Watson, G. E. (1962). Notes on the copulation and distribution of Aegean land tortoises. *Copeia* **1962,** 317-321.

Willemsen, R. E. (1987). De mogelijke aanwezigheid van de Moorse landschildpad (*Testudo graeca ibera*) op de Peloponnesos. *Lacerta* **45,** 169-171.

Wright, J., Steer, E. and Hailey, A. (1988). Habitat separation in tortoises, and the consequences for activity and thermoregulation. *Canadian Journal of Zoology* **66,** 1537-1544.

APPENDIX

Further records of the distribution and status of tortoises and other reptiles in Europe are always useful. These should be sent direct to:

DR BRIAN GROOMBRIDGE: IUCN Monitoring Centre, 219c Huntingdon Road. Cambridge CB3 0DL, UK.

and

DONATO BALLASINA: R.A.N.A. – Belgium vzw' Populierenlaan 17'B – 1980 Tervuren, Belgium.

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IMMUNOCYTOCHEMICAL AND QUANTITATIVE STUDY OF INTERSTITIAL CELLS IN THE HIGH MOUNTAIN TOAD *BUFO BUFO GREDOSICOLA* DURING THE SPERMATOGENETIC CYCLE

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ABSTRACT

The interstitial cells of the toad *Bufo Bufo gredosicola* were studied throughout the seasonal period of spermatogenesis (from April to October) by means of immunocytochemical detection of testosterone and quantitative histological studies. The total number of interstitial cells per testis did not vary during the spermatogenetic period. However, in April, May and October, there were many interstitial cells showing an abundant testosterone content, whereas from June to September poorly-differentiated interstitial cells with a scanty testosterone content are the most abundant interstitial cell type. Since the interstitial cells with abundant testosterone content are larger than the interstitial cells with scanty testosterone content, the volume occupied by interstitial cells decreased in June-September. The development of thumb pads coincides with that of testosterone-containing interstitial cells.

INTRODUCTION

It is generally accepted that, as in other vertebrates, the interstitial cells or Leydig cells represent the major source of testicular steroid hormones in anuran amphibia. This ideas has been supported by direct evidence from cytochemical (presence of cholesterol-rich cytoplasmic droplets and $\Delta^5$3ß-hydroxysteroid dehydrogenase activity), and ultrastructural studies (Lofts, 1974; Unsicker, 1975; Rastogi and Iela, 1980). However, unlike in higher vertebrates, no tissue cultures and incubation experiments with dissociated cell populations of the anuran testes have been performed and it cannot be excluded that other cells such as the Sertoli cells, which also show Δ^5-3ß-hydroxysteroid dehydrogenase activity, might be involved in steroid biosynthesis (Lofts and Bern, 1972).

A variety of studies have shown that the interstitial cells of the anuran testes show seasonal changes in their content in lipid droplets, Δ^5-3ß-hydroxysteroid dehydrogenase activity, and their ultrastructural steriod-synthesising characteristics (Van Oordt and de Kort, 1969; Lofts and Bern, 1972; Schulze, 1972, Chanda, 1982). These changes have been related with those observed in the development of thumb pads which have been considered as androgen-dependent structures (Miller, Obert and Schneider, 1977).

The aim of the present report was to investigate the evolution of interstitial cells in the hibernating toad of the 'Gredos' high mountains during the seasonal period of spermatogenesis by means of quantitative studies on histologic sections stained for immunocytochemical localisation of testosterone and to correlate these findings with the degree of thumb pad development. The results of this study provide new data concerning qualitative and quantitative circannual changes in the interstitial cells of this species of toad, data which can probably be extended to other anuran amphibia.

MATERIALS AND METHODS

Six adults male toads (*Bufo bufo gredosicola*) were collected during each of the months corresponding to the seasonal period of spermatogonesis (from April to October) in 'Laguna Grande', located at 2000 metres in the 'Gredos' mountains (Spain). During the period of testicular quiescence (from November to March) the toads were hidden under the snow and they could not be collected. In order to eliminate the influence of 'body weight' in the quantitative studies only toads weighing between 60 and 65 g were collected. The animals were anaesthetised with methane sulphonate (MS-222, Sandoz) and perfused throughout the aorta with 3 per cent phosphate-buffered glutaraldehyde-paraformaldehyde (30 minutes). Following this, both testes were removed and weighed; testicular volumes were calculated by water displacement. The right testes were sliced into small fragments which were processed for electron microscopy.

