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Original paper

Morphological and histological studies on the epididymis and deferent duct of the Sunda porcupine (*Hystrix javanica*)

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Abstract. The Sunda porcupine (*Hystrix javanica*) is an endemic species of Indonesia. The population of this species has been decreased by hunting and habitat loss. Understanding of reproductive characteristics is essential for the appropriate management of their population. However, there is little knowledge about reproductive features of this species. The epididymis and deferent duct are important organs for maturation, transport, and storage of sperm. In this study, therefore, the epididymis and deferent duct of Sunda porcupines were examined histologically, histochemically, immunohistochemically, and electron-microscopically to understand their structures and functions. Four adult wild porcupines that inhabit Central Java, Indonesia, were captured in June and September and euthanized. Then, the epididymides and deferent ducts were removed. Principal cells were the main cell type found in the epididymis with stereocilia and apical blebs, but both of these structures were absent in the deferent duct. These observations suggest high secretion and absorption capability by the epididymal epithelium. In the epididymis and deferent duct, PAS-positive principal cells were shown, and the immunoreactivities for 3β-HSD were found in the epithelium of the deferent ducts. These reults suggest effects on sperm by glycoproteins and steroid metabolites.

Key words: deferent duct, epididymis, porcupine, steroidogenesis.

The order Rodentia is subdivided into three major suborders: Sciuromorpha, Myomorpha, and Hystricomorpha, and porcupines are classified in Hystricomorpha. Furthermore, porcupines make up two families that are phylogenetically located apart from each other in Hystricomorpha: Hystricidae of the Old World and Erethizontidae of the New World (Roze 2012). Unlike the North American porcupine, *Erethizon dorsatum* (New World porcupines), which is arboreal and seasonal breeder mating with multiple females, the Cape porcupine, *Hystrix africaeaustralis* (Old World porcupine) is terrestrial and continuous breeder throughout the year with monogamy (Van Aarde and Skinner 1986; Roze 2012).

The Sunda porcupine, *Hystrix javanica*, is one of the Old World porcupines, which is endemic to Indonesia. Sunda porcupines have been hunted for food, medicinal purposes, and illegal trade by local people. The continuous hunting of the Sunda porcupine and also habitat loss by exploitation have caused a decline of their population (Mustikasari et al. 2019). Therefore, recently this animal is listed as a protected animal under Indonesian Law.

The porcupine is ecologically important because its activities greatly impact the environment, such as feeding competition among other herbivores, digging which increases plant biomass and species richness, and as potential prey for carnivores (Roze 2012). Porcupines also play a role as a dispersal agent of plants (Mori et al. 2017). Therefore, a decrease in their population will significantly

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disturb the ecosystem. Thus, knowledge about reproductive biology is essential for the management of the population in wild porcupines. However, little is known about the reproduction of the Sunda porcupine.

The epididymis and deferent duct (vas deferens) are important parts of the male reproductive system. In the epididymis, sperm are nurtured by epididymal secretions that help the sperm survive and mature (Richard and Kristin 2014). The deferent duct follows the epididymis and serves not only as a passage for sperm from the epididymis to the urethra but also secretes fluid for sperm transport and may function in the resorption of spermatozoan remnants (Koslov and Andersson 2013).

In the epididymal epithelium, the various cell types such as principal, apical, clear, narrow, and basal cells were recognized (Robaire et al. 2006; Joseph et al. 2011; Zhou et al. 2018). The principal cell is a major cell type, serving the reabsorption of the epididymal fluid, proteins secretion e.g., glycoprotein by merocrine via exocytosis and apocrine with apical bleb, and uptake of luminal substances by endocytosis (Robaire et al. 2006; Zhou et al. 2018). The nucleus of both narrow and apical cells is located in the upper half of the cytoplasm. Nevertheless, both cells are structurally distinguished. Narrow cells have the cytoplasm which reaches the basement membrane and show an apical bulge, and occasionally possess the nucleus protruding to the luminal area (Hermo et al. 2000; Hess et al. 2000; Cho et al. 2003; Robaire et al. 2006; Joseph et al. 2011; Arrotéia et al. 2012; Zhou et al. 2018). Meanwhile, apical cells which are related to pH regulation and endocytosis, do not contact the basement membrane (Hermo and Robaire 2002; Robaire et al. 2006; Arrotéia et al. 2012). Clear cells of which nucleus is located in the basal region, are endocytic large cells and involved in luminal acidification with narrow cells (Adamali and Hermo 1996; Hermo et al. 2000; Shum et al. 2011; Arrotéia et al. 2012; de Souza et al. 2017). Basal cells with cytoplasmic extensions are located in the basal part of the epithelium, regulate principal and clear cell function, as well as involved in the endocytosis from the blood and indirect acidification (Joseph et al. 2011; Shum et al. 2011; Arrotéia et al. 2012).

