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Spermatogenesis in *Callorhynchus callorhynchus* (Linnaeus) (Pisces: Holocephali), from Chile.

Espermatogénesis en *Callorhynchus callorhynchus* (Linnaeus) (Pisces: Holocephali), en Chile

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ABSTRACT

Spermatogenesis in reproductive active male *Callorhynchus callorhynchus* was investigated microscopically. Spermiocytogenesis as well as seven stages of spermiogenesis are described. Collecting ductules for the transport of sperm from the ripe spermatocytes to the vasa efferentia after spermiation, are described and illustrated. The histological description of spermatogenesis has not been described in Callorhynchidae before.

Key words: Spermatogenesis, spermiocytogenesis, spermiogenesis, Callorhynchidae.

RESUMEN

Se estudió microscópicamente la espermatogénesis en machos activamente reproductivos de *Callorhynchus callorhynchus*. Se describe la espermiocitogénesis, así como siete estadios de espermiogénesis. Se describen e ilustran los ductos colectores para el transporte de espermios desde los espermatocitos maduros hasta los vasa efferentia, después de la expulsión de los espermios. La descripción histológica de la espermatogénesis en Callorhynchidae no había sido descrita anteriormente.

Palabras clave: Espermatogénesis, espermiocitogénesis, espermiogénesis, Callorhynchidae.

INTRODUCTION

Testicular structure in elasmobranchs is polyspermatogenic and the unit of structure and function is the spermatocyst (Parsons & Grier 1992). Hoar (1969) first recognised morphologically different testes types among elasmobranchs. Pratt (1988) described three distinctly different types namely, radial, diametric and compound. Although there are differences in the patterns by which spermatocysts originate and develop in different elasmobranch species (Pratt 1988), spermatocyst development and spermatogenesis are similar in all the species examined thus far (Parsons & Grier 1992). In elasmobranchs spermatogenesis occurs in spermatocysts which are arranged in a manner that seems to be unique among the vertebrates (Hoar 1969).

Callorhynchidae, one of three living families of Chimaeriformes, are cartilaginous fishes confined to cooler waters off southern Australia, New Zealand, southern Africa and South America. *Callorhynchus callorhynchus* is distributed along the entire coast of Chile almost reaching the coast of Peru (Lorenzen *et al.* 1979) and also along the coast in Argentina (Menni *et al.* 1984).

Studies on the reproductive biology of Holocephali have been investigated by several authors (Garman 1904, Burlend 1910, Gorman 1963, Stanley 1963, Stahl 1967, Roosen-Runge 1977, Jones & Jones 1982 and Hara & Tanaka 1986) but detailed studies of the process of spermatogenesis have not been described before. Biological aspects, which included the reproduction of *C. milii* from New Zealand, have been described by Gorman (1963). Aspects of reproduction for chimaeroids have also been described by Garman (1904) and Burlend (1910).

Due to the confusion that exist in terms of testicular terminology in elasmobranchs (see Parsons & Grier 1992), the following terms will be used in this report (terms used by previous workers in brackets): spermatocyst (ampulla and follicle); spermatocytogenesis (spermiocytogenesis) and spermiogenesis (spermiohistogenesis).

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MATERIALS AND METHODS

C. callorhynchus were obtained from fishermen in Queule (40 °S), operating gillnets in the south-central area of the Chilean coast.

Specimens of *C. callorhynchus* were collected during February, March and April 1991. For the purpose of this study only adult males were selected. Males that possessed an erupted tenaculum and calcified claspers, were considered reproductively mature. The testis were dissected out and preserved in Bouin's fluid. After fixation, the samples were embedded in paraffin wax (Histosec) and sectioned on a rotary microtome. The sections were cut at 7-10 µm thickness and stained with eosin and Harris's haematoxylin.

