Study of the anatomy and histology of the male reproductive system of the Asian snake-eyed skink, *Ablepharus pannonicus* (Sauria: Scincidae)

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**Abstract**
This study examines and compares the anatomy and histology of the male reproductive system in *Ablepharus pannonicus* from Scincidae family during spring and autumn. Male specimens of the Asian-eyed skink, *A. pannonicus*, were collected from the northern mountain slopes of Sorkheh-Dizeh village, 125 km west of Kermanshah Province, west Iran. Post-anesthetization, specimens were lab-dissected and sections of the male reproductive system underwent tissue processing. Using a rotary microtome, serial sections were prepared and slides were stained with Hematoxylin-Eosin for histological study. The results showed that the lumen of each testicular spermatogenic tubule is abundant with free sperm in spring. However, in the autumn season, almost no free sperm can be seen, and more developing spermatids can be observed. Therefore, the reproductive pattern in the male lizard *A. pannonicus* is seasonal. Its gonads and reproductive ducts show the greatest change and increase in activity in spring (May). Simultaneously, with the decrease in ambient temperature and the end of the breeding season, the volume and activity of the gonads also decrease. No signs of sexual activity can be seen in this lizard in autumn (November). Given the findings of this research, there exists a possibility that the animal prepares itself for hibernation by feeding and storing fat.

**Keywords:** Reproductive pattern, Spermatogenic tubule, Seasonal reproductive pattern, Testis
Introduction
Understanding the origins of the diverse reproductive patterns among lizards is a fascinating subject for biologists. Lizards exhibit a wide range of reproductive behaviours. Some species lay eggs, while others give birth to live young; some produce many offspring in a single reproductive episode, while others produce only a few; some construct nests and attend to their eggs until hatching, while others do not nest at all. Among oviparous species, some lay eggs immediately after they are shelled, while others retain eggs for extended periods before oviposition. Viviparity has arisen multiple times within squamates, and some species reproduce asexually. These reproductive differences represent just a small sample of the intriguing aspects of lizard reproduction that have attracted the attention of biologists (Rheubert et al., 2014).

Three types of reproductive cycles are generally observed in lizards: constant, associated, and dissociated (Pough et al., 2001). In the constant reproductive cycle, the gonads are active almost year-round (Jenssen & Nunez, 1994). In both associated and dissociated reproductive cycles, the mating season is discontinuous. In the associated type, gonadal activity increases immediately prior to the mating period in both males and females simultaneously, eliminating the need for females to store sperm due to its availability during the reproductive season (Huang, 1997; Censky, 1995). In the dissociated type, gonadal activity is low during the mating period and peaks during the non-mating period. Male gonadal activity is shorter than that of females, and sperm is stored by the female genital system for later fertilization (Torki, 2006).

The male reproductive system in lizards consists of the gonads (testes), gonadoducts (testicular ducts), the sexual segment of the kidney (SSK), and the cloaca (Rheubert et al., 2010). In lizards, sperm develop in the germinal epithelium of the seminiferous tubules within the testes (Gribbins, 2011) and pass sequentially through the various portions of the gonadoducts: rete testis, ductuli efferentes, epididymis, ductus deferens, and, in some species, an ampulla ductus deferentis (Sever, 2010). Sperm then
usually mixes with secretions from the SSK in a variable ductus deferens-ureter complex and enters the cloaca through the urogenital papillae (Rheubert et al., 2014). The skink *A. pannonicus*, a member of Scincidae family. This species is found in Iran, Azerbaijan, Turkmenistan, Tajikistan, Uzbekistan, Afghanistan, Pakistan, Iraq, Syria, Jordan, Saudi Arabia and Türkiye (Vaissi. et al, 2023). Despite numerous studies, no research has been conducted on the anatomy and histology of the reproductive system of the Asian eyed skink. Therefore, considering the wide distribution of this species, this research investigates the anatomy and histology of the male reproductive system of this lizard for the first time.

**Martial and methods**

**Study Area and Sampling**
This research was conducted in the natural surroundings of the village of Sorkheh Dizeh, located 125 km from Kermanshah Province, west Iran. Sampling was carried out in spring (May) and autumn (November), with three adult specimens of *A. pannonicus* collected by hand in each season.

**Macroscopic Study of the specimens**
Upon arrival at the laboratory, the specimens underwent a preliminary assessment. Anaesthesia was administered through cotton saturated with diethyl ether. Morphometric parameters, including body length and mass, were meticulously documented. A longitudinal incision was performed on the ventral surface of the specimens, which allowed for the removal of the digestive tract. This procedure exposed the reproductive system, which was then photographed for subsequent analysis. The reproductive organs were carefully isolated, and their mass was precisely determined. This data was utilized to compute the gonadosomatic index, providing a quantitative measure of reproductive development (Darabitabar et al., 2021).

