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Leydig cells in *Myleus ternetzi* testes

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Abstract

Males of *Myleus ternetzi*, at various maturation stages were netted in a French Guyana river. Testes were histologically and ultrastructurally analysed. Leydig cells were always present in large numbers. In mature or almost mature testes, they were characterized by a large spherical nucleus and an extensively developed smooth endoplasmic reticulum (SER) composed of very narrow tightly packed tubules. In regressed testes, most of the Leydig cells had a smaller nucleus with an irregular shape, a disorganized SER and the fine structure of the mitochondria was greatly affected. These changes would reflect different phases throughout a metabolic cycle in relation to spermatogenesis.

Keywords : Fish, South America, testis, Leydig cell, ultrastructure.

Les cellules de Leydig chez Myleus ternetzi.

Résumé

Des mâles de *Myleus ternetzi* à différents stades de maturation, ont été pêchés dans une rivière de Guyane française et les testicules ont été analysés en microscopie photonique et électronique. Les cellules de Leydig sont toujours présentes en grand nombre. Dans les testicules matures ou prématures, beaucoup d'entre elles sont caractérisées par un gros noyau sphérique et un réticulum lisse très développé constitué de tubules très fins étroitement tassés. Dans les testicules régressés, la plupart des cellules de Leydig ont un noyau plus petit de forme irrégulière, un réticulum lisse désorganisé et des mitochondries dont l'ultrastructure est très modifiée. Ces changements reflètent vraisemblablement différentes phases d'un cycle d'activité en relation avec le déroulement de la spermatogenèse.

Mots-clés : Poisson, Amérique du Sud, testicule, cellule de Leydig, ultrastructure.

INTRODUCTION

Although the site of steroid production in the testis of teleosts has been a topic of controversy until recently, there is now more evidence that the Leydig cells are the main source of testicular steroids in fishes. These cells are usually distributed singly or in small groups in the interstices between the lobules. Their ultrastructure has been described for several fishes. However, at the present time, knowledge of their biology is limited and many questions dealing for instance with their origin, their renewal and changes through the annual reproductive cycle remain unanswered. One way to advance the study of fish Leydig cells is to apply *in vitro* techniques using enriched-cell populations (Loir, 1988), particularly making use of species in which the testis contains a high proportion of these cells.

In the course of an investigation on the reproductive biology of *Myleus ternetzi*, a Serrasalmidae which is common in French Guyana rivers (Le Bail *et al.*, 1989), we have focused our attention on the

interstitial cells. In this paper, we report some preliminary observations indicating that this species presents some interesting peculiarities as far as the number and the ultrastructure of the Leydig cells are considered.

MATERIAL AND METHODS

A total of thirty five males of *Myleus ternetzi* have been netted in the Kourou river in December and March (1984, 1985 and 1986). As soon as possible after netting, 2 to 3 cm^3 venous blood were collected from the living animals and, after centrifugation, plasma was stored in liquid nitrogen. Usually, one testis was fixed in Bouin Hollande and the other fixed for electron microscopy.

For histological analysis, the testes were embedded in paraplast and 5 µm thick sections were stained with Regaud hematoxylin, orange G and aniline blue. The diameter of the Leydig cell nuclei and the number of these nuclei per unit area were measured on photographs taken at a final magnification of 1 100. The percentage of testis section area occupied by the interstitium was determined using a steorological method (Le Bail *et al.*, 1989). The maturation stage of the testes was determined by a semi quantitative analysis of spermatogenesis using the steorological method of Le Bail *et al.* (1989). According to these authors, males at stage 2 were unpubescent, stage 3 corresponded to the start of the first gametogenesis and stages 5 and 6 to spermatogenesis (first one and following ones). Testes at stage 7 were mature or premature. Those at stage 4 were in a regressed state after spermiation. They were histologically intermediate between those at stages 3 and 5, *i.e.* they contained not only spermatogonia but also spermatocytes and spermatids. *Myleus ternetzi* is able to breed throughout the year but more animals breed (stage 7) just before the major wet season.

For electron microscopy, the best results were obtained after fixation followed by storage until further processing in 6% glutaraldehyde in 0.09 M cacodylate buffer pH 7.4 plus 5 mM $CaCl_2$. Before embedding, the samples were washed in 0.12 M cacodylate buffer then post-fixed in 1% osmium tetroxide in 0.08 M cacodylate buffer plus 1% potassium ferricyanide. After washing they were dehydrated in acetone and embedded in a mixture of Epon-Araldite. Sections were double-stained and observed with a 100 CX Jeol electron microscope.

