Turkish Journal of Zoology

Manuscript 3204

Determination of spermatogenesis activity using lectin histochemistry in water monitorlizards (Varanus salvator) harvested from the wild

ANDHIKA YUDHA PRAWIRA

NI LUH PUTU RISCHA PHADMACANTY

GONO SEMIADI

HELLEN KURNIATI

WAHYU TRILAKSONO

See next page for additional authors

Determination of spermatogenesis activity using lectin histochemistry in water monitorlizards (Varanus salvator) harvested from the wild

Authors
ANDHIKA YUDHA PRAWIRA, NI LUH PUTU RISCHA PHADMACANTY, GONO SEMIADI, HELLEN KURNIATI, WAHYU TRILAKSONO, NURHIDAYAT NURHIDAYAT, and SAVITRI NOVELINA

TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2024) 48: 664-674
© TÜBİTAK
doi:10.55730/1300-0179.3204

Determination of spermatogenesis activity using lectin histochemistry in water monitor lizards (*Varanus salvator*) harvested from the wild

Andhika Yudha PRAWIRA[1,*], Ni Luh Putu Rischa PHADMACANTY[1], Gono SEMIADI[2], Hellen KURNIATI[2], Wahyu TRILAKSONO[2], Nurhidayat NURHIDAYAT[3], Savitri NOVELINA[3]

[1]Research Center for Applied Zoology, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN), Cibinong, Indonesia

[2]Research Center for Biosystematics and Evolution, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN), Cibinong, Indonesia

[3]Department of Anatomy Physiology and Pharmacology, School of Veterinary Medicine and Biomedical Science, IPB University, Bogor, Indonesia

Received: 21.04.2024 • **Accepted/Published Online:** 14.09.2024 • **Final Version:** 05.11.2024

Abstract: Lectin is often used to investigate the differentiation and maturation of spermatogenic cells in the testes of several wild animals, facilitating the screening of mature individuals. Therefore, this study aimed to determine spermatogenesis activity using lectin histochemistry in water monitor lizards (*Varanus salvator*) harvested from the wild. Testes of 43 comprehensively examined water monitor lizards were collected from slaughterhouses in Palembang (Sumatra) and Banten (West Java), Indonesia, with snout–vent lengths (SVL) ranging from 39 to 96 cm. Additionally, histologic, histomorphometric, periodic acid-Schiff (PAS) staining, and lectin histochemistry (Con A, RCA, WGA, PNA, VVA, DBA, UEA-I) observations were performed. The results showed a positive correlation between SVL, morphometry, and histomorphometry at $p < 0.05$ with Pearson's correlation analysis. All specimens presented a spermatogenesis process with different activities among various SVL sizes, with larger animals having higher spermatogenic activity. Moreover, PAS-positive reactions in the testes were mainly observed in the cytoplasmic residues of spermatogenic cells, connective tissue, Leydig cells, and blood vessels. The most dominant-reacting lectin bindings were ConA, RCA, and WGA, distributed in nearly the entirety of the testes, including in spermatids and spermatozoa phases. PNA and VVA bindings were localized in Golgi and Cap stage spermatids, while UEA-I and DBA lectins showed no binding reaction. Although the larger animals had more spermatogenic cells, the lectin-binding patterns of all specimens were similar. The presence of lectin binding of PNA, VVA, and WGA in spermatids and spermatozoa in spermatogenic cells signified that male monitor lizards harvested from the wild exhibited active spermatogenic processes and were able to produce mature spermatozoa.

Key words: *Varanus salvator*, lectin binding, marker, maturation, spermatogenic cells, spermatozoa

1. Introduction

Lectin is a protein complex that specifically binds carbohydrates in biological materials as glycoconjugates (Spicer, 1993). These glycoconjugates play diverse roles in cells for monitoring differentiation, maturation, recognition, adhesion, disease, and interaction (Dias et al., 2015). Lectin histochemistry has been used to study the differentiation and maturation of spermatogenic cells in the testes of several wild animals, including tilapia (Tokalov and Gutzeit, 2007), banded bamboo sharks (Gewaily et al., 2021), Spanish newts (Sáez et al., 2000), *Tropidurus itambere* lizards (Ferreira and Dolder, 2003), deer (Wahyuni et al., 2016), Sunda porcupines (Budipitojo et al., 2020), and lesser mouse deer (Agungpriyono et al., 2009). Various studies have also found differences in lectin-binding patterns in the testes and spermatozoa between immature and mature animals, such as in *Tropidurus itambere* lizards (Ferreira and Dolder, 2003) and Sunda porcupines (Budipitojo et al., 2020).

Water monitor lizards (*Varanus salvator*) are the most traded species in Asia and are often hunted for their meat and skin and used as pets (Setyawatiningsih, 2018; Boscha et al., 2020; Sy and Lorenzo, 2020). Wild water monitor lizards are frequently harvested for the skin, with collectors preferring males owing to their larger skin size and better skin quality (Nijman, 2016; Setyawatiningsih, 2018; Arida et al., 2020; Boscha et al., 2020). Lectin histochemistry can characterize glycoconjugate distribution in testes, making

* Correspondence: taha

it a valuable tool for assessing the status of testicular development and maturation. Sufficient information about lectin-binding patterns in the testes of water monitor lizards is currently unavailable. Therefore, this study aimed to investigate the distribution characteristics of lectin binding and glycoconjugates and the developmental status of spermatogenic cells in the testes of wild water monitor lizards.

2. Material and methods

2.1. Animals and samples

The testes of 43 water monitor lizards obtained from Palembang (Sumatera) and Banten (West Java) slaughterhouses were used in this study. Samples were collected in Palembang in August 2022 and between May and June 2023 in Banten. All testes were collected from dead animals that the slaughterhouse considered waste, and no individual water monitor lizards were intentionally killed for or in this study. Ethical approval (no. 138/KE.02/SK/07/20230 was obtained from the National Research and Innovation Agency of Indonesia.

The snout–vent length (SVL) of the animals ranged between 39–96 cm, with body weights (BW) ranging from 1 kg to 14 kg. The samples were divided into four groups based on SVL size, namely groups 1 (39–50 cm), 2 (50.1–60 cm), 3 (60.1–70 cm), and 4 (>70 cm). The collected testes were separated from attached connective tissue, and the testes' length (LT) and width (WT) were measured using polypropylene tape and a microcaliper, respectively (Mitutoyo, Japan). These were fixed in a 10% buffered neutral formalin solution for 24–48 h and transferred to a 70% ethanol solution before conducting histological analysis.

2.2. Histologic preparation

Testes were cut into smaller sizes and processed into histological slides using the standard paraffin method. Tissue blocks were serially sectioned at a 4–5-µm thickness using a microtome rotary (Yamato-RV 240, Japan). Testicular tissue slides were stained with routine hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and lectin histochemistry was performed.