The left testes were fixed for an additional 6 hours in the same fixative, dehydrated and embedded in paraffin. Five sagittal sections (7μm in thickness) of each testis at points 1/6, 1/3, 1/2, 2/3, and 5/6 of the transverse testicular diameter were performed and stained with hematoxylin and eosin and the peroxidase-anti-peroxidase (PAP) method for cellular detection of testosterone (Chemes, Gottlieb, Pasqualini, Demenichini, Rivarola and Bergadá, 1985). Rabbit antitestosterone γ-globulin (Biogenex Laboratories, Dublin, Ireland) was used as first antibody at a dilution of 1/100. Goat anti-rabbit γ-globulin at a dilution of 1/300 was used as the second antibody. After the PAP incubation and washing, peroxidase activity was detected by diaminobenzidine and hydrogen peroxide. This stain seems to be specific for testosterone.

The interstitial cells were classified according to their testosterone content as measured with a cytophotometer (Wicker Instruments, York, England) in the PAP-stained sections. A scoring range from 0 (interstitial cells without testosterone) to 4 (cells with the highest testosterone content) was established. According to this range the interstitial cells were designated as T^- (from 0 to 1), T^+ (1-2), T^{++} (2-3), and T^{+++} (3-4).

In each PAP-stained section the following parameters were calculated:

1) Volume density of seminiferous tubules, testicular interstitium and each Leydig cell type (surface occupied by these structures divided by total surface of the section) by use of a semiautomatic image analyser.

2) Numerical density (number of cells per unit volume of the testis) of each interstitial cell type, by use of the Floderus (1944) equation: $N_v = N_a/(T+D-2h)$, where N_a is the number of cell nuclei per unit area, D is the average nuclear diameter (measured with a vernier ocular in at least 50 nuclei of each interstitial cell type), T is the average thickness of the section (6.9μm), and h is the height of the smallest recognisable cap section of the nucleus (about 10 per cent of the nuclear diameter), according to Wing and Christensen (1982).

3) Total number (per testis) of interstitial cells from each type by multiplying their numerical density by the testicular volume and by a correction factor (0.76) which results from transformation of testicular volume after perfusion in testicular volume after embedding. This factor was previously calculated using 50 testes from toads.

4) Average volume of each interstitial cell type by dividing the volume density of each cell type by its numerical density.

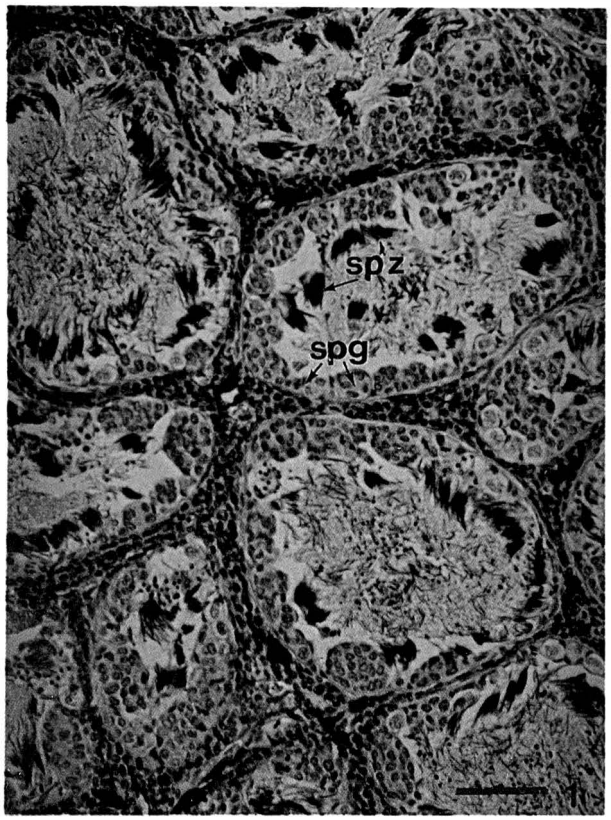
The means and standard deviations for each group (month) were calculated from the values for each animal. Comparison of the means between the different groups was carried out by the one-way ANOVA test and Scheffe's pairwise comparison.