Androstenedione, testosterone, 11-cetotestosterone (11 KT) and 17α,20β-hydroxyprogesterone were assayed in plasma after extraction with ethylacetate/-cyclohexane (1/1) by radio-immunoassays according to Sanchez-Rodriguez *et al.* (1978), Fostier *et al.* (1982) and Fostier and Jalabert (1986). Student's test was used to compare groups of values.

RESULTS

As shown in *table* 1, the percentage of the testis section area occupied by the interstitium was between 18 and 46%, according to the maturation stage.

Table 1. – Percentage (mean±SE) of testis section area occupied by the interstitium, as a function of the maturation stages, in *Myleus ternetzi.*

Stage	% of the area	Nos. of animals
2	46.8±9.1	5
3	17.6±3.6	3
5	23.1±5.7	3
6	31.2±7.8	6
7	26.1±5.0	2

Under electron microscope, myoid fibroblast-like cells were visible in the interstitium around the lobules, near the basal lamina. In addition to these cells and to blood vessels, the interstitium was almost exclusively composed by numerous cells, the ultrastructure of which identified them as Leydig cells.

Table 2. – Mean diameter (±SE) of the Leydig cell nucleus, as a function of the maturation stages, in *Myleus ternetzi* (2 animals per stage).

Stage	Nuclear diameter (µm)	Nos. of nuclei
2	3.50±0.35	70
3	3.66±0.40	47
4	3.51±0.32	120
5	3.70±0.54	40
6	4.05±0.12	40
7	4.13±0.17	70

Although the mean nuclear diameter (*table* 2) differed significantly only between stages 2 and 7, there was a progressive increase of this parameter throughout stages 2, 3, 5, 6 and 7. At stage 4, the Leydig cell nuclei had the same diameter as at stage 2. In addition, in stages 2 and 4 the nuclei often had an irregular shape, while they were clearly spherical in stage 7. The number of Leydig cell nuclei per 1 000 $µm^2$ was semi-quantitatively estimated. It decreased from 13 at stage 2 to 6 at stage 7.

In testes at stage 7, most of these cells had a large round nucleus (*fig.* 1). Their cytoplasm was filled with smooth endoplasmic reticulum (SER) organized in a mass of very narrow tightly packed tubules (189±25 Å, $n=25$) (*fig.* 2). Round or ovoid mitochondria were scattered in this mass. They had a dense matrix and tubulovesicular cristae. Other organelles were scarcely visible. Most of the other Leydig cells also had a round nucleus but they differed in having a more loosely organized SER. The diameter of the tubules was somewhat greater (220±67Å, $n=22$ to 440±91 Å, $n=20$, depending on the cells) and they were less tightly packed (*fig.* 2). At the same time, small dense granules which could be glycogen (maximal diameter around 300 Å) were sometimes scattered between these tubules. In these

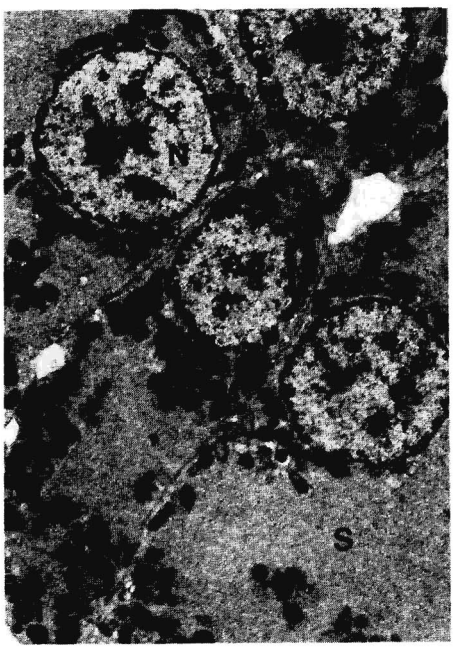


Figure 1. — Male *Myleus ternetzi* at maturation stage 7. Some Leydig cells of the type predominating at this stage. S, SER; N, nucleus; M, mitochondria × 7 000.

