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Histoarchitectural variations during Oocyte growth in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

During the present study changes in gonadosomatic index and hepatosomatic index in different reproductive phases of rainbow trout *Oncorhynchus mykiss* has been worked out. This study represents detail description of histological variations during developmental stages of ovarian follicles. In this study, oocyte growth has been described in 7 stages Chromatin-nucleolus, Perinucleolar, Cortical alveolus, Primary vitellogenesis, Secondary vitellogenesis, Maturation and Spent phase. The mean oocyte diameter increased from 183.5 μ m at perinucleolar phase to 2249.5 μ m in maturation phase, and nuclear dimensions changed from 73.5 μ m in perinucleolar phase to 184 μ m in secondary Vitellogenic stage. During this dramatic increase in the ooplasm and follicle wall revealed a numerous changes in morphological characteristics like nuclear extrusion, alveoli formation, vitellogenesis, and variations in thickness of zona pellucida. These morphological changes in growing oocyte have been discussed in relation to various physiological factors, including endocrine, exocrine and environmental cues.

Keywords: Rainbow trout, ovary, chromatin-nucleolus, perinucleolar, cortical alveoli.

1. Introduction

The success of any fish species is determined by its ability to reproduce successfully in changing environment and maintain its population. In recent past the reproduction in teleost has received a great attention because of the economic reasons. Eigdery (1981) [1] revealed that histological studies determine the peak period of spawning assessment, biological characteristics and life cycle of a fish species. West (1992) [2] demonstrated that the histological studies provide important information on gonadal development of a species.

Reproduction is a highly integrative function, which involves complex physiological changes at intracellular and intercellular level. The gonads show a series of developmental alterations with the onset of maturation which are closely accompanied by conspicuous cellular, biochemical, molecular and endocrinological changes [3]. As the gonads increase in size, somatic growth slows down and eventually stops. At this stage, proteins and lipids are mobilized from the somatic tissues and transferred to the gonads [4].

The Rainbow trout is one of the most important fish species commercially exploited in Jammu and Kashmir. Histological analysis is a must to demonstrate the most valuable information on spawning season and is thus essential for detecting details regarding the maturation cycle of the fish [5, 6]. The most suitable method for determining the reproductive success in female fish is to observe seasonal developmental changes in the oocytes [5].

Wallace, 1985; Celsius and Walter, 1998 [7,8] revealed that the Oocyte growth in female teleosts is mainly due to the incorporation of proteins such as vitellogenin (Vtg) and zona radiata proteins (Zrp) under the control of sexual hormones. These proteins are synthesized by the liver under the stimulation of estradiol and are then transported in the bloodstream to the follicular layer and then endocytated by oocytes and used for the formation of both yolk and vitelline envelope.

Trout are the most important cultivated fish of the world (FAO yearbook, 2009) [9]. In India trout was introduced by Mitchell in 1900 from Scotland in Kashmir valley. The trout species were reared in Harwan near Srinagar (Kashmir) and in 1905-1906 Mitchell successfully established trout hatchery at Harwan. Since then brown trout and rainbow trout has established throughout the Jammu and Kashmir. These two fish species have been survived well in the changed habitat over a century. Till date, no systematic study has been made to analyze on the environs of Kashmir. Only small fragmentary information is available on

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breeding biology including biochemistry among the trout strains of different countries. The trout species of Kashmir i.e., Rainbow trout and Brown trout are under severe threat of being wiped out from different water bodies because of extensive fishing round the year. It therefore becomes more imperative to analysis these fish species in terms of breeding biology. The studies on circa-annual gonadal changes in these fish species will help to devise strategies for conservation of these exotic fishes ensuring their survival in India. To minimize the losses at gamete level, fry and fingerling levels need to be addressed to ensure quality and quantity fish seed of cold water fishes to farmers of Kashmir. The aim of the present study was to provide the 1st information on the reproductive biology including oocyte and ovarian development of the fish. The present studies on histological analysis of Rainbow trout fish ovaries with the aim to describing the oocyte development stages and gonad development.

