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MORPHOLOGICAL CHARACTERIZATION OF PLANTAIN SQUIRREL (*Callosciurus notatus*) SPERM

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ABSTRACT

Sperm morphology is the key point in assessment of sperm quality. This research aimed to determine the morphological characteristics of the plantain squirrel (*Callosciurus notatus*) spermatozoa. This research consisted of several stages: anesthesia of animal samples, surgery, a maceration method, observation of the spermatozoa morphology using William's staining, and morphometry of sperm using ImageJ 1.46r software. The results show that the plantain squirrel sperm has a slightly flat head shape, falciform with one apical hook, and a not too long tail. The average sperm head length is 1.87 ± 0.30 µm, the average sperm tail length is 5.48 ± 0.95 µm. Further research is therefore needed to more clearly elucidate specific about head sperm analysis in the plantain squirrel, therefore, be able to improve the reliability of morphology assays in clinical and research laboratories especially reproductive biotechnology.

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1. INTRODUCTION

Indonesia has a diverse fauna. However, along with the times, human activities have reduced biodiversity and caused damage and loss of fauna habitat (Opoku, 2019; Scanes, 2018). Habitat destruction has increasingly threatened many animals and even exterminated some types of animals. Various efforts have been made to protect endangered wildlife. In general, there are two approaches taken: in-situ conservation by protecting animals' natural habitat and ex-situ conservation by maintaining and breeding wild animals outside their habitat. Even though Indonesia is listed as the fourth-largest country protecting forest areas (Sun et al., 2008), habitat destruction and population decline in this country occur faster than animal protection (Scanes, 2018).

The plantain squirrel (*C. notatus*) is a rodent of the family *Sciuridae* (Thorington Jr & Hoffmann, 2005). This animal could be found in the Malay Peninsula, Thailand, Sumatra, Kalimantan, Java, Bali, Lombok, and the surrounding islands at an altitude of 500-1100 below sea level. Studies have recognized 49 subspecies of *C. notatus* in Indonesia (Boddaert, 1785; Thorington & Hoffmann, 2005).

The plantain squirrel has a tricolor pattern; the upper side is brownish with blackish and yellowish spots. On the sides of the body, slightly down, between the forelegs and hind limbs, there is a pale yellowish and black line (Nor et al., 2001). Plantain squirrels are characterized by 150-225 mm of head and body lengths, 169-210 mm of tails, and 150-280 g of weight (Saiful & Nordin, 2004). In general, the plantain squirrels are arboreal animals that live in trees. Moreover, they live solitarily or in small family groups. The population of coconut squirrels have decreased every year. According to the 2019 Checklist of the mammals of Indonesia, the plantain squirrel has been in a low concentration status or received the least concern (Maryanto et al., 2020). Based on the previous explanation, it is necessary to accelerate efforts to conserve wildlife, including integrating technology with conservation efforts. One of these efforts is the application of reproductive technology. However, the development of biotechnology depends on proper knowledge of sperm morphology to understand male reproductive capability based on sperm physiology aspects because male fertility potential depends on different cellular sperm structures (Leao et al., 2020).

Fertilization levels in animal reproduction highly depend on the ability of sperm to enter the female reproductive tract; using sperm with high quality and quantity will easily obtain high-quality fertility. Several researchers have discovered physiological characteristics of animal reproduction by analyzing sperms (Oliveira et al., 2015, 2016; Roldan, 2020; Sampaio et al., 2017; Umami et al., 2020; Visco et al., 2010). Another study

has employed a seminal analysis or spermiogram that crucially helps the process of artificial insemination, in vitro fertilization, and embryo development (Ozkavukcu et al., 2008).

Sperm morphology is the key point in assessment of sperm quality. However, there is still a lack of information on the reproductive biology in sperm characteristics of plantain squirrel to help efficient reproductive technologies. This research aimed to determine the morphological characteristics of the sperm of plantain squirrels (*C. notatus*). This study is a valuable basis for understanding the physiology quality of sperm by micromorphologically describing *C. notatus*. This description is important to accurately predict the fertility capacity of semen rapidly produced by inexpensive techniques.