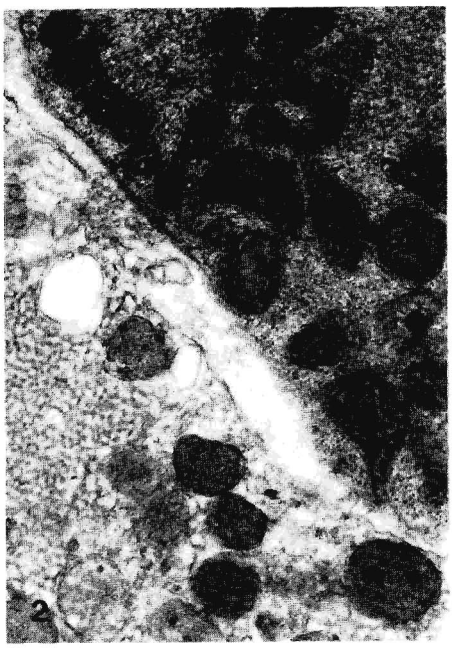
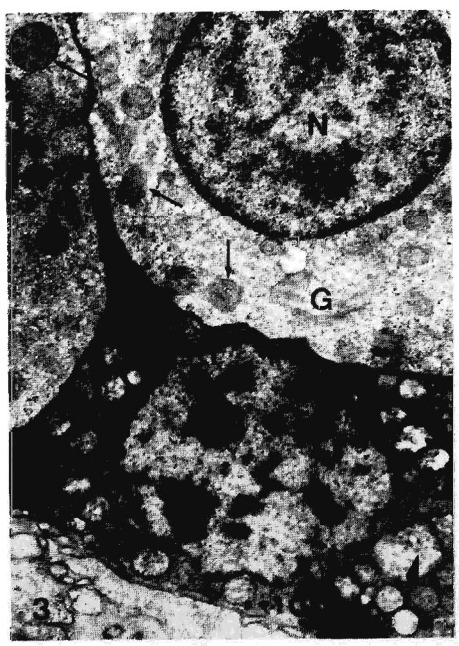


Figure 2. — Stage 7 testis. At the top, a Leydig cell of the predominating type. At the bottom, a Leydig cell with a loosely organized SER and with mitochondria the cristae of which are tubular × 19 900.



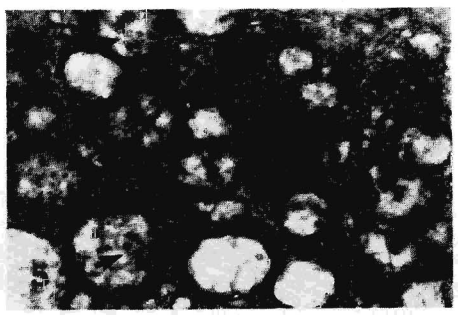


Figure 3. — Stage 4 testis. At the top, 2 Leydig cells with a spherical nucleus (N), mitochondria with narrow worm-like cristae (thin arrows), a loosely organized SER and an electron-translucent cytoplasm. At the bottom, a Leydig cell with a non-spherical nucleus containing large patches of dense chromatin, with mitochondria either filled with worm-like cristae (large arrows) or seeming "empty" (arrowheads) and with an electron-dense cytoplasm. G: golgi; C: collagen fibers in the intercellular space × 11 000.

Figure 4. — Stage 4 testis. Detail of cytoplasmic organelles in Leydig cells similar to that at the top of *figure* 3. Note the narrow tubular mitochondrial cristae, the unorganized SER and the presence of some glycogen granules × 21 000.

Figure 5. — Stage 4 testis. Detail of a Leydig cell similar to that at the bottom of *figure* 3. Some mitochondria are filled with worm-like cristae (arrows) while others have no cristae. Note that the outer membranes of mitochondria (arrowheads), the membranes of the SER and the cristae still present are well preserved × 15 300.

Leydig cells the electron density of the matrix was lowered in some mitochondria (*fig.* 2). The cristae were rather tubular and had a small diameter (510 ± 64 Å, $n = 18$). They then occupied a larger volume in the inside of the mitochondria. Some scarce Leydig cells with an electron-dense cytoplasm (*see* below) were also present in one male at stage 7.

