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Characteristics and morphology of sperm from Crossed Duroc boar at different frequencies of shelter time

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Abstract

This study aims to examine the characteristics and morphology of sperm from cross-bred Duroc boars with different shelter time frequencies. This research was conducted in Buahan Village, Payangan District, Gianyar Regency, Bali Province, Indonesia. This research was carried out for two months, using a completely randomized design (CRD), which consisted of three treatments, namely: sperm storage which was carried out every 2 days (P0), sperm storage which was carried out every 4 days (P1), and sperm storage which was carried out every 7 days (P0). Variables observed included sperm volume, viscosity, odor, color, pH, spermatozoa motility, spermatozoa concentration, spermatozoa viability, and spermatozoa morphology. The results showed that the semen volume P0 (165.33±11.02ml); P1 (254.33±10.02ml); and P2 (242.67±21.08ml) were not significantly different (P>0.05). The viscosity, color, odor and pH are in accordance with the Indonesian National Standard (SNI) regarding liquid boar sperm. Spermatozoa motility in treatments P1 and P2 was: 78.33±2.88% and 73.33±2.88% significantly different (P<0.05) from P0 (61.67±2.88 ml). The spermatozoa concentration at P1 and P2 was significantly (P<0.05) higher than at P0. Spermatozoa morphology (spermatozoa abnormalities) at P1 (4.23±1.06%) showed the lowest results compared to P0 (8.52±2.55%) and P2 (5.95±0.6%). From the results of this research, it can be concluded that sperm collection carried out every four days shows the best results in the characteristics and morphology of spermatozoa in cross-bred Duroc boars.

Keywords: Boar Duroc-cross; Sperm; Shelter frequency; Motility; Viability

1. Introduction

In an effort to improve the quality and quantity of pig farming, several aspects have been carried out by the government, one of which is the development of Artificial Insemination (AI) technology. Through AI, breeders are more practical in the mating process for pigs, so they can encourage good pig production. There are important things to pay attention to in AI activity programs in pigs, namely not only the quality and quantity or handling of semen from boar ejaculation, but also depends on the ability to maintain quality and increase the volume of semen that can be stored for a longer time after ejaculation [1]. Based on the research results of [2] obtained the AI success rate in pigs reaching 67.50%. However, this condition does not necessarily have a positive impact on breeders in its application. Artificial insemination is still doubted by breeders regarding the quality of the sperm produced through the AI process [3].

Apart from genetic factors, the quality of sperm produced from the AI process can be seen from the characteristics and morphology of spermatozoa after sperm storage. Characteristics and morphology are important benchmarks as indicators for determining the quality and quantity of a good shelter. However, research on the characteristics and morphology of spermatozoa in crossbred Duroc pigs has not been widely reported. The research results of [4] stated that the percentage of spermatozoa motility of Landrace male pigs in the young age group was higher than that of male

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pigs in the older age group, and the average fresh sperm production of males in the young age group was 273.60 ml, while that of the older age group as much as 107.66 ml. This difference is caused by the reproductive organs of males in the mature age group being in a high productivity phase, so that the number of spermatozoa cells and the production of accessory sex glands produced in one ejaculate is higher than that of males in the older age group.

The characteristics and morphology of spermatozoa in males can be influenced by several factors, such as feed, libido, age of the animal, and frequency of sperm storage. According to [3], the frequency of sperm collection is generally twice a week. The high frequency of sperm storage will force spermatozoa to move quickly from the head to the cauda epididymis, so that they do not have enough time to mature. Research on the characteristics and morphology of spermatozoa in Duroc-crossed boar has not been widely reported, therefore it needs to be studied further in order to obtain maximum results.

2. Material and methods

2.1. Material

This research was carried out in Buahan Village, Payangan District, Gianyar Regency, Bali Province, Indonesia. This research was carried out for two months starting with preparation, data collection, observation and data analysis. The boar cage used was an iron cage measuring 2.5m^2 with a height of 1.20 meters, the floor of the cage uses a concrete floor. Each male cage was equipped with an automatic drinking water feeder (water nipple). The feed given to males is Comfeed feed produced by PT. Japfa Comfeed Indonesia Tbk., Indonesia. The total feed was 2.5 kg/head/day and drinking water was provided *ad libitum*. The equipment used in this research was a dummy sow, pig sperm collection device, water heater, measuring cup, glass cover, preparation box, microscope-binocular, cold box, and other tools used according to their function.