The degree of thumb pad development was evaluated measuring the thickness of the epidermis in histological sections of the thumb pads. According to these measurements, the progressive degrees of development were designated as + (less than 100μm), ++ (from 100 to 134μm), +++ (from 135 to 174μm), and +++ (more than 175μm).

RESULTS

The testicular volume and the volume density of seminiferous tubules increased during the period of germ cell proliferation up to round spermatids (from April to August) and decreased during the period of spermiogenesis (September and October) (Table 1).

The numbers of cells corresponding to each different interstitial cell type (Figs. 1-4) varied during the spermatogenetic cycle (Table 2). In April and May there were numerous interstitial cells that showed an abundant testosterone content. These cells decreased in number in June; were absent in July and August, and reappeared in October. The contrary occurred with the interstitial cells showing scanty testosterone content. The total number of interstitial cells (all types) did not change during the period studied.



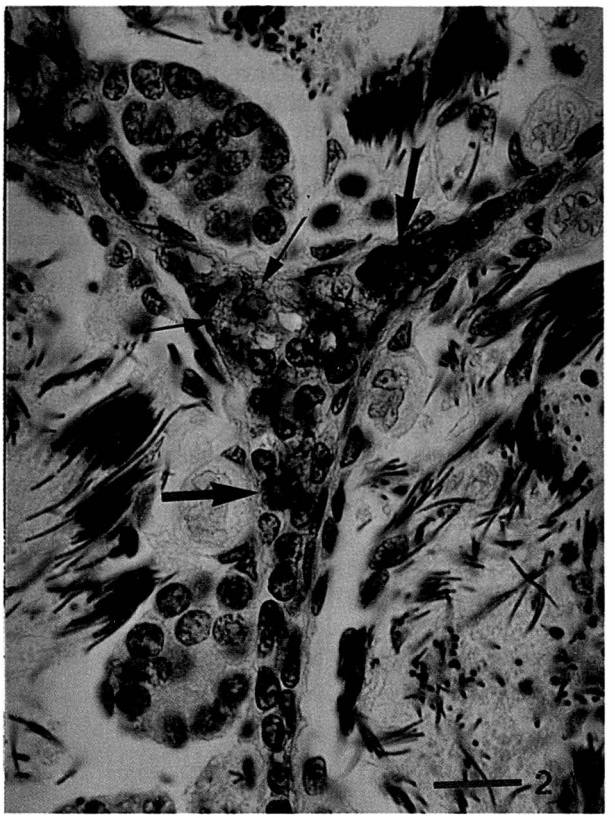
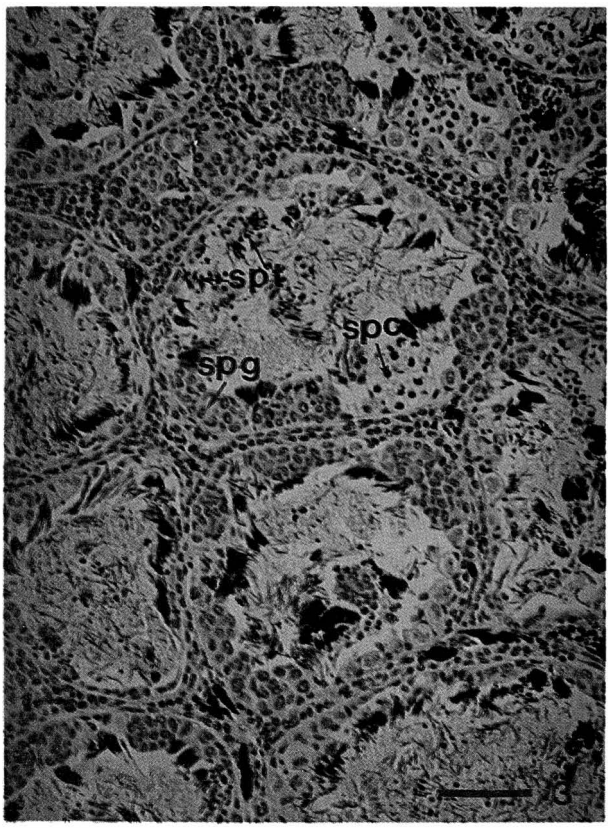


Fig. 1 Testis of *Bufo bufo gredosicola* in April. The testicular interstitium appears intensely stained with the PAP method for cellular detection of testosterone. spg: spermatogonia. The spermatozoa (spz) in the tubular lumen were formed in the preceding cycle. Bar = 80μm.

