spermatozoa within the epidermis resulting into successful maturation of the sperm and occurrence of better sperm quality having increased motility especially in the breeding season. Finally, choline can trigger the successful ovarian maturation depicting better yield, causing substantial profit to fish farmers.

Keywords: Indian major carps, air-breathing teleosts, semi-intensive culture, choline chloride, ovary, testis

Introduction

Choline, a rediscovered vitamin B_4 that mostly exists in the form of phospholipids, plays a critical role in several biological functions. It is essential for building and maintaining the cell membranes and organelles, such as mitochondria and microsomes, and is also needed for normal maturation of the cartilage matrix of the bone (Calderano et al., 2015). It is also an essential component of acetylcholine, involved in the transmission of nerve impulses across synapses (Wauben & Wainwright, 1999). The distinguished structural feature is the presence of biologically active methyl groups labile as a methyl donor in the formation of methionine from homo-cysteine after being oxidized to

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betaine (Zhang et al., 2012). It is a lipotropic factor preventing the abnormal accumulation of lipid and development of fatty liver (Halver, 2002). Unlike other vitamins, choline can be synthesized through *de novo* synthesis, and its deficiency resulting in growth retardation and perosis in developing animals; moreover, the bioavailability of native choline varies largely and its abundance is also restricted (NRC, 1994; Ghazalah, 1998; Workel et al., 2002), while, a substantial amount of choline in the animal diets results in hygroscopicity, acceleration of oxidative loss of vitamins, and the formation of trimethyl amine (TMA) in the gastrointestinal tract of the animals (Zeisel et al., 1989). Direct application of choline chloride into the pond water under a semi-intensive culture system resulted into increased yield, quality food-fish comprising less fat in liver and muscle, decreased muscular cholesterol and finally making a conducive aquatic body for sustainable aquaculture (Das et al., 2020, 2021, 2022).

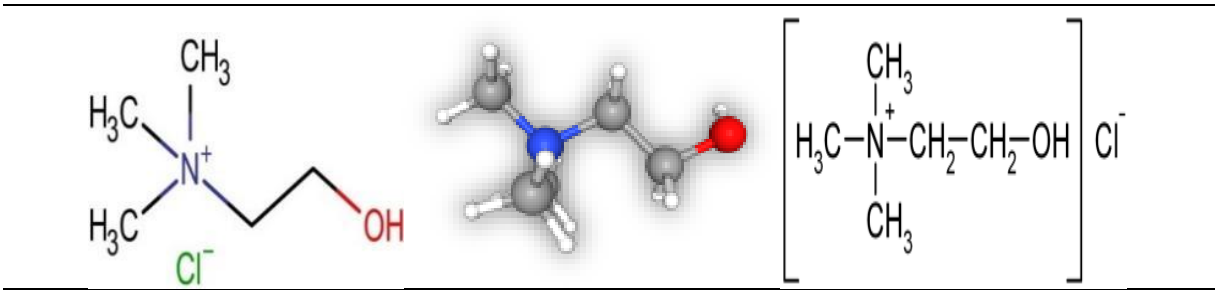

Figure 1. The Chemical structure of choline chloride (Sheard & Zeisel, 1989)(3D structure of choline chloride: iStock by Getty images).

So, there are number of studies on dietary supplementation of choline on growth and development of all animals starting from human to fish, e.g., in yellow perch (*Perca flavescens*) (Twibell & Brown, 2000), in juvenile cobia (*Rachycentron canadum*) (Mai et al., 2009), in channel catfish (*Ictalurus punctatus*) (Zhang & Wilson, 1999), in lake trout (*Salvelinus namaycush*) (Ketola, 1976), in juvenile Atlantic salmon (*Salmo salar* L.) and in juvenile Jian carp (*Cyprinus carpio*) (Wu et al., 2011) etc.

Interestingly, study on gonadal maturation due to the direct application of choline into the pond water is scanty, so, the present work had been framed to meet up this research gap for not only exploring the gonadal maturation but also the upgradation of reproductive quality of experimental fish species under the choline exposure into a semi-intensive aquaculture condition by using histotechnology.

