MORPHOLOGICAL ASPECTS OF GONADAL MATURATION IN THE HAKE, MERLUCCIUS GAYI GAYI

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ABSTRACT. Morphological aspects of gonadal maturation in the hake, **Merluccius gayi gayi**.

The histological structure of ovaries and testes was described based on the study at the light microscope of gonads from hake specimens in different stages of maturity. Besides, some ultrastructural features were analysed, with special emphasis on possible sites involved in steroidogenesis.

The results led to characterize histologically the macroscopic reference scale of gonadal maturity previously proposed for hake. Particularly, the histological structures representatives of the multiple spawning of this species were analysed.

Key words: reproduction, histology, ultrastructure, hake, **Merluccius gayi gayi**.

RESUMEN. Aspectos morfológicos de la maduración gonadal en la merluza, **Merluccius gayi gayi**.

Sobre la base del estudio de las gónadas de ejemplares de merluza en diferentes estadios de maduración sexual, se describió la estructura histológica de los ovarios y testículos a nivel de microscopía de luz. Además, se analizaron algunos

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aspectos ultraestructurales con especial énfasis en los posibles sitios involucrados en la esteroidogénesis.

Los resultados permitieron caracterizar histológicamente la escala macroscópica apreciativa del estado de maduración gonadal propuesta previamente para la merluza. En particular, se analizaron las estructuras histológicas representativas del desove múltiple de esta especie.

INTRODUCTION

The biological and physiological characteristics of the reproductive cycle of the hake ("merluza") Merluccius gayi gayi (Gulchenot 1848) point out this species as a peculiar case study. Females spawn the eggs in batches and a variable percentage of the adult population is sexually mature throughout the year (Balbontín & Fischer 1981; Goldberg 1985). Thus, physiological aspects of the reproduction can be studied quite independently from factors influenced by the season of the year (Balbontín et al. 1978; Herrera 1986).

Since the development of the egg production method for estimating spawning biomass of fish (Parker 1980, Lasker 1985), the importance of histological criteria to characterize sexual stages of maturity in fish resources has increased. An illustrated description of the histological characteristics of the gonads of the hake has not yet been published, except for postovulatory follicles (Hunter & Macewicz 1985).

This paper is mainly concerned with the histological structure of ovaries and testes of the hake at the light microscopical level. Besides, some ultrastructural features are described with special emphasis on possible sites involved in steroidogenesis in the gonads. Other aspects of the reproductive physiology of the hake related to sex steroids and calcium plasmatic levels will be published elsewhere.

MATERIALS AND METHODS

Hakes were collected aboard commercial bottom trawling boats along the coast of Central Chile, between Los Vilos (31º56'S) and Algarrobo (34º30'S). Previous to the histological study, gonadal maturity was macroscopically classified into six stages for females and five for males (Balbontín & Fischer 1981). These stages are: I, virginal gonads; II, beginning of gonadic acti-

vity, including those gonads recovering from a recent spawning; III, maturing gonads, ovaries with oocytes visible to the naked eye; testes whitish but not flowing sperm; IV, ripe gonads, oocytes translucent, testes with flowing sperm; V, spent gonads, ovaries with resorbing oocytes of various sizes; IIIa (only for females), ovaries in a stage similar to III that return to stage IV after spawning a batch of eggs.

For light microscopy analysis, gonadal samples were fixed in Bouin's fluid and embedded in paraffin. Histological sections were cut at 5 um and stained with hematoxylin-erithrosine.

To determine if the deep staining seen in the cytoplasm of previtellogenic oocytes truly corresponded to a basophilic reaction, the histochemical technique of galocianin-chromalum was used for sections of mature and immature ovaries. The staining solution was employed at pH 1.65. Sections incubated in ribonuclease (pH 6.8) at 37º for 4 hours were used as controls, with enzymatic digestion of cytoplasmic RNA.