Similar to the epididymis, the main cell type in the deferent duct is the principal cell. The principal cells of the deferent duct also perform the secretion of proteins by merocrine and apocrine, as well as endocytosis (Hermo et al. 2002). These cells were also involved in the synthesis and secretion of steroids and in water transport to eliminate water from the lumen of the deferent duct (Andonian and Hermo 1999).

In this framework, this current study examined the morphological and histological features of the epididymis and deferent duct in the Sunda porcupine to better understand their reproductive functions.

Materials and methods

Animals and tissue collection

The epididymides and deferent ducts of four wild Sunda porcupines were utilized in this study. All samples were considered as adult based on the presence of sperm in the lumen. Samples were obtained in June, August and September from their habitat in Tawangmangu, Central Java, Indonesia with official permission. A collection permit (number: SK.56/KSDAE/SET/KSA.2/2/2018) was issued by the Directorate General of Natural Resources and Ecosystem Conservation (Direktorat Jenderal Konservasi Sumber Daya Alam dan Ekosistem, Jakarta, Indonesia) under the Ministry of Environment and Forestry of the Republic of Indonesia. This study was also conducted in accordance with the ethical principles for animal experimentation of the Ethics Committee for the Use of Animals of the Faculty of Veterinary Medicine at the Gadjah Mada University (Number: 0014/EC-FKH/Eks/2017).

After euthanasia by ketamine HCl (10–15 mg/kg) and xylazine HCl (0.10–0.15 mg/kg) intramuscularly, the epididymides and deferent ducts were collected from each animal. The tissue samples were removed and immediately fixed in Bouin's fixative. Then, the tissues were dehydrated in serial ethanol, cleared in xylene and embedded in paraffin. The paraffin blocks were cut at 4-µm thickness and mounted on aminopropyl-triethoxysilane-coated slides (S9226, Matsunami Glass, Osaka, Japan). Deparaffinized sections were stained with hematoxylin and eosin (HE) for general observation of histological structure, periodic acid-Schiff (PAS) to detect glycoproteins and glycolipids, or Alcian blue (AB, pH 2.5) to detect acid glycoproteins, sulfated mucopolysaccharides (glycosaminoglycans) and proteoglycan.

Immunohistochemistry

To examine the localization of steroidogenic enzymes in the epididymides and deferent ducts, the sections were immunohistochemically stained using the avidin-biotin peroxidase complex (ABC) method (Hsu et al. 1981). The sections were microwaved in high-pH target retrieval solution (1:10, S3307; Dako Cytomation, Inc., CA,

U.S.A) for antigen retrieval. Then, the endogenous peroxidase activity was blocked with methanol containing 0.3% H_2O_2 for 10 min at room temperature (RT), and the sections were incubated with normal goat serum (1:50, S-1000, Vector Laboratories, Burlingame, CA, USA) for 30 min at RT to prevent nonspecific staining. The samples were next incubated with each primary antibody overnight at 4°C. For primary antibodies, rabbit polyclonal anti-rat cytochrome P450 side-chain cleavage enzyme (P450scc; 1:200, AB-1244, Chemicon International, Temecula, CA, USA) and rabbit polyclonal anti-human 3β-hydroxysteroid dehydrogenase (3β-HSD; 1:500, Doody et al. 1990) were used. After being incubated with the primary antibodies, the sections were incubated with the biotinylated anti-rabbit IgG (1:200, BA-1000, Vector Laboratories) for 30 min at RT, and then with the Vectastain *Elite* ABC Kit (1:2, PK-6100, Vector Laboratories) for 30 min at RT. The immunoreactive sites were visualized using Tris HCl buffer (pH 7.4) containing 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.006% H_2O_2. The negative control sections were stained, omitting each primary antibody.

Scanning electron microscopy (SEM)

Small pieces of the samples fixed in 10% formalin were washed in 0.1 M phosphate buffer (PB, pH 7.4), post-fixed in 1% osmium tetroxide in 0.1 M PB and dehydrated in a graded series of ethanol for SEM. The specimens were then freeze-dried with t-butyl alcohol in a freeze drier (ES-2030, Hitachi High-Technologies, Tokyo, Japan) and sputter coated with Pt using an ion sputter (E-1045, Hitachi high-Technologies). The samples were then observed by SEM (S-3400N, Hitachi high-Technologies) at an accelerating voltage of 5.0 kV.

Results

Epididymis

The epithelium lining of the epididymal ducts in the Sunda porcupine was a pseudostratified columnar epithelium composed of principal cells with an elongated nucleus and stereocilia, basal cells with a circular or oval nucleus, narrow cells with an apical bulge and apically positioned nucleus, and apical cells with a rounded nucleus located in the upper half of the cytoplasm (Figs. 1, 2C). In the histological observations, however, we could not distinguish the clear cells.