In the testes at stage 4, only a few Leydig cells of the type predominating at stage 7 were observed, but many had a loosely organized SER, similar to those described in stage 7 testes (*fig.* 3 and 4). In addition to these Leydig cells, others (*fig.* 3 and 5) were present in more or less equal numbers. They were characterized by a nucleus with an irregular profile and containing numerous patches of dense chromatin, mitochondria either filled with worm-like cristae or seeming "empty" *i. e.* without cristae (*fig.* 3 and 5) and either a loosely organized or an electron-dense cytoplasm where SER tubules are still visible. All these different types were usually mixed in the same area within the interstitium (*fig.* 3).

Table 3. — Plasma levels (mean ±SE) of 3 androgens in male *Myleus ternetzi* at different maturation stages. In brackets: number of animals.

Stage	Androstenedione ng/cm³	Testosterone ng/cm³	11 cetotestosterone pg/cm³
2 (4)	0.645±0.408	0.030±0.052	0.4±0.2
3 (3)	0.710±0.122	0.208±0.049	3.5±0.8
5 (3)	0.946±0.255	0.314±0.044	5.3±0.9
6 (6)	1.815±0.695	0.461±0.148	8.3±5.1
7 (3)	0.961±0.665	0.472±0.305	9.3±10.9

Steroid assays have been carried out on a small number of animals (3 to 6 animals/stage). Nevertheless, it appears that (*table* 3):

— 17α, 20β-hydroxyprogesterone has not been significantly detected in plasma;

— individual testosterone concentrations varied between 0.01 and 10 ng/cm³ plasma while 11 KT concentrations were never higher than some pg/cm³ plasma;

— the highest levels of androgens were found in animals in stage 7 and the lowest ones in animals in stage 2.

DISCUSSION

Numerous papers have dealed with Leydig cells in teleost testes (for review *see* Nagahama, 1983). From this point of view, and in the present state of knowledge, it appears that *Myleus ternetzi* presents two original features.

Firstly, no teleost has been documented with such a high concentration of Leydig cells in the testis at every stage of maturation.

Secondly, in no gonochoristic teleost, morphometric and ultrastructural changes of Leydig cells have been evidenced in correlation with the reproductive cycle. Such changes have been reported only for hermaphrodites (Yeung *et al.*, 1985). It should be noted that the interaction between spermatogenesis and the morphology of the Leydig cells has been particularly well-documented also for an amphibian (Pudney *et al.*, 1983).

Although the results of the steroid assays were obtained with small numbers of animals, they support data obtained, for instance, for the trout (Sanchez-Rodriguez *et al.*, 1978; Fostier *et al.*, 1981; Scott and Baynes, 1982) indicating that the androgen levels in plasma rise with the progression of spermatogenesis. The changes in nuclear diameter and in number of nuclei/1 000 μm² suggest that the size, and probably the metabolic activity, of the Leydig cells increase at the same time.

At the peak of their steroidogenic activity during stage 7, the Leydig cells have a cytoplasmic ultrastructural organization which, apparently, has not been observed previously in teleosts. In particular, the fine structure of these cells for a neighbour species, *Mylossoma aureum* (Cruz-Hofling and Cruz-Landim, 1984) is rather similar to that observed in most teleosts. In *Myleus ternetzi*, the SER is extensively developed and the tubules which are very tightly entwined have a particularly small diameter. In mammals, the abundance of the SER in steroidogenic cells has been correlated with their capacity to secrete testosterone (Zirkin *et al.*, 1980).

After spermiation, when the testes have regressed to stage 4, a higher number of Leydig cells displays a loosely organized SER and modified mitochondria. The disorganization of the SER and the transformation of the tubulovesicular cristae to worm-like cristae and their subsequent disappearance accompanied by the clearing of the matrix, may be interpreted as reflecting a restriction of the steroidogenic potential. This restriction coincides with an apparent decrease in the nuclear activity.

Thus, our observations suggest that in *Myleus ternetzi* the structure of the Leydig cells would undergo a cycle in relation to spermatogenesis. What happens with the Leydig cells at the end of the cycle? Do they die, being then replaced by new ones at the beginning of the next spermatogenic cycle, or do they recover the ability to synthesize steroids actively? Do some Leydig cells die to be replaced throughout the spermatogenic cycle? This could explain the structural heterogeneity of the Leydig cells observed in stage 7 testes. If Leydig cells disappear, what is the origin of the new ones? At the present time these questions, which concern all fishes, remain unresolved. Undoubtedly, *Myleus ternetzi* could constitute an interesting model to try to answer them.

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