Sperms motility of Ongole Crossbreed (OC) Bull at various age

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Abstract. The purpose of this study was to identify the effect of different ages on characteristics of sperms motility of Ongole Crossbreed (OC) bulls. The material used was 12 PO bulls aged 4–6 years old, distinguished into 2 age groups, namely 4–5 years old (6 heads) and 5–6 years old (6 heads). Collecting semen was done by using an electro ejaculator. Examination of motility (kinetic parameters) used SCA v. 2.1. The parameters measured include: progressive motility, motility, velocity curve linear (VCL), velocity straight linear (VSL), velocity average pathway (VAP), linearity (LIN), straightness (STR), wobble (WOB), amplitude lateral head (ALH), beat cross frequency (BCF) and hyperactivity (H) of sperms. Data were analyzed using a T-test (IBM SPSS 16). The results showed that the value of BCF in the 5–6 years old group was significantly higher (P<0.05) than the 4–5 years old group. The age of the bull affected the sperm's kinetic parameters (BCF). The 5–6 years old group has a more regular pattern of sperms movement than the 4–5 years old group.

1. Introduction
One of the factors that determine the productivity of cows is the performance of bulls. The fertile bull that can fertilize the cow is the main measure of bull performance. However, it was not all the bulls can be used for mating. Breeding Soundness Evaluation (BSE) is a bull selection method with several selection criteria, including body conformation, scrotal circumference and semen quality (motility and morphology), and health status of bulls [1].

Several factors affecting the quality and amount of semen production in beef cattle, including temperature, season, frequency, genetics, breed, age, male body weight, feed, and ejaculation [2]. The parameters of sperms motility and morphology (membrane integrity) greatly influenced fertilization. Motility allows sperms to move towards the ovum, through the cumulus and the zona pellucida. Meanwhile, the integrity of the membrane is a parameter of sperms for making binding with zona pellucida [3].

Sperms motility is an important parameter in assessing semen quality. The assessment process (analysis) of sperms motility can be done subjectively (biased) and objectively (unbiased). Two factors influence the sperm's motility, namely internal and external factors. External factors that affect the sperm's motility include; physiological and biophysical factors, stimulation-inhibition, suspending fluid. Meanwhile, the internal factors including; energy reserves, maturation of sperms, surface-active agents, and age of bull [4]. The purpose of this study was to identify the effect of different ages on characteristics of sperms motility of Ongole Crossbreed (OC) bulls.
2. Materials and methods

The materials used in this research activity were 12 heads PO bull with a range of 4-6 years old. Research activities were carried out at the Cage and Livestock Reproduction Laboratory of Beef Cattle Research Station in 2019. The method of collecting fresh semen was used as an electro ejaculator. There are 2 treatment groups, namely group I was a group of PO bulls aged 4–5 years old (6 heads) and group II (6 heads) was a group of PO bulls aged 5-6 years.

The motility of fresh semen was analyzed by using SCA v.2.1. The extender for semen used was CEP-2. The dilution ratio was determined by the consistency of fresh semen. Fresh semen with medium consistency was diluted in the ratio 1:2. Meanwhile, fresh semen with a thin consistency was diluted in a ratio of 1:1. The setting of SCA for motility analysis includes using a green filter and pH1 contrast phase. A total of 3–4 µl mixture of semen and diluent samples were placed on a prewarmed glass object and covered with a glass cover. The number of fields was taken and analyzed is 5 fields.

The parameters measured in this research were spermatozoa motility and its kinetic parameters, including progressive motility, motility, VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF, and hyperactive spermatozoa. Data were analyzed by using a T-test (IBM SPSS 16).

3. Results and discussion

3.1. Sperms motility

Observations of sperms motility and kinetic parameters of fresh semen PO bulls are listed in table 1, 2, 3, and 4.

Table 1. Progressive motility and motility of fresh semen PO bulls.

| Motility parameter of sperms | Age        | Sig.     |
|-----------------------------|------------|----------|
|                             | 4-5 years old | 5-6 years old | Sig. |
| Progressive motility (%)    | 60.8±9.7   | 67.9±12.1 | P>0.05 |
| Motility (%)                | 97.4 ± 0.9 | 92.4 ± 6.0 | P>0.05 |

In table 1, it is known that the values of progressive motility and motility of sperms between groups did not show a significant difference. The value of sperms motility is the percentage of moving sperms. Meanwhile, progressive motility of sperms is the percentage of sperms moving at a speed (VAP) >25 µm/s [5]. The motility value of sperms in all age groups was in a good category and in accordance with the motility standards of bulls. These bulls can be optimized as breeding bulls with the consideration of sperms motility >50% [4]. Sperms with good motility show the motility of the sperms to reach the ovum and penetrate the zona pellucida for fertilization [2]. Sperms motility is one of the factors that determine sperms fertilization [6].

