Meta data analysis of conception rate in relation to sperm motility in Madura superior bulls

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Abstract. Madura bulls are Indonesian germplasm with a very high capacity to adapt to dry environments. Madura bulls come from a crossbreed between Zebu (Bos indicus) and banteng (Bos javanicus). One of the breeding strategies of Madura cattle is the use of artificial insemination (AI) with frozen semen. Regarding sperm motility as one of the standard parameters of good semen quality, it is good to know the reliability of sperm motility with the bull fertility rate. This study aimed to determine the conception rate percentage (%CR) relation to sperm motility in Superior Madura bulls. The frozen semen from eight Madura bulls belonging to the National Singosari and Lembang AI centre were used. They were classified based on the selected field reproductive efficiency data from the year 2018 until 2020. Sperm motility was evaluated using Computer Assisted Sperm Analysis (CASA). The data were analyzed using one-way ANOVA and Pearson correlation. The data showed that %CR was significantly higher (P<0.05) and positively correlated with sperm motility. It is proved that sperm motility represents good quality sperm as one of the fertility parameters in Madura bulls.

1. Introduction
On the island of Madura, one of Indonesia’s indigenous cattle is the Madura cattle. Madura cattle are a breed of beef cattle that thrives in arid environments and barren terrain. These cattle have a good tolerance for poor feed, heat stress, and a tiny body with good reproductive capacity. Madura cattle are native Indonesian cattle thought to be a cross between Zebu cattle (Bos indicus) and banteng cattle (Bos javanicus). Zebu cattle have contributed genetic traits such as climate stress tolerance, tick resistance, and long-term natural and environmental selection. Thus, allowing Madura cattle to become a cattle nation that can grow and has an excellent capacity to adapt to the environment, particularly in Madura Island [1]. Madura cattle can be grouped into three groups based on their uses: Karapan cattle (race cattle), Sonok cattle, and beef cattle [2,3]. Due to natural isolation and environmental factors, Madura
cattle have evolved into a native beef cattle breed. They have distinct features that set them apart from other Indonesian beef cattle varieties. Adverse selection, such as productive cattle slaughter and inbreeding variables, has resulted in a decline in Madura cattle production. One of the breeding strategies to increase and maintain the Madura cattle is artificial insemination (AI) with frozen semen. AI can help increase livestock genetic quality and efficiency in reproduction [4]. The success of AI is influenced by many factors, one of which is bulls fertility [5]. Superior bulls are selected based on good quality frozen semen and proven by reproductive efficiency in the field [6]. Production of Madura frozen semen in Lembang and Singosari AI Centre is based on Indonesian National Standard (SNI) number 4869.1:2017 for Bull Frozen Semen. The post thawing motility (PTM) score is more excellent than 40% when the individual value is greater than 2, and the sperm concentration is more significant than $2 \times 10^6$ AI dosage [7]. However, frozen semen from superior bulls that pass the breeding soundness examination (BSE), and quality control processes still showed various reproductive performances [8,9]. This study aimed to determine the conception rate percentage (%CR) relation to sperm motility in Superior Madura bulls. Thus, it could determine the reliability of sperm motility parameters as a fertility marker for superior bulls as evidenced by the presence or absence of correlation with the conception rate data in the field.

2. Materials and methods

2.1. Experimental animals
The Madura bulls were chosen from the national AI centres in Lembang and Singosari, based on the statistics from the field conception rate. Then, the frozen semen from eight Madura bulls were divided into three fertility levels which were high fertile (HF), medium fertile (MF), and low fertile (LF). The data was then gathered and analyzed. All animals were in good condition, the bulls were appropriately handled, and the frozen semen output was standardized between the two AI centres.

2.2. Sperm motility evaluation
A study of sperm motility was carried out objectively using computer-assisted sperm analysis (CASA) (Sperm Vision 3.7, Minitube, Germany). Ten microliters of the thawed semen were diluted into ninety microliters of saline solution. Then, ten microliters of the aliquots dripped onto the object-glass and then covered with a cover glass. The evaluations were conducted in four different optical fields. The measured parameters were sperm motility: total motility, progressive motility, and sperm kinematic: curvilinear velocity (VCL), straight-line velocity (VSL), average pathway velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), hyperactivity, the amplitude of lateral displacement (ALH) and beat cross frequency (BCF). The data were analyzed using IBM SPSS statistics 26 with One Way ANOVA between three groups of conception rate.

2.3. Statistical analysis
A completely randomized design was employed in this study with different replications. The collected data were analyzed using analysis of variance at a 95% significance level and 99% for the correlation analysis. If the groups differed significantly, Duncan's Multiple Range Test was used for additional analysis. The correlation between %CR or fertility rate and sperm motility was analyzed using Pearson correlation. The data were examined using SPSS Version 26.0 (IBM Corp., New York, USA) and showed as mean ± standard deviation (SD).

3. Results and discussions

3.1. The grouping of Madura bull based on the conception rate data
The Madura cattle used in this study can be grouped into three fertility groups, namely high, medium and low fertile, as shown in Table 1. Classification is carried out based on the results of the %CR.
calculation using the metadata of ISIKHNAS from the year 2018 until 2020. The range of AI for each Madura bull is 100 to 1500 times AI. Each group of Madura cattle semen was evaluated for motility characteristics.