Materials and Methods

Experimental design

The experiment was conducted under field conditions in two seasons (Dry: November to January and Breeding: June to August) for the period of ninety days in three semi-intensive ponds, where the experimental fish species (*Catla catla, Labeo rohita*, *Clarias batrachus* and *Anabas testudineus* reared in a ratio of 2:5:1:1) in the two ponds (P1 and P2) were treated with choline chloride along with 'farm-made aqua-feed, in short, 'farm-feed' and another pond was considered as control (C: fed only with 'farm-feed'). The experimental diet, study area, culture pond preparation, procurement of fish species, culture procedure, and the dose of choline chloride (feed grade: 98 % pure) directly into the treatment ponds had been followed as described earlier (Das et al., 2021, 2022). While, the analytical part of the present study was carried out in the Eco-toxicology laboratory, the University of Burdwan, Burdwan, West Bengal, India. Moreover, the present experimental protocol has also been summarized in the Figure 2.

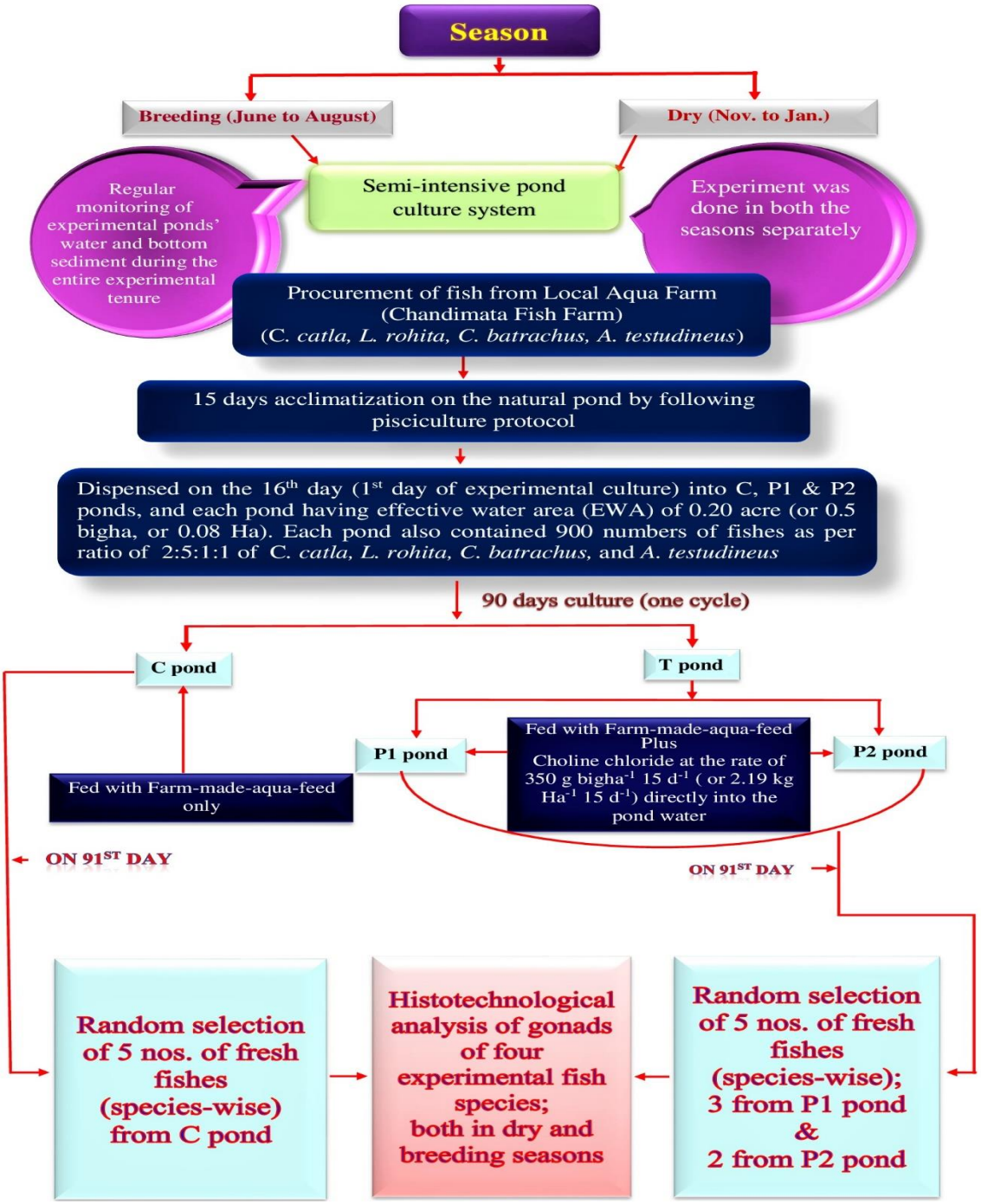

Figure 2. Experimental design at field.