RESULTS AND DISCUSSION

Cross-sections that were made of the testes of *C. callorhynchus*, reveal a diametric pattern of spermatocyst development as described by Pratt (1988). Spermatogenesis or the development of male germ cells has been divided into several stages by previous authors. Roosen-Runge (1977) divided spermatogenesis into three stages: a) spermatocytogenesis, the "multiplication stage" or mitotic stage; b) spermatocytic or meiotic stage and c) spermiogenesis or spermatid stage.

Teshima (1981) divided spermatogenesis into two stages: (a) spermiocytogenesis (which is a combination of the mitotic and meiotic stages), where spermatogenic cells repeatedly divide to become spermatids,

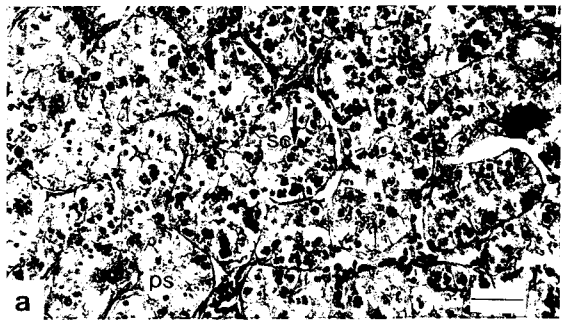

Fig.1.a. Aspects of spermatogenesis of *Callorhynchus callorhynchus*. Primary spermatocytes showing a central lumen and Sertoli cells arranged around the center of the spermatocyte ; sc -Sertoli cell. Bar scale = 100 µm.

Aspectos de la espermatogénesis de *Callorhynchus callorhynchus*. Espermatocitos primarios mostrando un lúmen central y células de Sertoli ordenadas alrededor del centro de los mismos. ps- espermatocito primario; sc- célula de Sertoli. Escala de la barra = 100 µm.

Fig.1.c. Stage 2 vermiform spermatids (indicated by arrow head). Bar scale = 100 µm.

Espermátidas vermiformes, estadio 2 (indicadas por la flecha). Escala de la barra = 100 µm.

Fig.1.b. Stage 1 spermatids. Bar scale = 100 µm.

Espermátidas estadio 1. Escala de la barra = 100 µm.

Fig.1.d. Stage 3 spermatids (indicated by arrow head). Bar scale = 100 µm.

Espermátidas, estadio 3 (indicadas por la flecha). Escala de la barra = 100 µm.

and (b) spermiohistogenesis, where the spermatids develop into spermatozoa.

The author of this paper prefers the term spermiogenesis, which is more widely used to describe the latter stage (Bols *et al.* 1980, Stanley 1971).

Spermatocytogenesis

The onset of spermatocytogenesis is indicated by the appearance of primary spermatogonia confined to the germinal zone (Parsons & Grier 1992). These cells are in close association with seminiferous epithelial cells and are not contained in spermatocysts. Stanley (1966) considered these epithelial cells homologous with mammalian Sertoli cells and I will refer to these cells as Sertoli cells. Seminiferous epithelial cells surround the primary spermatogonia. A close association between secondary spermatogonia and Sertoli cells develop as the early spermatocyst develops (Parsons & Grier 1992). A single layer of Sertoli nuclei occupies the area next to the lumen while the nuclei of the primary spermatogonia are situated at the periphery (Fig.1.a). The same situation was described for *Hydrolagus colliei* (Stanley 1963). Spermatogonia develop, through mitotic division, between the basement membrane and the seminiferous epithelium. Proliferation of the spermatogonia push the nuclei of the seminiferous epithelium towards the lumen. The mitoticaly active spermatogonia continue to proliferate and consequently the spermatogonial spermatocysts develop from a unilayered to a multilayered stage. The seminiferous epithelium or Sertoli cells now occupy an adluminal position (Fig.1.a).