2.2. BoarDuroc-cross

This research used three Duroc-crossed boar animals that were 3 years of age at sexual maturity, and were in good health and had good sperm quality, namely spermatozoa motility averaging above 60%. Sperm collection was carried out in the morning, namely every two days, every four days, and once every seven days using the manual method (glove hand method) assisted by a collection tube. Separation of the gelatin fraction was carried out by lining gauze at the mouth of the collection tube. The diluent used was life semen, while the dye used was 2% eosin with the aim of observing the viability of spermatozoa from Duroc-cross boars.

2.3. Experimental design

This research was carried out for two months, using a completely randomized design (CRD), which consisted of three treatments, namely: sperm storage which was carried out every 2 days (P0), sperm storage which was carried out every 4 days (P1), and sperm storage which was carried out every 7 days (P0), respectively.

2.4. Macroscopic Evaluation

Macroscopic evaluation of Duroc-crossed boar sperm which includes: (i) sperm volume: obtained from the results of collecting Duroc-crossed boar sperm which was then put into a sperm storage tube; (ii) sperm viscosity: checked by gently shaking the sperm storage tube; (iii) sperm odor: determined by positioning the sperm tube upright, then bringing the top of the tube under the nose and inhaling slowly until you can smell the sperm odor; (iv) sperm color: obtained by directly observing sperm in a holding tube made of transparent glass; and (v) sperm acidity (pH): evaluated using indicator paper. The pH value was considered normal if the pH of the sperm produced has a range between 6.0-7.8 (showing green) and if the pH is acidic then the indicator paper will be yellow or red, whereas if the pH is alkaline then the pH paper will be blue or purple.

2.5. Microscopic Evaluation

Spermatozoa motility was the mass of spermatozoa observed according to the quote from [5], namely by placing one drop of sperm on a glass object without covering it with a cover glass, then observing it under a microscope with a magnification of 100 times. Sperm quality can be determined based on the assessment of mass movements as follows:

- Very good (+++) if the movement of spermatozoa was seen forming large, numerous, dark, thick and active waves and moving quickly from place to place.
- Good (++) if the spermatozoa movement looks like small waves, thin, rare, unclear and moving slowly

- Sufficient (+) if no wave-like movement of spermatozoa was seen but only progressive active individual movement is visible.
- Poor (necrospermia, 0) if there was little or no visible movement of individual spermatozoa at all.

Spermatozoa concentration: determining the concentration of spermatozoa by counting the number of spermatozoa present using a haemocytometer by diluting 10µl of semen in 990µl of formolsaline in an Ependorf tube. The solution was homogenized, then 1µl was taken using a micropipette and placed on top of the counting chamber, then covered using an object glass. Observations were carried out under a microscope with a magnification of 40×10. The number of spermatozoa was counted in 5 rooms diagonally or in four corners and one in the middle [6].

Spermatozoa viability: can be calculated by dropping one drop of sperm onto an object glass, then adding one drop of eosin solution and mixing evenly, then drying over a Bunsen flame until dry and forming a smear preparation, then observed using a microscope with a magnification of 10x40 times. Live spermatozoa will not absorb the eosin solution, so their heads were clear, while dead spermatozoa will absorb the eosin solution so their heads are red.

Spermatozoa morphology: Abnormality test using the results of eosin-negrosin review for viability testing, then continued observation under a light microscope with a magnification of 40×10. Forms of spermatozoa abnormalities include large heads or small heads, short, wide heads, double tails, folded tails, acrosomal sheaths that were detached from the head without a tail, and severed tails [7].

2.6. Data Analysis

The data obtained will be analyzed using the SPSS program, if the treatments are significantly different (P<0.05) it will be followed by Duncan's multiple range test [8].

3. Results and discussion

In general, different shelter frequencies had a significant influence on the characteristics and morphology of Duroc-cross boar spermatozoa. More details are presented in Table 1.