Fig. 2 Detail of Figure 1 at higher magnification. Intensely stained cells of types T^{++} (small arrows) and T^{+++} (large arrows) predominate in the testicular interstitium. Bar = 20μm.



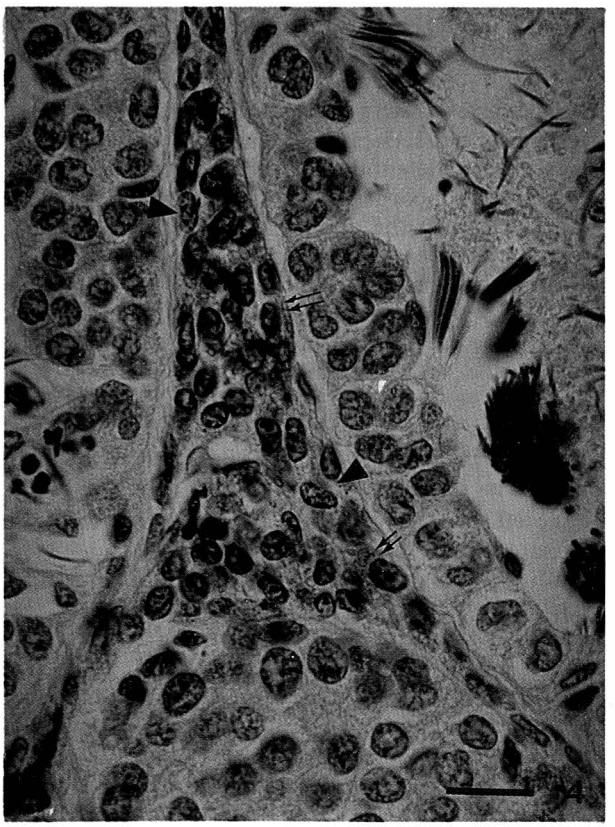


Fig. 3 Testis of *Bufo bufo gredosicola* in August. The testicular interstitium appears less stained than in April. spg: spermatogonia. spc: spermatocytes. spt: spermatids. Bar = 80μm.

Fig. 4 Detail of Figure 3 at higher magnification. Interstitial cells with a scanty (arrowheads) or moderate (double arrows) testosterone content predominate in the testicular interstitium. Bar = 20μm.

	April	May	June	July	August	September	October
Body weight (g)	62 ± 1	63 ±1	61 ± 1	62 ± 1	60 ± 1	63 ± 1	63 ±1
Left testis volume (mm^3)*	99 ± 12^{a}	108 ± 22^{a}	120 ± $17^{a,b}$	141 ± 24^{b}	156 ± 24^{b}	126 ± $18^{a,b}$	117 ± $18^{a,b}$
Volume density of seminiferous tubules (%)	77 ± 6	79 ± 6	85 ± 7	87 ± 8	88 ± 8	86 ± 8	84 ± 8
Volume density of testicular interstitium (%)	23 ± 2^{a}	21 ± 2^{a}	15 ± 2^{b}	13 ± 1^{b}	12 ± 1^{b}	14 ± 2^{b}	16 ± 2^{b}
Seminiferous tubule volume per testis	76 ± 9^{a}	85 ± $11^{a,b}$	102 ± $14^{b,c}$	123 ± $18^{c,d}$	137 ± 21^{d}	108 ± $15^{b,c}$	98 ±$14^{a,b,c}$
Interstitium volume per testis	23 ± 3^{a}	23 ± 3^{a}	18 ± 2^{b}	18 ± $3^{a,b}$	19 ± $3^{a,b}$	18 ± 2^{b}	19 ± $3^{a,b}$
Thumb pad development#	++++	++++	++	+	+	++	+++

