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Short note/Kurze Mitteilung

Immunohistochemical localization of androgen receptors in two different types of teleostean testes*

Immunhistochemische Lokalisation von Androgenrezeptoren in zwei unterschiedlichen Hodentypen von Teleosteern

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Zusammenfassung: Im Hoden adulter Männchen von *Poecilia reticulata* (Cyprinidontiformes), bei dem die Spermatogonien nur auf den distalen Teil von Lobuli beschränkt sind („restricted spermatogonial testis"), und von *Ancistrus* sp. (Siluriformes), bei dem die Spermatogonien auf der ganzen Länge von anastomosierenden Hodentubuli vorkommen (Terminologie nach PARENTI & GRIER 2004)), lassen sich Androgenrezeptoren immunhistochemisch in den Spermatogonien und Spermatocyten sowie in den Sertolizellen und wohl auch in den Boundary-Zellen nachweisen. Eine starke positive Reaktion findet sich auch im efferenten Gangsystem des Guppyhodens.

For male sexual differentiation and spermatogenesis fish testes mainly produce the androgens 11-oxygenated androgens (11-KT; BORG 1994, for review see SCHULZ 2003) and testosterone (T). Their physiological actions are mediated by the androgen receptors (AR), i.e. nuclear receptors that function as ligand-activated transcription factor. Androgen receptors are expressed in Sertoli cells and in other testicular somatic cells, while germ cells probably do not express known AR forms (IKEUCHI et al. 2001; for review see SCHULZ 2003). However, immunohistochemical localization of two distinct ARs in the testis of rainbow trout performed after microwave treatment revealed immunoreactivity in various somatic cells as well as in germ cells (TAKEO & YAMASHITA 2001).

In teleosts different types of testes can be distinguished; anastomosing tubular testes found in basal teleost taxa, e.g. the Siluriformes, and lobular testes found in the Neoteleostei. Lobular testes can be divided in (i) the "unrestricted spermatogonial testis", in which spermatogonia and spermatocysts formed by Sertoli cells are located along the length of the lobule, and (ii) the "restricted spermatogonial testis", in which spermatogonia are located at the distal end of the lobule; here spermatocysts are displaced towards the efferent duct system. Since all germ cells within one spermatocyst are clonal descendents of one stem cell, a given Sertoli cell contacts germ cells that are all in the same stage of development. During spermiation Sertoli cell processes of the unrestricted type separate and cyst and tubule lumina fuse, whereas Sertoli cells of the restricted type transform and become efferent duct cells. In both types of testis, a discontinuous layer of boundary cells borders the lobules (for review and terminology see PARENTI & GRIER 2004).

We examined the distribution of androgen receptor in the restricted lobular testis of the