Sampling

After the end of the experiments in both the seasons the experimental fish species both from the treatment and control ponds were taken after hauling and selected randomly (species-wise) [For treatment (T): n = 5 (3 number fishes from P1 and 2 number of fishes from P2) while, for control (C): n = 5, 5 number of fishes from C]. Finally, those were anesthetized with tricaine methanesulphonate (MS 222) and sacrificed for collecting desired tissues of gonads. Moreover, during the end of the dry season, both (treatment and control) of the gonads were collected on the 1st day of February, while in the breeding season, the desired tissues were taken on the 1st day of September.

Tissue preparation, fixation, and staining

For the purpose of general histological study, desired tissues were being fixed for about overnight in aqueous Bouin's fluid solution. Then, those were processed for paraffin sections and were cut into at 3-4 micron. The tissue sections were stained by using Haematoxylin-Eosin [(H-E: for histological study (Ghosh, 1991)] for understanding the histological orientation of cells. Finally, the stained slides were mounted with the help of the DPX solution for future use of image capturing.

Image capturing and image processing

Tissue histological studies were captured under a Leica microscope (Model no. DM 2000) with an attachment of EC3 digital camera and finally processed under 'Adobe photoshop 7' for presentation.

Results and discussion

Histotechnological observations

Ovary

In the natural condition, there are almost six stages of oogenesis (development of oocytes) depending on time, season, age and sex. Usually, according to the changes in size, nucleus, ooplasm and egg membranes of the developing ova, the developmental stages are being categorized as follows: (a) Stage 1 (oogonia), (b) Stage 2 (early oocyte or chromatin nucleus stage), (c) Stage 3 (late oocyte or perinucleolar stage), (d) Stage 4 (vacuolated follicles or yolk vesicle or cortical alveolar stage), (e) Stage 5 (yolk globule stage or vitellogenesis), (f) Stage 6 (mature follicles). During dry season we have seen up to stage 4 and in breeding season the ovary undergoes stage 5 and stage 6. Here, observations were also categorized according to developmental stages.

During dry season under farm-feed condition (CD), in the ovary of *C. batrachus* (Plate 1) revealed the presence of cortical alveolus (CA) oocytes with the content of cortical alveoli in the cytoplasm. Nucleus (N) in the cytoplasm was noticed. Nucleoli (NU) were observed in the periphery of the nuclear membrane. Cortical vacuoles appeared and those were gradually increased. A thin layer of zona radiata (ZR) was formed and was noticed under observation. Moreover, yolk vesicles (YV) were also noticed in the cytoplasm and fish under this cortical alveolar (CA) stage depicted quite thin and less condensed yolk vesicles (YV) in the cytoplasm under farm-feed exposure with the presence of small yolk globules (YG) appeared in the ooplasm around the nucleus (Photo 1.1). Under the choline exposure in the dry season (TD) the fish displayed a large number of yolk vesicles (YV) and also a moderate number of yolk globules (YG) in the cytoplasm around the nucleus. Zona radiata (ZR) was prominent and distinct in nature and the oocytes under this cortical alveolar stage (CA) the ZR was well-compacted, well-articulated, quite thickened and well-bordered (Photo 1.2). On the other hand, in *C. batrachus*, the ovary under breeding season in farm-feed condition (CB) showed a greater number of residual primary oocytes (RPO) and atretic follicle (AF) as the experimented fish were harvested in the spawning phase/stage, i.e., under the stage 6 (mature follicles). The extra vascular space (EVS) was found to be maximum during farm-feed condition. The ova were held together by the stroma (vascular collagenous connective tissue with a few smooth muscle fibres). The stroma (ST) was consisted of finger-like ovarian (ovigerous) lamellae, which contained ovarian follicles of different stage of oogenesis. Ovigerous lamellae (OL) protruded into ovocoel (ovarian cavity) from the ovarian wall and the oogenesis generally occurred in this lamellae. The stroma was found in this condition extremely pressed due to enlargement of ova (Photo 1.3).