Epithelium of the epididymal duct was surrounded by sub-tubular myoid cells and stromal cells. In the lumen of all epididymal ducts, spermatozoa were visible (Fig. 1A, B). The apical part of the cytoplasm of principal cells occasionally bulged out to form an apical bleb (Fig. 1C). The extrusion of nuclei was also observed in several cells of the epithelium of the epididymal ducts (Fig. 1D). Periodic acid-Schiff- and AB-positive stainings were observed in the stereocilia of the epithelium of the epididymal ducts (Fig. 2A, D). However, the apical bleb of principal cells was PAS-negative (Fig. 2B). Occasionally, the cytoplasm of principal cells showed PAS-positive, and PAS-positive substance regarded as the secretion was accumulated in the lumen (Fig. 2C).

The structure of the epididymal ducts was also confirmed using SEM (Fig. 3). The stereocilia, which are long cytoplasmic projections (microvilli), were laid over the luminal surface of the epididymal ducts (Fig. 3A, B). Apical blebs connected with stalk-like structures to the apical part of principal cells were also noted (Fig. 3C).

Immunolocalization of steroidogenic enzymes in the epididymis was confirmed in this study. However, the result showed that in the epididymides of all samples, no immunoreactivities of steroidogenic enzymes were detected.

Deferent duct

The histological features of the deferent duct are shown in Fig. 4. The epithelium of the deferent duct was a pseudostratified columnar epithelium consisting of principal and basal cells (Fig. 4B). No stereocilia and apical blebs were noted in the epithelium (Figs. 4B, 5), although short microvilli were observed by SEM (Fig. 7B). The mucosa and submucosa of the deferent duct were surrounded by three thick muscle layers, namely the inner longitudinal, middle circular, and outer longitudinal layers (Fig. 4A). The epithelium of the deferent duct occasionally exhibited numerous vacuoles located among principal cells (Fig. 4B), which were negative for PAS and AB staining (Fig. 5). On the other hand, the cytoplasm of the principal cells had PAS-positive small particles (Fig. 5B).

Immunoreactivity for 3β-HSD was strong in the supranuclear cytoplasm of principal cells (Fig. 6). For P450scc, however, immunoreactivity was not detected in the deferent duct. The architectural features of the deferent duct, which is a tubular structure with crimped lumen surrounded by a thick muscle coat, were visualized by SEM (Fig. 7). The absence of stereocilia and apical blebs on the surface of the epithelium was confirmed using a higher magnification (Fig. 7B).

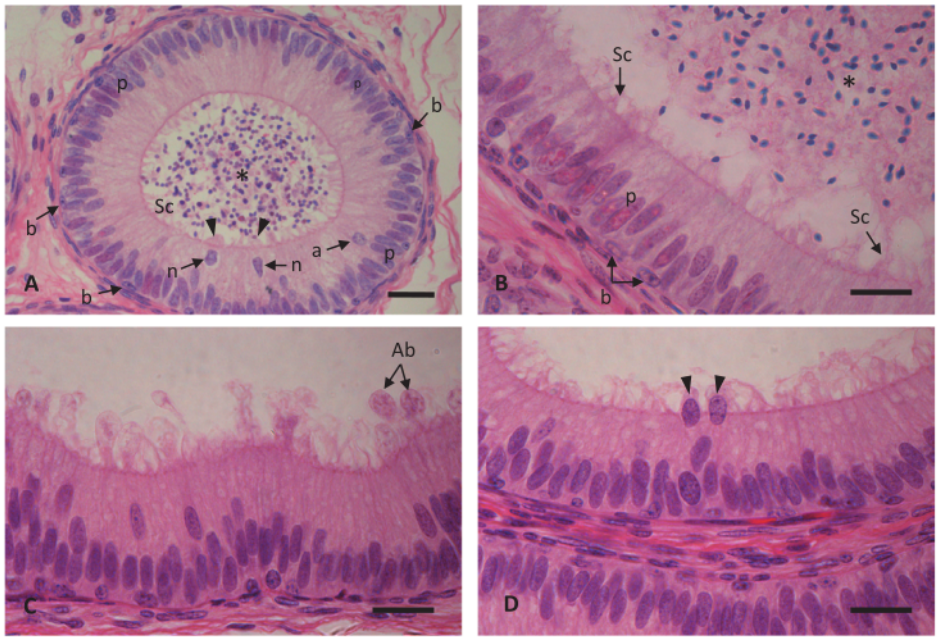

Fig. 1. Histological observation of the epididymides of the Sunda porcupine. A, B: The epithelium of the epididymal duct was a pseudostratified columnar epithelium consisting of principal cells (p) with stereocilia (Sc), basal cells (b), narrow cells (n), and apical cells (a), and the lumen of the epididymal duct contained spermatozoa (asterisk). Arrowheads show apical bulges in narrow cells. C: Apical blebs (Ab) were present in the luminal part of the epithelium. D: Extruded nuclei (arrowheads) approached the lumen. Scale bar = 20 μm. HE staining.