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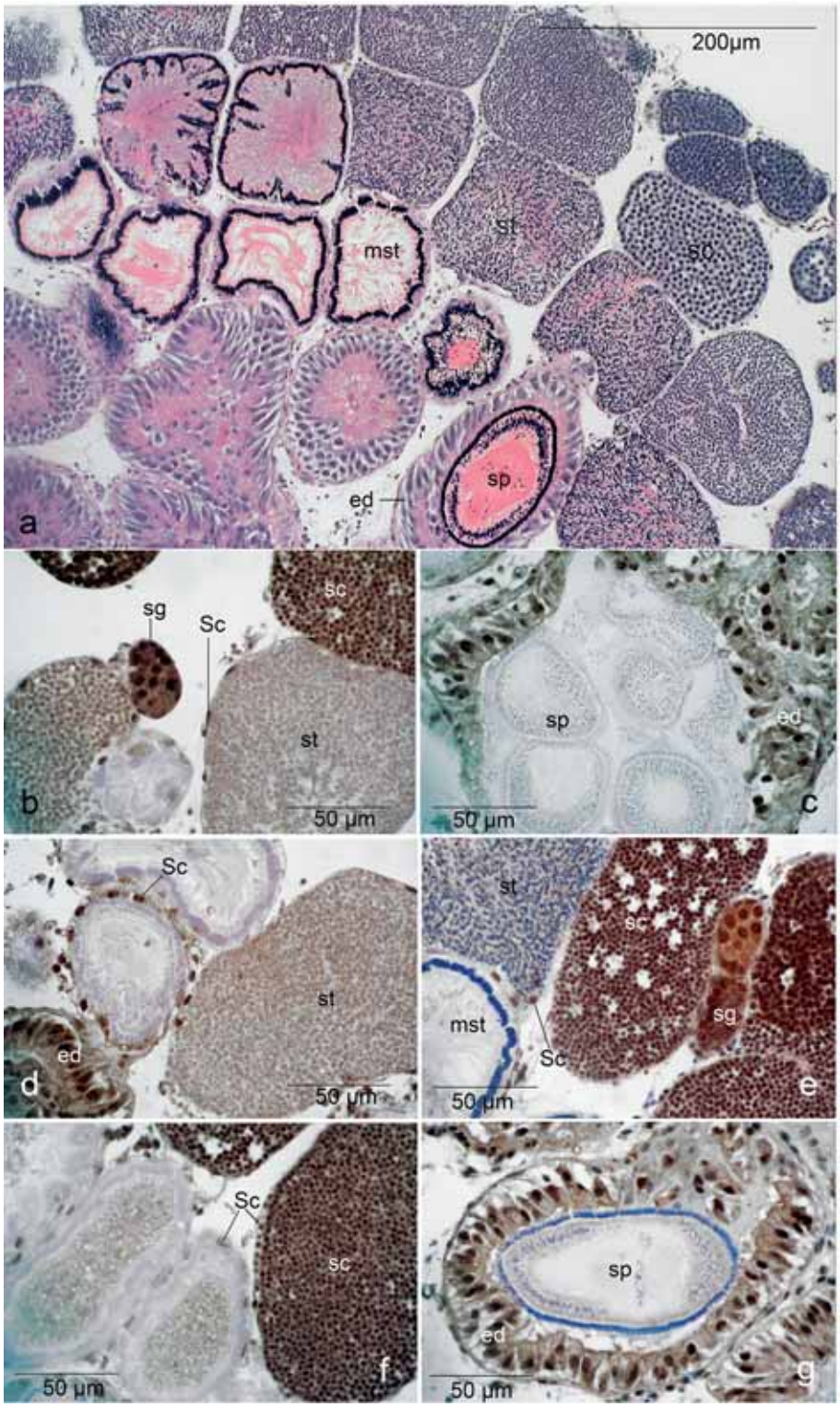
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viviparous guppy, *Poecilia reticulata* (fig. 1a), and the anastomosing tubular testis of the catfish *Ancistrus* sp. (Siluriformes) (fig. 2a). The guppy discharges spermatozeugmata instead of free spermatozoa (fig. 1a).

Immunohistochemical staining of the sections (3-5 µm thick) followed a protocol, which included heating in 0.01 mol/l citrate buffer, pH 6.1, in a microwave oven for antigen retrieval, treating with 3% hydrogen peroxide (Sigma) to inactivate endogenous peroxidase, washing in phosphate-buffered saline (PBS) and blocking of unspecific staining with normal goat serum (Vector Lab. Inc.). For androgen receptor detection a polyclonal anti-androgen-receptor antibody (Rabbit Polyclonal IgG; ABR / Dianova GmbH, Hamburg), diluted 1:400 in PBS (incubation 12 h at 4 °C) was used. After PBS-washing a biotinylated secondary antibody (goat-anti-rabbit; Vector Lab. Inc.) was employed for 30 min in a dilution of 1:200 in PBS. After washing in PBS the sections were treated with ABC ELITE Reagent Vectastain (Vector Lab. Inc.) for 30 min, followed by washing in PBS. After a final wash the antigen-antibody complex was detected by incubation with NovaRed (Vector Lab. Inc.).