**Microscopic Study of the Samples**
After the genital organ was removed from the body of the lizard, its parts were immediately placed in 10% formalin for 72 hours; The samples were dehydrated in a
series of ethanol solutions: 60% for 60 minutes, 70% for 30 minutes, 80% for 30 minutes, and 96% for 120 minutes; For clarification, the samples were placed in three containers containing xylene, each for 30 minutes; The samples were then embedded in paraffin in an incubator set at 58°C; In this step, three containers of paraffin were used, and the capsules containing the tissue samples were placed in each container for 120 minutes to allow the paraffin to replace the xylene in the tissue. L-shaped metal molds, known as Leuckhard molds, were used for molding; The mold was adjusted to the appropriate size, and then molten paraffin was poured into them; The samples were then placed vertically inside the molds; In the next step, the blocks were separated from the mold, resulting in a molded block for each part; A rotary microtome (CUT SLEE 4060) was used to cut the blocks; After the slices were affixed to the slide, the samples were prepared for staining; Finally, the slides were stained using hematoxylin-eosin staining (Suvarna et al., 2018).

**Results**

In male *A. pannonicus*, the right testis is positioned higher than the left testis within the abdominal cavity (Fig. 1).

![Image of testes and kidney](image)

**Figure 1.** Position of the testes (T) and kidney (K) in *A. pannonicus* during spring (May).

Each testis is encased in a capsule, the branches of which extend into the testis, dividing the interior space into several pyramidal compartments. Within each compartment, a seminiferous tubule and numerous interstitial cells can be observed at the border between them. The diameter of these tubules significantly increases during the animal's sexual activity. Within the tubule wall, several Sertoli cells and various
types of germ cells can be seen at different stages of development, from spermatogonia to sperm (Fig. 2).

Figure 2. Transverse section of the testis of *A. pannonicus* in spring (A) and autumn (B) with magnifications of 20 μm and 50 μm, respectively. As depicted in the figure, the lumen (L) of the seminiferous tubules is filled with various stages of sperm cells in spring, including spermatogonia (SP), spermatocytes (SC), round spermatids (S1), elongating spermatids (S2), and mature sperm (S3). In contrast, the lumen is almost empty in autumn.

The lumen of each tubule in the spring season (May) is abundant with free sperm, but in the autumn season (November), almost no free sperm can be seen, and developing spermatids are more visible. In the spring, due to the activity of the testicles and the increase in spermatogenesis, the weight of the testicles and the diameter of the seminiferous tubules increase. The data related to these changes are shown in Table 1.

Table 1. Seasonal Changes in Body Weight (W), Testes Weight (TW), Seminiferous Tubules Diameter (STD), Gonadosomatic Index (GI), and Snout-Vent Length (SVL) in Male *Ablepharus pannonicus* (Values are Mean ± Standard Deviation).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spring</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SVL (mm)</td>
<td>31.3±2.4</td>
<td>30.2±3.1</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>0.58±0.05</td>
<td>0.5±0.04</td>
</tr>
<tr>
<td>Testes weight (g)</td>
<td>0.004±0.001</td>
<td>0.003±0.001</td>
</tr>
<tr>
<td>STD (μm)</td>
<td>156.16±12.31</td>
<td>135.22±6.85</td>
</tr>
<tr>
<td>GI</td>
<td>0.68±0.05</td>
<td>0.6±0.03</td>
</tr>
</tbody>
</table>
The ductuli efferentes in *A. pannonicus* consist of several ducts with a spherical cross-section, which are adjacent to the epididymis. The wall of these ducts is composed of cuboidal epithelium, with the majority of the cell volume occupied by a very large nucleus. Several microvilli-like appendages can be seen on the inner surface of these ducts (Fig. 3).

![Figure 3](image)

**Figure 3.** Transverse section of the ductuli efferentes (De) and epididymis (Ep) of *A. pannonicus* in autumn (A) and spring (B) with magnifications of 50 μm and 20 μm, respectively.

The ductuli efferentes lead to a convoluted tube known as the epididymis. The diameter of the epididymis gradually decreases as it approaches the ductus deferens. The tissue lining its inner surface is composed of columnar cells, with the nucleus of these cells located on their basal surface. In the spring, the inner space of this tube is filled with sperm and secretions, but in the fall, coinciding with the decrease in sexual activity of the testes, a very small number of sperm can be seen in the lumen of this tube (Fig. 4).
Figure 4. Transverse section of the epididymis of *A. pannonicus* with magnifications of 20 μm and 100 μm. Figures A, B, and C were taken in the spring (May), showing the epididymis filled with sperm. Figure D, taken in the autumn (November), shows the epididymis without visible sperm. Abbreviations: De represents the ductuli efferentes, Ep represents the epididymis, and S represents sperm.

