Seasonal changes in the testicular activity of the Iranian Mountain Viper, *Montivipera albicornuta* (Nilson & Andrén, 1985) (Reptilia: Viperidae)

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The Iranian Mountain Viper, *Montivipera albicornuta*, is a venomous snake endemic to Iran. We compared the seasonal cycle of testicular activity of 74 male specimens during a one-year period in two cold mountain climate habitats: Tarom (in Zanjan Province) and Bostanabad (in Eastern Azerbaijan Province) in northwestern Iran. The maximum testicular volume was observed in both habitats during autumn. The diameter of seminiferous tubules reached a maximum length in winter and a minimum in summer. The number of spermatids increased in autumn. Spermiogenesis occurred in spring and the maximum number of spermatozoa was reached then. In *M. albicornuta* in Iran, the testes were more active in autumn and spring, and spermatogenesis started in autumn and continued until mating in spring.

Keywords: *Montivipera albicornuta*; testis; spermatogenesis; cold regions

Introduction

The Iranian Mountain Viper (Zigzag Mountain Viper), *Montivipera albicornuta* (Nilson & Andrén, 1985), is a venomous snake endemic to Iran and found in parts of the Elburz, Talyszh and Zanjan mountains (Mallow, Ludwig, & Nilson, 2003). It is generally associated with rocky habitats, including rock walls, screed and cliff edges, and is found at elevations of around 2,500 m above sea level (McDiarmid, Campbell, & Touré, 1999). This is a viviparous species that breeds in summer and gives birth to 3 to 8 young (Latifi, 2000).

Vipers have a slow metabolism during hibernation (see e.g. Lourdais et al., 2002) and no growth and no change in body size occurs during this period. Males have a pair of testicles usually located cranially to the kidneys in the body cavities. The shape and location of the testes vary in different species. The right testicle is usually located cranial to the left testicle. The colour and shape of the testes may change during the mating season. Sperm production in snakes usually takes place in two stages: once prior to mating after hibernation, and a second one that starts after mating in spring. The second period of sperm production starts after the first period in spring and ends in late summer; these sperms are stored in the ductus deferens and used in the following spring. Testes regress in late summer/autumn and spermatogenesis generally ends before hibernation (Aldridge & Duvall, 2002). Snake species from temperate areas show a seasonal sexual behaviour. Some species mate twice per year, usually in the spring and autumn, and some are annuals, which means that they mate in the summer (Goldberg, 1999;
Schuett et al., 2006; Lind, Husak, Eikenaar, Moore, & Taylor, 2010). Most of the mating season in snakes in temperate zones is temporally dissociated from fertilization or, in other words, the mating season is temporally separated from spermatogenesis.

Previous work on the reproductive cycle of *M. albicornuta* has been confined to sperm reproduction (Moshiri, Todehdehghan, & Shiravi, 2014). We studied the male reproductive cycle of *M. albicornuta* during a one-year period to evaluate the testicular activity of this species.

**Material and Methods**

**Study Area.** The study area is located in two cold-climate mountainous habitats in the northwestern regions of Iran: Bostanabad (46°30’-47°14’E and 37°32’-38°04’N) in East Azerbaijan Province, and Tarom (48°30’-49°14’E and 36°38’-37°13’N) in Zanjan Province. The mean temperatures are 8°C in Bostanabad and 17.5°C in Tarom. Bostanabad is located at 1740 m a.s.l., Tarom at 750 m. The mean annual precipitation in Bostanabad is 320 mm and in Tarom 200 mm. The seasons are characterised as summer (late June–late September), autumn (late September–late December), winter (late December–late March), and spring (late March–late June).

**Sampling.** Sampling was done in the course of a medical study aiming at preparing antidotes for snake venoms. In the period from early January 2011 to late January 2012, a total of 74 adult males of *M. albicornuta* was collected: from Bostanabad 11 in spring, 10 in summer, 14 in autumn and 11 in winter, and from Tarom 10 in spring, 4 in summer, 8 in autumn and 6 in winter. The specimens were transferred to the Venomous Animals and Antivenin Production Department of Razi Vaccine and Serum Research Institute, Karaj, Iran.