2. RESEARCH METHOD

Experiment object: The plantain squirrel (Callosciurus notatus) with testes length was 2.63 cm, testes width was 1.94 cm and penis length were 1.34 cm. Materials: NaCl 0.9%, eosin 0.2%, William's dye, alcohol 70%, immersion oil, aquadest, light microscope, petri dish, object glass, cover glass, Pasteur pipette, stirring glass, hemocytometer, micropipette and tip, straw, erythrocyte pipette, pH indicator, thermometer, stopwatch, clean paper, dissecting set, label, and ImageJ 1.46r software.

Anesthesia of animal samples

The animal was caught, put in a calico bag, and held tightly. The sample was anesthetized intramuscularly with 0.08 ml of ketamine and 0.01 ml of xylazine 10mg/kg. Afterward, it was monitored by the operator until it could be handled (Semiadi & Nugraha, 2005).

Semen collection with the maceration method

The anaesthetized sample was dissected, and the cauda epididymis was isolated from its testes and placed in a petri dish. The sample had been cut using a scalpel until smooth, then it was added with NaCl 0.9%. Afterward, it was homogenized by agitation in the petri dish. The liquid was sucked using a Pasteur pipette, and a few drops were placed above the object glass. Finally, the sample was observed under a microscope.

Morphology of spermatozoa with William's stain

Fresh semen was smeared and then air-dried. After that, the semen was washed with alcohol for four minutes until dried. The sample was washed in 0.5% chloramine solution for 1-2 minutes until the mucus layer disappeared. Therefore, the smear was clean and easy to observe. The sample was washed with distilled water and alcohol 95% and subsequently soaked in William's dye for 8-10 minutes. Finally, it was washed with water, dried, and observed under a microscope.

Morphometry of spermatozoa

The sperm was measured using the ImageJ 1.46r software. At least 200 spermatozoa cells were measured using the parameters of head length and tail length. Afterward, the results were recorded.

Method of analysis

The collected data including morphological data were analyzed descriptively.

3. RESULTS AND DISCUSSION

The plantain squirrel (*Callosciurus notatus*) sperms have a slightly flat head shape, falciform with one apical hook, and a not too long tail. *Callosciurus notatus* is one of the rodents that exhibit the widest range of sperm sizes among eutherian mammals. Breed (2004) asserts that rodents show considerably different sperm head shapes, from simple oval to falciform heads with one or several apical hooks or elongations in the base of the head. Based on the observation from samples spermatozoa cells no abnormal morphology was found.

The head of a mammalian sperm consists of a nucleus, surrounding plasma membrane, and acrosome. Chromatin is the main constituent of the sperm head; as a result, the morphometric parameters of the sperm nucleus will correspond to that of the sperm head (Ostermeier et al., 2001). The measurement using the ImageJ 1.46r software reveals that the average sperm head length is 1.87 ± 0.30 µm, and the average sperm tail length is 5.48 ± 0.95 µm. These findings support the theory that spermatozoa have the most diverse size and shape among taxa. Like other mammalian species, primate species have a relatively small sperm head area (<20µm^2). On the opposite, bovines, pigs, and rabbits have relatively large sperm head areas (>25 µm^2) (Sanchez et al., 2013; Yániz et al., 2015).

The size and shape of the head would determine the volume and total surface of the spermatozoa so that they affect the exchange of water, ions, cryoprotectants, and resistance to cryopreservation. To discover the size and shape of the spermatozoa's head, many scientists have measured them micromorphologically using a microscope. The microscopic measurement of spermatozoa is influenced by various factors: fixation techniques,

staining, semen handling, microscope quality, and personal skills (Esteso et al., 2006; Hidalgo et al., 2006; Rijsselaere et al., 2004)