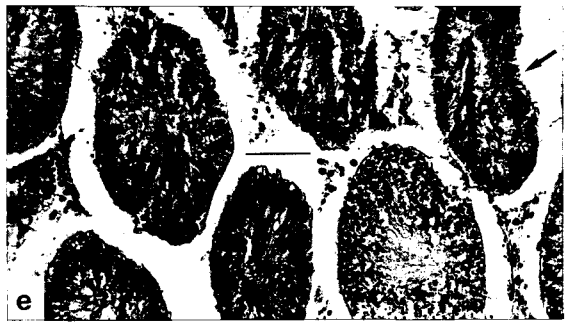

Fig.1.e. Stage 4 spermatids. A prominent hook is visible at the base of the spermatid (indicated by arrow head). Bar scale = 100 µm.

Espermátidas, estadio 4. En la base de la espermátida se observa un gancho prominente (indicado por la flecha). Escala de la barra = 100 µm.

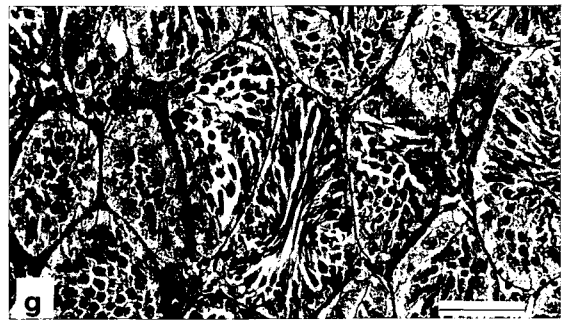

Fig.1.g. Stage 6 spermatids. Bar scale = 100 µm.

Espermátidas, estadio 6. Escala de la barra = 100 µm.

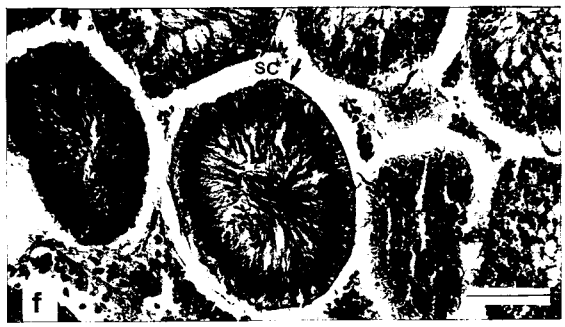

Fig.1.f. Stage 5 spermatids. These spermatids are loosely arranged. sc: Sertoli cell. Bar scale = 100 µm.

Espermátidas, estadio 5. La ordenación de estas espermátidas no es compacta. sc: célula de Sertoli. Escala de la barra = 100 µm.

Fig.1.h. Stage 7 spermatids and collecting ductule (indicated by arrow head). Bar scale = 100 µm.

Espermátidas, estadio 7 y ductos colectores (indicados por la flecha). Escala de la barra = 100 µm.

Continuous division of spermatogonia fill the entire spermatocyst, with the lumen much reduced (Fig.1.b). After repeated divisions, the secondary spermatogonia are transformed into primary spermatocytes that are delineated by a basement membrane (still engulfed by Sertoli cells) and which are the largest spermatogenic cells in the development process. The first meiotic division of the primary spermatocytes results in the formation of secondary spermatocytes, which are smaller.

Spermiogenesis

This developmental process with different stages of spermatid development can be observed in the topographic separated spermatocysts. Van der Horst & McClusky (1986), employing scanning electron microscopy, described spermiogenesis in the soupfin shark, *Galeorhinus galeus*. Their study complements the earlier transmission electron microscopic and light microscopic studies of chondrichthtyan spermiogenesis, as reviewed by Stanley (1971).

On the basis of nuclear shape and spermatocyst organization, Bols *et al.* (1980), recognized eight stages of spermiogenesis in *Raja rhina*, and seven stages in *Squalus acanthias*. Parsons & Grier (1992) examined the seasonal cycling in the testis of *Sphyrna tiburo* and classified the spermatocysts into seven spermatogenic stages. In *Galeorhinus galeus* (Van der Horst & McClusky 1986), *Mustelus palumbes* (Rossouw & Van Essen 1993) and in the present study on *C. callorhynchus*, seven stages were observed. Bols *et al.* (1980) state that the sixth and seventh stages observed in *R. rhina*, have both completed spiralization, but differ in their organization within the spermatocyst. The only difference being a tighter grouping of the spermatids, the author of this study believe this does not warrant a seventh stage.