Under the farm-feed condition in the dry season (CD), the *A. testudineus* (Plate 2) was also characterized by the appearance of cortical alveoli (CA) and small amount of yolk globules (YG). The centrally positioned nucleus (N) in the cytoplasm appeared as oval in the stage of cortical alveolus (CA) with the abundance of numerous peripherally located nucleoli of various sizes. A thin layer of ZR was appeared in this stage (Photo 2.1). Whereas, under choline-exposed condition in the same season (TD), the *A. testudineus* depicted condensed cytoplasmic and nuclear volumes which were comparatively higher. The yolk vesicles (YV) of nucleus were increased under choline exposure rather

than fishes under farm-feed exposure. The nucleoli were tending to lie close to the nuclear envelop. The yolk globules (YG) were deeply noticed in this CA stage. The ZR was quite thick and predominant (Photo 2.2). Moreover, the *A. testudineus* under aqua-feed situation in the breeding condition (CB) depicted maximum number of RPO and AF. The stroma (ST) was reduced between distended ovigerous lamellae (OL) under this condition. Some yolk nucleus (YN) was found at this stage of experiment. The nucleus was found to begin to move to the animal pole just beneath the surface of oocytes and breakdown of germinal vesicles (GVBD) indicating the end of vitellogenesis, which had resulted into rapid growth of oocytes (Photo 2.3).

Catla catla (Plate 3) in farm-feed condition in the dry season (CD) depicted nucleoplasm with acidophilic reaction as revealed in the H-E stain and the nuclear membrane was irregular. Ooplasm became slightly acidophilic and few of small yolk vesicles were arranged in the periphery of the cytoplasm. Later these became cortical alveoli and took part in the formation of perivitelline space. Few small yolk globules were also observed in the ooplasm around the nucleus. Nucleus was not densely arranged in presence of nucleoli, having different sizes (Photo 3.1). But in *C. catla* under choline exposure during the same season (TD) showed a dense arrangement of yolk vesicles in presence of lager amount of small yolk globules in the cytoplasm. Zona radiata became thick and persistent. Nucleus was densely arranged and well-articulated in presence of nucleoli, having uniform sizes (Photo 3.2), whereas, in the breeding season under farm-feed condition (CB) ovary produced a sufficient number of RPO and AF. The ovocoel was noticed under the same condition. Content in the yolk vesicles was found to become thinner in presence of some nucleolus into the oocytes with a thin layer of the theca. The matured oocytes under this condition displayed slight distortion and damage (Photo 3.3).

Distortion in the membranes of the cells in most of the cortical alveolar oocytes was observed under the only farm-feed supplemented *L. rohita* (Plate 4) in the dry condition (CD). Nucleus was not arranged compactly and the nucleoli were bigger in size, but altered and deformed as noticed in the present experiment (Photo 4.1). Whereas, in the choline-supplemented condition in the same season (TD) the number and size of the nucleoli decreased in the yolk globule and thus leading to the maturing phase to form matured oocytes. A large number of yolk vesicles along with the presence of higher quantity of yolk globules were also dominant under exposure of choline in this cortical alveolar stage (Photo 4.2). Under the farm-feed condition in the breeding season (CB) the experimental *L. rohita* depicted mature oocytes, characterized by its large size and absence of nucleus. Ovigerous lamellae (OL) were prominent and distinct, but the presence of atretic follicle was maximum in absence of choline and the non-fertile residual primary oocytes (RPO) were abundant during only farm-feed condition in the present experimental species. A thin fibrous layer of basal lamina (BL) was noticed between follicular epithelium and theca layer (Photo 4.3).

Testis

Testes possess almost five developmental stages, viz., (a) resting phase or early immature condition, (b) late immature phase, (c) maturing phase, (d) mature phase, and (e) spent phase; present study intended to focus on it.