3.2. Sperms velocity

Sperms movement speed (velocity) is one of the sperms kinetic parameters that deserves to be considered. The observations of sperms velocity from PO bull are shown in table 2.

Table 2. VCL, VSL, and VAP sperms fresh semen of PO bulls.

| Motility parameter of sperms | Age        | Sig.     |
|-----------------------------|------------|----------|
|                             | 4-5 years old | 5-6 years old | Sig. |
| VCL (µm/s)                  | 42.9±4.2   | 44.2±7.4 | P>0.05 |
| VSL (µm/s)                  | 21.9±4.7   | 26.1±9.2 | P>0.05 |
| VAP (µm/s)                  | 31.9±5.6   | 33.4±9.4 | P>0.05 |

Sperms velocity between groups did not show any significant difference (P>0.05). The sperms velocity parameter is related to the speed of sperms to reach the ovum. The parameters of progressive motility and velocity are very influential on fertilization [7]. The VSL parameter describes a more progressive sperms movement than VCL [8]. The VCL value more describes the vigor of the sperms.
movement strength. Many factors that affect the velocity of sperms include osmolarity, pH, an energy source of sperms, viscosity of diluents, etc [7].

3.3. Linearity (LIN), Straightness (STR), Wobble (WOB)

The swimming patterns of sperms between groups of PO bulls are shown in table 3.

| Motility parameter of sperms | 4–5 years old | 5–6 years old | Sig. |
|-----------------------------|---------------|---------------|------|
| LIN (%)                     | 50.7±6.4      | 57.9±13.0     | P>0.05 |
| STR (%)                     | 68.4±3.5      | 76.8±9.8      | P>0.05 |
| WOB (%)                     | 74.0±6.8      | 74.7±8.9      | P>0.05 |

In table 3, it is known that between groups, the values of LIN, STR, and WOB did not show a significant difference (P>0.05). The parameters LIN, STR, and WOB are an indication of a good swimming pattern of sperms movement. These three parameters are derived values of the velocity parameter. The LIN and STR values indicate sperms progressively moving. Several previous studies stated that the swimming pattern parameter showed a low correlation with the fertility of bulls [5, 9].

3.3.1 Amplitude lateral head and beat cross frequency

The movement pattern of the spermatozoa head can be seen from ALH and BCF parameters. During the observation, the head movement patterns of the sperms are shown in table 4.

| Motility Parameter of Sperms | 4–5 years old | 5–6 years old | Sig. |
|-----------------------------|---------------|---------------|------|
| ALH (µm)                    | 1.7±0.2       | 1.7±0.2       | P>0.05 |
| BCF (Hz)                    | 8.2±1.2       | 10.0±1.1      | P<0.05 |
| Hyperactive (%)             | 4.1±1.8       | 3.3±2.7       | P>0.05 |

In table 4, it is known that the BCF value between groups of bulls 5–6 years old is significantly higher than group 4–5 years old. This is probably caused by bulls aged 5–6 years more mature in their reproductive function so that the quality of the semen is better. The BCF value indicates the frequency of sperms passed their average movement trajectory. A high BCF value indicates a regular pattern of sperms movement and a good indication of the quality of sperms. Meanwhile, ALH is the farthest distance (side) of the sperms head movement. This is not a good parameter for quality sperms. A high ALH value indicates a sharp deviation of the sperm's head so that it tends to form a star-shaped pattern. The results of previous studies stated that ALH and BCF parameters are not correlated and cannot predict the fertility of bulls [9,10]. However, other studies suggested that this parameter correlates with the fertility of bulls [11].

The sperms hyperactivity parameter is a motility parameter that can describe other motility parameters. The criteria for hyperactive sperms are showing high VCL and ALH, but low in LIN. Each CASA has a different setting that can be determining the criteria for sperms hyperactivity. The tool was used in this research activity is SCA v. 2. 1, which has criteria hyperactive sperms; VCL >150 µm/s, ALH >5 µm, and LIN <50%. In table 4, it is known that there is no difference in the value of sperms hyperactivity between the age groups of bulls. In vitro hyperactivity is a good indication of semen quality. However, if it is associated with cryopreservation semen during storage, an increasing hyperactive sperm is an indication of poor (low) quality of sperms. Hyperactive sperms cannot survive last long because they run out of energy for moving [3].
4. Conclusion
Age factor influences the sperms kinetic parameter (BCF). The group 5–6 years old bull has a more regular pattern of sperms movement than the 4–5 years old group.

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