Routine HE staining was performed with deparaffinization and rehydration of the tissue sections, followed by Mayer's hematoxylin for 1 min and immersion in running tap water for 5 min. Eosin was added for 2 min, followed by dehydration, clearing, and mounting.

PAS staining is often used to visualize the glycoprotein in tissues, including the spermatozoa acrosome (Hess, 1990; Hess and de Franca, 2008). This was conducted through deparaffinization and rehydration, followed by treatment with 1% periodic acid for 5 min and rinsing with distilled water. Schiff's solution was added and incubated for 5–10 min at room temperature, then washed with distilled water. The slides were counterstained with hematoxylin for 10 s, immersed in tap water for 5 min, dehydrated, cleared, coverslipped, and mounted before observation.

2.3. Lectin histochemistry

Dewaxed sections were incubated with 0.3% H_2O_2 in methanol for 20 min at –30 °C and washed with phosphate buffer saline (PBS) pH 7.58. These sections were further incubated with 10% donkey normal serum (Abcam ab7475, Abcam, Waltham, MA, USA) for 30 min at room temperature. The sections were then incubated overnight at 4 °C with seven biotinylated lectins (Table 1; BK-1000, Vector Lab. Inc. Burlingame, CA, USA). Subsequently, the sections were washed with PBS (pH 7.58) and incubated with an Avidin–Biotin Complex kit (PK-6100, Vector Lab. Inc. Burlingame, CA, USA) for 30 min at room temperature. The reaction product was visualized by incubation in 3,3¢- diaminobenzidine (Abcam ab64238, Abcam, Waltham, MA, USA) for 1–3 min. In addition, the sections were counterstained with Mayer hematoxylin for 5 s, dehydrated in graded ethanol, cleared in xylene, and mounted with Entellan (Merck, Rahway, NJ, USA). The sections were then observed under a light microscope (Olympus CX 31, Japan) at 200× magnification, and the intensity was compared by a qualitative scoring graded as negative (–), weak (+), moderate (++), strong (+++), and very strong (++++). Qualitative scoring was conducted by comparing the intensity of the reaction to the negative control section and the darkest brown color in all sections. Negative controls were present in the sections treated with PBS only as a lectin substitute.

2.4. Histomorphometric measurement

HE-stained testes were examined under a microscope (Olympus CX 31, Japan) at 300×. magnification. The histomorphometric characteristics of the testes were observed by measuring the cross-sectional surface area of the seminiferous tubules (Cat), the thickness of the epithelial layer in the seminiferous tubules (Tet), and the number of spermatogenic cells in the seminiferous tubules (Nsc) in ten randomly selected tubules showing a cross-section (circular in the histological section). Measurements were performed using ImageJ (Schneider et al. 2012).

2.5. Data analysis

Histomorphometric data were analyzed using one-way analysis of variance, nonparametric Kruskal–Wallis analysis, principal component analysis (PCA), and Pearson correlation using MiniTab version 20. Lectin-binding data were analyzed descriptively by observing the binding pattern and distribution of lectins to tissue components in testes.

Table 1. Lectin and glycoconjugate specificity.

Lectin	Abbreviation	Sugar specificity	Concentration
Concanavalin agglutinin	ConA	α-D-Man, > α-D-Glc	10 µg/mL
Wheat germ agglutinin	WGA	βGlcNAc > > NeuNAc	20 µg/mL
Peanut agglutinin	PNA	Galβ1,3GalNAc	20 µg/mL
Vicia villosa agglutinin	VVA	βGlcNAc > > NeuNAc	20 µg/mL
Riccinus communis agglutinin	RCA	Galβ1,4GlcNAc	10 µg/mL
Ulex Europaeus agglutinin I	UEA I	α-L-Fuc	10 µg/mL
Dolichos biflorus agglutinin	DBA	α-D-N-GalNAc	10 µg/mL

3. Results

The results showed that the testicular structure of the water monitor lizards was primarily composed of seminiferous tubules, connective tissues, and interstitial Leydig cells (Figure 1). All examined samples had a complete structure, with only histomorphometric variation observed among the SVL groups (Figure 2). The seminiferous tubules consisted of a basement membrane, Sertoli cells, and spermatogenic cells, including spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, elongated spermatids, and spermatozoa in the lumen. Furthermore, spermatogenic epithelial cells in the form of spermatogonia to early-stage spermatozoa were loosely arranged and attached to the Sertoli cell cytoplasm. Many immature sperm cells were observed attaching to the apex of the Sertoli cell cytoplasm. This structure was present in all samples, although larger samples (SVL >70 cm) occasionally showed spermatogenic cells in the lumen. Spermatogonia cells were observed in the basal region of the tubules, whereas actively proliferating primary spermatocyte (I) cells were identified through condensed chromatin. Secondary spermatocytes (II) were numerous and smaller than primary spermatocytes. The elongated spermatids had a compacted nucleus, an oval shape without a tail, and residual cytoplasm. The spermatozoa had a long head with a pointed tip, a short body in the middle section, and a visible tail. Some sperms were found attaching to the cytoplasm of Sertoli cells, while others had separated and were located in the lumen.

The bright magenta color of PAS staining signified a strong positive reaction in the cytoplasm remaining from spermatogenesis, suggesting that residual glycoproteins were wasted (Figure 1d and 1e). However, no positive reaction was found in the head or tail of spermatozoa, and a PAS-positive reaction was observed in the connective tissue. The basement membrane of tubules and blood vessels in the interstitial tissue had a strong PAS-positive reaction. Moreover, Leydig cells in the interstitial tissue showed moderate positive reactions.

Based on morphometric analysis (Table 2), the body size of the water monitor lizards and testicular morphology had a substantial positive association with the Nsc. The PCA described approximately 89.2% of data variance in principal component 1 (PC 1: 78.7%) and principal component 2 (PC 2: 10.5%). PC 1 represents the morphometric variables, including SVL, BW, WT, Cat, and Tet, while PC 2 represents the Nsc variables. According to the PCA plot, the size of the testes, seminiferous tubules, and Nsc increased proportionally with body size (Figure 3). The plot graphic showed that most animals with an SVL size smaller than 60 cm were plotted on the left side, while animals with an SVL size larger than 60 cm were plotted on the right side of the graphic (Figure 3). Analysis confirmed a significant difference ($p < 0.05$) among the SVL groups in several variables (Table 3). For nearly all variables, no significant difference was detected between SVL groups 1 and 2. However, significant differences ($p < 0.05$) were found between SVL groups 1 and 2 compared to groups 3 and 4.