C. batrachus under choline exposure at breeding season (TB) [Plate 1]: Histologically, Testis is enclosed completely by a thin tunica albuginea. Histologically it is consisted of few collagenous fibres, some smooth muscles and elastic fibres. The testicular parenchyma is consisted of branching tubular seminiferous tubules and interstitial tissues and the seminiferous tubules contained the germinal epithelium which gives rise to spermatozoa (SP). Under this condition the spermatozoa were observed to remain in the lumens of seminiferous tubules. These seminiferous tubules were found to be lager under the breeding condition and also had a central lumen surrounded by the germinal epithelium. The sertoli cells (SC) were situated near the outer rim of the seminiferous tubules. The spermatocytes were formed by the cytoplasmic projections of sertoli cells. The spermatocytes contained primary spermatocytes (PS), secondary spermatocytes (SS) and spermatids (SD), while spermatozoa (SP) were found in the lumen of the seminiferous tubules and the content of secondary spermatocytes (SS) was quite dense and thick, spermatogonia were less and dense number of spermatozoa was also observed under choline exposure in the mature phase. The decreased volume of interstitial tissues (IT) was noticed in the triangular space between the seminiferous tubules, contained interstitial cells (IC), fibroblasts, blood vessels (BV) and collagen fibres. Interstitial cells of Leydig was bigger in size during the breeding season under choline exposure and these were located in the fibrous supporting connective tissues and formed the groups near the blood capillaries in-between the seminiferous tubules (Photo 1.4).

A. testudineus under choline exposure at breeding season (TB) [Plate 2]: Histological observations revealed presence of interstitial cells (IC), a thick bulbous content of sertoli cells (SC). Interstitial tissues were also noticed, where IC was observed as small or large clusters and contained spherical nuclei and maximum development was found in this stage of maturation under choline exposure. Moreover, abundance of SS in higher quantity with huge

spermatozoa (SP) in larger volume depicted the maximum maturity of the fish under the choline exposure. Occurrence of spermatid and well-articulated sperm (S) also displayed the gross development of testis under this mature phase. A thick layer of basement membrane (BM) was also noticed under choline supplemented condition (Photo 2.4).

C. catla under choline exposure at breeding season (TB) [Plate 3]: Fish under choline-supplemented condition depicted a well-organized content of spermatogonial cells within tunica albuginea. A thick layer of basement member was also observed which was surrounded by each testicular lobe. SC, PS, SS, IC and SP were clearly visible and well-organized under choline exposure. A large number of matured SS, having dense basophilic nuclei (as observed under H-E stain) were produced by the meiotic division and the SS were somewhat smaller than PS. The spermatids metamorphosed into spermatozoa (Photos 3.4, 3.5 & 3.6).

L. rohita under choline exposure at breeding season (TB) [Plate 4]: Under choline exposure in breeding season *L. rohita* revealed that the seminiferous tubules were comprised of primary spermatocytes, secondary spermatocytes, and spermatids. The lobules of the testis were well-articulated bearing a thick layer of basement membrane. The volume of the interstitial tissues was decreased where interstitial cells were noticed (Photo 4.4).

Histological conditions of gonads due to the application of dietary feed supplements viz., cotton seed meal (free from gossypol), commercial feed additives, e.g., Therigon®; Nuvisol Hatch®; gibberellic acid; L-carnitine, energy diets, containing soya-acid oil etc., were studied in many fishes like Nile tilapia (*Oreochromis niloticus*), juvenile common carp (*Cyprinus carpio*), sharp tooth catfish (*Clarias gariepinus*) by various researchers (Tope-Jegede et al., 2019; Wang et al., 2014; Abdelhamid et al., 2013; Cek & Yilmaz, 2008).