2. Materials and Methods

For the present study mature Rainbow trout (*Oncorhynchus mykiss*) specimen were procured from different water bodies and hatcheries especially from Verinag (33.55° N and 75.25° E) and Kokernag (33.69° N and 75.22° E) hatcheries of Kashmir valley (Jammu and Kashmir). Fish specimens were collected during different months i.e., from March to December 2013. Soon after capturing gravimetric data of the fish specimens were recorded and thereafter the specimens were sacrificed and ovaries were dissected out. For histological studies the tissue was fixed in Bouin’s fixative (75:25: 5 picric acid: formalin: acetic acid) for 24 h then transferred to 70% ethanol. These samples were processed through an ethanol for dehydration, cleared in xylene and embedded in wax for sectioning. Sections were cut of about 5 μm thickness and were stained with Hematoxylin and eosin. Slides were examined under light microscopy. The results were analyzed by one-way ANOVA.



Fig 1 and 2: Vitellogenic oocyte in Ovary and Mature Ripe Ova at spawning phase (4X).

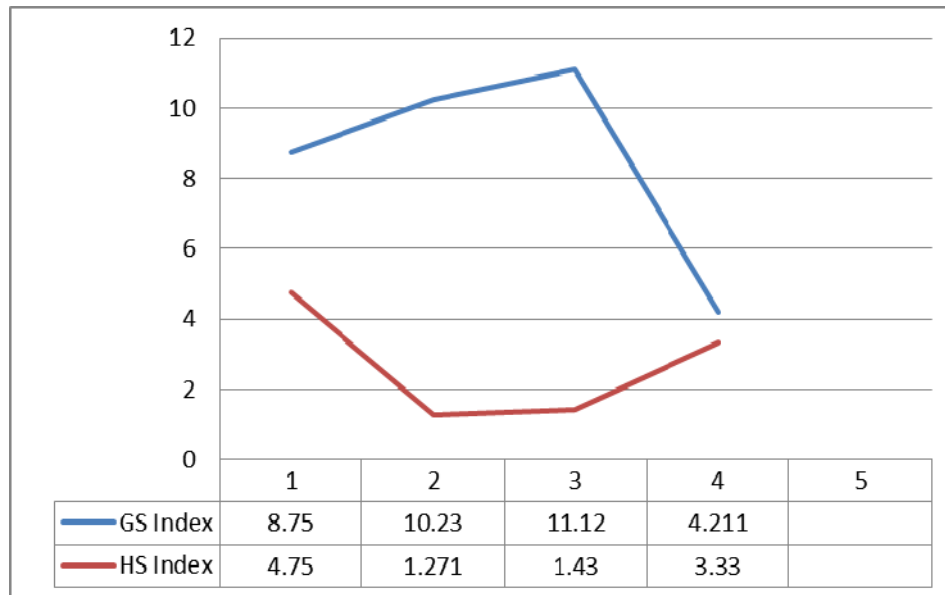
3. Results

The gravimetric data revealed that the gonadosomatic index was maximum (11.12) during the spawning season followed by (10.23) in early spawning it was (8.75) in developing stage while minimum 4.21 in the spent phase. (Table 1, graph 1).

Contrary to it’s the maxima of hepatosomatic index (HSI) was maximum at developing stage and the minimum was recorded in early spawning stage (Table 1, graph 1).

Table 1: Gonadosomatic Index GSI and Hepatosomatic index HSI in different seasons of ovary.

Group 1	Body weight (g)	Ovary weight (g)	Liver weight (g)	GSI	HIS
Developing Stage	240	21	11.4	8.75	4.75
Early spawning stage	606	62	7.70	10.231	1.271
Spawning stage	1115	124	16	11.121	1.435
Spent stage	285	12	9.5	4.211	3.333



Graph 1: Mean value of GSI and HSI through breeding cycle of fish.

3.1 Histological studies

The growth of Rainbow trout oocytes was divisible in seven stages, based on morphological basics. Stage I- Chromatin-nucleolus stage, Stage II- perinucleolar stage, Stage III- Cortical alveolus stage, Stage IV- Primary Vitellogenic Stage, Stage V- Secondary Vitellogenic stage, Stage VI- Maturation stage and Stage VII- Spent stage.