Table 1 Characteristics and morphology of Duroc-cross boar sperm at different frequencies of shelter time

Variable	Frequency of Sperm Storage (days)			SEM[2)]
	2	4	7	
Sperm				
Volume (ml)	165.33±11.02[b]	254.33±10.02[a]	242.67±21.08[a]	0.38
Consistency	watery	watery	watery	-
Smell	typical smell	typical smell	typical smell	-
Color	milky white	milky white	milky white	-
pH	7.73±0.35	7.13±0.31	7.73±0.32	0.07
Motility (%)	61.67±2.88[b]	78.33±2.88[a]	73.33±2.88[a]	0.78
Concentration (x 10^6sel/ml)	231.00±34.69[b]	313.67±5.68[a]	288.00±12.52[a]	0,12
Viability (%)	80.95±10.01	87.87±7.08	78.59±5,69	0.21
Abnormalities (%)	8.52±2.55[a]	4.23±1.06[b]	5.95±0.64[b]	0.10

Note: SEM: *Standard Error of the Treartment Mean;* Values with different letters [a,b] on the same row are significantly different (P<0.05)

The average sperm volume of Duroc-cross boars at a holding frequency of once every 4 days (P1) and a holding frequency of once every 7 days (P2), namely: 53.83% and 46.78% significantly (P<0.05) higher than P0. The frequency of shelter did not have a significant effect (P>0.05) on the degree of acidity (pH) of sperm. The average motility of Duroc-

cross boar spermatozoa in treatments P1 and P2 was: 27.01% and 18.91% significantly (P<0.05) higher than the control (P0).

The average concentration of Duroc-cross boar spermatozoa in treatments P1 and P2 was: 35.79% and 24.68% significantly (P<0.05) higher than P0. Meanwhile, boar sperm viability did not show any significant differences (P>0.05). Abnormalities of Duroc-cross boar spermatozoa in treatments P1 and P2 were: 50.35% and 30.16% significantly (P<0.05) lower than P0.

In this study, there was an increase in the sperm volume of Duroc-cross boars in groups P1 and P2. The increase in the results of this study is not in accordance with [9] who stated that the volume of boar sperm without gelatin ranged from 200-250 ml. The semen volume at P0 was the lowest compared to treatments P1 and P2, which was due to more frequent storage times, where the production of spermatozoa cells in the testicles takes time from formation to maturation of spermatozoa cells.

A larger sperm volume can increase the chances of successful fertilization and pregnancy in female animals. Apart from that, increasing sperm production can support breeding programs (artificial insemination) to select superior genetic sources to improve the quality and productivity of pigs. Several factors that influence sperm volume, color, consistency and pH are variations in age, level of stimulation, ejaculation frequency and feed quality [10]. A larger semen volume can increase the chances of successful fertilization and pregnancy in female animals.

The main characteristic of good spermatozoa is their motility or movement power which is used as a benchmark or the simplest way to assess sperm for artificial insemination (IB). Waves of sperm movement moving in the same direction can be seen with the help of a light microscope in undiluted sperm [6]. The factors that can influence the volume, motility and amount of liquid sperm production in boars include: feed, temperature and season, ejaculation frequency, disease, libido and physical factors, livestock transportation, age and exercise. Low feeding levels and feed quality can inhibit boar growth, reduce the number of spermatozoa per ejaculate, and reduce libido. This is confirmed by the statement of [11] which stated that the addition of *Moringa* leaf supplementation to Landrace-cross boar feed was able to increase the length of ejaculation so that it had a positive correlation with increasing the volume of sperm produced. Therefore, male pigs must be given proper and sufficient feed, both in quality and quantity. Boars that are too often and continuously tasked with mating with females or collecting their sperm can reduce the number of sperm and spermatozoa concentration [12].

Consistency of Duroc boar sperm from the results of this study, it was found that Duroc boar sperm in each treatment had a watery consistency. This is in accordance with [13] who stated that pig semen has a watery consistency, the percentage of abnormalities is less than 20% while the average pH is 6.7. Sperm consistency is closely related to sperm color, because the darker the sperm color affects the sperm consistency. If there is a decrease in sperm consistency, this will result in the color of the sperm appearing clearer. Sperm color is closely related to the consistency and concentration of spermatozoa. Sperm that is dark in color indicates the consistency and high concentration of spermatozoa [2]. Sperm odor is the aroma produced by sperm after it has been deposited. The odor of sperm generally has a distinctive and fishy smell, resembling the smell of milky white egg yolk and a watery consistency, and with an average pH of 7.40±0.2.