TABLE I: Body weight and testicular parameters in *Bufo bufo gredosicola* during the period of spermatogenesis.
Valued are expressed as means ± standard deviation. For each parameter, the results of the comparison between the averages values corresponding to each month are indicated using superscript letters. Values coinciding in some superscript letter do not differ significantly between them; and those with different superscript letters differ significantly between them ($P<0.01$). *This represents volume after fixation, dehydration, and embedding. # This was evaluated according to the thickness of the epidermis in histological sections of the thumb pads; + not developed; ++ semideveloped; +++ developed; ++++ very developed.

Interstitial cell type	April	May	June	July	August	September	October
T^{-}	195 ± 25^{a}	253 ± 38^{a}	437 ± 67^{b}	1181 ± 189^{c}	1099 ± 168^{c}	475 ± 76^{b}	385 ± 56^{b}
T^{+}	270 ± $35^{a,b}$	234 ± 35^{a}	650 ± 100^{c}	253 ± $41^{a,b}$	321 ± 51^{b}	781 ± 117^{c}	415 ± 67^{d}
T^{++}	692 ± 90^{a}	759 ± 113^{a}	306 ± 47^{b}	—	—	152 ± 25^{c}	394 ± 67^{b}
T^{+++}	202 ± 27^{a}	148 ± 22^{b}	—	—	—	—	145 ± 26^{b}
All cell types	1359 ± 177	1394 ± 208	1393 ± 216	1434 ± 230	1420 ± 223	1420 ± 220	1339 ± 212

TABLE 2: Number of cells ($X10^3$) per testis of the different interstitial cell types classified according to their testosterone content in *B. b. gredosicola* during the period of spermatogenesis.
Values are expressed as means ± standard deviation. The interstitial cells are classified as T^{-}, T^{+}, T^{++}, and T^{+++} according to their increasing testosterone content as measured with a cytophotometer in the sections stained with the PAP method for testosterone detection. For each interstitial cell type, the results of the comparison between the average values corresponding to each month are indicated using superscript letters. Values coinciding in some superscript letter do not differ significantly between them; and those with different superscript letters differ significantly between them ($P<0.01$).

The testicular volumes occupied by each different interstitial cell type changed from April to October in a similar way to the interstitial cell numbers (Table 3). The total testicular volume occupied by interstitial cells (all types) was greater in April, May and October than in June-September. This is due to the larger average volume of testosterone-rich cells than that of testosterone-poor interstitial cells (Table 4).

The progressive development of thumb pads (Table 1) coincided with that of testosterone-containing cells.

DISCUSSION

The spermatogenetic cycle in the high mountain toad *B. b. gredosicola* is similar to that of other anuran amphibia, with the characteristic of being shorter, due to the high altitude at which these toads live. Like in other anurans (Schulze, 1972; Unsicker, 1975; Koskela, Pasanen and Vaananen, 1979; Chanda, 1982; Jorgensen and Billeter, 1982; Jorgensen, 1984) the interstitial cells of this toad also have a seasonal cycle, and they become prominent at the end of the spermatogenetic period.

Four types of interstitial cells have been observed in ultrastructural studies on anuran testes: (1) undifferentiated, fibroblast-like cells, which were found throughout the cycle, mainly in summer; (2) semidifferentiated cells containing numerous lysosomes, a few lipid droplets, mitochondria with tubular cristae, and a moderately developed smooth endoplasmic reticulum; these cells appeared in autumn; (3) well-differentiated cells with more abundant smooth endoplasmic reticulum, and more numerous and larger mitochondria than the semidifferentiated cells; these cells were found in winter and spring; and (4) involuting cells with scanty smooth endoplasmic reticulum, and abundant lipid droplets and secondary lysosomes; these cells were observed in summer (Schulze, 1972; Unsicker, 1975; Cavicchhia and Moviglia, 1982). These four cell types have been interpreted as successive stages of interstitial cell development, from undifferentiated to involuting interstitial cells.