3.2 Developmental stages of the oocytes

a. Chromatin-nucleolus stage

In this stage the follicles were of the smallest size. The nucleus was large and occupied a large portion of the follicle, which was surrounded by thin follicular layer. A large number of nucleoli were present in the nucleus. The diameter of the follicle and the nucleus was 183.5 ± 9.68 and 73.5 ± 3.01 respectively (Fig. 3 and 4).

b. Perinucleolar stage

This stage was characterized by increased size in follicle and increased nuclear dimensions. The mean oocyte diameter was 320 ± 28.901 and the mean nucleus diameter was 95 ± 6.62 . Numerous large, basophilic nucleoli were found at the periphery of the nucleus which indicates increasing activity. In this phase the zona radiata was comparatively thin (Fig. 5).

c. Cortical alveolus stage

In this stage the cortical alveoli appeared initially in the peripheral zone and with the advancement in size these alveoli were distributed throughout the ooplasm. The characteristic feature of the cortical alveolus stage is the presence of large unstained vacuoles present in the ooplasm. This stage was representing the oocytes with mean diameters of $617 \pm 21.10 \mu\text{m}$ and nucleus mean diameter $135 \pm 7.76 \mu\text{m}$. The nucleoli remained at the periphery of the oocyte. The zona pellucida become visible during this stage and its mean thickness was found to be 4.5 ± 0.429 (Fig. 6 and 7).

d. Primary Vitellogenic stage

The cortical alveoli appeared in early stages starts accumulating yolk of the ooplasm. In the early stage alveoli appears in a central position but migrated towards the periphery with the advancement or growth in this phase. Nuclessar morphology was not altered during this stage. However the nucleoli were extruded into ooplasm. Significant increase in the thickness of zona pellucida was found. The diameter of the follicles and the nucleus was 657.5 and $151 \mu\text{m}$ respectively. The diameter of the nucleus increased significantly (Fig. 8).

e. Secondary Vitellogenic stage

In this stage the yolk spheres moved toward the center of ooplasm and cortical alveoli and lipid droplets moved to the periphery. The thickness of zona pellucida was found to be $14.625 \pm 0.89 \mu\text{m}$ and was higher during this stage. The diameter of the oocytes was $807.5 \pm 21.64 \mu\text{m}$ and the nucleus diameter was $184 \pm 4.72 \mu\text{m}$. In this stage the oocytes reached a maximum diameter (Fig. 9 and 10).

f. Maturation phase

In this stage the follicle showed a marked increase in size and reached the final growth stage. The mean diameter of the follicles was 2249.5 ± 28.72 . During the mature phase the organelles are difficult to see as they are displayed and are covered with huge amount of yolk stored in the cytoplasm (Fig. 11).

g. Spent phase

In this phase follicles show the irregular structure having folds in the zona pellucida. This stage composed of a large number of ruptured post ovulatory and atretic follicles. There was a significant decrease in the size of the follicles during this phase (Fig. 12).