Observations of glycoconjugate distribution, identified through lectin binding, showed specific patterns in the spermatogenic cells of the water monitor lizard testes. Generally, the distribution patterns of lectin bonds were present in all SVL groups, with differences detected only in specific samples based on variations in binding reaction intensity (Table 4).

Basal spermatogonia and Sertoli cells reacted positively to ConA, RCA, and WGA lectin binding, with the highest intensity observed for ConA lectin binding (++++), while the others reacted with moderate to strong intensity (++~+++). These binding reactions were observed in the cytoplasm and cell membrane. Spermatocyte cells showed positive reactions to ConA, RCA, and WGA lectin binding, with a strong intensity (+++) observed for ConA binding and a weaker to moderate intensity (+~++) for RCA and WGA (Figure 4). Spermatids reacted positively to ConA, PNA, RCA, WGA, and VVA, with binding intensity varying from weak to strong. The PNA binding reaction was strong (+++), with distribution around the cell nucleus at the Golgi-cap stage, characterized by dense bodies that reacted more strongly than the nucleus. The VVA binding reaction was similar to the PNA binding but at an intensity varying from moderate to strong (++~+++) (Figure 4). The ConA binding reaction was observed to

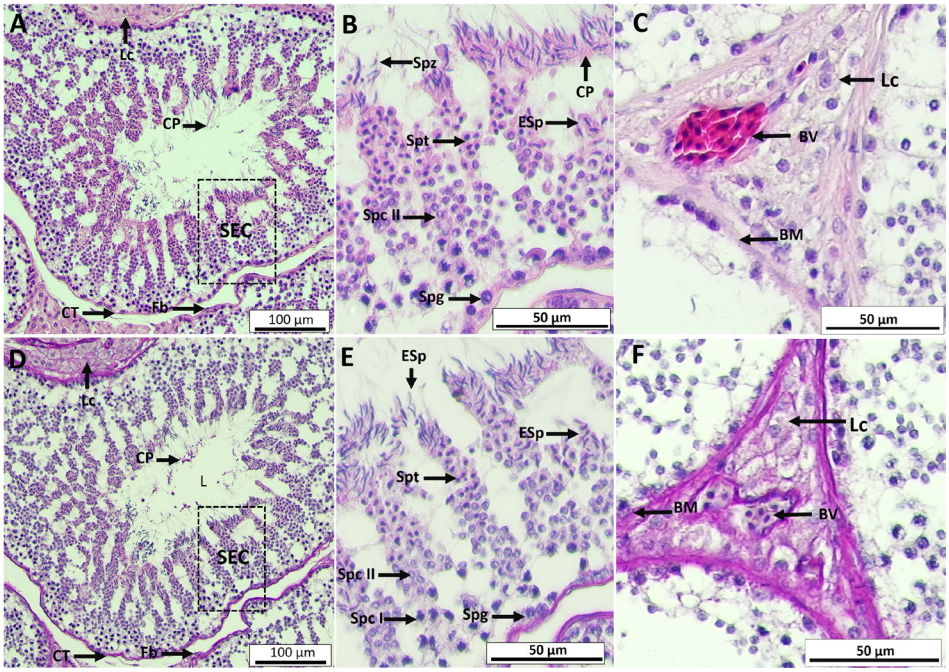

Figure 1. Representative images of the tubulus seminiferous of water monitor lizards (*Varanus salvator*). (a) HE staining of general structure of testes; (b) higher magnification of spermatogenic epithelial cells (SEC) of seminiferous tubules; and (c) interstitial tissue of seminiferous tubules. (d) PAS staining of general structure of testes; (e) higher magnification of SEC; and (F) interstitial tissue of seminiferous tubules. BM: basement membrane; BV: blood vessels; CT: connective tissue; CP: residual cytoplasm; ESp: elongated spermatozoa; Fb: fibroblast; L: Lumen; Lc: Leydig cells; Spg: spermatogonia cells; Spc I: spermatocyte I; Spc II: spermatocyte II; Spt: spermatid.

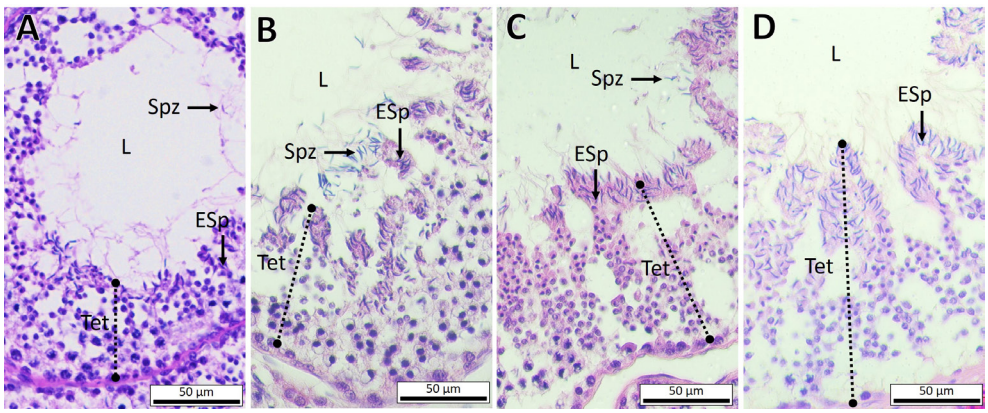

Figure 2. Histological comparison of spermatogenic cells among SVL groups of water monitor lizards (*Varanus salvator*). (a) Group 1 SVL: 39–50 cm; (b) Group 2 SVL: 50.1–60 cm; (c) Group 3 SVL: 60.1–70 cm; and (d) Group 4 SVL: >70 cm. ESp: elongated spermatids; Spz: spermatozoa; Tet: thickness of epithelial tubules; L: Lumen.

have a strong intensity—although it lacked specificity—and was distributed uniformly throughout the spermatocyte and spermatid cells. RCA and WGA binding reactions were observed at weak to moderate intensity (+~++), with binding distribution in the cell membrane and nucleus of spermatocyte and spermatid cells.

Elongated spermatids were observed to bind to lectins ConA, PNA, RCA, WGA, and VVA (Figure 5). The binding intensity was weak to moderate (+ ~ ++), with a variable distribution of binding (Table 4). ConA binding was observed in all cell (early-stage spermatozoa) parts, including the nucleus, cytoplasm, and membrane, while

Table 2. Correlation coefficient among the variables.

Variables	SVL	BW	LT	WT	Sat	Tet
Body weight (BW)	0.949					
Testes length (LT)	0.789	0.756				
Testes width (WT)	0.847	0.797	0.850			
Cross-sectional surface area of tubules (Cat)	0.781	0.722	0.671	0.738		
Thickness of epithelial tubules (Tet)	0.706	0.616	0.700	0.730	0.882	
Numbers of spermatogenic cells (Nsc)	0.644	0.600	0.636	0.638	0.882	0.830

Note: All the correlation coefficient values are significant at $p < 0.05$ with Pearson's Correlation analysis.