**Laboratory Procedures.** Individuals were de-parasitized, quarantined and sacrificed by alcohol injection into the eye cavity. All procedures were performed according to the animal ethics guide lines ISIRI 7216-2 (ISIRI, 2008). For each snake, the following data were collected: (1) body weight, (2) snout-vent length (SVL), (3) tail length (TL), (4) right testis weight, (5) left testis weight, (6) right testis length, (7) left testis length, (8) left testis volume, (9) right testis volume, (10) seminiferous tubule diameter, and (11) thickness of the tunica. The volume of the testes was calculated by the ellipsoid formula \(4/3 \pi a.b.c\), in which \(a = \text{length}/2\), \(b = \text{width}/2\), \(c = \text{thickness}/2\) (Pleguezuelos & Feriche, 1999). Necropsy was performed, and the left and right testes, kidneys and hemipenes were removed (Zacariotti et al., 2007). The Gonadosomatic Index (GSI) was calculated by the formula: right testis mass/body mass \(\times 100\) (Clesson, Bautista, Baleckaitis, & Krohmer, 2002). The testes diameters were measured using a digital caliper (resolution 0.02 mm) and weighed with a digital scale (precision Swiss 0.0001 g). Organs were fixed in 10% formalin solution and then transferred to Bouin’s solution for histological studies as a standard procedure. From each specimen, a section was obtained from the mid-region of the testis and the distal region of the ductus deferens (portion between the kidney and the cloaca). The tissues were sectioned, mounted and stained with haematoxylin-eosin. Also, sections 5 \(\mu m\) thick were cut.

**Results**

Morphometric parameters of the male *Montivipera albicornuta* from Bostanabad and Tarom including body weight, body length from snout to vent and tail length are given in the Annex (Table 1). The peak of mean body weight of specimens was noted in autumn both in Bostanabad and Tarom. The testicular weight, length and volume of Bostanabad and Tarom specimens are presented in Figures 1–3. The testicular size and volume reach their peak in autumn.

The reproductive system in this snake is located along with the gastrointestinal tract and is attached to the dorsal surface of the animal body. The male reproductive system consists of a pair of testes, a portion of the kidneys, a pair of ductus deferens and a pair of hemipenes. Testes are pink in colour and oval-shaped, while the right testis is located slightly higher than the left one and is generally larger than the left one. Microscopic
Figure 1. Annual cycle of the mean weight of the testes of *Montivipera albicornuta* in two areas in Iran.

Figure 2. Seasonal change in the mean length of the testes in *Montivipera albicornuta* in two areas in Iran.

Figure 3. Seasonal change in the mean volume of the testes in *Montivipera albicornuta* in two areas in Iran.
testicular characters of *M. albicornuta* are presented in the Annex (Tables 2–3) and our data have shown the numbers of spermatogonia, spermatocytes, spermatids, spermatozoa, seminiferous tubule diameter and the tunica thickness in different seasons. Table 4 (see Annex) gives the hemipenis length of *M. albicornuta* in Bostanabad and Tarom. Figure 4 shows the mean number of germ cells in *M. albicornuta* during one year. Figure 5 shows the seminiferous tubules in *M. albicornuta* in spring (mid-June) and summer (mid-July), respectively.

The majority of primary and secondary spermatocytes and spermatids were observed in autumn. In the winter, some colloidal materials, degenerated cells, sertoli cells and spermatogonia cells were observed in a number of tubules with the largest possible diameter, which showed that spermatogenesis was dormant. Since spermiogenesis started in autumn, some spermatozoa were formed and this activity declined in winter and increased again in spring.

In April, the maximum length of right and left ductus deferens was 19.75 and 15.50 cm in Bostanabad, and 18.16 and 16.50 cm in Tarom. The mean weight of the right and left testis was 1.12 and 0.85 g in Bostanabad specimens, and 0.96 and 0.83 g in Tarom specimens. The maximum volume of the right and left testis was 896.5 and 779.7 mm$^3$ in Bostanabad specimens and 772.9 and 643.1 mm$^3$ in Tarom specimens.

The maximum sperm motility was 52.73 % in Bostanabad specimens (October) and 21.69 % in Tarom specimens (December). The average sperm concentration was 0.81 $\times 10^6$, 0.48 $\times 10^6$, 0.12 $\times 10^6$ ml in spring, autumn and winter respectively in Bostanabad specimens. The average sperm concentration was 0.30 $\times 10^6$, 0.73 $\times 10^6$, 0.46 $\times 10^6$ ml in spring, autumn and winter respectively in Tarom specimens.

