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Surface Morphology of Germ Cells in Pre breeding Testis of Himalayan Newt

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Abstract

Pre breeding testis (March – May) of the first year specimen exhibited the lobular arrangement of germ cells in different groups. The peripheral lobules are dominated by rough surfaced gonial cells, and smooth walled spermatocytes. The inner lobules exhibited more advanced gonial cells. However, no sperm were observed in the lobules suggesting the progression of the spermatogenic activities in these months. The empty lobules which are found in March – April testes suggest the release of the sperm from sperm nests (spermatophores) before the onset of breeding season.

Abstrait

Le testicule pré-reproducteur (mars-mai) du spécimen de la première année a exposé l'arrangement des cellules germinales dans les groupes différents. Les lobules périphériques sont dominés par les cellules goniales à la surface rougmeuse, et les spermatocytes à la paroi lisse. Les lobules intérieures ont exposé des cellules goniales plus avancées. Pourtant, aucun sperme n'était observé dans les lobules, ce qui suggérait la progression des activités sperma-togéniques à ces mois-ci. Les lobules vides trouvées dans les testicules de mars-avril suggèrent l'écoulement du sperme des spermatophores avant le commencement de la saison d'accouplement.

Various workers have studied annual testicular cycles of the amphibians, particularly of anura and urodela. (2, 3). On the basis of histological studies have identified that the annual testicular cycle of Himalayan Newt (*Tylototriton verrucosus* Anderson) can be subdivided into four distinct phases *viz*, Pre Breeding (March–May), Breeding (June–September), Postbreeding (October–November), and Regression (December– February).

In the present communication an attempt has been made to co-relate the histological features observed in a pre-breeding testis under light microscopy with that of surface morphological architecture revealed from Scanning Electron Microscopy. (SEM).

Material and Methods

Material

The male Himalayan newt *Tylototriton verrucosus* Anderson (Urodela : Amphibia) era collected from natural habitat of Darjeeling hill, (Altitude-1660 mts., Latitude-26-95 East and Longitude-88.27 North) and a culture was established in our laboratory. They were feed with normal as well as fish meal.

Methods

Testes of pre breeding specimens (April–May) were surgically removed and medially cut into two halves. One half was kept into the Bouin's solution (aqueous) for routine histological technique. Remaining half was fixed in 2.5% glutaraldehyde with 0.1M sodium cacodylate buffer (pH7.1) for 4 hours at 4°C. After fixation the materials were transferred 1% osmium tetroxide solution in same buffer for 2 hours. After 2 hours the tissue was washed with cacocylate buffer and dehydrated through ascending graded alcohol, and was treated with a mixture of absolute alcohol and amyl acetate (1:1) for 30 minutes.

The surface morphology was studied under Hitachi – S530 Scanning Electron Microscope at Central Instrumentation Center of Burdwan University. CPD and gold coating were done before the study at the same institute.

Results

Light Microscopy Study

The cross-section of the pre-breeding testis (April–May), seminiferous tubules (cysts) shows different stages of spermatogenesis. Primary and secondary spermatogonial cells, primary and secondary spermatocytes, and spermatids are found in different cysts.

Primary spermatogonial cells (PSG) are distributed singly and located adjacent to the basement membrane of the seminiferous tubule. These cells are bigger in size, lightly stained and chromatin material finely distributed throughout the nucleus in a reticular fashion.

Secondary spermatogonial cells (SSG) are comparatively smaller than that of primary spermatogonial cells (PSG). The nucleus bears condensed chromatin materials. These cells contain very small amount of cytoplasm.

Primary spermatocytes (PSC) are larger in size and found either in the central parts of the cross-section of the cysts, or attached to the well of the tubules. In the cross-section of the PSC cyst, various types of meiotic stages are also seen. After the completion of the first meiotic division secondary spermatocytes (SSC) are formed. Secondary spermatocytes are smaller in size and found near the primary spermatocytes. A group of spermatids formed spermatid nest. This type of spermatid nest often found at the wall of the seminiferous lobules. There are some interstitial cells found in between the seminiferous lobules. They are large, compact and ovoid in shape The nucleus is large and dense chromatin granules seen in the nucleus.