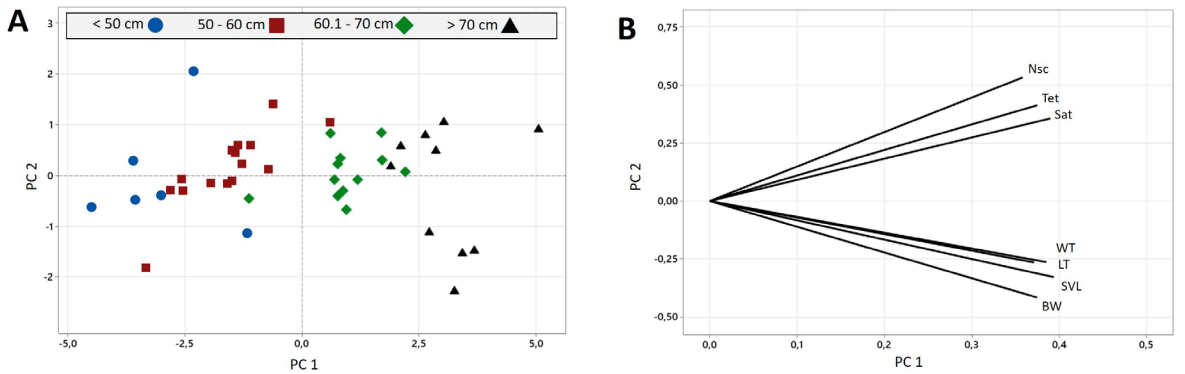

Figure 3. Plot graphic of (a) sample distribution based on variables and (b) variable correlation of water monitor lizard (*Varanus salvator*) reproductive organs and body weight. PC 1 represents the testes' morphometric variables, while PC 2 represents the number of spermatogenic cells variable. In the (a) sample distribution graphic, animals with an SVL size less than 60 cm were plotted on the left side, while samples larger than 60 cm were plotted on the right side. In the (b) variable correlation graphic, positive correlations were observed and clustered into two main variables: histomorphometric and macromorphometric. (Tet: thickness of epithelial tubules; Nsc: number of spermatogenic cells; Cat: cross-sectional surface area of tubules; BW: body weight; SVL: snout–vent length; LT: testes length; WT: testes width).

Table 3. Morphometric comparison among the SVL groups.

Variables	SVL Group			
	<50 cm (n = 6)	50–60 cm (n = 15)	60.1–70 cm (n = 12)	>70 cm (n = 10)
BW (kg)	1.55 ± 0.38 a	2.49 ± 0.44 a	5.43 ± 0.91 b	9.73 ± 3.12 c
LT (cm)	2.44 ± 0.66 a	3.18 ± 0.58 a	4.24 ± 0.73 b	4.72 ± 0.61 b
WT (cm)	1.11 ± 0.35 a	1.61 ± 0.29 a	2.14 ± 0.31 b	2.47 ± 0.24 c
Cat (mm^2)	0.054 ± 0.03 a	0.066 ± 0.03 a	0.109 ± 0.02 b	0.144 ± 0.03 c
Tet (mm^2)	0.062 ± 0.02 a	0.085 ± 0.02 b	0.111 ± 0.01 c	0.118 ± 0.01 c
Nsc (cells/surface area)	769.0 ± 497.62 a	862.0 ± 388.69 a	1511.3 ± 340.23 b	1806.06 ± 650.82 b

Note: Different fonts in a row show a significant difference at $p < 0.05$.

PNA, RCA, WGA, and VVA binding occurred mainly in the residual cytoplasm. Spermatozoa comprising the nucleus, midpiece, and tail reacted to ConA, RCA, and WGA lectins, with bindings of weak to moderate intensity (+~++) observed in the tail. The cytoplasm at the tip of the spermatogenic tubule epithelium, which was a combination of the residual cytoplasm of spermatid cells and Sertoli cell processes, showed varying reactions to ConA, PNA, RCA, WGA, and VVA lectins (Figure 5). ConA lectin exhibited the highest intensity, followed by WGA and RCA and PNA and VVA.

Leydig cells in the interstitial tissue had strong to very strong (+++~++++) ConA, RCA, and WGA lectin-binding reactions, with scattered distribution in the cytoplasm

Table 4. Lectin scoring of water monitor lizard testes.

Lectin	Testicle part								
	Lc	CT	SC	Spg	Spc	Spt	ESp	Spz	CP
ConA	++++	++++	++++	++	+ ~ ++	+++	++	++	+++ ~ ++++
PNA	-	-	-	-	-	+++	+ ~ ++	-	+ ~ ++
RCA	+++	+++	++ ~ +++	+ ~ ++	+ ~ ++	+ ~ ++	+ ~ ++	+	++ ~ +++
WGA	++++	+++	++ ~ +++	+ ~ ++	+ ~ ++	+	+ ~ ++	+	++ ~ +++
VVA	-	-	-	-	-	++ ~ +++	+ ~ ++	-	+ ~ ++
DBA	-	-	-	-	-	-	-	-	-
UEA	-	-	-	-	-	-	-	-	-

Note: Lc: Leydig cells, CT: connective tissue, SC: Sertoli cell, Spg: Spermatogonia, Spc: spermatocyte, Spt: spermatid, ESp: elongated spermatids, Spz: Spermatozoa, CP: residual cytoplasm.

