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SOME SEASON-RELATED MORPHOMETRIC, STRUCTURAL AND WEIGHT CHARACTERISTICS OF TESTES IN THE VIPER (*VIPERA AMMODYTES*)

D. S. DIMITROV, K. V. HRISTOV & A. ANGELOV
Trakia University, Stara Zagora, Bulgaria

Summary

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The study was performed on testes, obtained from 14 vipers (*Vipera ammodytes*), inhabiting various regions of Bulgaria, collected during the four seasons of the period 1996-1999 inclusive. The weight and the macrometric parameters of testes were measured. The outer diameter of seminiferous tubules and season-related microstructural features were determined via light microscopy. Data from weight, metric and micrometric measurements were statistically processed. Their analysis showed that from winter to the end of spring (when the breding period of vipers in Bulgaria occurs), the weight of testes increased more than thrice, the diameter of seminiferous tubules - more than twice and afterwards the values of both parameters decreased gradually and reached the highest values for the winter season by the end of the autumn. The seasonal variations in both weight and diameter correlated with the histological status of testes. The involution processes within seminiferous tubules, starting by the beginning of winter, were still active also in the middle or end of spring, when vipers were ready again for another reproductive period.

Key words: season-related characteristics, testes, viper

INTRODUCTION

The viper (*Vipera ammodytes*) has been subject of a considerable number of studies. They are mainly with a general biological or popular character. The reports on the seasonal activity of *Vipera ammodytes* are relatively few and the existing data – partial or fragmentary. According to some investigators, venomous snakes are characterized by species-related specificity in their mode of living, seasonal changes in the function of various organs and systems, dependent also on the geographical area they are inhabiting. Turner (1919) has studied for the first time the seasonal cycles in the spermatogenesis of the perch while Herland (1933) investigated them histologically in the testes of various reptiles. Further data about the seasonal characteristics of reproductive organs of several reptile species were presented by Wilhoft and Reiter (1985), Lofts (1989), Unsicker and Burnstock (1995) and Wyk and Mouton (1998). Data for changes in reproductive organs and the influence of various factors on them in unvenomous snakes and other reptiles were reported by Search-Link (1984; 1995; 1998; 1999). There are no data about the seasonal influence upon the reproductive system of the viper living in semi-farm conditions.

The breach of some ecological rules from the part of men caused not only the severe reduction in viper populations but also a general reduction in the number of venomous snakes and impaired the balance of natural ecosystems. This makes the reproduction of vipers in laboratory conditions a question of present interest and requires a complete knowledge of their way of living in nature and its dependence on seasons.

The present studies aimed to determine the season-dependent particularities, if any, of morphometric, structural and weight characteristics of testes in *Vipera ammodytes* in Bulgaria.

MATERIALS AND METHODS

Testes from 14 vipers, living in semi-farm conditions, inhabiting various regions in Bulgaria, collected during all four seasons within the period 1996–1999, were used (Table 1). All representatives were sexually mature, with a length of 55-60 cm[1].

After anaesthesia, euthanasia, decapitation and laparotomy, the testes were carefully separated for surrounding structures. The weight and morphometric parameters were determined on unfixed testes using a Sartorius electronic balance (precision 0.0001 g), micrometer and plotting paper. Following fixation with 10% neutral formaldehyde, dehydratation and clearing, the testes were embedded in paraffine. The 5 μm cross sections were stained with hematoxylin (according to Erlich)-eosin and durable (permanent) histological preparations were made (Pearse-Everson, 1962). The outer diameter of seminiferous tubules was determined via light microscope (Ergaval) and an eyepiece (Zeiss). For each viper and each histological preparation, the tubules were measured in 10 randomly selected microscopic fields (Avtandilov, 1990). The weight, metric and micrometric values were statistically processed by the Student's t-test and Mann-Whitney test at a level of significance $p<0.05$ (StatMost for Windows, DataMost Co., 1994). The structural features of testes were studied and microphotographied using a NU-2 universal light microscope.

RESULTS AND DISCUSSION

Data for the weight, length, circumference and the outer diameter of seminiferous tubules in vipers during the four seasons are presented in Table 1.

Our experiments revealed distinct seasonal variations in the *weight of testes* in *Vipera ammodytes*. The lowest values were measured in winter (55.6 ± 6.92 mg) and the highest (157±8.60 mg) – in spring, when the breeding period of vipers occurs. After reaching the peak values, the weight of testes began to decrease gradually and evenly. At the beginning of summer the values remained unchanged for several weeks and then, a monthly decrease by about 10–15 mg was observed – to 109 mg in the middle and 96 mg in the end of summer. This tendency persisted in the beginning (87 mg) and the middle (68 mg) of autumn. Our data showed that the weight of viper's testes increased more than thrice from the winter to the middle of the spring.