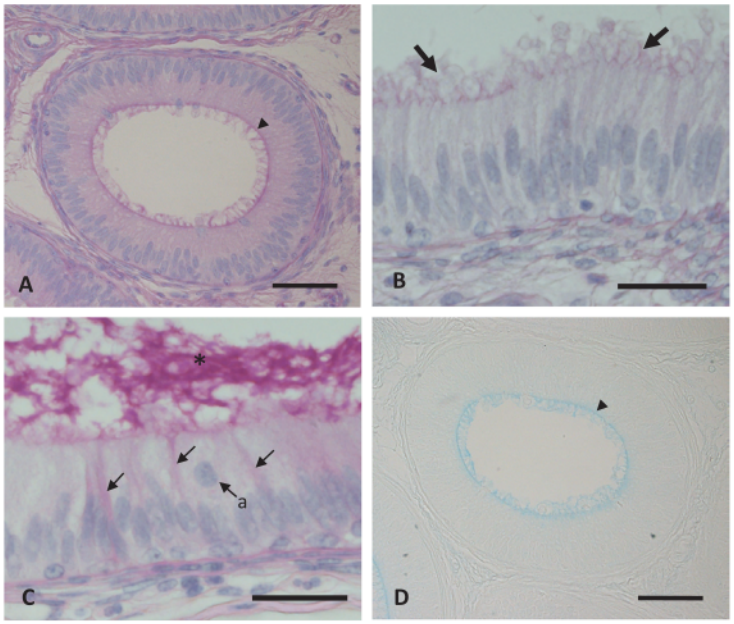

Fig. 2. Periodic acid-Schiff (PAS) (A–C) and Alcian blue (AB) (D) staining in the epithelium of the epididymal duct. A, D: The positive staining of PAS and AB was significantly noted in the stereocilia of the epithelium (arrow heads). B: PAS-positive reaction was not shown in the apical bleb (large arrows) of principal cells. C: The principal cells occasionally had a PAS-positive cytoplasm (small arrows). a, apical cell; *, PAS-positive substance. Scale bar = 50 μm (A, D); 25 μm (B, C).

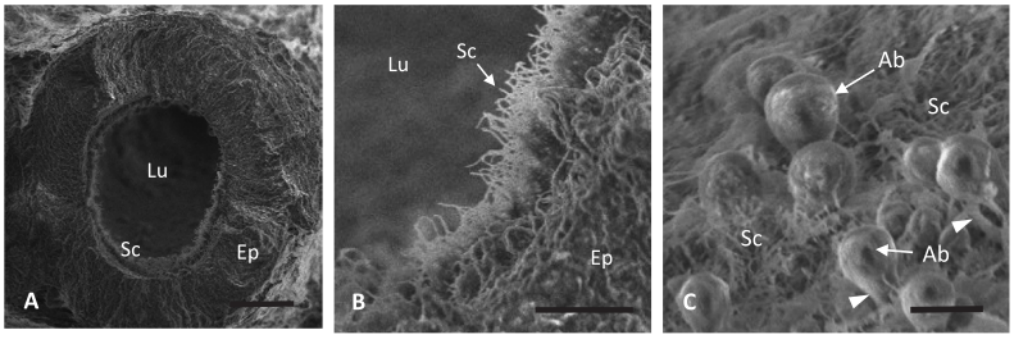

Fig. 3. Scanning electron micrographs showing the presence of stereocilia (Sc) and apical blebs (Ab) in the luminal part of the epithelium (Ep) of the epididymal duct. A: Cross-section of the epididymal duct. B: High magnification of the stereocilia in the principal cells. C: Apical blebs connected by stalk-like structures (arrowheads) were visible in the luminal surface of the epithelium in the epididymal duct. Lu, lumen of the epididymal ducts. Scale bar = 30 μm (A); 12 μm (B); 3 μm (C).