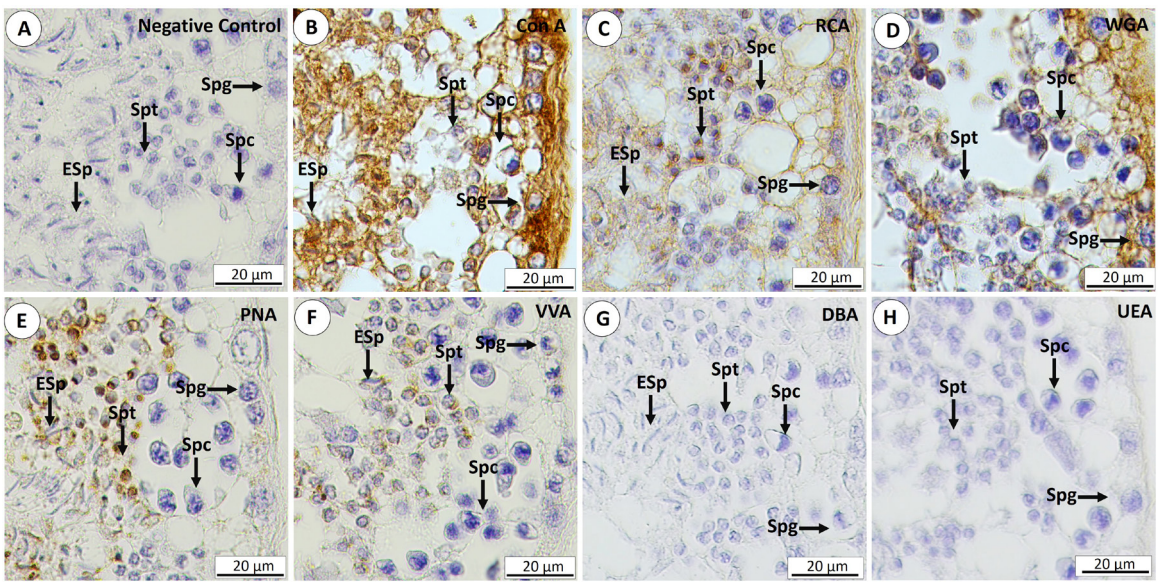

Figure 4. Representative image of lectin-binding distribution pattern in spermatogenic tubule epithelium of water monitor lizards (*Varanus salvator*).Various distribution and intensities of each lectin binding in spermatogonia (Spg), spermatocyte (Spc), spermatid (Spt), and elongated spermatozoa (ESp)

(Figure 6). Additionally, ConA, RCA, and WGA induced reactivity in connective tissues in the testes, including the basement membrane and interstitial tissues. UEA-I lectin interacted with Leydig cells in only one specimen, but this reaction was determined to be nonspecific, and DBA lectin showed no binding reaction to tissues in the testes.

4. Discussion

Lectin binding in animal testes was characterized by several scientists to determine the distribution pattern of lectin binding in each species and identify specific lectins serving as markers for certain spermatogenesis conditions (Spicer, 1993; Dias et al., 2015; Nakata et al., 2017; Wakayama et al., 2022). Information on the general morphological, anatomical, and histological characteristics of male reproductive organs in water monitor lizards was reported by Mahfud et al. (2015a, 2015b, 2016). However, no further investigation has been conducted to analyze spermatogenesis-specific characteristics.

The testes used in this study were obtained from adult water monitor lizards, as indicated by the long SVL. Sumatran water monitor lizards are considered mature when an SVL of 40 cm is attained for males and 47 cm for females (Shine et al., 1996); the Javan water monitor lizard juveniles have an SVL of between 14–20 cm (Kurniati et al., 2023). In this study, the smallest specimen had an SVL of 39 cm and could be categorized as an adult since the process of spermatogenesis was observed as active. The testes of water monitor lizards with spermatogenic activity were characterized by spermatogenic cells actively

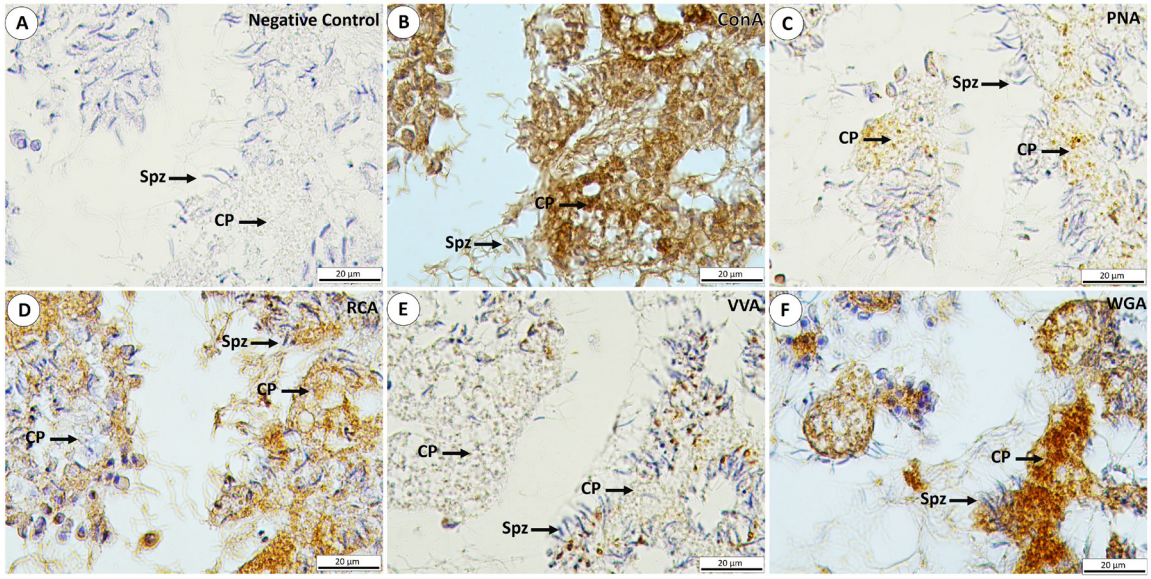

Figure 5. Representative image of lectin binding distribution patterns in spermatozoa (Spz) and residual cytoplasm (CP) in the lumen of tubules of water monitor lizards (*Varanus salvator*).

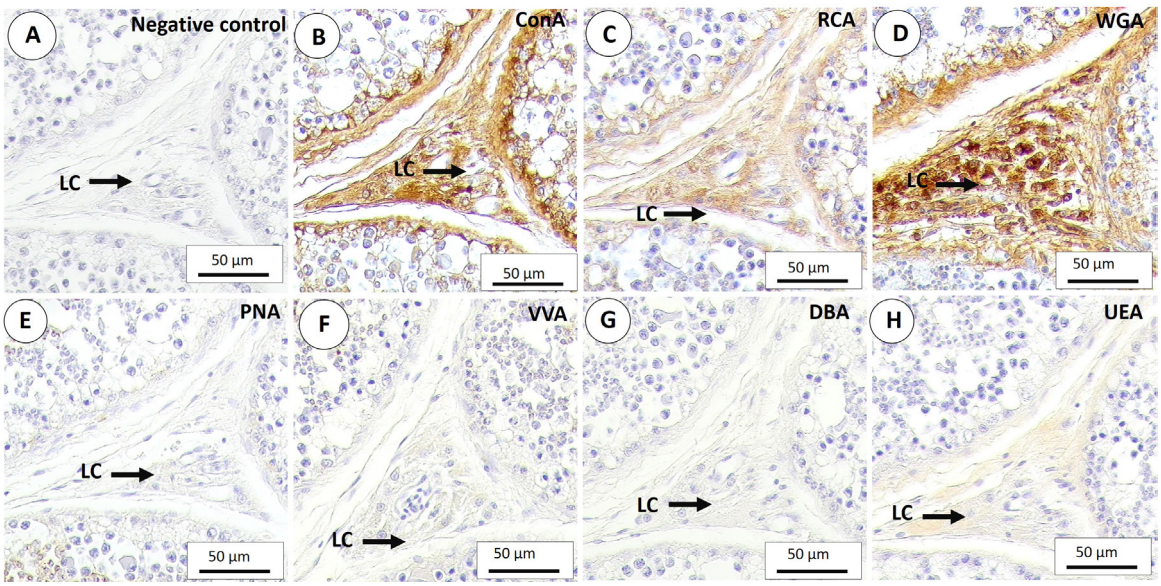

Figure 6. Representative image of lectin binding patterns and distribution of Leydig cells (Lc) in testes of water monitor lizards (*Varanus salvator*).