Interstitial cell type	April	May	June	July	August	September	October
T^-	224 ± 30^a	300 ± 44^b	553 ± 85$^{c.d}$	1308 ± 209^e	1364 ± 209^e	597 ± 96^d	462 ± 73^c
T^+	447 ± 60^a	404 ± 59^a	1101 ± 172^b	473 ± 77^a	544 ± 85^a	1431 ± 229^b	759 ± 119^c
T^+	1778 ± 240^a	1900 ± 287^a	776 ± 119^b	—	—	401 ± 65^c	998 ± 159^b
T^{+++}	725 ± 97^a	502 ± 74^b	—	—	—	—	501 ± 81^b
All cell types	3204 ± 433^a	3106 ± 466^a	2430 ± 379$^{a.b}$	1781 ± 288^c	1908 ± 298$^{b.c}$	2429 ± 396$^{a.b}$	2720 ± 438$^{a.b}$

TABLE 3: Volume ($mm^3 X10^{-3}$) per testis occupied by the different interstitial cell types classified according to their testosterone content in *B. b. gredosicola* during the period of spermatogenesis.
Values are expressed as means ± standard deviation. The interstitial cells are classified as indicated in Table 2. For each interstitial cell type, the results of the comparison between the average values corresponding to each month are indicated using superscript letters. Values coinciding in some superscript letter do not differ significantly between them; and those with different superscript letters differ significantly between them ($P<0.01$).

Interstitial cell type	April	May	June	July	August	September	October
T^-	1149 ± 101^a	1186 ± 108^a	1264 ± 118^a	1241 ± 117^a	1241 ± 119^a	1257 ± 112^a	1206 ± 111^a
T^+	1767 ± 157^b	1726 ± 141^b	1694 ± 148^b	1695 ± 131^b	1695 ± 141^b	1832 ± 153^b	1829 ± 157^b
T^{++}	2569 ± 229^c	2503 ± 210^c	2536 ± 211^c	—	—	2638 ± 224^c	2533 ± 210^c
T^{+++}	3589 ± 291^d	3392 ± 275^d	—	—	—	—	3589 ± 294^d

TABLE 4: Average volume (volume density/numerical density, μm^3) of the different interstitial cell types classified according to their testosterone content in *B. b. gredosicola* during the period of spermatogenesis.
Values are expressed as means ± standard deviation. The interstitial cells are classified as indicated in Table 2. For each interstitial cell type, the results of the comparison between the average values corresponding to each month are indicated using superscript letters. Values coinciding in some superscript letter do not differ significantly between them; and those with different superscript letters differ significantly between them ($P<0.01$).

A comparison of ultrastructural studies and the present immunocytochemical observations suggests a close correlation between the degree of cytological differentiation and that of active testosterone synthesis in the interstitial cells. Well-differentiated interstitial cells, similar to mammalian Leydig cells, seem to correspond to the interstitial cells with the highest testosterone content. In summer, these cells initiate their regression until they become undifferentiated interstitial cells. These involuting and undifferentiated cells probably correspond to the interstitial cells with a scanty testosterone content. In autumn, the undifferentiated cells begin to differentiate again and gradually increase their testosterone synthesis; they would correspond to the cells with an intermediate testosterone content. These results agree with those of endocrine studies in *Rana esculenta* which reveal a pronounced drop in the serum levels of testosterone in the period of germ cell proliferation and a recovery of high testosterone levels during spermatozoa formation and spermiation (Rastogi, 1976; Rastogi, Tammaro, di Meglio, Iela, di Matteo and Chieffi, 1981).

Changes in interstitial cell morphology in relation to the stage of the seminiferous epithelium have also been reported in the urodele amphibia *Necturus maculosus* (Ucci, 1982; Pudney, Canik, Mak and Callard, 1983; Pudney and Callard, 1984) and *Salamandra salamandra* (Lecouteux, Garnier, Bassez and Joby, 1985). In these animals only undifferentiated interstitial cells are found around the cysts showing spermatogenesis, whereas differentiated interstitial cells appear around tubules with mature spermatozoa and reach their greatest differentiation after spermination. This led Ucci (1982) to suggest the occurrence of a local feedback control of interstitial cell development by the seminiferous epithelium. This author has postulated that a diffusable product of the seminiferous epithelium acts locally to regulate androgen biosynthesis or to modulate the sensitivity of interstitial cells to luteinising hormone. This agent might enhance or inhibit interstitial cell development during part of the cycle. A similar idea has been put forward for the rat testis by Aoki and Fawcett (1978) and Bergh (1982) who attributed the production of this agent to the Sertoli cells. Recently, a nongonadotropic Leydig cell stimulating factor has been isolated in rat testicular interstitial fluid (Risbridger, Jenkin and De Kretser, 1986).