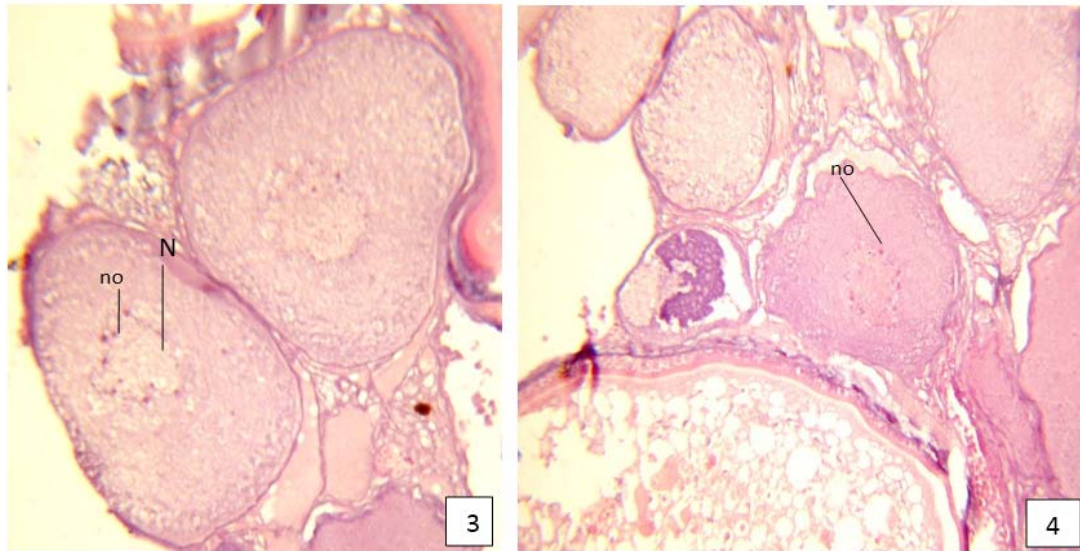


Fig 3 and 4: Chromatin-nucleolus stage showing a large nucleus (N) and nucleolus (no) evenly distributed in the nucleus. Hematoxylin & Eosin (100X).

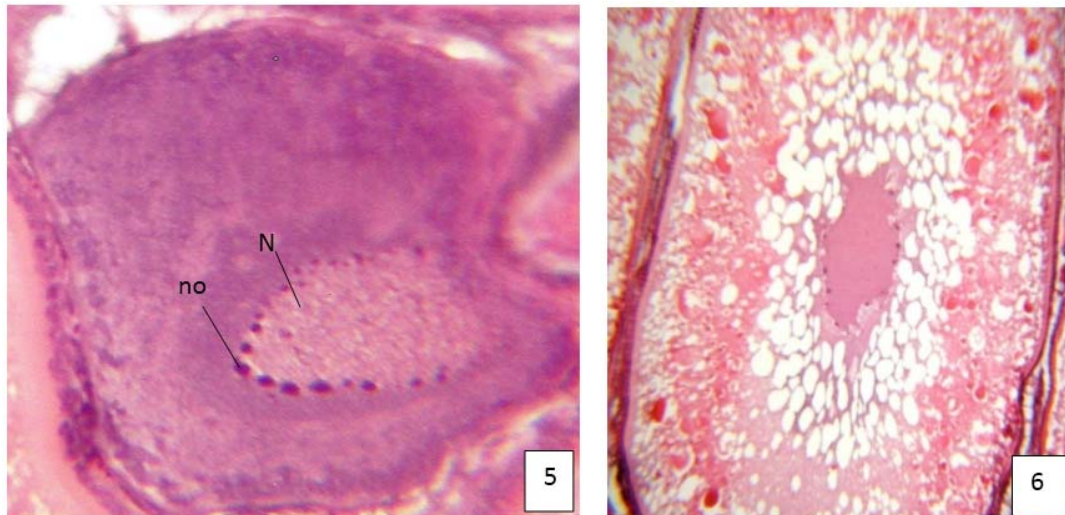


Fig 5: Perinucleolar stage of the primary oocyte having several nucleoli (no) appearing at the periphery of the nucleus (N). Hematoxylin & Eosin (100X).