[1] The reproduction of vipers in Bulgaria occurs in the spring (Beshkov, 1993). They are capable of reproducing having reached a length of 60 cm. This dimension is probably appropriate to 5-year old snakes. Beshkov (1993) reported data from Bruno (1968) and Biella (1983) about vipers from South Tyrol, where female vipers were sexually mature at the age of 5 years while male ones - at 4 years. Greek representatives living in the Halkidiki peninsula are considered sexually mature at a smaller length – 30 cm.

Table 1. Seasonal features of testicular parameters in the viper (*Vipera ammodytes*)

Season, month	Weigth (mg)	Length (mm)	Circumference (mm)	Outer diameter of seminiferous tubules (μm)
Winter				
January	50.6	38.0	2.6	41.18
February	52.7	38.1	2.6	42.50
February	63.5	40.0	2.9	44.02
Mean ± SD	**55.60 ± 6.92**	**38.70 ± 1.13**	**2.70 ± 0.17**	**42.77 ± 1.14**
Spring				
March	151.5	50.0	2.9	84.15
March	150.3	50.1	2.8	83.78
April	158.0	50.5	2.9	84.49
April	169.1	51.8	3.5	90.88
Mean ± SD	**157.23 ± 8.60***	**50.60 ± 0.83***	**3.03 ± 0.32**	**85.83 ± 3.38***
Summer				
June	109.3	50.8	3.0	68.87
June	105.8	50.6	2.9	68.16
August	96.8	48.9	2.8	60.35
Mean ± SD	**103.97 ± 6.45**	**50.10 ± 1.04***	**2.90 ± 0.10**	**65.79 ± 4.73***
Autumn				
September	87.3	48.0	2.6	52.54
October	79.0	46.9	2.5	48.28
October	79.3	43.8	2.6	41.15
October	68.3	41.9	2.9	32.66
Mean ± SD	**78.48 ±7.80***	**45.15 ± 2.80***	**2.65 ± 0.17**	**43.66 ± 8.71**

* Level of significance at $p< 0.05$ versus winter, according to both Student's t-test and Mann-Whitney test.

The *length* and *circumference* of viper's testes also changed during the four seasons: by ≈ 10 mm and ≈ 1 mm, respectively, for the whole year. Significant elevations in length vs winter values (38.7 ± 1.13 mm) were present in all other seasons with maximum in spring (50.6 ± 0.83 mm). Similar changes were observed for the circumference but without a statisticall significance.

Apparent seasonal variations were observed in the *outer diameter of seminiferous tubules*. It increased more than twice from winter (42.77 ± 1.14 μm) to spring (85.83 ± 3.38 μm) ($p<0.05$), afterwards decreased progressively and by the middle of autumn was similar to the peak diameter, measured in winter.

The seasonal variations in the weight of testes and the diameter of seminiferous tubules correlated to data from the microstructural analysis of testes. The highest testicular weight and the highest outer diameter of seminiferous tubules in *spring* matched with histological findings, characterized by cells in all stages of the spermatogenesis, and a big number of free spermatozoa in the lumen of seminiferous tubules and the lumen of tubules of epididymes. Those findings were observed until the first few weeks of *summer*. Afterwards, the number of developing and free spermatozoa in the lumen of tubules progressively declined. Later, until the spring of next year, no spermatozoa were observed. At the end of summer, besides the characteristic partial or total degenerative changes occuring in separate cells from the spermatogenous range during the spermatogenesis in various vertebrate species (Dalcq, 1921; Roosen-Runge, 1962; 1980; Clermont, 1967; Holstein, 1970), we observed early destructive changes in seminiferous tubules. In *autumn*, when the weight of testes was extremely reduced and the peak values for the winter were almost reached, seminiferous tubules continued to change actively. The architectonics of the spermatogenous range in their wall was entirely impaired, with most different generations of reproductive cells being so damaged, that they could hardly be typified (Fig. 1). In the most actively changing tubules, the wall was severely thinned and the lumen filled with basophil-like stained detritus, filling the lumen of epididymal tubules too (Fig. 2). The *winter* testes besides the low weight, were characterized by a testicular regression expressed in involution of seminiferous tubules. The latter were with a small diameter, with a very clearly defined wall from the extensively grown peritubular zone (Fig. 3). Our results suggest that the attitude of Unsicker and Burnstock (1995) was valid for vipers. The authors prove that the myoid cells from the peritubular regions in reptiles differentiate in spring when the breeding period begins, while in late autumn most of them are replaced by a fibroblastoid cell elements. The lumen of those tubules has no free cell elements, but in the wall, a spermatogenous epithelium and single differentiating huge spermatocytes are clearly observable. The presence of such organized seminiferous tubules (as a result from their involution) suggests that from the end of winter to the middle of spring, the spermatogenesis would reach its final phase and the vipers would be ready for reproduction (Fig. 4).