For transmission electron microscopy, gonadal samples of 1 mm in thickness were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4ºC for 3 hours, followed by post-fixation in 1% OsO_4 at 4ºC in phosphate buffer for 2 hours. Tissues were dehydrated in ethanol and embedded in Epon 812. Semithin sections were stained with Toluidine blue-borax for light microscopy whereas ultrathin sections, contrasted with lead citrate and uranyl acetate were inspected and photographed in a Zeiss 109 electron microscope.

RESULTS

Histological description of gonadal stages.

Testes: The testis is formed by lobules, delimited by connective tissue strands. The parenchyme of the immature testis (stage II) is formed chiefly by small spermatogonial cysts lying over connective tissue septa. This gonadal stage is characterized by a branched trabecular disposition, where cysts of zygotene primary spermatocytes are intermingled with cysts of primary and secondary spermatogonia (Fig. 1a, b). During testicular maturation (stage III), cysts of zygotene and pachytene spermatocyte are more prominent, with a reduction of the spermatogonial compartment (Fig. 1c). When maximal maturity is reached (stage IV), the testicular tissue contains mostly cysts of spermatids and spermatozoa (Fig. 1d, e). After spermiation and during gonadal regression (stage V), the testis exhibits thick connective tissue septa lined by elongated cells and limiting spaces where spermatozoa can be found (Fig. 1f).

Ovaries: The virginal parenchyme (stage I) is represented by oogonia and primitive, previtellogenic oocytes with a scarce, slightly basophilic cytoplasm and a large germinative vesicle with numerous peripherically located nucleoli. It is also possible to see previtellogenic oocytes further developed, with a highly basophilic cytoplasm rich in cytoplasmic RNA (Fig. 2a, b).

When immature (stage II), the ovarian tissue shows predominance of previtellogenic oocytes and oocytes in endogenous vitellogenesis, being the oogonia less numerous. The oocytes have enlarged cytoplasm and less basophilia (Fig. 2c). These oocytes are surrounded by a layer of flattened thecal cells (Fig. 2d). On the contrary, during vitellogenesis (stage III), oocytes in the process of exogenous vitellogenesis are more abundant, with a central or displaced germinative vesicle. The follicular envelope and the layer of thecal cells are more prominent (Fig. 2e, f). During this phase, a chorion can be found between the oocyte and the follicular cells.

During gonadal maturation, and after recent partial egg laying (stage IIIa), the aspect (Fig. 3a, b) is similar to the preceding stage (III), but post-ovulatory follicles of different ages are found. They are bilaminar structures, comprising follicle and theca. In the vitellogenic oocytes a striated aspect of the chorion is noticeable (Fig. 3c).

In mature ovaries (stage IV), the tissue shows large vitellated oocytes which represent the more advanced cell type, with the cytoplasm fully loaded with vitelline globules. The nucleus may be displaced towards the cell periphery or even be absent, according to the degree of progress of the vitellogenesis and oocyte maturation. The follicle-thecal layer is well developed (Fig. 3d, e). On the contrary, gonadal regression (stage V), is characterized by great lytic activity in the oocytes and follicles, evidenced by follicular atresia, which is frequently found in this ovarian stage (Fig. 3f).

Gonadal steroidogenic tissue.

The testis contains irregularly shaped interstitial cells. They are found in the areas adjacent to the cysts and their distribution is not uniform. In the ovary, the oocytes are surrounded by a layer of cuboidal or cylindrical follicular cells, which are disposed outside the chorion, making up a bilayer together with the thecal cells; the latter are elongated in shape.

Ultrastructural analysis of the interstitial cells of the testis (Fig. 4a, b) as well as the follicular and thecal cells of the ovary (Fig. 4c,d,e,f) reveals that these cells present a large nucleus, with marked indentations of the nuclear envelope, the chromatin being condensed in large, peripheral clumps. In the cytoplasm, organelles associated to synthesis of sex steroids, such as a well developed smooth endoplasmic reticulum and mitochondria with laminar cristae, are seen.