The farm-feed fed diet in the present experimental aliquots during dry (CD) and breeding (CB) conditions disclosed similar observations as on Nile tilapia (*Oreochromis niloticus*) under the feeding arrangement of 1.0 g Therigon®/kg basal diet showing coagulated necrosis in yolk granules, follicular layer separation, follicular atresia, resulting into non-fertile oocytes, and ovarian tissue necrosis. Degenerative changes like atretic follicles and declination of yolk granules were also noticed when the fish fed with 2.0 g Therigon® along with per kg basal diet, but 0.5 g Therigon® with per kg basal diet did not affect the fish ovary too much alike the fish of the present experiment in TD condition during choline-supplementation (Abdelhamid et al., 2013). Whereas, Nuvisol Hatch® at the rate of 1.0 g per kg basal diet depicted ripe and developed oocytes with few atretic follicles which showed resemblance to the TD condition of the present experiment in choline exposure of the experimental fish. The present experiment in the CB condition under farm-feed exposure showed similar results when they were fed with 2.0 g and 3.0 g of Nuvisol Hatch® with per kg basal diet (Abdelhamid et al., 2013). In the present experiment, in the CD and CB conditions, the vitellogenic oocyte depicted several alterations, e.g., cell wall erosion in some oocytes, yolk sphere liquefaction, containing vacuoles in the ripe oocyte of *O. niloticus* when fishes fed with 60 mg gibberellic acid with per kg basal diet (Abdelhamid et al., 2013). Moreover, the occurrence of such alternations as well as degradation of ovary in farm-feed fed system both in the CD and CB conditions might be resulted from the environmental stress, thrust of agricultural waste and bacterial invasion, which caused disruption and development of germ cells, finally, reproductive ability of the experimental fish became reduced (Cek & Yilmaz, 2008; Lye et al., 1998). Furthermore, choline into the water [as GnRH (Gonadotropin releasing hormone) stimulant] might result in an excellent reproduction performance in females and the hypothalamic neurosecretory decapeptide exhibited gonadotropin secretion, steroidogenesis and ovulation in breeding season alike in salmon as studied by Van Der Kraak et al. (1984); Haraldsson and Sveinsson (1993); El-Sebai et al. (2003). But the dietary gossypol (a toxic crystalline compound present in cotton-seed oil) present in the cotton-seed meal (CM) did not affect the ovary (development or degradation) and gonado somatic index (GSI) of *C. carpio* and *O. niloticus* (Wang et al., 2014; Rinchard et al., 2002) when fed with the basal diet, even in the high level of the dietary CM (52 %). Whereas, dietary gossypol caused a negative impact on the gonad maturity of male fish, viz., decreased sperm concentration with the increase of dietary gossypol (0.22 - 0.95 %) in rainbow trout (Dabrowski et al., 2001), depletion of sperm cells in seminiferous tubule at dietary CM of 24 % in tilapia (Salaro et al., 1999), lowered sperm cell ratio at a level of 54 % CM in *C. carpio* (Wang et al., 2014). A gross development of testes under the choline-supplemented condition in the breeding season was observed in Nile tilapia, fed with L-carnitine (dipeptide amino acid), synthesized from methionine (a metabolite of choline) and lysine (Zeyner & Hameyer, 1999). On the other hand, extensive seminiferous tubules degeneration, necrosis in the seminiferous tubules and also in the focal areas with major haemolysis in testicular tissue were observed when the same fishes (Nile tilapia) were fed with 1100 mg with per kg of basal diet (Msiska, 2002; Abdelhamid et al., 2013). Actually, choline may procure requisite substrate for the spermatozoa within the epidermis; it can trigger the successful maturation of the sperm in the breeding season and also initiated the motility of the sperm and thus, resulted in better sperm quality (Chatzifotis et al., 1995; El-Damrawy, 2007). On the other hand, the ovarian development was sharply affected by the exposure of high energy

diets, containing soya-acid oil in higher concentration in the sharptooth catfish, depicting a smaller number of yolky oocytes and follicular atresia by hindering the metabolic function in the liver, while the development of vitellogenic follicles is directly related with the synthesis of yolk (vitellogenin) done by the liver (Cek & Yilmaz, 2008; Guraya, 1986). But an optimum concentration of the energy diet resulted in the best weight gain as well as best gonadal maturity, with the highest mean number of yolky oocytes in the ova in sharptooth catfish (Cek and Yilmaz, 2008), similar to the present experiment in choline-supplemented condition. Moreover, the choline into pond water along with the basal diet improved the seminiferous tubules of testis as the number of sertoli cells increased a lot which has a direct co-relation with sperm production as also reported by Tope-Jegede et al. (2019) in Nile tilapia.