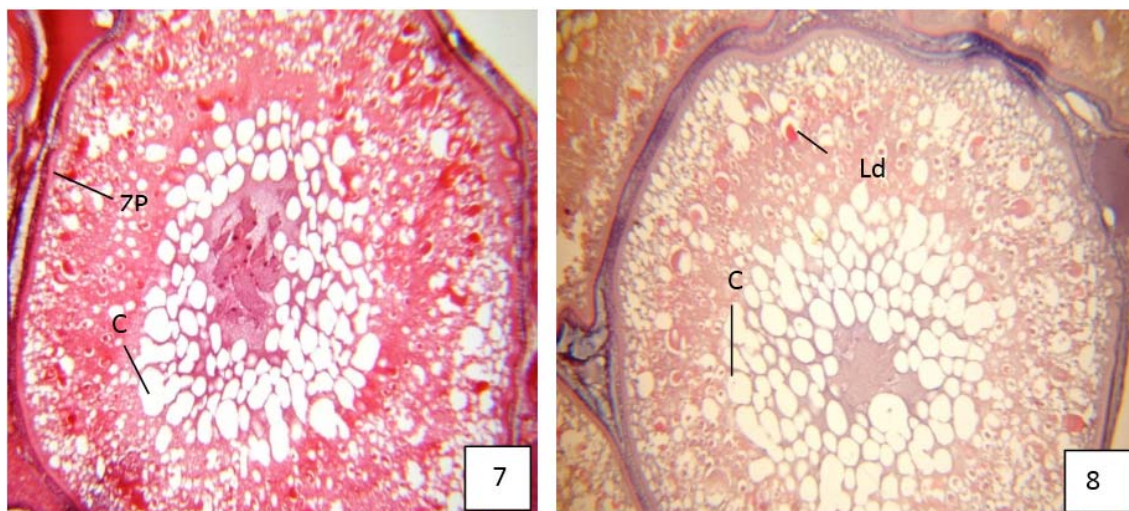


Fig 6 and 7: Oocyte in Cortical alveolus stage. The alveoli fill the oocyte (C). The nucleus enlarges and becomes irregular in shape and thin zona pellucida (ZP). Hematoxylin & Eosin (100X).

Fig 8: Oocyte in primary vitelliogenesis stage showing lipid droplets (Ld), nucleus (N), Cortical alveoli (C) and zona pellucida (ZP). Hematoxylin & Eosin (100X).

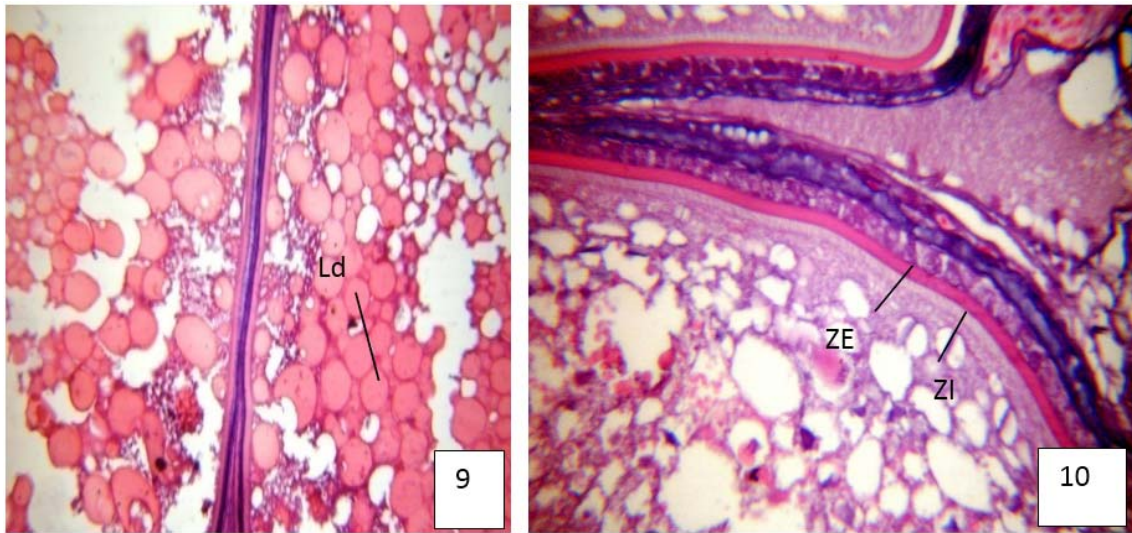


Fig 9 and 10: Oocytes in secondary Vitellogenic follicles showing increase in the thickness of zona pellucida, zona interna (ZI), zona externa (ZE) and the accumulation of the lipid droplets (Ld) in the peripheral region of oocyte. Hematoxylin & Eosin (100X).