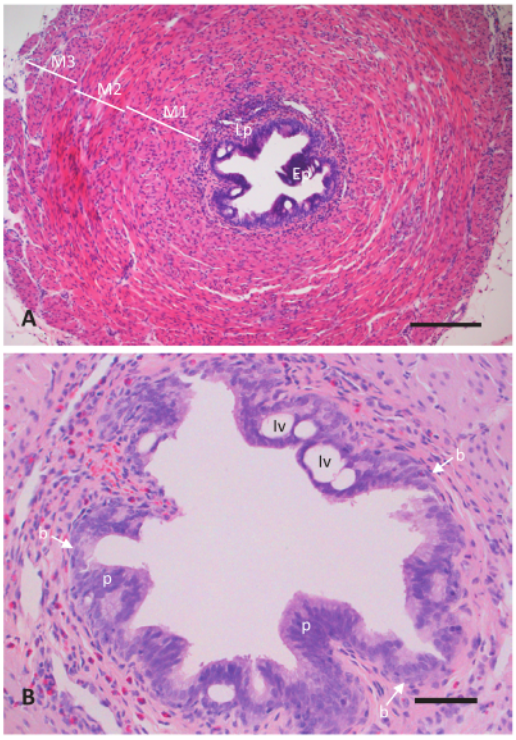

Fig. 4. Histological features of the deferent duct in the Sunda porcupine. A: The entire deferent duct. B: Higher magnification of the epithelium. The epithelium (Ep) of the deferent duct was a pseudo-stratified columnar epithelium with principal (p) and basal (b) cells surrounded by three thick muscle layers, the inner longitudinal (M1), middle circular (M2) and outer longitudinal (M3) layers. Numerous intraepithelial vacuoles (Iv) were also observed. Lp, lamina propria. Scale bar = 200 μm (A); 50 μm (B). HE staining.

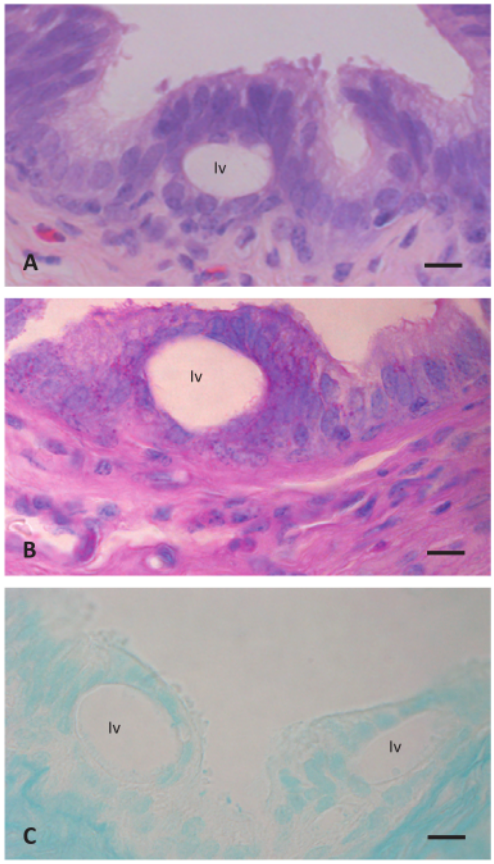

Fig. 5. Periodic acid-Schiff (PAS) and Alcian blue (AB) staining of the intraepithelial vacuoles (Iv) of the deferent duct of the Sunda porcupine. A: HE staining of vacuoles. B: Vacuoles were negative for PAS staining, and the cytoplasm of principal cells had PAS-positive small particles. C: Vacuoles were also negative for AB staining. Scale bar = 10 μm.

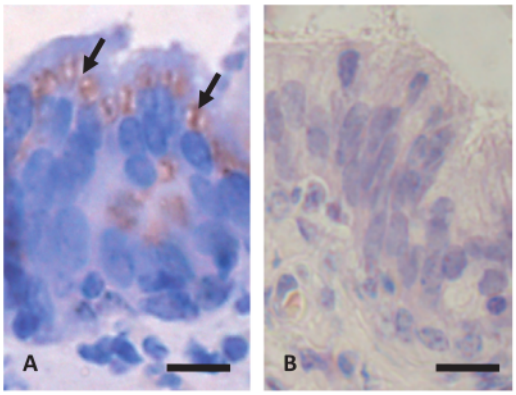

Fig. 6. Immunohistochemical staining of 3β hydroxysteroid dehydrogenase (3β-HSD) in the deferent duct of the Sunda porcupine. Immunoreactivity for 3β-HSD was localized to the supranuclear cytoplasm of principal cells (arrows). B: Negative control. Scale bar = 10 μm.