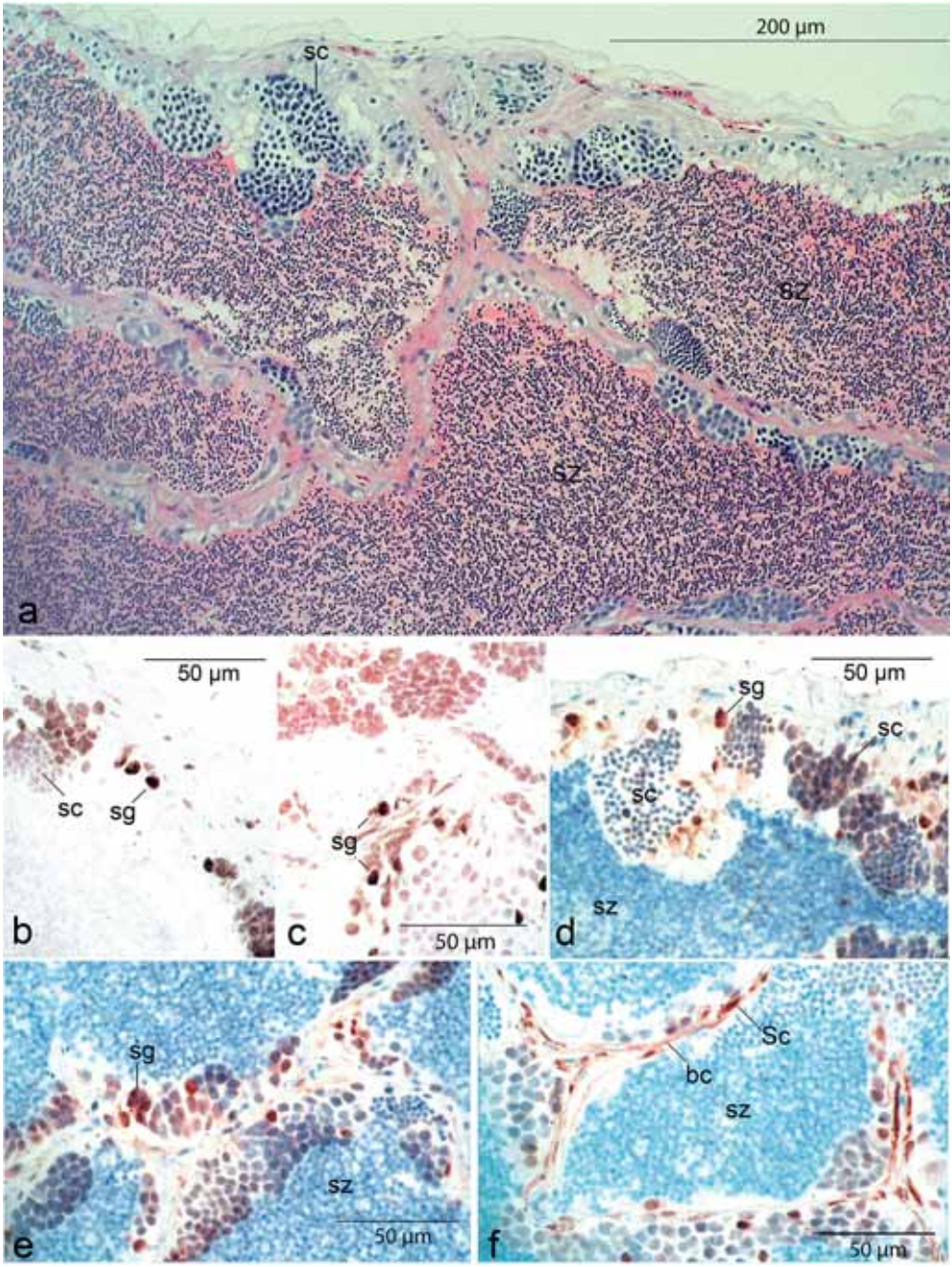
As negative control the immunoreactions was performed by omitting the incubation of the sections in the primary antibody. Sections were viewed either without counterstaining or were counterstained with haematoxylin. Slides were mounted with Super Frost plus mounting medium (Menzel GmbH, Braunschweig) for permanent preservation.