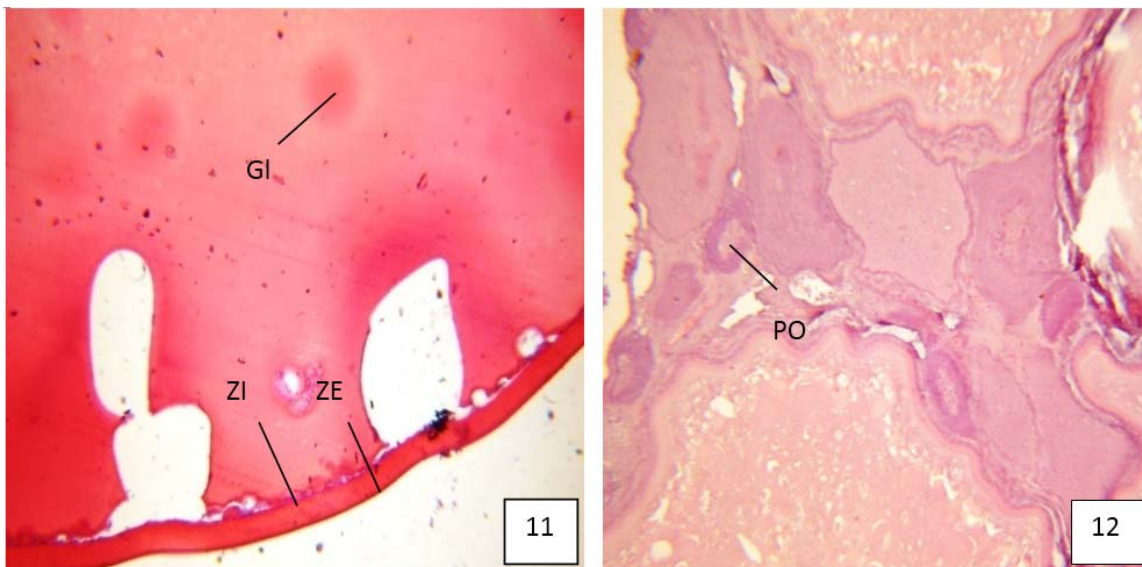
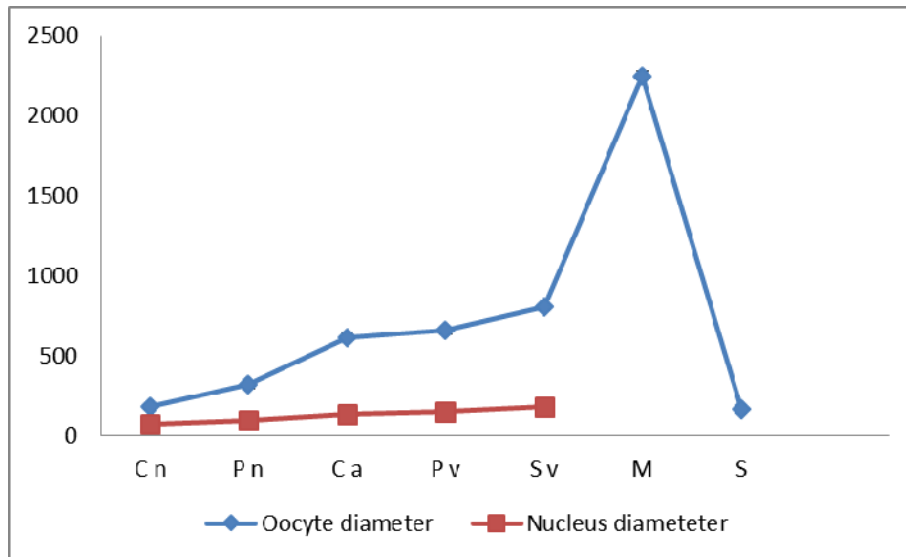


Fig 11: Oocytes in maturation phase, the ooplasm is completely filled by yolk globules (Gl). Hematoxylin & Eosin (100X).

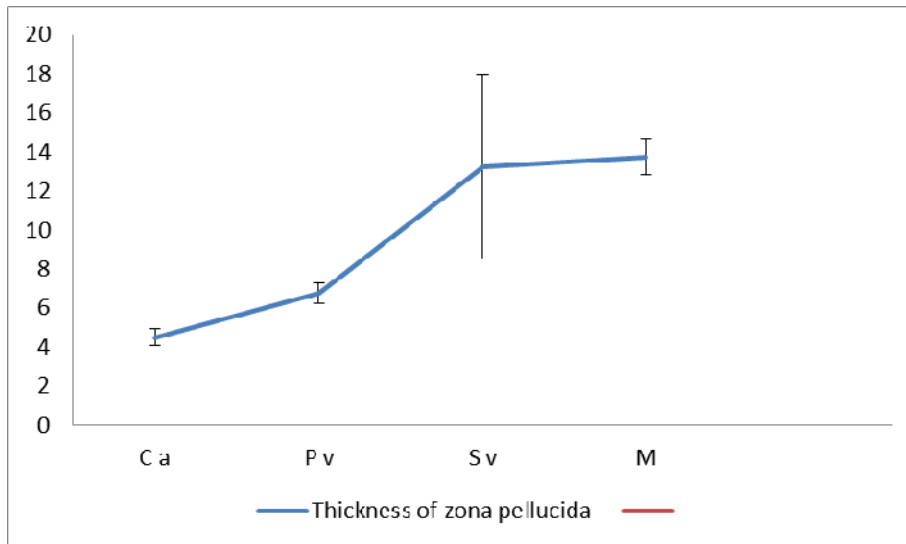
Fig 12: Oocyte in spent phase showing irregular folds in zona pellucida and post ovulatory follicles (PO).Hematoxylin & Eosin (100X).