Scanning Electron Microscope Study

Under the Scanning Electron Microscope the peripheral lobules of the testis shows primary and secondary gonial cells. The surface of the gonial cells are rough. Spermatocytes and spermatids are absent or rarely present in this lobule. The inner lobules of the testis showsmooth electron dense spermatocytes and smaller spermatids. (Fig.–3B). In higher magnification a few mature spermatids with tail are also seen. (Fig–3C). There are some are Leydig cells in interstitial region. The middle portion of the testis some empty lobules are found (Plate 1 A–F).

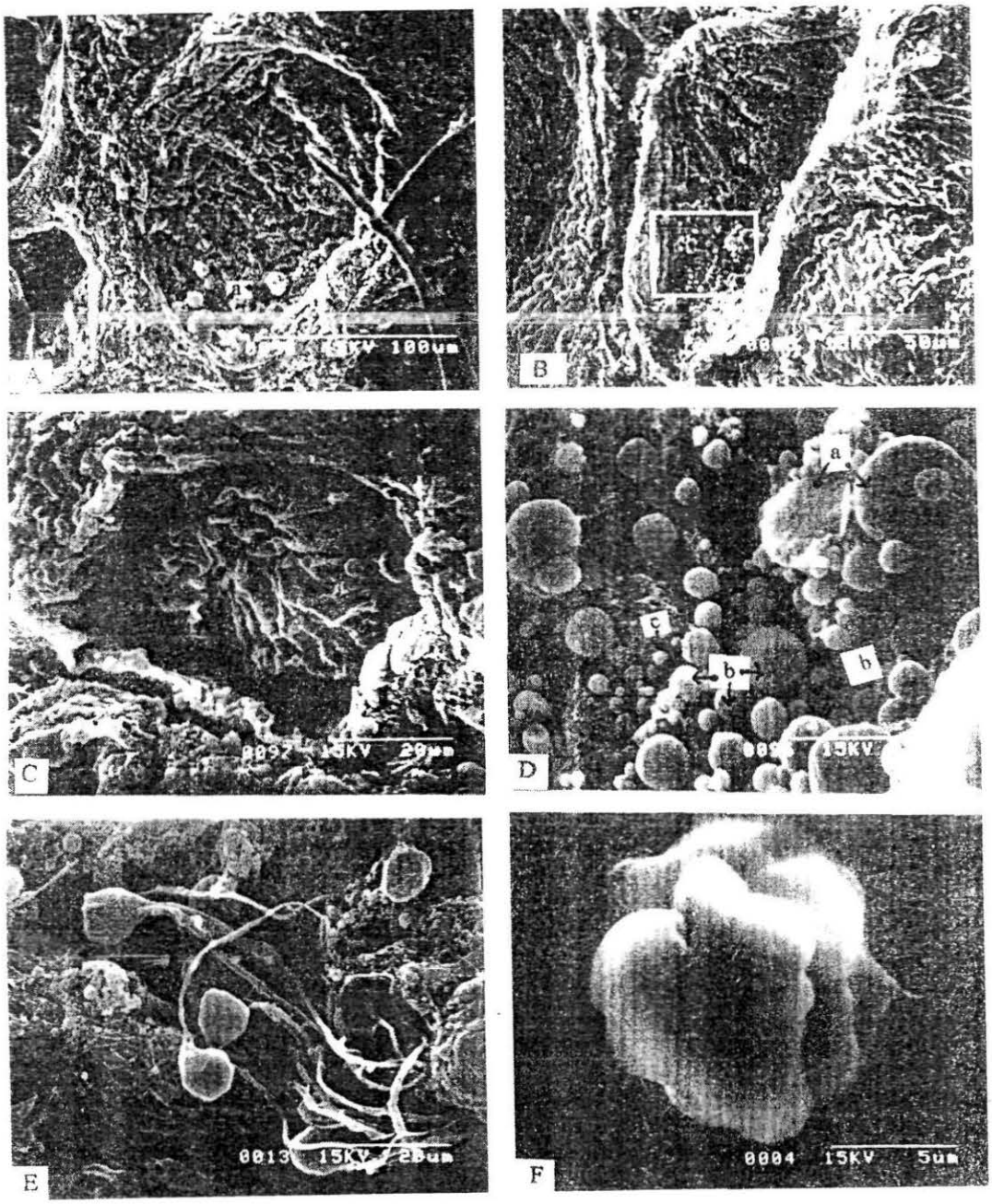
Discussion

Among the Urodels, two distinct spermatogenic patterns are recognized :

(a) Classical annual spermatogenic pattern found in aquatic breeders viz. Ambystomatidaes, Amphiumidaes, and most Salamandridaes. In these species spermatogenesis begins in the Spring.