DISCUSSION

Histological analysis of the male gonad in **Merluccius gayi gayi** revealed that the testis is formed by lobules, which are delimited by thin connective tissue strands, a disposition typical for most teleosts. After proliferation of primary spermatogonia, spermatogonial cysts develop. They give rise to cysts containing different stages of the spermatogenic process up to the final steps of spermiogenesis. When final spermatids are formed, the cysts expand and disrupt, thus releasing spermatozoa into the sperm ducts.

These observations agree with those by Barr (1963) in **Pleuronectes platessa**; Bara (1969) in **Fundulus heteroclitus**; Schulz (1984) in **Salmo gairdneri**; Alexandrino **et al.** (1985) in **Prochilodus scrofa**; Srivastava (1984) in **Chama striatus**, who have described the topographical anatomy of the testis, with emphasis in the histological variations of the organ according to the gonadal cycle.

In the interlobular compartment interstitial cells were found. Their ultrastructural traits, with mitochondria with lamellar cristae and a well developed smooth endoplasmic reticulum, correspond to the observations by Marshall & Lofts (1956) and O'Halloran & Idler (1970) in **Salmo salar**; Yaron (1966) in **Tilapia mossambica**; Bara (1969) in **Fundulus heteroclitus**; Nicholls & Graham (1972) in **Cichlasoma nigrofasciatum**, among others. These authors have described the presence of large polygonal, clustered or isolated cells. In their cytoplasm there is a prominent smooth endoplasmic reticulum and mitochondria provided with large, tubular cristae, observations that are endorsed also by Sugimoto & Takahashi (1979) and Eckstein **et al.** (1982) (both quoted by Nagahama 1983). These authors found morphological changes associated to increased steroidal synthesis (increased number of mitochondria with tubular cristae and development of smooth membranes of the endoplasmic reticulum) after stimulation of the interstitial cells of immature testis of **Anguilla japonica** and **Anguilla anguilla**, using chorionic gonadotropin (HCG).

In the females, histological analysis demonstrated typical asynchronic ovaries in **Merluccius gayi gayi**, in correspondance to the fact that it is a multiple spawner, a situation pointed out by Balbontín & Fischer (1981) and Goldberg (1985) for this species.

As ovaric development progresses, adcquisition of vitellum by the primitive oocytes is more evident. This process is at its maximum when the oocyte starts maturation. Together with oocytic evolution, there is a noticeable thickenning of the egg investment, which is hyperthrophic by the time of egg laying, when is invaded by blood vessels originated in the theca and accompanied by lysis of the oocytes and follicular atresia.

Similar findings have been reported by Christiansen (1971) in **Merluccius merluccius hubbsi**; Zanuy **et al.** (1973) in **Paracentropristis cabrilla**; Cerisola

et al. (1978) in **Basilichtys australis**; Wallace & Selman (1981); Sarano (1986) in **Merluccius merluccius**. These authors describe ovarian histology with a characterization of the gonadal morpho-physiological changes during the sexual cycle, with emphasis on the events occurring in the oocyte, germinal vesicle and yolk platelets. Vand der Hurk & Peute (1979) described the ovarian histology in **Salmo gardnieri** and identify three periods: ovulatory and pre-vitellogenic; in active vitellogenesis and final phase of oocyte maturation, characteristics that agree with the observations reported here for **Merluccius gayi gayi**.

Concerning the post-ovulatory follicles, their identification in the ovary is useful to ascertain the incidence of egg laying and thus, to estimate the fraction of females spawners per night in the case of multiple spawner fishes (Hunter & Goldberg 1980; Hunter & Macewicz 1980, 1985; Alarcón et al. 1984; Retamales & González 1983; Herrera & Claramunt (unpublished data). The temporary presence of these structures in mature ovaries stands for the multiple spawning characteristic of the hake, a situation reported also by Goldberg (1985). Hunter & Macewicz (1985) analyzed the structural characteristics of the post-ovulatory follicles of **Engraulis mordax**; **Sardinops sagax** **Merluccius gayi gayi**; **Scomber japonicus**; **Euthynnus lineatus**, and found very little differences among them. On these premises, Alarcón et al. (1984) described the histology of post-ovulatory follicles in **Sardinops sagax** and characterized the remmants of ovarian follicles of different ages on the basis of the criteria originally used by Hunter & Goldberg (1980) in **Engraulis ringens**.