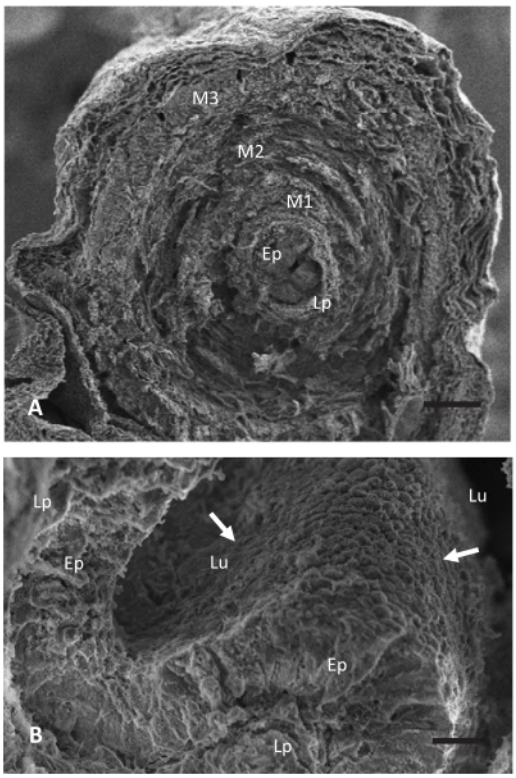

Fig. 7. Scanning electron micrograph of the deferent duct of the Sunda porcupine. A: Middle circular (M2), inner (M1) and outer (M3) longitudinal muscle layers constituted the tunica muscularis of the deferent duct. B: Stereocilia were unable to be observed in the deferent duct of the Sunda porcupine, although short microvilli were found on the luminal surface (arrows). Ep, epithelium; Lp, lamina propria; Lu, lumen. Scale bar = 150 μm (A); 10 μm (B).

Discussion

The general structure of the epididymis in the Sunda porcupine has many similarities to that in other mammalian species. Four types of epithelial cells were distinguished in the epididymal ducts of the Sunda porcupine, such as principal, basal, narrow, and apical cells. The principal cells were the main cell type and were recognized by the appearance of stereocilia and blebs on their apical surface. They serve many functions such as protein secretion associated with the physiological maturation of spermatozoa, receptor-mediated endocytic uptake of materials from the lumen, absorption or secretion of fluid for altering the composition of luminal fluid and concentration of sperm, and phagocytosis of dead and/or defective spermatozoa (Lea and French 1981; Hermo 1995; Hermo and Robaire 2002; Akbarsha et al. 2015).

The second largest cell population of the epididymal epithelium was the basal cells. The basal cells were located in the basal part of the epithelium and closely contacted the principal cells. These cells may play a role in regulating electrolyte and water transport carried out by the principal cells. This process is mediated by the local formation of prostaglandins synthesized by basal cells (Leung et al. 2004). The apical cells were found in the epididymal epithelium in fewer numbers. Apical cells were reported to take in substrates from the lumen that will be degraded by enzymes within their lysosomes and to protect spermatozoa from harmful compounds (Adamali and Hermo 1996).

In this study, an extruded nucleus was occasionally noted in the epithelium of the epididymis. These nuclei advanced towards the lumen of the epididymal duct, causing the cytoplasm to bulge into the lumen. It is thought that this cell type is the narrow cell of which the nucleus is remarkably protruding to the luminal area. This cytological feature has been a characteristic for the narrow cells (Hermo et al. 2000; Hess et al. 2000; Cho et al. 2003; Robaire et al. 2006; Joseph et al. 2011; Arrotéia et al. 2012; Zhou et al. 2018).

Clear cells can not be distinguished in this study. To identify these cells, it is needed to use the vacuolar H^+-ATPase (V-ATPase) as a clear cell marker (Hughes and Berger 2015). Clear cells express V-ATPase in their apical membrane (Shum et al. 2011). Labelling of V-ATPase to identify clear cells in the epididymis of the Sunda porcupine will be conducted in the further examination.

The presence of stereocilia and apical blebs in the principal cells may be related to their important function in

the epididymis. Stereocilia in the epididymis are long microvilli that have no motility. Their function is similar to that of microvilli in other epithelial cells for absorption. These extensions of microvilli increase the surface area, enabling greater absorption and secretion (Patni and Kothari 1984). A large surface area of stereocilia enables stereocilia to absorb excess testicular fluid and supply nutrients to sperm stored temporarily in the lumen (Moini 2015). Moreover, it has been reported that aquaporin 9 (AQP9) and AQP5, which are related to the uptake of water into the cytoplasm, are co-expressed in the long apical stereocilia of the epididymal principal cells, suggesting active water reabsorption by the stereocilia of the epididymis principal cells (Pastor-Soler et al. 2001; Da Silva et al. 2006).

In the present study, PAS and AB staining were positive in the stereocilia of the Sunda porcupine. In a previous study, stereocilia of the principal cells in the rat epididymis were PAS- and AB-positive (Martan 1969). In camels and stallions also, a similar staining pattern was reported in the stereocilia of the principal cells (Lòpez et al. 1989; Zayed et al. 2012). This staining pattern was suggested to be caused by the localization of glycoproteins and/or glycolipids, and acid glycoproteins and/or proteoglycans in the stereocilia of the porcupine. This content may be related to their function in absorption and secretion as described previously.