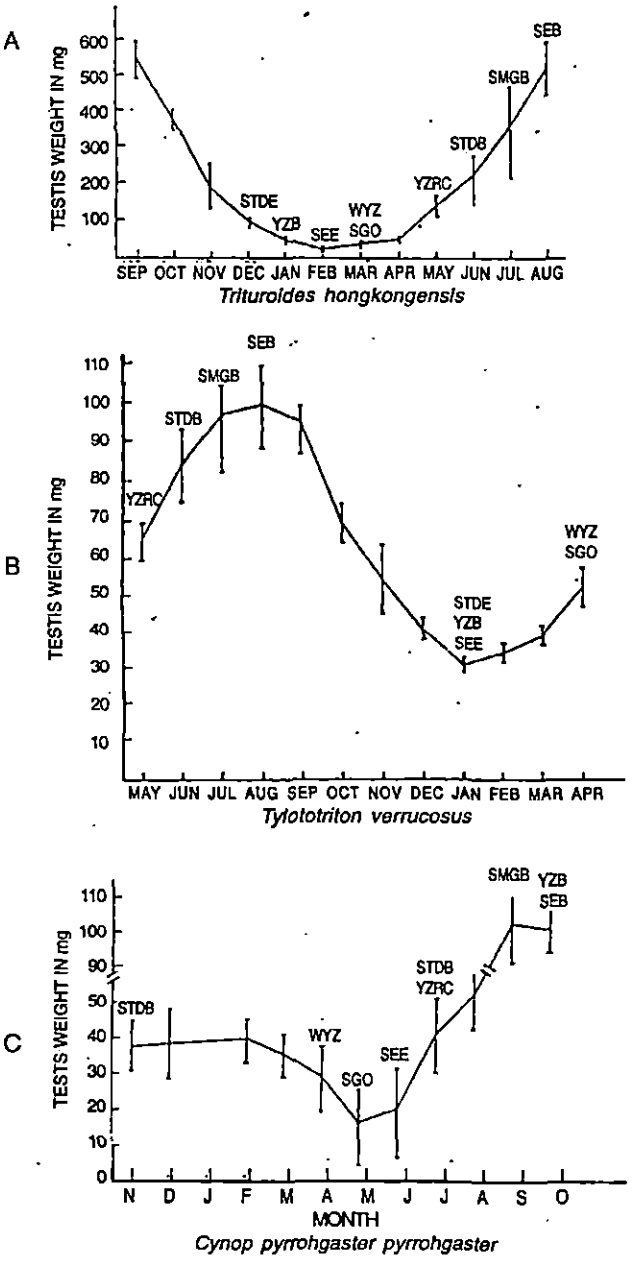
(b) Biennial pattern, the characteristics of Plethodontids salamanders, where gametogenesis recorded biannually.

Tylototriton follows a classical annual pattern as found in *Triturodies hongkongensis* (7) and *Cynopyrrhogaster*



1. (Figs. A–F) Scanning Electron Microscopic photograph of pre-breeding testis from Himalayan Newt. Fig-A Peripheral lobules (500X) (a) rough gonial cells, Fig-B Inner lobule (600X) Showing gonial cells, spermatocytes, and spermatids. Fig-D Magnified view of block portion of Fig-B (a) gonial cells, (b) spermatocytes, (c) spermatids, (4000X), Fig-C Empty lobule (1500X) Fig-E mature spermatid with tail. (2000X), Fig-F Interstitial cell (6000X).

pyrrhogaster (6). However, testicular composition and events are differ among these three species and events have been summarized in the diagram – 1. In *Tylototriton*, the on set of spermatogenic activity is palpable in April testis. In the first year specimen the spermatogenic activity is restricted as spermatocytes are found to some particular lobules only. Meiosis initiates in May testis where spermatogonial cells present in peripheral lobules while primary spermatocytes are restricted in the inner lobules of the testis. Meiotic upsurge is restricted in June and July specimens, where about 80% of the lobules are studed with spermatocytes at different divisional stages.



SEB – sperm evacuation begins, SEE – sperm evacuation ends, SGO – sperm ontogenic onset, SMGB – spermiogenesis begins, STDE – spertid formation ends, STDB – spermatid formation begins, WYZ – well developed yellowzone, YZB – yellow zone formation begins, YZRC – yellow zone resorption complete.