The labelling of the androgen receptors is visible as dark staining inside the cells. All controls lack positive immunohistochemical staining. In both species AR immunoreactivity was detected in the nuclei of spermatogonia arranged in nests (guppy: figs. 1 b, e) or more or less singly along the tubules (*Ancistrus* sp.: figs. 2 b-e) and spermatocytes (figs. 1 b, e, f). The intensity of staining in the nuclei of spermatocytes in *Ancistrus* sp. was weaker than in the spermatocytes of the guppy (compare figs. 1 e, 2 b). Specific staining of early and late spermatids of the guppy is questionable (figs. 1 d, e). Late spermatids were not clearly recognized in our *Ancistrus* sp. preparations. Positive AR immunoreactivity was detected also in the nuclei of the Sertoli cells (guppy: figs. 1 b, d, e f; *Ancistrus* sp.: fig. 2 f). We could not clearly show the localization of AR in the nuclei of Sertoli cells in course all stages of sperm development, because it was difficult to identify their position in all cases. In the guppy, the Sertoli cells enclosing spermatocytes (figs. 1 f), mature spermatids (figs. 1 d, e) and those transformed to the efferent ducts (figs. 1 c, g) were always positive. Further, immunoreactivity was found at the level of the cells of the boundary tissue in *Ancistrus* sp. (fig. 2 f; not clearly identified in the guppy). Leydig cells could not be distinguished unequivocally. Mature spermatozoa, free as in *Ancistrus* sp. or packaged into spermatozeugmata as in *Poecilia reticulata*, did not show a positive reaction.

In brief: (i) The positive immunostaining in boundary cells clearly demonstrated in the testis of *Ancistrus* sp. suggests that these cells are targets for androgens; these cells contain actin (unpublished) and may play a role in spermiation. (ii) The positive staining in Sertoli cells, which are generally considered to be target cells of androgens, was expected and indicates their involvement in the androgen control of spermiogenesis and, at least in the guppy, of spermiation, i.e. detachment of spermatozoa

Figs. 1 a-g: Spermatocysts in the testis of *Poecilia reticulata*, haematoxylin-eosin (**a**). Immunohistochemical staining of androgen receptors without (**b, c, d**, **f)** and with (**e**, **g**) hematoxylin counterstain. Efferent duct (ed), Sertoli cells (Sc), spermatocytes (sc), spermatogonia (sg), spermatozeugmatum (sp), spermatids (st), mature spermatotids (mst).

Abb. 1 a-g: Spermatocysten im Hodens von *Poecilia reticulata*., Haematoxylin-Eosin (**a**). Immunhistochemischer Nachweis von Androgenrezeptoren ohne (**b**, **c**, **d**, **f**) und mit (**e**, **g**) einer Hämatoxylin-Gegenfärbung. Ductus efferentes (ed), Sertolizellen (Sc), Spermatocyten (sc), Spermatogonien (sg), Spermatozeugmatum (sp), Spermatiden (st), reife Spermatiden (mst).



Figs. 2 a-f: Testis periphery of *Ancistrus* sp., haematoxylin-eosin (**a**). Immunohistochemical staining of androgen receptors without (**b**, **c**) and with (**d-f**) haematoxylin counter stain. Boundary cells (bc), Sertoli cells (Sc), spermatocytes (sc), spermatogonia (sg), mature spermatozoa (sz).

Abb. 2 a-g: Peripherie des Hodens von *Ancistrus* sp., Hämatoxylin-Eosin (**a**). Immunhistochemischer Nachweis von Androgenrezeptoren ohne (**b**, **c)** und mit (**d-f**) einer Hämatoxylin-Gegenfärbung. „Boundary"-Zellen (bc), Sertolizellen (Sc), Spermatocyten (sc), Spermatogonien (sg), Spermien (sz).

from the Sertoli cells. The distribution of positive and negative cells in the cysts containing different stages of spermatogenesis has to be studied in detail. (iii) The positive immunostaining in the various stages of germ cells (spermatogonia, spermatocytes) is consistent with the findings of TAKEO & YAMASHITA (2001) suggesting that androgens can directly act on germ cells and that action of androgen during spermatogenesis occurs both at the level of somatic cells and germ cells.

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