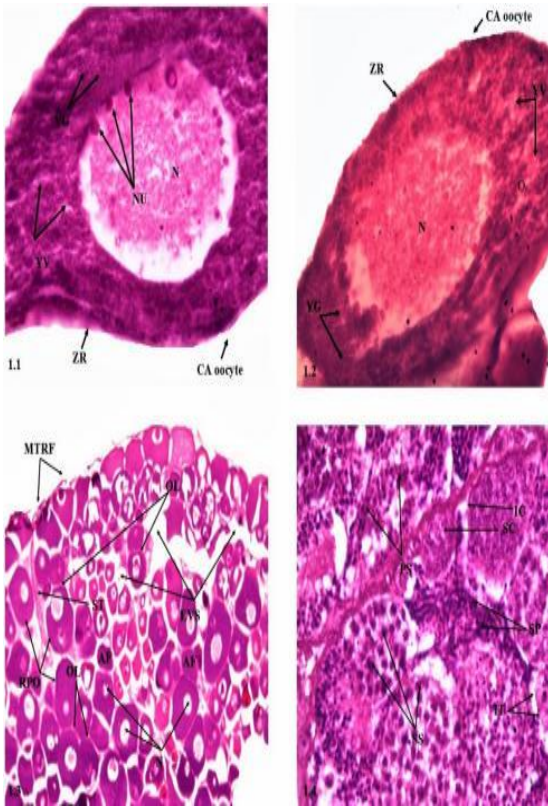

Plate 1: Gonads of *C. batrachus*

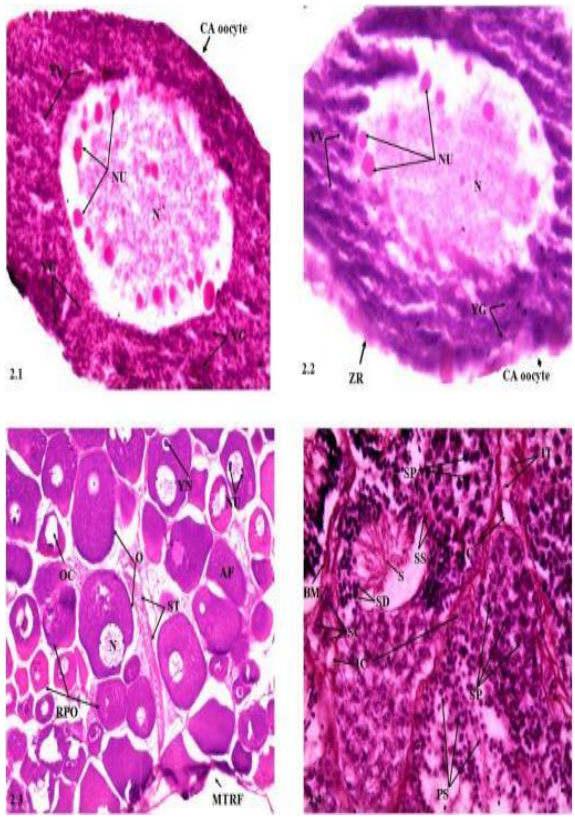

Plate 2: Gonads of *A. testudineus*

PLATE 1

Photos 1.1 - 1.4: Photomicrographs of transverse section of gonads in *C. batrachus* under control-dry (CD), treatment-dry (TD), control-breeding (CB) and treatment-breeding (TB) conditions under field exposure showing cortical vacuoles of cortical alveolus (CA) oocytes of ovary, thin layer of zona radiata in the ovary; well-compacted, well-articulated and thickened zona radiata; residual primary oocytes and atretic follicle, stroma consisting of finger-like ovarian lamellae in the ovary; dense number of spermatozoa in the lumens of seminiferous tubules, sertoli cells, primary spermatocytes (PS), dense and thick secondary spermatocytes (SS), spermatids (SD), interstitial tissues (IT) and interstitial cells of Leydig in the testis. [H-E; X 400, 1000]

PLATE 2

Photos 2.1 - 2.4: Photomicrographs of transverse section of gonads in *A. testudineus* under CD, TD, CB and TB conditions under field exposure showing deformed appearance of cortical alveoli stage of ovary; well-compacted and densely arranged yolk globules and thick layer of zona radiata of ovary; residual primary oocytes with atretic follicle in mature follicular stage of the oocyte, reduced stroma in the ovary; interstitial cells and interstitial tissues, bulbous

content of sertoli cells, secondary spermatocytes in higher quantity with huge spermatozoa, healthy spermatid and well-articulated sperm under mature follicular stage of the oocyte in the matured testis. [H-E; X 200, 400, 1000]