dividing and differentiating into spermatozoa (Hess et al., 2008; Mahfud et al., 2016). The loosely arranged structure of the spermatogenic epithelium resembled the testicular structure, as observed in *Tropidurus itambere* lizards (Ferreira and Dolder, 2003). In this study, animal subjects with SVL sizes between 39–60 cm had fewer spermatogenic cells than those between 60–90 cm, suggesting that larger water monitor lizards have a more active proliferation of spermatogenic cells.

PAS can stain neutral polysaccharides and oligosaccharides, including glycogen and glycoproteins, in tissues (Kumar, 2010). Since carbohydrates are specifically dispersed in the form of glycoconjugates, which play various roles in cellular processes, PAS staining is a valuable tool for visualizing these cellular components. A major application of PAS staining is determining the reactivity of spermatogenic cells to this stain. Gewaily et al. (2021) reported the distribution of neutral polysaccharides

in interstitial tissues across spermatogonial, spermatocyte, and spermatid zones, as well as Sertoli cells in the spermatozoal cysts of the testes of banded bamboo sharks. The neutral carbohydrate content found in testes or spermatozoa signifies the process of spermatogenesis and acrosome maturity as the acrosome is rich in glycoproteins (Martínez-Menárguez et al., 1992; Verini-Supplizi et al., 2000). Visualization of the acrosome using a conventional method, such as PAS staining, is occasionally challenging. Therefore, lectin staining is used to visualize acrosome development in the spermatid to spermatozoa (Hess, 1990; Chiarini-Garcia and Russell, 2001; Hess and de Franca, 2008; Meistrich and Hess, 2013; Nakata et al., 2017). This study did not obtain a positive PAS reaction in spermatozoa from any of the samples, which might be a unique characteristic of water monitor lizards. This result was further verified through lectin-specific staining because of glycoprotein's potential to bind lectin.

Most carbohydrates found in glycoconjugates, specifically glycoproteins and glycolipids, are polysaccharides or oligosaccharides containing linear or branched sugar chains, mainly composed of mannose (Man), fucose (Fuc), galactose (Gal), glucose (Glc), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), and sialic acid (NeuAc) (Gabius, 2001). This composition provides the unique properties and functions of carbohydrates observed in biological systems. Lectin is a protein complex that selectively binds to carbohydrates as glycoconjugates in biological materials (Spicer, 1993).

Since lectin-binding analysis of testicular organs in nonmammalian animals, specifically reptiles in Indonesia, has not been comprehensively investigated, this study determined that lectin binding was positive for ConA, WGA, RCA, PNA, and VVA at various sites in the testes. Although larger water monitor lizards showed more spermatogenic cells, the lectin-binding characteristics were similar in all lizard sizes. The differences in the number of spermatogenic cells might be influenced by other factors, such as hormones and growth factors (Schlatt and Ehmcke, 2014). The spermatogenic cell population tends to be controlled by proliferation and antiapoptotic activities (de Rooij and Russell, 2000).

The results showed that Sertoli and Leydig cells were positive for ConA, WGA, and RCA lectin, indicating the presence of the glycoconjugates D-mannose, D-glucose, N-acetyl-d-galactosamine, N-acetyl-d-glucosamine, and sialic acid. Furthermore, glucose and mannose sugar residues were present in the glycoconjugates responsible for ion transport (Spicer and Schulte, 1992; Blackmore and Eisoldt, 1999). ConA lectin has been found to label spermatogonia, Sertoli cells, and Leydig cells in certain wild mammals, including the lesser mouse deer (Agungpriyono et al., 2009), muntjaks (Wahyuni et al., 2016), and Japanese shrew moles (Mizukami et al., 2001). Additionally, ConA lectin binding was observed in the Sertoli cells of tilapia, banded bamboo sharks, and Caspian turtles (Ballesta et al., 1991; Tokalov and Gutzeit, 2007; Gewayli et al., 2021). Several mammals, including lesser mouse deer and muntjacs, present different WGA lectin bindings in Leydig cells, which is classified as a strong reaction (Agungpriyono et al., 2009; Wahyuni et al., 2016). There is a difference in these reactions compared to the type observed in water monitor lizard cells. RCA lectin binding in the Leydig cells of water monitor lizards has a strong similarity to the binding observed among several mammals, including babirusas (Agungpriyono et al., 2007) and muntjacs (Wahyuni et al., 2016). However, this binding pattern was not observed in fish species, such as tilapia and medaka fish (Tokalov and Gutzeit, 2007).

Spermatogonia and primary spermatocytes showed positive reactions to ConA, WGA, and RCA, suggesting the presence of glycoconjugates such as D-mannose, D-glucose, N-acetyl-d-galactosamine, N-acetyl-d-glucosamine, and sialic acid on the cell membrane. These glycoconjugates are presumed to participate in cell adhesion and interaction, as evidenced by the attachment to the cytoplasmic extension of members of Sertoli cells. In other animals, such as tilapia, medaka fish, the Caspian turtle, babirusas, and lesser mouse deer, spermatogonia did not show any binding reaction to RCA lectin but had reactivity to ConA and WGA lectins (Ballesta et al., 1991; Tokalov and Gutzeit, 2007; Agungpriyono et al., 2007; 2009).

Spermatids and spermatozoa pass through a series of transformations during spermiogenesis, resulting in changes from round to oval and compact cell shapes. Furthermore, the process starts with the Golgi and cap stages in early spermatids, followed by the acrosome stage (elongated spermatozoa) and maturation (spermatozoa). PNA and VVA lectins specifically label spermatids at the Golgi and cap stages, characterized by a dense body resembling an acrosome cap, but cannot label the subsequent stages. However, ConA, WGA, and RCA lectins are able to label several parts of sperm, including the tail. Some studies have reported that PNA lectin could bind to the acrosome until maturation, making it a useful marker of sperm maturity. Visualization of the acrosome is crucial for determining the spermiogenesis stage. PNA lectin is commonly used to label the acrosome because it can recognize intraacrosomal glycoproteins (Nakata et al., 2017; Wakayama et al., 2022). The results obtained in the present study contradict observations reported in various studies on the spermatozoa of lizards and fish. For instance, a study by Ferreira and Dolder (2003) on spermiogenesis found that PNA could label spermatozoa in the nucleus and midpiece of *Tropidurus itambere* lizards, and PNA

produced effective labeling in banded bamboo sharks (Gewaily et al., 2021). The current study's results require further confirmation regarding the binding of PNA lectin in the spermatozoa of water monitor lizards since the binding pattern was different than in other lizards.