Another possible explanation for the seminiferous epithelium-interstitial relationship cells is that these cells would be involved in the regulation of the spermatogenetic cycle through a direct supply of testosterone to the seminiferous epithelium (Ritzén, Boitani, Parvinen, French and Feldman, 1982). An interesting finding is that the stage of the rat seminiferous epithelium (spermatozoon formation and release) showing the maximum volume density of Leydig cells correspond to the highest concentration of intratubular testosterone (Parvinen and Ruokonen, 1982). This increased testosterone concentration at the end of spermatogenesis might be responsible for sperm formation and release or the preparation of the seminiferous epithelium for the initiation of spermatogensis in the following cycle. A series of experiments in

anurans suggests that sperm formation and release are androgen-dependent processes (Lofts, 1974; Rastogi *et al.*, 1981).

The number of interstitial cells apparently decreases from May to September if quantitations are expressed as the number of cells per unit of testicular volume. However, when these values are transformed into absolute values, it is seen that the interstitial cell number per testis remains constant throughout the cycle. This suggests that the interstitial cells do not degenerate yearly but they only undergo cyclic changes in their morphology and activity. If well-differentiated interstitial cells degenerated and died in summer, the rapid increase in the number of undifferentiated interstital cells in this short period could only be explained in terms of a proliferation of these cells or their precursors; however, no mitoses were seen in the testicular interstitium.

The close association of developed thumb pads and the presence of numerous interstitial cells with an abundant testosterone content agrees with the notion that thumb pads are androgen-dependent structures (Müller *et al.*, 1977; Jorgensen and Billeter, 1982).

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REFERENCES

Aoki, A. and Fawcett, D. W. (1978). Is there a local feedback from the seminiferous tubules affecting activity of Leydig cells? *Biol. Reprod.* **19**, 144-158.

Bergh, A. (1982). Local differences in Leydig cell morphology in the adult rat testis: evidence for a local control of Leydig cells by the adjacent seminiferous tubules. *Int. J. Androl.* **5**, 325-330.

Cavicchia, J. C. and Moviglia, G. A. (1982). Fine structure of the testis in the toad (*Bufo arenarum* Hensel): A freeze-fracture study. *Anat. Rec.* **203**, 463-474.

Chanda, S. (1982). A histometric comparison of Leydig cells in hibernating and non-hibernating toads (*Bufo mealnosticus*). *Endokrinologie* **80**, 361-363.

Chemes, H. E., Gottlieb, S. E., Pasqualini, T., Domenichini, E., Rivarola, M. A. and Bergadá, C. (1985). Response of acute hCG stimulation and steroidogenic potential of Leydig cell fibroblastic precursors in humans. *J. Androl.* **6**, 102-112.

Floderus, S. (1944). Untersuchungen über den Bau der menschlichen Hypophyse mit besonderer Berücksichtigung der quantitativen mikromorphologischen Verhältnisse. *Acta Pathol Microbiol. Scand. (Suppl.)* **53**, 1-276.

Jorgensen, C. B. (1984). Testis function in the toad *Bufo bufo* (L.), (Amphibia, Anura) at organ and subunit levels. *Vidensk. Meddr. dansk. naturh. Foren.* **145**, 117-130.

Jorgensen, C. B. and Billeter, E. (1982). Grow, differentiation, and function of the testes in the toad *Bufo bufo bufo* (L.), with special reference to regulatory capacities: effects of unilateral castration, hypophysectomy, and excision of Bidder's organs. *J. Exptl. Zool.* **221**, 225-236.