Electron microscopical studies have provided ultrastructural landmarks of steroidogenic activity in both the follicular as well as the thecal cells of the bilamellar investment of the ovarian follicle in the hake. These landmarks are the abundant smooth endoplasmic reticulum and the outstanding tubular cristae of the mitochondria. These observations are similar to the results published by Iwamatzu & Ohta (1981, cited by Nagahama 1983) and Nagahama et al. (1982). These authors point out to the above mentioned cytoplasmic structures as a good proof for synthesis of sexual steroids by the follicular-thecal envelope.

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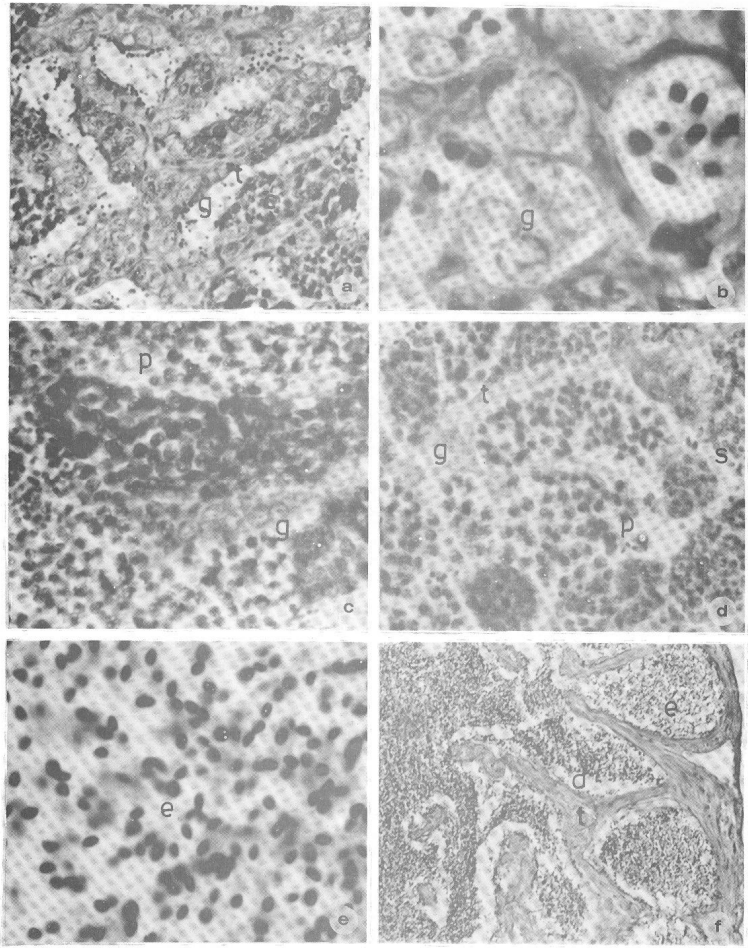
Figs. 1a (150 X) and 1b (1500 X). Immature testis. Gonadal stage II. The spermatogonial compartment is the most predominant. 1a: small spermatogonial (g) cysts (c) adjacent to connective tissue strands (t). Fig. 1c (520 X). Testicular maturation. Gonadal stage III. Cysts of pachytene (p) spermatocytes are abundant whereas spermatogonial (g) cysts are reduced in number. Figs. 1d (150 X) and 1e (1500 X). Maximal testicular maturation. Gonadal stage IV. Cysts with pachytene (p) spermatocytes and spermatids (s) are seen, separated by thin layers of connective tissue (t). Scarce spermatogonia (g) are found. In 1e, cysts filled with spermatozoa (e) are seen. Figs. 1f (150 X). Testis during spermiation and starting to regress. Gonadal stage V. Sperm ducts (d) filled with spermatozoa (e) and layered by thick connective strands (t) are observed.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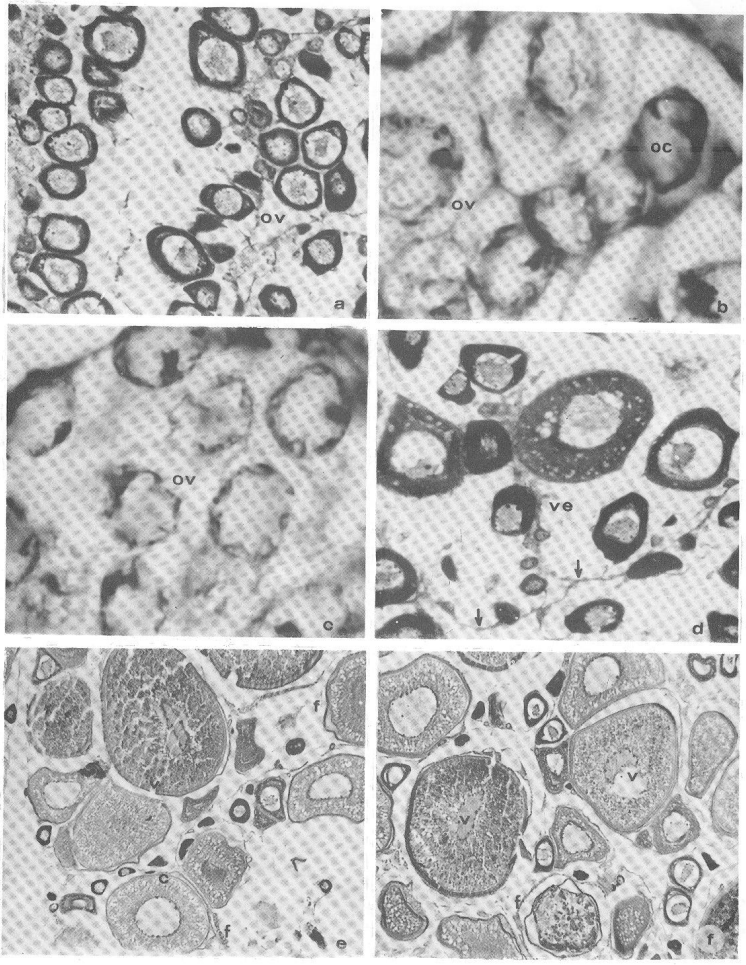