The principal cells of the camel and common vampire bat possessed PAS-positive granules in the basal part of the cytoplasm (Zayed et al. 2012; Castro et al. 2017). On the other hand, the PAS-positive cytoplasm was occasionally noticed in the principal cells of the porcupine, although there were no PAS-positive large granules and apical blebs. It is thought that glycoproteins and glycolipids may be also secreted from the principal cells by the merocrine manner in the Sunda porcupine. This staining pattern is similar to that of epididymal principal cells in mice (Abe and Takano 1988).

In the present study, we observed PAS-positive reaction in the cytoplasm of the epithelium of the deferent duct as small particles, although the positive reaction was not detected in the vacuoles, suggesting that glycoproteins and glycolipids may be secreted from the deferent duct as well as the epididymis by merocrine. It has been reported that glycoproteins secreted from the epididymal epithelium are incorporated into the sperm plasma membrane and offer the fertility and forward motility in the female reproductive tract to sperm passing through the epididymis (Caballero et al. 2011; Tecle and Gagneux 2015). In the Sunda porcupines, PAS-positive secretion from both epididymis and deferent duct may contribute to the acquisition of these abilities in sperm.

The presence of apical blebs in the epididymis of the Sunda porcupine may be related to the sexual maturity period. Apical blebs are a mature phenotype because they appear at puberty and increase during sexual maturity (Hughes and Berger 2015). In the current study, the presence of spermatozoa in the epididymal ducts supported that the porcupines used in this study had reached sexual maturity.

The apical blebs observed in the Sunda porcupine were spherical and elongated but greatly varied in size. According to previous studies, the formation of apical blebs is regulated by hormones because the epithelium of coagulating glands in the rat lost secretory blebs after castration (Holterhus et al. 1993). All blebs disappeared after androgen deprivation, whereas the estrogen-treated animals still exhibited blebs, although their outline became irregular (Aumüller et al. 1999). In the rat prostate, the number of apical blebs also decreased after castration or estrogen treatment, but they are a salient feature in castrated rats administered testosterone (Aumüller 1979). However, in the boar epididymis, Hughes and Berger (2015) reported that estrogen might not be related to the increase or decrease in apical blebs. The difference in the regulation of apical blebs may be due to the developmental origin of the epididymis and accessory glands, and/or species differences (Hughes and Berger 2015).

Apical blebs have been suggested to play a role in apocrine secretion. Apocrine secretion has been demonstrated in the epididymis of many species, such as the boar (Hughes and Berger 2015, 2018), mouse, rat (Jack 2001), and bull (Caballero et al. 2011; D'Amours et al. 2012). The apical cell cytoplasm of principal cells bulges out to form an apical bleb that is filled with an accumulation of polysomes in the cytoplasm. Then, apical blebs containing proteins synthesized on the polysomes detach from the apical cell surface, subsequently rupture in the lumen and release the small membranous vesicles (epididymosomes) including proteins and small non-protein coding RNA (Hermo and Robaire 2002; Girouard et al. 2009; Zhou et al. 2018). In this manner, the liberated proteins then can interact with spermatozoa and perform their specific functions for sperm maturation in epididymides (Hermo and Robaire 2002).

The morphological features of the deferent duct were similar to those reported in other mammalian species, excluding the absence of stereocilia (Kennedy and

Heidger 1979; Murakami et al. 1982; Hermo et al. 2002). Principal and basal cells were the main cells along the deferent duct. Principal cells function in endocytosis, water transport and steroid synthesis (Andonian and Hermo 1999; Hermo et al. 2002). In this study, the stereocilia were unable to be detected in the principal cells of the deferent ducts by histological and SEM observations, unlike in epididymides, although short microvilli were detected. Thus, the deferent duct of porcupines may differ from that of the mammals possessing the stereocilia regarding the function for fluid absorption. In the Sunda porcupine, the deferent duct with short microvilli may show a low ability for the absorption of the testicular and epididymal fluid.

The role of the deferent duct in steroid synthesis has been demonstrated in previous studies. The localization of 3β-HSD was immunohistochemically detected in the cytoplasm of principal cells of the deferent duct from the rabbit, hamster, marmoset monkey, and rat (Prakash and Moore 1982; Andonian and Hermo 1999). In the present study, the immunoreactivity of 3β-HSD in the principal cells was observed in the deferent duct of the Sunda porcupine. The presence of this enzyme in the deferent duct enables androgens to be metabolized and interconverted into steroid metabolites that will be utilized by sperm (Prakash and Moore 1982; Hermo et al. 2002), which will ensure a constant supply of androgens for metabolism by sperm (Hamilton and Fawcett 1970; Hamilton 1971; Andonian and Hermo 1999).