Reported by [2] that the color and consistency of boar sperm depends on the fraction contained, namely the pre-spermatozoa fraction is watery with a white-grey color, and the spermatozoa-rich fraction is milky-nonviscous with a creamy white color and is in line with the opinion of [14] who stated that pigs have watery sperm and are milky white in color.

The degree of acidity or pH (Potential of Hydrogen) is an important indicator in determining the level of acid or base in a liquid. The degree of acidity (pH) of sperm obtained during this research was pH 7.53. The results of the research on semen pH are still in the range of sperm pH research results from [13], namely: 6.4-7.8. However, it is lower than [2] research results, namely: 7.78. Factors that influence pH changes occur due to age, level of stimulation, ejaculation frequency, environment and feed quality [15]. The lower or higher the normal pH, the faster the spermatozoa die [2].

Spermatozoa motility is an assessment of the movement of progressively moving spermatozoa visually using a microscope or using an automated system with computer assistance (computer assisted semen analysis/CASA). Previous researchers have confirmed the close relationship between spermatozoa motility and viability and sperm fertilization ability in livestock [16]. In this study, the best motility percentage was obtained at 78.33 ± 2.88% at P1. This

is in accordance with [17] which states that liquid sperm comes from fresh sperm with a minimum progressive spermatozoa motility of 70%.

Good motility allows sperm to swim quickly and efficiently towards the egg, while high viability ensures that the sperm has sufficient strength and endurance to survive the long journey to the uterus and egg [18]. Research by [19] found that increasing spermatozoa motility and viability was positively correlated with the success rate of fertilization in livestock, indicating the importance of optimal sperm quality in achieving pregnancy.

Sperm progressive movement or motility has a major role in the fertilization process. In order for the egg to be fertilized, it is very important for the spermatozoa to move forward in the female genital tract. Spermatozoa motility of less than 60% and spermatozoa concentration of less than $200x10^6$ cells/ml have a short shelf life. Assessment of spermatozoa concentration is essential because this factor describes the number of spermatozoa contained in one ejaculate. The spermatozoa concentration in the results of this study showed the best value at P2, namely 313.67±5.68 (10^6 cells/ml). This result is different from research by [13] which said that the concentration of spermatozoa ranged from 200-300 million cells/ml. According to the statement by [20], factors that can influence spermatozoa concentration include: type of boar, age of the boar, environment, frequency of shelter, and health status of the animal. Different holding frequencies can have an influence on the concentration of spermatozoa because a holding frequency that is too short results in the maturity level of spermatozoa cells in the testicles being less than optimal, thus affecting the concentration of spermatozoa produced. Likewise, vice versa, the holding process carried out for a long time will also have a negative effect on the concentration of spermatozoa produced.

Spermatozoa viability is the viability of spermatozoa which is known by observing the number of live and dead spermatozoa with eosin negrosine staining [21]. Different frequencies of shelter time have no effect on spermatozoa viability. Tamoes et al. [22] stated that the viability of boar spermatozoa differs from motility because spermatozoa are not motile but are actually still alive, while motile spermatozoa are definitely alive.

Spermatozoa abnormalities are an indication of reduced fertility, because they reduce the capacitation of spermatozoa during fertilization and affect the development and maintenance of pregnancy [23]. Abnormalities in this study decreased at P1 and P2. Abnormalities of spermatozoa in boar sperm were observed, such as folded or coiled tails, broken heads, or broken tails and enlarged heads. Garner and Hafez [13] stated that the percentage of abnormal boar spermatozoa per ejaculate should not exceed 20%. This is in accordance with [17] regarding fresh sperm having a maximum abnormality value of 20%. Factors that cause this abnormality include genetics, age, breed, light and temperature, maintenance management, shelter frequency, dilution and environment. The lower the percentage of abnormalities, the better the sperm quality.

4. Conclusion

It was concluded that the frequency of sperm collection every four days was able to provide the best quality for the characteristics and morphology of Duroc-cross boar spermatozoa. It can be recommended to Duroc-cross boar breeders to carry out sperm collection every four days to maintain the quality and quantity of spermatozoa produced and maximize the success of the artificial insemination program.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that there is no conflict of interest.

Statement of ethical approval

The animals used in experiments in this study were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University Denpasar, Indonesia.

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