Figs. 2a (50 X) and 2b (1500 X). Virginal ovary. Gonadal stage I. 2a: general ovarian histology, with oogonia (ov) and small oocytes with a deeply basophilic cytoplasm. 2b: small clusters of oogonia (ov) and previtellogenic oocytes (oc). Figs. 2c (520 X) and 2d (150 X). Immature ovary. Gonadal stage II. Oocytes in previtellogenesis and endogenous vitellogenesis are the more abundant. 2c: Small group of oogonia (ov). 2d: general aspect of the ovary during endogenous vitellogenesis (ve); a probable thecal cell layer (arrows) surrounds the oocytes. Figs. 2e and 2f (50 X). Ovarian maturation. Gonadal stage III. Histological aspects of an asynchronic ovary. The most noticeable elements are oocytes in exogenous vitellogenesis (V), with their follicular envelope (f) and chorion (c).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Figs. 3a (150 X), 3b (520 X) and 3c (1500 X). Ovary in maturation with recent partial egg spawning. Gonadal stage IIIa. Oocytes in different stages are seen; post-ovulatory (po) follicles stand out in the stroma. 3a: oocytes in exogenous vitellogenesis (V) and post-ovulatory follicles (po). 3b: oocytes in exogenous vitellogenesis (V), covered by a chorion (c) and a layer of tall cubic follicular cells. A post-ovulatory follicle (po) is seen, surrounded by follicular and thecal cells (ct). 3c: chorion (c) and folliculo-thecal layer. Tall cubic follicular cells are seen layered by flat thecal cells (ct). 3d: (50 X) and 3e (520 X). Maximal ovarian maturation. Gonadal stage IV. Vitelline oocytes and oocytes in exogenous vitellogenesis (V) are seen. Chorion (c); folliculo-thecal layer (ft); vitelline globules (gv). 3f: (150 X). Ovarian regression. Gonadal stage V. Lysis of oocytes (lo) and follicular atresia (fa) are found together with folliculo-thecal hyperthrophy. Some macrophages can be seen in the neighbourhood.