**Discussion**

Studies on the testicular parameters in many species of reptiles show an increase during active spermatogenesis, and such parameters are significant in adult animals (Ramirez-Bautista, Guillette, Gutiérrez-Mayén, & Uribe-Peña, 1996). However, in testicles with a
larger size or volume, more sperms exist during active spermatogenesis (Estrada-Flores et al., 1990).

The results of the body size of *M. albicornuta* in the present research were similar to previous studies: The total body length of *M. albicornuta* was recorded as 82 cm (Latifi, 2000) and 65 cm (Farzanpey, 1990) compared to a maximum total body length in this study of 81 cm in Tarom and 82 cm in Bostanabad. In previous records, *M. albicornuta* grows to a maximum total length of 66 cm (Mallow et al., 2003). The mean tail length was recorded as 5 cm by Latifi (2000) compared to 6 cm in our specimens. The mean body weight of specimens in autumn just before hibernation was 260 in Bostanabad and 195.8 g in Tarom, which was due to fat storage and preparing for hibernation, while the mean body weight of males of this species was recorded as 193.25 g by Moshiri et al. (2014).

Our results show that the mean length of the right ductus deferens is more than the mean length of the left one during the reproductive season. Bilateral asymmetry has also been reported in other species of snakes, and the right ductus deferens may be about 30% longer than the left one (Sever, 2004). The right testis weight is more than the left one in most studied snakes (Johnson, Jacob, Torrance, 1982; Faghiri, Shiravi, Hojati, & Kami, 2011). The right testis volume is more than the left one in most studied snakes (Goldberg, 1999; Faghiri et al., 2011). The mean volume of the left and right testes of the Grass Snake, *Natrix natrix*, was reported as 525.5 and 569.1 mm$^3$, respectively (Faghiri et al., 2011). The maximum sperm concentration was found in Bostanabad specimens in spring and in Tarom specimens in autumn. Morphological studies of sperms in this research showed that the number of normal sperms is much greater than that of abnormal ones. The maximum number of normal sperms was in spring and summer, and the maximum number of abnormal sperms was in winter and related to tail defects such as crooked tails in both regions. The spermatogenic cycle was completed in spring and the peak of spermatogenesis was in late May to late June. Moreover, an increase in primary spermiogenesis or sperm formation was observed in late December. Also, an increase in testis volume was observed.

The morphology of the reproductive systems of *M. albicornuta* is similar to other vipers such as *Crotalus durissus terrificus* (Selma, 2004). Testosterone rises to a peak during mating in *Cerastes montivipera*, during autumn and spring and declines in early summer, and spermatogenesis is active from April to October. Studies in Brazil with a tropical climate showed that vipers mated in spring and the peak of spermatogenesis
was in summer and autumn, while their reproduction was also in the summer (Rojas, Barros, & Almeida Santos, 2013).

Two spermatogenetic cycles, vernal and aestival, have been described in temperate colubrid snakes. In both cycles, mating occurs in spring. Although vernal species produce spermatozoa in spring just prior to mating, aestival species yield spermatozoa from the previous summer. Aestival spermatogenesis is common in snakes from temperate to cold regions whereas the vernal cycle is found in species inhabiting the warm regions where the longer activity period allows the completion of a complete reproductive cycle within a calendar year (Feriche, Pleguezuelos, & Santos, 2008). The peak of sperm production is directly related to the parameters associated with puberty including SVL, and the highest quantity of sperms is found in specimens from Bostanabad in autumn and the specimens from Tarom in spring. The maximum diameter of the seminiferous tubules of *M. albicornuta* was observed during winter and the minimum diameter was observed during summer. The diameter of the ductus deferens was high in winter. According to our results, the maximum amount of normal mature sperms and testicular activity was recorded in May, October and November. This species has abundant motile sperms in spring and autumn. Mating occurred in spring (one case was observed in May in the vivarium). The results show that the spermatogenic cycle of *M. albicornuta* is aestival and that there are two peaks of spermatogenesis, in spring (May) and autumn (December).

**Supplementary Material**

The tables are given as a Supplementary Annex, which is available via the “Supplementary” tab on the article’s online page (http://dx.doi.org/10.1080/09397140.2016.1144284).

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No potential conflict of interest was reported by the authors.

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