The literature data that could be compared to ours as to the seasonal weight and metric parameters of viper's testes are very few and refer to unvenomous snakes or to other reptile species. Wyk and Mouton (1998) present data about the seasonal changes in the weight of testes and the status of the spermatogenesis in male lizards (*Pseudocordylus capensis*) having studied the sexual dimorphism in the

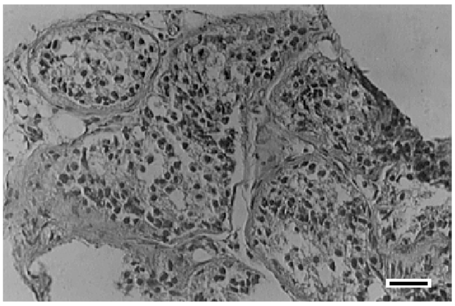


Fig. 1. Area of seminiferous tubules in autumn with impaired architectonics and destructively changed reproductive (sexual) cells within. H/E, bar = 5 µm.

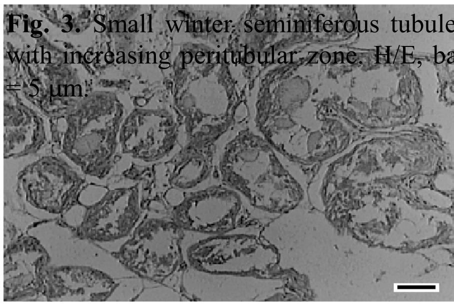
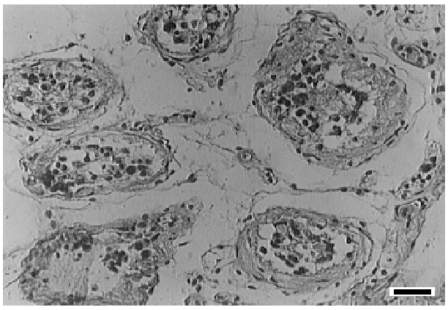


Fig. 3. Small winter seminiferous tubules with increasing peritubular zone. H/E, bar = 5 µm.

Fig. 2. Extremely changed seminiferous tubules whose lumen is filled with detritus in autumn. H/E, bar = 10 µm.



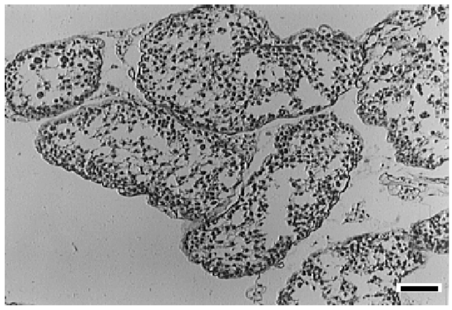


Fig. 4. Involuting seminiferous tubules from early spring days. H/E, bar = 10 µm.

mountain viviparous lizard. Only Search-Link (1995) reports few data about the weight of testes in the male gartner snake (*Thamnophis sirtalis parietalis*) without pointing out any season-related influence. Although limited, the reported data correspond to our results.

Summarizing the data from our weight, morphometric and microstructure studies on testes from vipers (*Vipera ammodytes*) living in semi-farm conditions in Bulgaria, performed during the four seasons, we could conclude that viper's testes, likewise other wild animal species, exhibited a clearly defined season-related changes. The most consistent changes were those in the weight, the diameter of seminiferous tubules and testicular microstructure while those in testicular length and circumference were less manifested. It could be hypothesized that the multifold increase in testicular weight without relevant changes in the length and especially the circumference of those organs was probably due to hypertrophic structural changes in seminiferous tubules and peritubular zones, observed by us, as well as to partial hyperplasia affecting some cells of the spermatogenous range, reaching giant dimensions.

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Correspondence: Dr. D. Dimitrov
Dept. of Veterinary Anatomy, Histology and Embryology,
Faculty of Veterinary Medicine,
Trakia University, Student Campus,
6000 Stara Zagora, Bulgaria