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Sperm morphological assessments of Bali boar semen collected from three area in Bali Island, Indonesia

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Abstract
Bali pigs is one of the original germplasm of Bali Island. This study assessed the sperm morphology of Bali boars collected from three areas in Bali Island. Sperm morphology is known to be closely related to boar fertility and sterility. Total of 15 Bali boars were used in this study and sperm observations for each ejaculation. One drop of semen was placed on two or three glass slides at each ejaculation for examination. Sperm staining used was the carbolfuchsin-eosin (Williams staining method). Observation of sperm abnormality was carried out on 200-250 spermatozoa in each semen sample. The results showed that the sperm morphology of Bali boars in the three districts was not significantly different (P>0.05). The types of abnormality found in Bali boar semen were bent tail and coiled tail. The percentage of sperm abnormality in Bali boars was less than 20%.

Keywords: Bali boars, Williams method, sperm morphology

Introduction
Bali pigs (*Sus vittatus*) is one of the original germplasm of Bali Island. Some of the advantages of Bali pig livestock is easy to maintain, adaptive, fast productivity, and used to complete traditional ceremonies for the Hindu community on Bali Island, so it has the potential to be developed. The development of Bali pigs can be done through improving the quality of males for breeding. Males who are physically and reproductively healthy will produce good spermatozoa to produce good offspring [1]. Bali boars have a manly posture, excellent libido and aggressive. Bali boars are not affected by the mating season and have the character of being able to give birth to ten or more piglet [15, 16, 20]. Moreover Bali pork is very popular with local people and foreign tourists as a processed product such as roast pork or suckling pigs [13]. Now day, the presence of Bali pigs on Bali Island is very small and only occurs in certain areas with low rainfall and limited availability of forage.

Spermatozoa is part of the semen ejaculated by males. The spermatogenesis occur in the seminiferous tubules of the testes, includes the spermatocytogenesis phase, namely the process of forming spermatogonia into spermatids, and the spermiogenesis phase is the process of forming spermatids into spermatozoa. The structure of the spermatozoa consists of a head, neck and tail. The head contains the cell nucleus, the neck connects the head to the middle, and the tail functions to move forward. The length of the tail is about 10x the head [10].

Evaluation of semen (namely macroscopic and microscopic evaluation) is needed to determine boar fertility and sterility. Semen analysis is limited to sperm concentration and motility (sperm movement), while sperm morphology (abnormalities and normal structure) has not much been formed, and no information about Bali boar sperm morphology. Sperm morphology is one of the important parameters in determining the quality of semen because high abnormalities of spermatozoa can affect fertility. Morphological observation is to observe normal and abnormal spermatozoa shape. According to [4, 3], abnormalities in spermatozoa can be classified into two main groups, namely primary abnormalities (head and acrosome spermatozoa), and secondary abnormalities (midpiece cytoplasmic droplets and damage to the tail). Boars semen has a high volume but low sperm concentration, and boar semen consists of three fractions, namely gelatin fraction, low sperm fraction, and rich sperm fraction.

Research on boar semen has been done a lot. However, specifically research done on Bali boars is not available, particularly about the characteristics and morphology of sperm.

Therefore, this research is very necessary in order to observe the sperm morphology of Bali boars on Bali island. The results of this study are expected to provide important information about Bali boars semen for the purpose of sustainable development of Bali pigs.

Material and Methods

Research material

Bali boar semen were collected from five boars in each observation area, namely Buleleng, Karangasem, and Nusa Penida. Staining and observation of smeared semen samples were performed at Reproductive Rehabilitation Unit Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University.

Research procedure

The semen sample were collected in each area, then the samples delivered to the laboratory in Bogor. The preparation of the semen sample was made following procedure: a drop of semen was placed on a glass slide, mixed with one drops of physiological saline (NaCl) and homogenized by stick; thin smears were then made 3-4 different glass slide. Smeared semen samples were air-dried and stored in the preparation storage box until staining. Smeared semen samples were stained with carbolfuchsin-eosin (Williams) dye according to Kayak *et al.,* 2004 cited by [3]. The steps taken were air-dried patch smeared fixed with Bunsen fire, then washed with absolute alcohol for 4 minutes, and air dried. Next, the smears were treated with 0,5% chloramine solution for 2 minutes until the mucous had disappeared and the smears looked fairly clear. The smears were washed in distilled water, rinsed in 95% alcohol, and stained with Williams solution for 10 minutes. Finally, the smears were washed in running water, allowed to dry and ready to be observed.

Fig 1: Bali Boar

Observation of abnormal spermatozoa used a light microscope (Olympus CH 20) at 400x magnification. It was observed at least 200 spermatozoa in smear. Abnormalities of sperm can be classified into two main groups, namely primary abnormalities (head and acrosome spermatozoa), and secondary abnormalities (midpiece cytoplasmic droplets and damage to the tail). The sperm morphology of Bali boars and variations in sperm morphology between areas of Bali boars rearing were presented in mean ± standard deviation (SD) which was calculated using Minitab software version 19.2020.

Results

The fresh semen of Bali boars consisted of three fractions, namely the gelatin fraction, the low sperm fraction, and the rich sperm fraction. The characteristics of Bali boar fresh semen compared to Landrace fresh semen are show in Table 1.