Stage 1 spermatids show round nuclei and are not loosely arranged, but show clumps or groups of spermatids (Fig.1.b). This phenomenon was also observed in *M. palumbes* (Rossouw & Van Essen 1993). According to Stanley (1971), this is a clear indication of spermatocyst formation, thus each group of spermatids originate from a single spermatogonium. Vermiform and spindle shaped nuclei, are recognized in stage 2 (Fig.1.c). It is difficult to distinguish tails at this stage. Stage 3 spermatid nuclei are in the process of elongation, still showing a vermiform nature (Fig.1.d). Spermatids are still arranged at random throughout the spermatocyst at this stage.

In Stage 4 the spermatids have elongated further, the waved shape is lost except for the basal end which now shows a prominent hook (Fig.1.e). Spermatid heads are also forming definite groups arranged on the periphery of the spermatocyts.

Stage 5 spermatids show clearly discernable tails, corkscrew shaped heads and Sertoli cell nuclei are visible on the peripheral spermatocystic wall (Fig.1.f). The heads are loosely arranged and do not intertwine at this stage. The position that the Sertoli cell nucleus now occupies, implies that it probably migrated peripherally during spermiogenesis. Simpson and Wardle (1967), observed this migration in *Squalus acanthias*. Migration could not be observed in *M. palumbes* (Rossouw & Van Essen 1993) or in *C. callorhynchus* (this study).

Intertwined, grouped spermatids with elongated tails are designated to stage 6 (Fig.1.g). All heads orientate towards the basement membrane.

Stage 7 spermatids or physiologically immature spermatozoa represent the final stage in spermiogenesis before spermiation. The sperm heads are tightly packed, and arranged spirally in the spherically shaped spermatocyst (Fig.1.g). Due to the spherical shape of the spermatocyst, cross sections as well as longitudinal sections of the sperm heads are visible in the individual spermatocyst sections (Fig. 1.g).

Spermiation

The release of spermatozoa from the individual spermatocysts has been described for several species of elasmobranchs (Stanley 1963, Stanley 1971, Teshima 1981, Rossouw 1983, Callard et.al. 1989 and

Rossouw & Van Essen 1993), but an accompanying illustration has only been described for *M. palumbes* (Rossouw & Van Essen 1993). The process of sperm release in *C. callorhynchus* was found to be similar to that in *R. annulatus* (Rossouw 1983) and *M. palumbes* (Rossouw & Van Essen 1993). The duct system for conveying the spermatozoa is not patent until the spermatocysts have reached the final stage of spermiogenesis (Grier 1992). Collecting ductules appear in the matrix between the spermatocysts and are connected to those spermatocysts ready for sperm release (Fig. 1.h). After an opening is established in the spermatocystic wall, the sperm is conveyed in the ciliated lumen of the collecting ductule. The mechanism for opening of the wall and consequent release of sperm, has yet to be established. Networks of these collecting ductules terminate in the vasa efferentia on the medial side of the testis. Degeneration of the spermatocysts and final resorption follows spermiation.

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LITERATURE CITED

BOLS NC, SA BOLISKA, JB RAINVILLE & HE KASINSKY (1980) Nuclear basic protein changes during spermiogenesis in the longnose skate and the spiny dogfish. Journal of Experimental Zoology Supplement 2: 23 - 34.

BURLEND TH (1910) The urogenital organs of *Chimaera monstrosa*. Procceedings of the Zoological Society of London 1910. 510 - 534.

CALLARD IP, LL KLOSTERMAN, LA SORBERA, LA FILETI & JC REESE (1989) Endocrine regulation of reproduction in elasmobranchs: archetype for terrestrial vertebrates. Journal of experimental Zoology 2: 12 - 22.