It has been reported previously that sperm express aromatase, a steroidogenic enzyme that converts androgen to estrogen, and sperm also express estrogen receptors (ER α and β) (Hess and Carnes 2004; Carreau et al. 2008). Moreover, the deferent duct also expresses ER α and β (Hess and Carnes 2004). Androgens synthesized and released by the principal cells of the deferent duct may be converted to estrogen by aromatase expressed in sperm passing the lumen of the deferent duct and/or stored in the tail of the epididymis. Then, the estrogen produced by sperm may act on ERs in sperm and/or the epithelium of the deferent duct to induce sperm maturation and sperm motility and/or functional adaptations of the deferent duct for complete sperm activity. In our recent study on the testes of the Sunda porcupine, we reported the possibility that only Leydig cells produce and secrete both androgen and estrogen (Nurliani et al. 2019). However, these steroid hormones may not react to sperm in the lumen of the deferent duct directly. Therefore, the steroid secreted by the principal cells of the deferent duct may be necessary for sperm functions in the Sunda porcupine.

Nevertheless, immunoreactivities of 3β-HSD were not detectable in the epithelium of epididymal ducts of the Sunda porcupine. It may be assumed that, therefore, only the principal cells of deferent ducts provide steroid metabolites to sperm in the Sunda porcupine. Although localization of 3β-HSD were observed in the epididymides of the rabbit, hamster, and marmoset monkey (Prakash and Moore 1982). The regulation of 3β-HSD has been reported to be very complex, affected by several compounds and most likely different between tisssues, different ages of organisms and species (Rasmussen et al. 2013). About the existence of 3β-HSD in the epididymides, species difference seems to responsible for this occurence.

On the other hand, in several mammals such as the mouse, rat, dog, monkey, and human, steroid 5α-reductases was located in the epithelium of epididymal ducts, suggesting that testosterone derived from testes may be converted to dihydrotestosterone (DHT) by this enzyme (Robaire and Hinton 2015). In the epididymal cells of the Sunda porcupine, DHT may be produced from testis-derived testosterone by steroid 5α-reductases, although the testosterone is not produced in the epididymides. In the further study, we will examine the localization of steroid 5α-reductases in the epididymal cells to confirm this estimation.

In this study, several vacuoles were found in the epithelium of the deferent duct located among principal cells. Zayed et al. (2012) reported similar structures within the epididymal epithelium of camels, which were considered as an intraepithelial gland due to their positive reactivities to PAS staining that may be formed by accumulations of secretions that should be released into the lumen. In the present study, the vacuoles of the deferent duct in the Sunda porcupine were negative for AB and PAS staining. Thus, the vacuoles in the deferent duct of the Sunda porcupine may not contain glycoproteins (both neutral and acidic) or sulfated mucopolysaccharides and/or proteoglycans, and it may be not related to the intraepithelial gland. On the other hand, similar structures were found in the epididymal epithelium of rats and mice, referred to as pale vacuolated epithelial cells. These structures were considered to result from the degeneration of a principal cell that leads to fistula formation (Agnes and Akbarsha 2001; Akbarsha et al. 2015). Therefore, the vacuoles found in the epithelium of the deferent duct of the Sunda porcupine may be considered as fistula which is a gland-independent structure.

Three thick muscle layers were also found as a prominent structure in the deferent duct of the Sunda porcupine. In the agouti, there is a difference in the number of muscle layers in the deferent duct depending on the stage of sexual development. In the prepubescent and prepubertal animals, there were two muscle layers, whereas in pubertal and adult animals, three muscle layers were observed (Arroyo et al. 2014). The difference in layer development with age suggested that the number of muscle layers is related to the presence of sperm. Sympathetic neurons activate the rapid peristaltic contractions of the muscle layer in the deferent duct, which are responsible for inducing sperm transfer during the brief period of ejaculation (Berridge 2008). In the Sunda porcupine, these muscle layers were considered to be less developed in the prepubertal period when spermiogenesis is incomplete.

In summary, the principal cells with stereocilia and apical blebs, basal cells, apical cells, and the narrow cells with an extruded nucleus were distinguished in the epididymal epithelium of the Sunda porcupine. The presence of principal cells with stereocilia and apical blebs suggests that the epididymal epithelium is highly capable of epididymosome secretion and absorption of fluid from the lumen. The epithelium of the deferent ducts did not have stereocilia and apical blebs, but possessed numerous vacuoles considered to be a gland-independent structure. Principal cells of deferent ducts exhibited 3β-HSD immunoreactivity, suggesting that sperm activity in the lumen of deferent ducts is locally controlled by several steroid metabolites in the Sunda porcupine. Moreover, the principal cells of the epididymides and deferent ducts show PAS-positive, suggesting the secretion of glycoproteins. In our current study, we suggest that epididymides and deferent ducts of the Sunda porcupine play important roles for sperm function through glycoproteins and steroid metabolites.

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