The ductus deferens is an almost straight duct that continues from the epididymis. In the spring, the space inside the ductus deferens is filled with sperm and its wall diameter is thin, but in the autumn, it is almost devoid of sperm and its wall is thick (Fig. 5).
Figure 5. The ductus deferens of *A. pannonicus* in spring (A) and autumn (B) with magnifications of 100 μm and 50 μm, respectively. (A) Longitudinal section of the ductus deferens in spring (May), where a large number of sperm are observed in its lumen. (B) Transverse section of the ductus deferens in autumn (November), where a very small number of sperm can be seen in its lumen. Abbreviations: K represents the kidney, Dd represents the ductus deferens, and S represents sperm.

At the posterior end of the ductus deferens, there is an ampulla ductus deferentis, which has many folds on its inner surface. The thickness of the wall of this area of the ductus deferens is almost twice that of other areas, due to the presence of dense connective tissue in the wall (Fig. 6).

Figure 6. Transverse section of the ampulla of the ductus deferens of *A. pannonicus* in autumn (November), with a magnification of 20 μm.

In the transverse sections taken from the kidneys of *A. pannonicus*, it was found that in addition to observing the cross-section of the renal corpuscle, the proximal convoluted tubule, the distal convoluted tubule, and the collecting duct, another cross-section of a part called the Sexual Segment of the Kidney (SSK) can be observed. In the spring, the lumen of this part is filled with substances secreted from the cells of its
wall. The columnar cells of its wall are longer and more elongated compared to the wall cells of the proximal and distal convoluted tubules (Fig. 7).

**Figure 7.** Transverse section of the kidney of *A. pannonicus* with a magnification of 20 μm. Abbreviations: DCT represents the distal convoluted tubule, PCT represents the proximal convoluted tubule, SSK represents the sexual segment of the kidney, and GL represents glomeruli.

**Discussion**

The testes in lizards usually have a round to slightly oval shape (Fox, 1975). In *A. pannonicus*, the testes are not only elongated but also positioned uniquely; the right testis is situated above the left one and at a relatively large distance from it. This arrangement, along with their elongated shape, allows the testes to align with the body's length. This alignment ensures the slim and snake-like appearance of the body is maintained, enabling the animal to make quick movements for burrowing into small ground holes or escaping from predators.

Studies conducted on the ductuli efferentes of *Scincella lateralis* reveal that the epithelium of these ducts is cuboidal to slightly columnar, with an abundance of cilia on the cell surface (Sever et al., 2013). In the species *Sitana ponticeriana*, two distinct areas can be distinguished in the ductuli efferentes (Akbarsha et al., 2007). However, in all other studied species (e.g., *Hemidactylus turcicus*, *Scincella lateralis* and *Seminatrix pygaea*), it has not been possible to separate these ducts into two completely distinct areas (Rheubert et al., 2010; Sever, 2010; Sever et al., 2013). In *A. pannonicus*, similar to *Scincella lateralis*, sections taken from different areas of ductuli efferentes show only two types of cells: with and without cilia. The uniform distribution of these two cell types throughout the ducts indicates that they cannot be divided into two separate areas.
The epididymis of *Hemidactylus turcicus* contains two types of cells, principal and basal, without any regionalization, along the entire length of this duct (Rheubert et al., 2010). Sever et al. (2013) found three cell types (light and dark principal cells and basal cells) in *Scincella lateralis* and differentiated the anterior from the posterior regions based on the amount of secretory material in the lumen.

The epididymis in *Tiliqua scincoides* was identified as a convoluted duct with a wall composed of a row of columnar cells (Shea, 2006). In *A. pannonicus*, similar to *Tiliqua scincoides*, the epithelium covering the inner surface of the epididymal duct consists of a row of columnar cells. Due to the presence of a large number of sperm, it likely acts as a sperm storage place.

In *Scincella lateralis*, the ampulla ductus deferentis, as the last part of the ductus deferentis, is responsible for connecting this duct to the orodeum of the cloaca, and its thickness is greater than other parts of the ductus deferentis (Sever et al., 2013). In *A. pannonicus*, like the lizards *Scincella lateralis, Cophosaurus texanus, Sceloporus consobrinus, Holbrookia propinqua*, and *Phrynosoma cornutum*, this ampoule has a thick wall and many epithelial folds, and a number of sperms are also observed in its lumen (Rheubert et al., 2014). Examinations of the kidney in *Scincella lateralis* (Sever & Hopkins, 2005), *Notomabuya frenata*, and *Aspronema dorsivittatum* (Novelli et al., 2018) species showed that the SSK region has a columnar epithelium and its lumen is filled with abundant secretions. The epithelium of the SSK area in *A. pannonicus* has columnar cells, like other relatives, and in the spring, it has some substances secreted from its wall.

**Conclusion**
This research showed that the reproductive pattern in the male lizard *A. pannonicus* is seasonal. The gonads and the genital ducts connected to them show the most changes and increased activity in spring (May). As the ambient temperature decreases, the volume and activity of the gonads also decrease, such that no signs of sexual activity can be observed in this lizard in the autumn season (November).
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References