Table 2: The morphometric data of oocyte, nuclear and zona pellucida in different stages of growing follicle (mean and standard error of mean).

Type of follicle	Follicle diameter (µm)	Nucleus diameter (µm)	Zona pellucida thickness (µm)
Chromatin-nucleolus stage	183.5±9.68	73.5±3.01	-
Perinucleolar stage	320±28.90	95±6.62	-
Cortical alveolus stage	617±21.10	135±7.76	4.5±0.42
Primary vitelliogenesis stage	657.5±23.82	151±9.05	6.76±0.51
Secondary vitelliogenesis stage	807.5±21.10	184±4.72	13.25±4.72
Maturation stage	2249.5±28.72	-	13.75±0.91
Spent stage	163±8.14	-	-



Graph 2: Change in mean diameter of oocytes and nucleus during oogenesis.



Graph 3: Change in mean diameter of zona pellucida in different stages of oocyte development.

4. Discussion

In the present study GSI was found to be maximum in spawning stage (11.12) and minimum in spent stage (4.21). Similar types of results were also reported in many teleost such as *Clarias fossilis* [10]. In the present study HSI was found to be lower in spawning season. Similar types of results were also reported in *C. arel*. [11]. The results of the present study strongly endorse the earlier findings. GSI increases gradually from spent stage to spawning stage and HSI increases from early spawning stage to developmental stage and the increase was statistically significant ($P < 0.0005$). Rae and Calvo (1995) [12] who have stated that GSI is correlated with gonadal development and it is therefore an important indicator of maturity of the fish. While HSI is used as an indicator of energy status [13]. The ovary of the Rainbow trout is synchronous type and it was found to consist of at least two populations of oocytes at different developmental stages. In present study the oocyte growth in rainbow trout has been studied in seven distinct phases. The oocyte development in teleost fishes and divided into five to eight stages [3, 2, 14, 15, 16]. Microscopic examination of ovaries revealed five developmental stages in sword fish [17], seven phases in *Mystus*

tengara [18], seven stages in *Dicentrarchus labrax* [19], nine stages in *Hemiodus* species [20]. In present study morphology of follicles and follicular diameter, nuclear diameter and zona pellucida diameter were analyzed in different stages of the development of the oocytes. Similar pattern changes were also studied in growing oocytes in teleosts [21, 22, 23]. The follicular diameter increases during the advancement of the development of the oocyte it increased from chromatin-nucleolus stage 183.5 ± 9.68 to maturation stage 2249.5 ± 28.723 , the increase was statistically significant ($P < 0.0005$) however the diameter of the oocyte was drastically reduced in spent phase 163 ± 8.14 . Shirali *et al.*, (2011) [24] studied similar results in common carp the minimum follicle diameter was in the Chromatin nucleolus stage and reached maximum in secondary Vitelliogenic stages. Similarly nucleus diameter also shows an increase in its dimensions during the developmental stages, it was found to be lower in chromatin-nucleolus stage 73.5 ± 3.01 and maximum in secondary vitelliogenic stage 184 ± 4.72 and the increase was statistically significant ($P < 0.0005$). Shirali *et al.*, (2012) [25] in common carp studied that the nuclear dimensions increased during the development of the oocytes which was minimum in Chromatin-nucleolus and reaches

maximum in primary vitellogenesis thus present observations reveal similar pattern of oocyte and nucleus growth as reported in common carp. In the present study the thickness of zona pellucida varied with developmental stages it begins to increase from Cortical alveolus stage and attains maximum thickness in maturation phase and the increase was statistically significant ($P < 0.0005$). El-Saba *et al.*, (2013) [26] found similar types of results in *Oreochromis niloticus*.

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