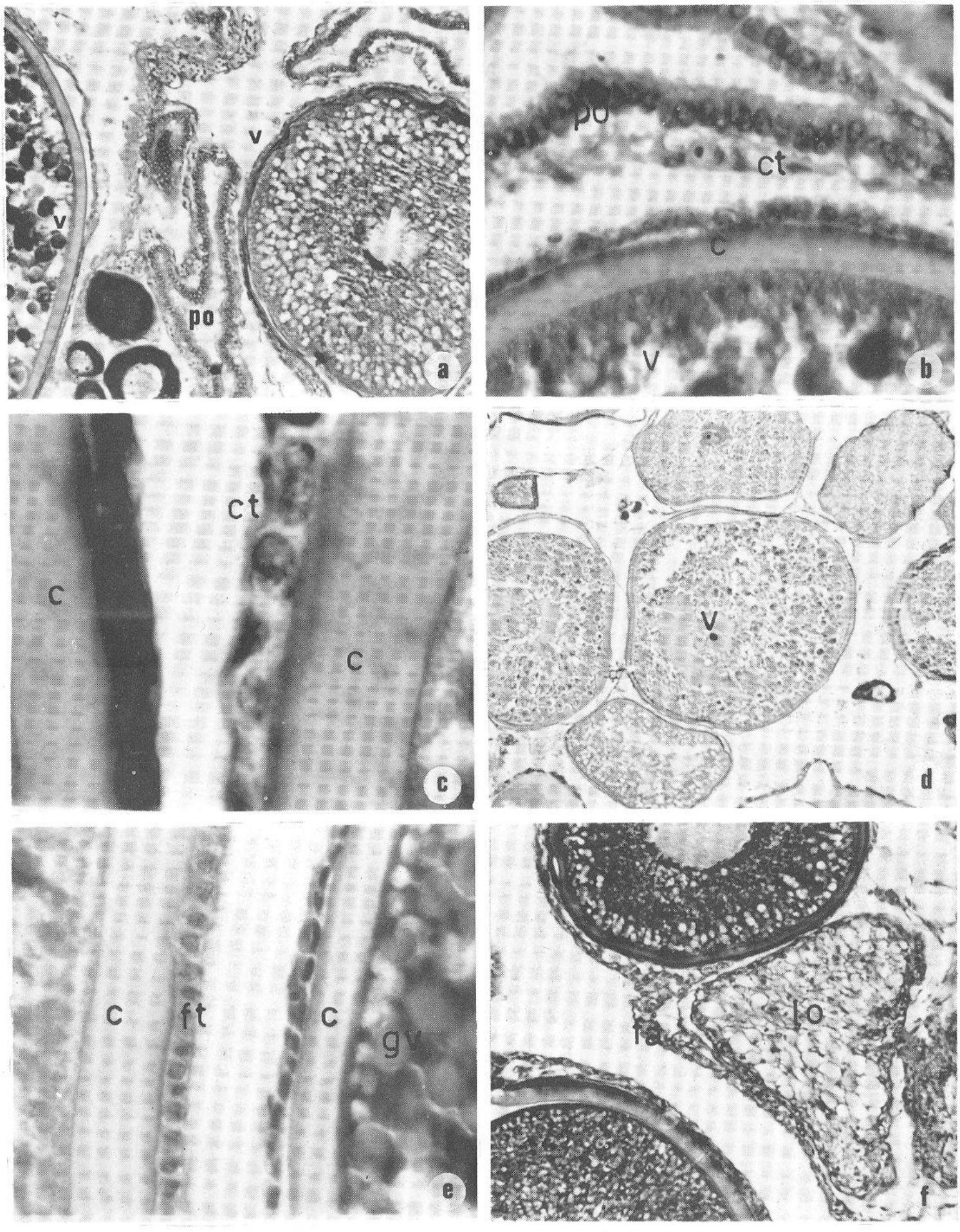
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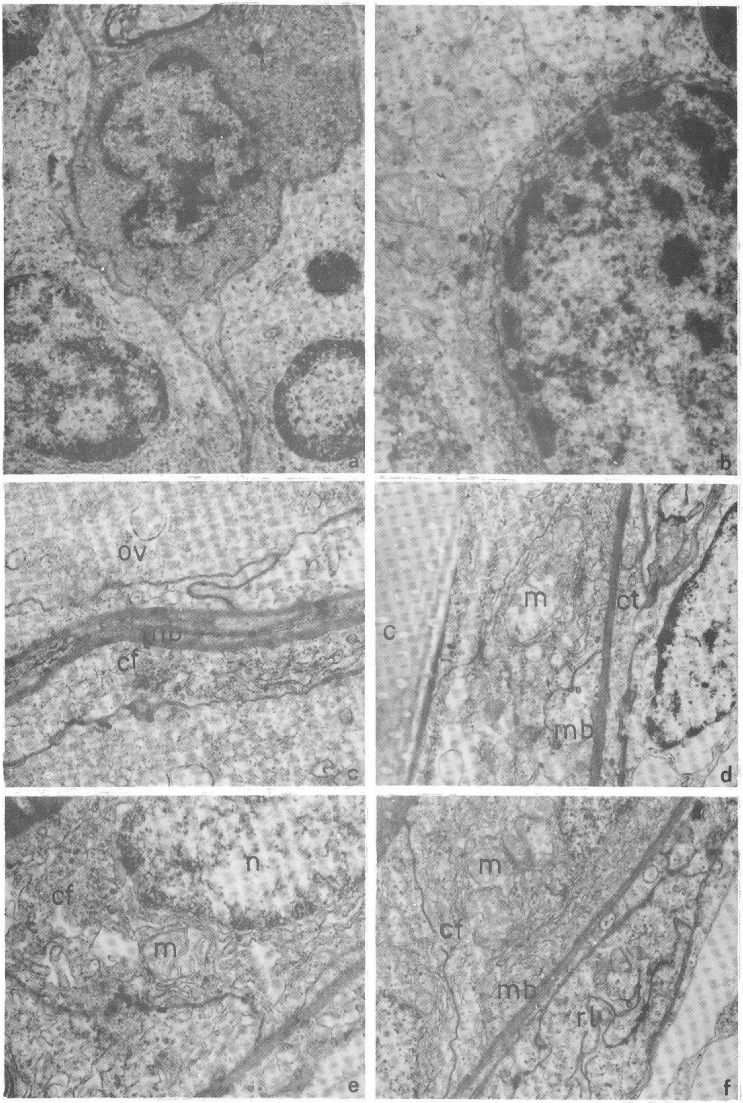
Fig. 4. Electron microscopical pictures of hake$ gonads. 4a: Interstitial testicular cell adjacent to two spermatocytic cysts. The cytoplasm is electron dense and the nucleus, of irregular shape, shows clusters of chromatin (4200 X). 4b: Mitochondria with numerous tubular cristae fill up the cytoplasm of a testicular interstitial cell (7000 X). 4c: Immature follicles with no organelles in large cytoplasmic areas (10000 X). 4d (7500 X), 4e (14000 X) and 4f (7000 X). Follicles showing exogenous vitellogenesis. Both in the follicular and thecal cells there is plenty of smooth membranes of the endoplasmic reticulum (rl) and numerous mitochondria (m) with tubular cristae. Cf: follicular cell; ct: thecal cell, mb: basal membrane; c: chorion; ov: oogonia; n: nucleus.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