Koskela, P., Pasanen, S. and Vaananen, R. (1979). The annual cycle in the testes of the common frog, *Rana temporaria* L. in Northern Finland. *Aquilo Ser., Zool.* **19**, 17-22.

Lecouteux, A., Garnier, D. H., Bassez, T. and Joby, J. (1985). Seasonal variations of androgens, estrogens, and progestrone in the different lobules of the testis and in the plasma of *Salamandra salmandra*. *Gen. Comp. Endocrinol.* **58**, 211-221.

Lofts, B. (1974). Reproduction. In Physiology of Amphibia, vol. 2, 107-218. Lofts, B. (Eds). New York: Academic Press.

Lofts, B. and Bern, H. A. (1972). Steroids in Non-mammalian Vertebrates, 37-126. New York: Academic Press.

Müller, B., Obert, H. J. and Schneider, H. (1977). Untersuchungen zur hormonale Steuerung der Ausbildung der Brunstschwielen bei der Riesen-Endkröte *Bufo bufo spinosus* Daudin, 1803 (Bufonidae, Anura). *Zool. Jahrb. Abt. Allg. Zool. Physiol Tiere* **81**, 90-111.

Parvinen, M. and Ruokonen, A. (1982). Endogenous steroids in the rat seminiferous tubules. Comparison of the stages of the epithelial cycle isolated by transillumination-assisted microdissection. *J. Androl.* **3**, 211-220.

Pudney, J. and Callard, G. V. (1984). Organization of interstitial tissue in the testis of the salamander *Necturus maculosus* (Caudata: Proteidae). *J. Morphol.* **181**, 87-95.

Pudney, J., Canick, J. A., Mak, P. and Callard, G. V. (1983). The differentiation of Leydig cells, steroidogensis, and the spermatogenetic wave in the testis of *Necturus maculosus*. *Gen. Comp. Endocrinol.* **50**, 43-66.

Rastogi, R. H. (1976). Seasonal cycle in anuran (amphibia) testis: the endocrine and environmental controls. *Boll. Zool.* **43**, 131-172.

Rastogi, R. H. and L. Iela, L. (1980). Steroidogenesis and spermatogensis in anuran amphibia: a brief survey. In Steroids and their Mechanism of Action in Non-mammalian Vertebrates, 131-146. Dehio, G. and Brachet, J. Eds). New York: Raven Press.

Rastogi, R. K., Tammaro, L., di Meglio, M., Iela, L., di Matteo, L. and Chieffi, G. (1981). Circannual testicular rhythm in the green frog *Rana esculenta*. *Boll. Zool.* **48**, 97-105.

Risbridger, G. P., Jenkin, G. and de Kretser, D. M. (1986). The interaction of hCG, hydroxysteroids and interstitial fluid on rat Leydig cell steriodogenesis *in vitro*. *J. Reprod. Fertil.* **77**, 239-245.

Ritzén, E. M., Biotani, C., Parvinen, M., French, F. S., and Feldman, M. (1982). Stage dependent secretion of ABP by rat seminiferous tubules. *Mol. Cell Endocrinol.* **22**, 25-33.

Schulze, C. (1972). Saisonbendingte Veränderungen in der Morphologie der Leydizellen von *Rana esculenta*. *Z. Zellforsch.* **142**, 367-386.

Ucci, A. A. (1982). A fine-structural study of interstitial cell changes in the testes of *Necturus maculosus* during a part of the annual cycle, and possible evidence for local control by seminiferous epithelium. *Am. J. Anat.* **165**, 27-38.

Unsicker, K. (1975). Fine structure of the male genital tract and kidney in the anura *Xenopus lavis* Daudin, *Rana temporaria* L. and *Bufo bufo* L. under normal and experimental conditions. I. Testicular interstitial tissue and seminal efferent ducts. *Cell Tissue Res.* **158**, 215-240.

van Oordt, P. G. W. J. and de Kort, E. J. M. (1969). La Specificité zoologique des hormones hypophysaires et de leurs activités. *Coll. Int. Centre Nat. Rech. Scie.* **177**, 345-350.

Wing, T. Y. and Christensen, A. K. (1982). Morphometric studies on rat seminiferous tubules. *Am. J. Anat.* **165**, 13-25.