Ferreira and Dolder (2003) reported that WGA and UAE-I lectins can bind to the fibrous sheath of the flagellum of late spermatids and mature spermatozoa in *Tropidurus itambere* lizards. Budipitojo et al. (2020) found similar outcomes in the testes of Sunda porcupines, with immature testes showing an adverse reaction to WGA lectin, while mature testes responded positively. In our study, a WGA lectin-binding reaction was detected in all testes samples of water monitor lizards, suggesting that the spermatozoa components were mature, but no binding of UAE-I lectin to spermatozoa was found.

Lectins UAE-I and DBA did not bind to various parts of the testes of the water monitor lizards, signifying the absence of the glycoconjugates l-fucose and αN-acetyl-d-galactosamine. This absence of UAE-I lectin binding was also observed in tilapia and medaka fish (Tokalov and Gutzeit, 2007), *Xenopus laevis* (Valbuena et al., 2010), babirusas (Agungpriyono et al., 2009), and muntjacs (Wahyuni et al., 2016). However, DBA lectin binding was also detected in the spermatogonia or Leydig cells in certain animals, including lesser mouse deer (Agungpriyono et al., 2009) and babirusas (Agungpriyono et al., 2007).

In conclusion, this study showed the specific structure of the testes and its correlation with animal size. The water monitor lizard testes showed reactivity to different lectins, such as ConA, RCA, WGA, PNA, and VVA. Varying binding distributions were observed among testicular cells, with ConA, RCA, and WGA lectins predominantly expressed in Leydig, Sertoli, and spermatogenic cells. PNA and VVA lectins were specifically bound to spermatids at the Golgi and acrosome cap stages and in the residual cytoplasm. The identified lectin-binding patterns suggest that water monitor lizards harvested from the wild were at an active stage of spermatogenesis, producing mature spermatozoa. Therefore, future studies should comparatively test lectin bindings in immature testes for a more comprehensive understanding of the process and to identify specific markers of spermatogenic activity and testicular maturation, which could be used to determine reproductive maturity in water monitor lizards.

Acknowledgment

The authors are grateful for the financial assistance provided by the National Research and Innovation Agency of Indonesia under funding scheme No 39/III.5/HK/2022. The authors would like to thank GS for contributing to the Results of Indonesian Biodiversity Disclosure and Utilization and HK, who acquired support from the Indonesian Reptile and Amphibian Trade Association for the sample collection and publication process. AYP, NLPRP, GS, and HK contributed equally to this study.

Conflict of interest

The authors declare no conflicts of interest.

References

Agungpriyono S, Kurohmaru M, Kimura J, Wahid AH, Sasaki M et al. (2009). Distribution of lectin-bindings in the testis of the lesser mouse deer, *Tragulus javanicus*. Anatomia Histologia Embryologia 38 (3): 208-213. https://doi.org/10.1111/j.1439-0264.2008.00923.x

Agungpriyono S, Kurohmaru M, Prasetyaningtyas WE, Kaspe L, Leus KYG et al. (2007). A lectin histochemical study on the testis of the babirusa, *Babyroussa babyrussa* (Suidae). Anatomia Histologia Embryologia 36 (5): 343-348. https://doi.org/10.1111/j.1439-0264.2007.00778.x

Arida E, Hidayat A, Mulyadi, Maireda NL, Subasli DR et al. (2020). Consumption and Trade of Asian Water Monitor, Varanus salvator as Reliance on Wildlife for Livelihoods among Rural Communities in North Sumatra, Indonesia. Journal of Tropical Ethnobiology 3 (2): 81-92. https://doi.org/10.46359/jte.v3i2.40

Ballesta J, Martínez-Menárguez JA, Pastor LM, Avilés M , Madrid JF et al. (1991). Lectin binding pattern in the testes of several tetrapode vertebrates. European Journal of Basic and Applied Histochemistry 35 (2): 107-117. PMID: 1768726.

Blackmore PF, Eisoldt S (1999). The neoglycoprotein mannosebovine serum albumin, but not progesterone, activates T-type calcium channels in human spermatozoa. Molecular Human Reproduction 5: 498-506. https://doi.org/10.1093/molehr/5.6.498

Boscha E, Arida E, Yudha DS (2020). Dorsal colour patterns of asian water monitor, *varanus salvator* collected for trade in Cirebon, Indonesia. Journal of Tropical Ethnobiology 3 (2): 133-138. https://doi.org/10.46359/jte.v3i2.43

Budipitojo T, Padeta I, Yulianti BU, Masithoh DB (2020). Distribution profile and function of carbohydrate residues in testes of immature and mature sunda porcupine (*Hystrix javanica*). World's Veterinary Journal 10 (1): 53-59. https://dx.doi.org/10.36380/scil.2020.wvj7

Chiarini-Garcia H, Russell, LD (2001). High-resolution light microscopic characterization of mouse spermatogonia. Biology of Reproduction 65: 1170-1178. https://doi.org/10.1095/biolreprod65.4.1170

de Rooij DG, Russell LD (2000). All you wanted to know about spermatogonia but were afraid to ask. Journal of Andrology 21 (6): 776-798. https://doi.org/10.1002/j.1939-4640.2000.tb03408.x

Dias RDO, Machado LDS, Migliolo L, Franco OL (2015). Insights into animal and plant lectins with antimicrobial activities. Molecules 20 (1): 519-541. https://doi.org/10.3390/molecules20010519

Ferreira A, Dolder H. (2003). Cytochemical study of spermiogenesis and mature spermatozoa in the lizard *Tropidurus itambere* (Reptilia, Squamata). Acta Histochemica 105 (4): 339-352. https://doi.org/10.1078/0065-1281-00731

Gabius HJ (2001). Glycohistochemistry: the why and how of detection and localization of endogenous lectins. Anatomia Histologia Embryologia 30: 3-31. https://doi.org/10.1046/j.1439-0264.2001.00305.x

Gewaily MS, Noreldin AE, Dawood MA, Hegazy YM, Kassab M (2021). The distribution profile of glycoconjugates in the testis of brown-banded bamboo shark (*Chiloscyllium punctatum*) by using lectin histochemistry. Microscopy Microanalysis Microstructure 27 (5): 1161-1173. https://doi.org/10.1017/S1431927621012113

Hess RA (1990). Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fxed and plastic-embedded testes. Biology of Reproduction 43: 525-542. https://doi.org/10.1095/biolreprod43.3.525

Hess RA, de Franca LR (2008). Spermatogenesis and cycle of the seminiferous epithelium. In: Cheng CY (editor). Molecular Mechanisms in Spermatogenesis. Advances in Experimental Medicine and Biology, vol 636. New York, NY, USA: Springer, pp. 1-15. https://doi.org/10.1007/978-0-387-09597-4_1.