Spermatogenesis commences in the June specimen and continues during the month of July to December (2,3). In the second year specimen spermatogenetic activity have been recorded in the both apical and caudal lobule but is not synchronous in all lobules and occurs in a wave like fashion. (2,3).

The Scanning Electron Microcopy observations have strongly supported the earlier observations (2,3) and on the basis of testicular compositions and the activity of yellow zone region, it can be strongly concluded that the spermatogenesis of *Tylototriton verrucosus* follows an annual pattern.

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SEXUAL DIMORPHISM IN ENZYME ACTIVITY PATTERN OF *Canis lupus chanco* (TIBETAN WOLF)

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A biochemical study was undertaken to reveal enzyme activity pattern of male and female captive wolves reared at Padmaja Naidu Himalayan Zoological Park, Darjeeling. The quantities of total protein, serum albumin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase were determined following standard biochemical techniques. The study indicated a strong sexual dimoephism in enzyme activity at normal physiological condition.

Introduction

Wolves are of generally two types – the Red wolves and the Grey wolves. The Tibetan wolf – *Canis lupus chanco* is one of the grey wolves. This Himalayan creature is found at an altitude between 7000 and 13000 feet in the Western Himalayas. These canines prey upon larger mammals, such as wild goats, sheeps and other high altitude herbivores to smaller ones like rodents besides birds (Annual Report PNHZP, 1995–1996).

Lots of information are available on the blood cells and various blood parameters of diverse vertebrates. Several workers have stressed upon the possibility of employing haematological indices as aids to the diagnosis and assessment of diseases. In the present work blood was collected from some of the captive wolves and was tried to estimate the total protein, serum albumin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase were determined following standard biochemical techniques. A biochemical study was undertaken to reveal the enzyme activity pattern in male and female wolves.

Materials and methods

Experimental animals

The specimens under experiment were from Padmaja Naidu Himalayan Zoological Park, Darjeeling, situated at an elevation of 2133.5 mts. Three Tibetan wolves were taken for the experiment.

Serum collection

10 ml of blood was drawn from individual wolf and centrifused (1 hr, 37°C) when the blood cells precipitated down. The supernatant was the only source for the serum which thereafter was centrifused (3000 RPM, 5 mins.).Discarding the unwanted materials the collected serum was immediately used for the assay.

Experimental procedure

The commercial kits were used for getting the results without delaying time as because the enzyme activity changes with the change of time. The experiments were done according to the protocols as provided by the kits (Qualigens diagnostics from Glaxo and Dr. Reddy's laboratory).

Results

After using the kits in required proportions and incubating in the prescribed order we recorded the results obtained from the spectrophotometric observations.

	Male	Female
Total Protein	8.29 gm%	8.79 gm%
Serum Albumin	4.13 gm%	3.82 gm%
Alkaline Phosphate	16.22 U/ml	14.24 U/ml
SGOT	16.25 U/ml	14.50 U/ml
SGPT	12.16 U/ml	10.92 U/ml

Discussion

From the observations recorded we noticed that all the blood parameters, except that of the total protein, show a greater value in male than that of the female wolf.

SGPT catalyses transfer of amino group from L–alanine to α–ketoglutarate with formation of pyruvate and glutamate. SGPT catalyses transfer of amino group from L–aspartate to α–ketoglutarate with formation of oxaloacetate and glutamate. Formed products are then used for citric acid cycle and protein biosynthesis (Das, 1995). The experimental result suggests that male wolves are metabolically more active than the females under captive condition.

SGOT, SGPT and alkaline phosphatase are enzymes which are globular in nature and supply a mass to the total protein.

A higher total potein level in female than the male may be due to the fact that the female bears the baby and so she feeds both for herself and the baby (Chatterjee, 1992). Again, a high total protein content in such carnivorous wild animals in respect to a normal human being (6.0–6.9 gm/100 ml, Chatterjee, 1992) suggests that it may help them during starvation period (there may be a chance of not getting adequate food everyday) as protein reservoir.

The serum albumin content in Tibetan wolves is in normal level (54–60% of the total plasma protein, Das, 1995) but the female shows a declined value than the male. We know that when the body fluid discharge or decreases the albumin level also decreases (McMurray, 1982). As the female in our experiment was pregnant, so the decreased albumin may be due to the placental supply to the baby.

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