3.2. Sperm motility and kinetics in Madura frozen semen using CASA in a different conception rate
Sperm motility and kinematics of Madura cattle's frozen semen with different conception rates using CASA are presented in Table 2. For the total motility and progressive motility of frozen semen, there were significant differences between HF and MF and LF bulls (p<0.05). Meanwhile, the percentage of total motility and progressive spermatozoa in Madura cattle with MF and LF groups were not significantly different. Total sperm motility ranged from 50.90 ± 1.58% to 56.33 ± 4.24%. Progressive sperm motility ranged from 45.57 ± 2.07% to 50.32 ± 3.18%. The total motility and progressive motility between MF and LF was not significantly different because the %CR between MF and LF was not much different. Thus, this group of Madura cattle classified as low fertility based on the %CR data that we obtained in this study may have fertility that is actually still quite good. In addition, it was also caused by the semen of Madura bull from the AI centre, which was semen that has passed the SNI for frozen semen production. According to the requirements of SNI 4869-1: 2017, the standard of the minimum sperm motility is 40% for post thawing frozen semen. Thus the result of sperm motility in this study is within normal motility [7]. Nalley et al. [10] stated that post thawing motility for Madura cattle is around 40% to 59%. Karunakaran et al. [11] also showed that, in general, the motility of spermatozoa decreased by about 34%-46% after the freezing and thawing processes.

### Table 2. Sperm motility and kinematics Madura bull's frozen semen using CASA in different conception rate

| Parameter             | Treatment | HF            | MF            | LF            |
|-----------------------|-----------|---------------|---------------|---------------|
| Total motility (%)    |           | 56.33 ± 4.24a | 51.54 ± 2.75b | 50.90 ± 1.58bc |
| Progressive motility (%) |         | 50.32 ± 3.18a | 45.60 ± 2.60b | 45.57 ± 2.07bc |
| VCL (µm/s)            |           | 140.85 ± 28.69 | 139.45 ± 17.82 | 132.45 ± 12.70 |
| VAP (µm/s)            |           | 83.76 ± 8.68  | 85.10 ± 11.28 | 86.31 ± 13.40 |
| VSL (µm/s)            |           | 56.07 ± 8.31  | 61.35 ± 12.46 | 65.56 ± 19.51 |
| BCF (Hz)              |           | 31.40 ± 5.73  | 29.65 ± 2.39  | 29.48 ± 1.58  |
| ALH (µm)              |           | 5.10 ± 0.63   | 4.78 ± 0.36   | 4.10 ± 0.36   |

Data show all mean±SD. Means in lines with different superscripts differ significantly at p<0.05.

This study showed that for the total motility and progressive spermatozoa of the semen of Madura cattle in the HF, MF, and LF groups, the percentage value was above 40%. It is caused by the production of adenosine triphosphate (ATP) in the mitochondria of spermatozoa that is a source of energy for spermatozoa movement. High motility of spermatozoa is caused by the proper functioning of mitochondria of spermatozoa. Mitochondrial damage is known to reduce the percentage of sperm survival [12]. A matrix helix is found in the middle of the sperm-mitochondria (mid-piece). Mitochondria's primary function is to create ATP through oxidative phosphorylation and give energy.
for spermatozoa to move around. A mitochondrial malfunction that affects the structure or genome of the mitochondria can decrease spermatozoa motility [13].

There was no significant difference in sperm kinematics in terms of conception rate (p>0.05). Some sperm kinematics characteristics are linked to fertility [14], including VCL, VAP, VSL, BCF, and lateral head displacement amplitude (ALH). The VCL, VSL and VAP values required for sperm to penetrate the ovum are VCL >70 m/s, VSL >45 m/s, and VAP >45 m/s, respectively [15]. BCF values of >20 Hz and ALH values of 2.5 to 6.5 µm suggest that sperm mobility is excellent and fertility is high [16]. The VCL, VAP, and VSL values of all group fertility rates were higher than the minimal criteria for fertilization in this study. The ALH value indicates the width of the sperm head movement to the side of the track. The high ALH value shows the sperm head movements that tend to rotate and hyperactivity. Conversely, BCF is a good indication in the analysis of semen quality. This parameter describes the number of spermatozoa movements across the average pathway. The higher the BCF showed the progressive and regular sperm trajectory (not rotating) [17].

3.3. Relationship between fertility rate and the sperm motility

The correlation between fertility rate based on the %CR and sperm motility was presented in Table 3. The result revealed that fertility rate and sperm motility were significantly different (P>0.01). This study showed that %CR has a strong positive correlation to sperm motility. Thus, the higher sperm motility showed the higher fertility rate. Rosyada et al. [9] also stated that fertility and sperm motility are correlated.

| Parameter                  | Fertility rate | Total Motility | Progressive Motility |
|----------------------------|----------------|----------------|----------------------|
| Fertility rate             | 1              | 0.532**        | 0.514**              |
| Total Motility             | 1              | 0.780**        |                      |
| Progressive Motility       |                |                | 1                    |

Significant: p<0.05*, p<0.01 **

4. Conclusion

There is a significant relationship between the conception rate (%CR) and sperm motility in Superior Madura bulls. Thus, motility is reliable as a marker of bull fertility, as evidenced by the correlation with the conception rate data in the field. Besides being commonly used to assess semen quality, sperm motility is the best parameter to evaluate or select superior bull fertility.

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