GARMAN S (1904) The Chimaeroids (Chismopnea Raf, 1815; Holocephala Mull, 1834), especially Rhinochimaera and its allies. Bulletin of the Museum of Comparative Zoology 41: 251 - 263.

GORMAN TBS (1963) Biological and economical aspects of the elephant fish, *Callorhynchus milii* Bory in Pegasus Bay and the Canterbury Bight. New Zealand Marine Department Fisheries Technical Report 8: 14 - 35.

GRIER HJ (1992) Chordate testis: the extracellular matrix hypothesis. Journal of Experimental Zoology 261 :151 - 160.

HARA M & S TANAKA (1986) Fine structure of spermatogenesis and mature spermatozoa in elasmobranch and chimaera fishes: a systematic consideration. Development Growth and Differentiation p. 173.

HOAR W S (1969) Reproduction. In WS Hoar & DJ Randall (eds.). Fish physiology Vol 3:1 - 72 Academic Press, New York.

JONES N & RC JONES (1982) The structure of the male genital system of the Port Jackson shark, *Heterodontus portusjacksoni*, with particular reference to the genital ducts. Australian Journal of Zoology 30: 523 - 541.

LORENZEN S, C GALLARDO, C JARA, E CLASING, G PEQUEÑO & C MORENO (1979) Mariscos y peces de importancia comercial en el sur de Chile. Universidad Austral de Chile, Valdivia.

MENNI RC, RA RINGUELET & RH ARAMBURU (1984) Peces marinos de la Argentina y Uruguay. Ed. Hemisferio Sur, 359 pp., Buenos Aires.

NORMAN JR (1937) Coast Fishes. Part II. The Patagonian Region. Discovery Reports 16 :1 - 150.

PARSONS GR & HJ GRIER (1992) Seasonal changes in shark testicular structure and spermatogenesis. Journal of Experimental Zoology 261: 173 - 184.

PRATT HL (1988) Elasmobranch gonad structure: a description and survey. Copeia 3: 719 - 729.

ROOSEN-RUNGE EC (1977) The Process of Spermatogenesis in Animals. Cambridge Univ. Press, Cambridge, pp. 102 - 132.

ROSSOUW GJ (1983) The biology of the sandshark *Rhinobatos annulatus* in Algoa Bay with notes on other elasmobranchs. Ph. D. thesis, Univ. Port Elizabeth, South Africa. 180 pp.

ROSSOUW GJ & LD VAN ESSEN (1993) Spermatogenesis in the male whitespotted houndshark, *Mustelus palumbes* Smith, 1957. South African Journal of Science 89: 244 - 246.

STAHL BJ (1967) Morphology and Relationships of the Holocephali with special reference to the Venous System. Bulletin of the Museum of Comparative Zoology 135: 181 - 195.

STANLEY HP (1963) Urogenital morphology in the Chimaeroid fish, *Hydrolagus colliei* (Lay and Bennet). Journal of Morphology 112: 99 - 127.

STANLEY HP (1966) The structure and development of the seminiferous follicle in *Scyliorhinus caniculus* and *Torpedo marmorata* (Elasmobranchii). Z. Zellforsch. 75: 453 - 468.

STANLEY HP (1971) Fine structure of spermiogenesis in the elasmobranch fish *Squalus suckleyi*. II. Late stages of differentiation and structure of the mature spermatozoon. Journal of Ultrastructural Research 36: 103 - 108.

TESHIMA K (1981) Studies on the reproduction of Japanese smooth dogfishes, *Mustelus manazo* and *M. griseus*. Journal of the Shimonoseki University of Fisheries, 29: 113 - 199.

VAN DER HORST G & L McCLUSKY (1986) Scanning electron microscopy of the testicular ampullae and spermatocysts of the Soupfin shark, *Galeorhinus galeus*. Electron microscopy Society of Southern Africa. Proceedings Vol 16: 71.