Table 1: Characteristics of Bali boars sperm

Variable	Area[1]			
	A	B	C	Landrace
Macroscopic				
Sperm color	milky white	milky white	milky white	creamy white[2,3)]
Odor	smell of sperm	smell of sperm	smell of sperm	smell of sperm [2,3)]
Consistency	liquid	liquid	liquid	liquid[2,3)]
Volume (ml)	98,62±11,58	104,22±10,11	105,28±8,81	273,60±1,23[2)]
pH	6,96 ± 0,15	7,28 ± 0,24	7,26 ±0,17	6,0 – 7,0[2)]
Microscopic				
Motility (%)	67,6 ± 2,24	70,0 ±1,26	70,0 ± 1,26	73,86±1,18[2)]
Sperm mass movement	+++	+++	+++	+++
Sperm concentration (×10^6/ml)	177,0 ± 13,64	189,0 ± 5,22	189,4 ± 11,69	191,65±1,71[3)]

Note: 1) Area: A. Buleleng; B. Nusa Penida; C. Karangasem
2) Sumardani *et al.* (2020)
3) Sumardani *et al.* (2008)

Abnormality is an indicator in determining the quality of sperm because the abnormal structure of spermatozoa can cause fertilization disorders, reducing implantation and pregnancy rates.

Discussions

The characteristics of Bali boar sperm in Buleleng, Nusa Penida, and Karangasem areas are not significant (P>0,05). Observations on fresh sperm from Bali boars semen include milky white colour, smell of sperm, liquid consistency and an average pH of 7,16. The results of macroscopic observations on Bali boar sperm were not different those Landrace. Sumardani [18] reported thatsperm of Landrace has a cream white sperm colour, distinctive smell of sperm, pH 6-7, and the consistency is liquid. One indicator of boar fertility is sperm motility and viability. Progressive motility or motility of sperm to fertilize eggs is an important indicator in determining the quality of male sperm [4, 5, 8]. The percentage of boar sperm motility that can be further processed for production of liquid sperm in a series of artificial insemination has a minimum sperm motility of 65% [3, 4, 8, 19]. In Table 1, the sperm motility percentage of Bali boar is in the good range, namely above 65% and has average sperm mass movement of 3+ (+++) or very good. Genetically, Bali pigs

are different from purebred pigs, the body size and testicular size. So, that the production of sperm Bali boars per ejaculate is also very small.

Several factors that can affect the morphology of sperm, both macroscopic and microscopic are race, genetics, age variation, level of stimulation, frequency of ejaculation, quality of feed, fraction of the collected sperm, pH, temperature and nutrient [3, 4, 9, 10, 11, 17]. The completeness of nutrients in animal feed can maintain the quality of the sperm produced and maintain the normality of endocrine function.

Sperm viability has a positive correlation with sperm motility. The vitality of sperm is indicated by the strength of the sperm plasma membrane [14]. Sperm viability is closely related to sperm morphology (sperm normality and sperm abnormality) because the percentage of sperm normality and abnormality has a relationship with the ability to fertilize [2]. According to [8], high sperm abnormalities (>20%) can reduce fertility. High sperm abnormality (>20%) indicates symptoms of male infertility. However, there are many variation in the ejaculation process of male so that the determination of fertility requires analysis of several ejaculation [12].

Bali boar semen has an abnormality percentage of less than 20%. The mean value of abnormalities such of Bali boars semen in the Buleleng 7,11±1,07%, 15,32±2, 00% Nusa Penida, and Karangasem 10,8±0,55%, respectively. Thus, in general regardless of individual factors, the abnormal level of sperm in all boars in this study was good.

The level of abnormal sperm is an important factor because the number of normal sperm has a longer viability than abnormal sperm. Normal sperm have a high fertilization ability before losing their motility [6]. Some of the abnormalities found in Bali boars sperm include bent tail and coiled tail, which are secondary abnormalities (abnormalities that generally occur in the tail of spermatozoa). Forms of the secondary abnormalities include folded tail, presence of proximal or distal cytoplasmic droplets, and the acrosome membrane detaching from the head without a tail, and a severed tail.

Sperm abnormalities are always found in every ejaculation but have a different impact on fertility. Secondary abnormalities can occur during the storage process and are most likely caused by treatment at the time of staining in the process of making the preparations [3, 8]. In this study, it was found that there were secondary abnormalities in the form of bent tail and coiled tails which were suspected to be due to the influence of improper preparation. The use of eosin dye by heating table at 37 ^{0}C causes the preparations to dry slowly and the dye is too long to be exposed, resulting in a change in shape and size which is basically not to large. The most accurate test to measure the fertility of the decisive boars are pregnancy and live birth. Although there are many criterias that can determine the sub-optimal high quality status of sperm, there is no single invitro parameter that can be used to predict boars sperm quality [8, 19].

Several factors that can affect the morphology of sperm, both macroscopic and microscopic, are race, genetics, age variation, stimulation level, ejaculation frequency, feed quality, collected semen fraction, food and environment [3, 4, 9, 10, 11, 17]. The completeness of nutrients in animal feed can maintain the quality of the sperm produced and maintain the normality of endocrine function.

Conclusion

The conclusion of this study is the Bali boar sperm morphological abnormality rate in sample collected from three area in Bali island was in normal range, which was at less than 20%. The most frequent type of secondary sperm abnormality was bent tail and coiled tails.

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