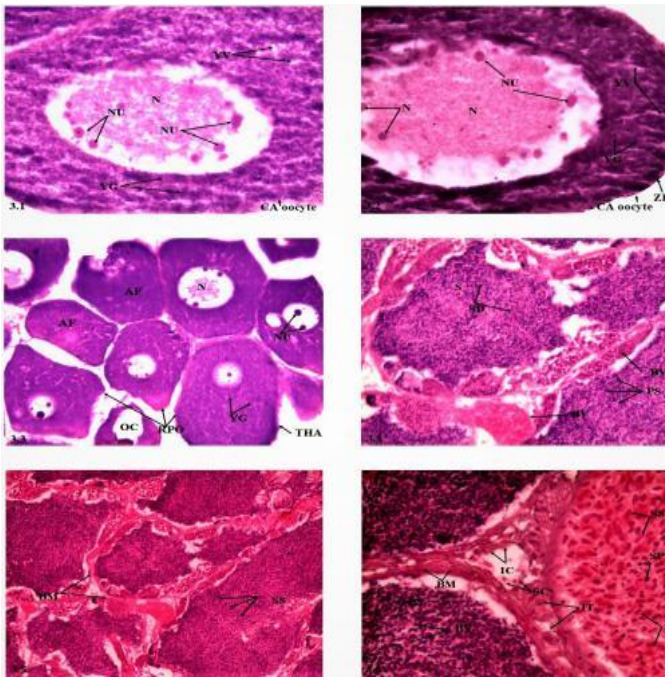

Plate 3: Gonads of *C. catla*

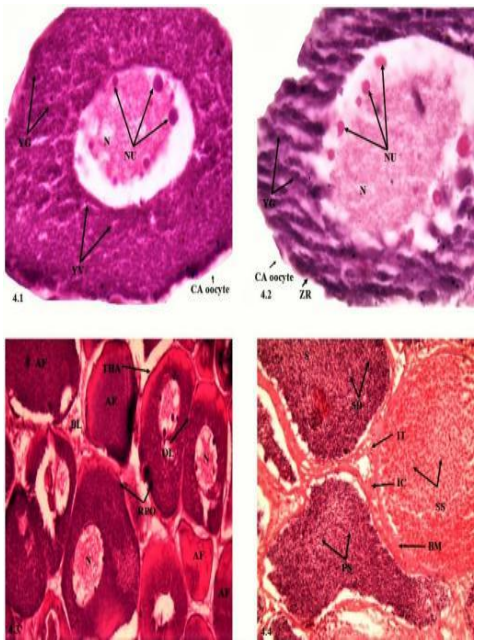

Plate 4: Gonads of *L. rohita*

PLATE 3

Photos 3.1 - 3.6: Photomicrographs of transverse section of gonads in *C. catla* under CD, TD, CB and TB conditions under field exposure showing small yolk vesicles and yolk globules in the ooplasm around the nucleus under cortical alveolar stage of ovary; presence of thick and persistent zona radiata, and densely arranged well-articulated nucleus in the ovary; occurrence of sufficient number of residual primary oocytes and atretic follicles, thinner content in yolk vesicles with a thin layer of theca under mature phase of ovary; occurrence of well-organized spermatogonial cells within tunica albuginea, well-articulated sertoli cells, secondary spermatocytes, primary spermatocytes, interstitial cells, spermatozoa and numerous blood vessels with thick layer of basement membrane (BM) in the testis. [H-E; X 200, 400, 1000]

PLATE 4

Photos 4.1 - 4.4: Photomicrographs of transverse section of gonads in *L. rohita* under CD, TD, CB and TB conditions under field exposure showing occurrence of non-compacted nucleus with altered and deformed nucleoli under cortical alveoli stage of ovary; decreased numbered and reduced sized nucleoli in the yolk globules, but presence of higher quantity of yolk globules in the ovary; presence of maximum number of atretic follicle and non-fertile residual primary oocytes, thin fibrous layer of basal lamina in the matured ovary; occurrence of numerous healthy primary spermatocytes, secondary spermatocytes, and spermatids, well-articulated lobules, and reduced volume of interstitial tissues with abundant interstitial cells in the matured testis. [H-E; X 200, 400, 1000]

Conclusion

Present investigations revealed the number of ripe and developed oocytes with few atretic follicles resulting into excellent reproductive performance in female, and the hypothalamic neurosecretory decapeptide exhibited gonadotropin secretion, steroidogenesis and ovulation especially in breeding season during additional choline supplementation. Moreover, choline can trigger the successful maturation of the sperm in the breeding season and it can also able to initiate the motility of the sperm in testis and thus, resulted in better sperm quality under its exposure as revealed in the present experiment. Thus, it can be assumed from the present observations that the maiden attempt of administrating choline chloride directly into the pond water under field condition resulted in better quality of brood fishes that can be utilised for spawn production with higher success rate under this semi-intensive pond culture system which may support rural, poor fish farmers to a greater extent.

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Conflict of Interests

The authors declare no conflict of interest with the contents of this article

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