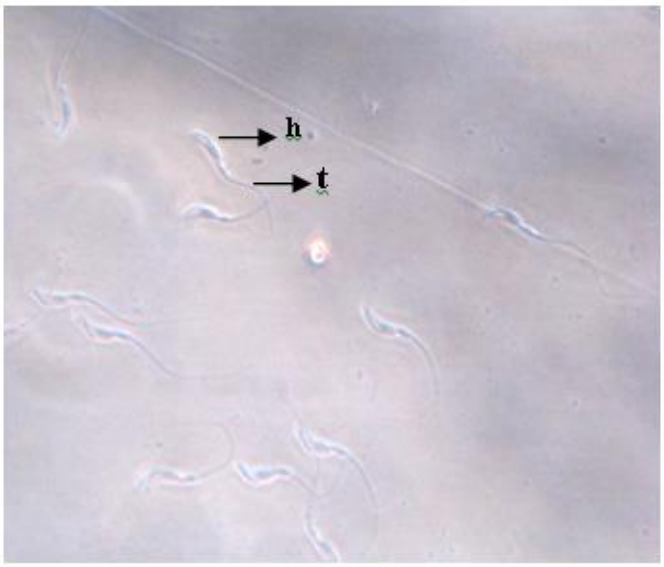

Figure 1. Sperm morphology of plantain squirrel (*Callosciurus notatus*) without staining.
h= sperm head; t = sperm tail

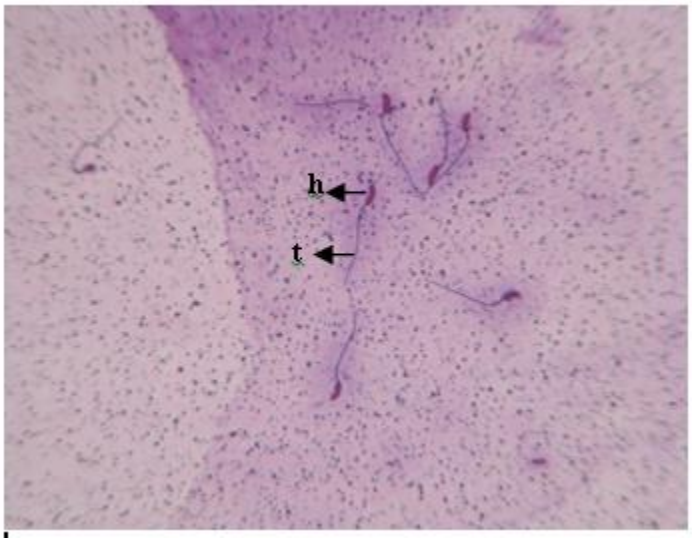

Figure 2. Sperm morphology of plantain squirrel (*Callosciurus notatus*) with William's staining.
h= sperm head; t = sperm tail

Spermatozoa in mammals are long, and the cells are motile. The spermatozoon has a head and tail. Its head consists of a nucleus with high density, thick chromatin enveloped by a perinuclear theca, an acrosome, and a plasma membrane. The main functions of the sperm head are penetrating the oocyte, carrying the haploid male genome, and initiating embryonic development after fertilization. The sperm tail can be divided into a connecting piece, a midpiece, a principal piece, and an end piece. The connecting part refers to a part of a short connecting circuit between the head and the tail and consists of segments, fibrous tissues, and a capitulum. The middle part serves as the protective membrane of the mitochondria, which is the energy regulator for sperm motility. This section starts from the distal connecting to the annulus (the structure that limits the middle to the main part). The main part of the tail is the area from the annulus to the tip of the tail. Overall, the sperm tail is useful for pushing spermatozoa to move through the uterus and fallopian tubes until they meet and penetrate the oocyte (Schatten & Constantinescu, 2008).

4. CONCLUSSION

The plantain squirrel (*Callosciurus notatus*) sperms have a slightly flat head shape, falciform with one apical hook, and a not too long tail. The plantain squirrel's average sperm head length is 1.87 ± 0.30 µm, and its average sperm tail length is 5.48 ± 0.95 µm. Further research is therefore needed to more clearly elucidate specific about head sperm analysis in the plantain squirrel, therefore, be able to improve the reliability of morphology assays in clinical and research laboratories especially reproductive biotechnology.

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