Sáez JF, Madrid JF, Alonso E, Hernández F (2000). Lectin histochemical identification of the carbohydrate moieties on N-and O-linked oligosaccharides in the duct cells of the testis of an amphibian urodele, the Spanish newt (*Pleurodeles waltl*). Histochemical Journal 32: 717-724. https://doi.org/10.1023/A:1004196926553

Kumar GL, Gill GW (2010). Introduction to special stains. In: Kumar GL, Kiernan JA (editors). Dako Education guide-special stains and H&E. 2nd ed. California, USA: Dako.

Kurniati H, Phadmacanty, NLPR, Semiadi G (2023). Skeletochronology in long tubular bones of the Javan water monitor lizard, *Varanus salvator bivittatus* in the juvenile stage (Lacertilia: Varanidae). Turkish Journal of Zoology 47 (1): 1-9. https://doi.org/10.55730/1300-0179.3107

Mahfud M, Nisa C, Winarto A (2015a). Anatomi Organ Reproduksi Jantan Biawak Air Asia, *Varanus salvator* (Reptil: Varanidae). Acta Veterinaria Indonesiana 3 (1): 1-7 (In Indonesian with abstract in English) https://doi.org/10.29244/avi.3.1.1-7

Mahfud M, Winarto A, Nisa C (2015b). Morfologi Kelenjar Aksesoris Kelamin Biawak Air (*Varanus Salvator Bivittatus*) Jantan. Jurnal Kajian Veteriner 3 (2): 83-91 (In Indonesian with abstract in English).

Mahfud M, Winarto A, Nisa C (2016). Micromorphological Structure of Primary Reproductive Organ of Male Water Monitor Lizard (*Varanus salvator bivittatus*). Indonesian Journal of Veterinary Sciences 10 (1): 72-76 (in Indonesian with abstract in English). https://doi.org/10.21157/j.ked.hewan.v10i1.3375

Martínez-Menárguez JA, Avillés M, Castells MT, Madrid JF (1992). Cytochemical characterization of glycoprotein in the developing acrosome of rats. An ultrastructural study using lectin histochemistry, enzymes and chemical deglycosylation. Histochemistry 97: 439-449. https://doi.org/10.1007/BF00270391

Meistrich ML, Hess, RA (2013). Assessment of spermatogenesis through staging of seminiferous tubules. Methods in Molecular Biology 927: 299-307. https://doi.org/10.1007/978-1-62703-038-0_27

Mizukami T, Kuwahara S, Ohmura M, Iinuma Y, Izumikubo J et al. (2001). Distribution of lectin bindings in the testes of the greater japanese shrew mole, *Urotrichus talpoides*. Journal of Reproduction and Development 47 (4): 205-210. https://doi.org/10.1262/jrd.47.205

Nakata H, Wakayama T, Asano T, Nishiuchi T, Iseki S (2017). Identification of sperm equatorial segment protein 1 in the acrosome as the primary binding target of peanut agglutinin (PNA) in the mouse testis. Histochemistry Cell Biology 147: 27-38. https://doi.org/10.1007/s00418-016-1478-8

Nijman V (2016). Perceptions of Sundanese men towards the consumption of water monitor lizard meat in West Java, Indonesia. Biawak 10 (1): 22-25. https://varanidae.org/10_1.pdf#page=23

Schneider CA, Rasband WS, Eliceiri KW (2012). NIH Image to ImageJ: 25 years of image analysis. Nature methods 9 (7): 671-675. https://doi.org/10.1038/nmeth.2089

Schlatt S, Ehmcke J (2014). Regulation of spermatogenesis: an evolutionary biologist's perspective. Seminars in Cell and Developmental Biology 29: 2-16. https://doi.org/10.1016/j.semcdb.2014.03.007

Setyawatiningsih SC (2018). The Indonesia's water monitor (*Varanus salvator*, Varanidae) trading. Journal of Physics: Conference Series 1116 (5): 052059. https://doi.org/10.1088/1742-6596/1116/5/052059

Shine R, Harlow PS, Keogh JS, Boeadi (1996). Commercial harvesting of giant lizards: the biology of water monitors *Varanus salvator* in southern Sumatra. Biological Conservation 77 (2-3): 125-134. https://doi.org/10.1016/0006-3207(96)00008-0

Spicer SS (1993). Advantages of histochemistry for the study of cell biology. Histochemical Journal 25: 531-547. https://doi.org/10.1007/BF00173051

Spicer SS, Schulte BA (1992). Diversity of cell glycoconjugates shown histochemically: a perspective. Journal of Histochemistry and Cytochemistry 40: 1-38. https://doi.org/10.1177/40.1.1370305

Sy EY, Lorenzo AN (2020). The trade of live monitor lizards (*Varanidae*) in the Philippines. Biawak 14: 35-44.

Tokalov SV, Gutzeit HO (2007). Lectin-binding pattern as tool to identify and enrich specific primary testis cells of the tilapia (*Oreochromis niloticus*) and medaka (*Oryzias latipes*). Journal of Experimental Zoology – B (JEZ-B): Molecular and Developmental Evolution 308 (2): 127-138. https://doi.org/10.1002/jez.b.21117

Valbuena G, Madrid JF, Hernández F, Sáez FJ (2010). Identification of fucosylated glycoconjugates in *Xenopus laevis* testis by lectin histochemistry. Histochemistry and Cell Biology 134: 215-225. https://doi.org/10.1007/s00418-010-0722-x

Verini-Supplizi A, Stradaioli G, Fagioli O, Parillo F (2000). Localization of the lectin reactive sites in adult and prepubertal horse testes. Research in Veterinary Science 69: 113-118. https://doi.org/10.1053/rvsc.2000.0398

Wahyuni S, Agungpriyono S, Adnyane IKM, Hamny H, Jalaluddin M et al. (2016). Studi histokimia lektin pada sel-sel spermatogenik testis muncak (*Muntiacus muntjak muntjak*). Indonesian Journal of Veterinary Sciences 10 (1): 82-85. https://doi.org/10.21157/j.ked.hewan.v10i1.3396

Wakayama T, Yokota S, Noguchi K, Sugawara T, Sonoda K et al (2022). Quantitative evaluation of spermatogenesis by fluorescent histochemistry. Histochemistry and Cell Biology 157 (3): 287-295. https://doi.org/10.1007/s00418-022-02080-6