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Kinematic Characteristics of Spermatozoa in Different Goat Breeds Using Computer Assisted Semen Analysis (CASA)

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ABSTRACT. Advances in semen quality analysis technology are developing rapidly. Computer-based semen analysis technology provides a more objective assessment of spermatozoa motility by measuring various kinematic movements of spermatozoa cells. This study aimed to analyze differences in spermatozoa movement patterns using Computer-Assisted Semen Analysis (CASA) in various goat breeds. This study used frozen semen from four goat breeds: Boer, Ettawa crossbred (PE), Saanen, and Anglo-Nubian. Parameter observations were conducted on thawed frozen semen from two straw samples for each group, consisting of six replicates from each breed. The observed sperm kinetic movement characteristics were VAP, VCL, VSL, DAP, DCL, DSL, STR, LIN, WOB, ALH, and BCF. The results showed that sperm movements did not differ significantly ($P>0.05$) in VAP, VCL, VSL, DAP, DCL, and WOB. There were significant differences in DSL, the highest in Saanen 21.99±3.01 µm/second, the highest STR and LIN values in Boer 0.69±0.04% and 0.45±0.03%, the longest ALH Anglo Nubian 6.19±0.39 µm, and the largest BCF Boer 25.56±1.87 Hz. This study concludes that differences in goat breeds affect the kinematic movement of spermatozoa as measured by CASA in the parameters of DSL, STR, LIN, ALH, and BCF.

Keywords: buck, CASA, kinematic movement, spermatozoa

INTRODUCTION

Goats are one of the most widely distributed animal species worldwide, offering a variety of uses and benefits (Pribadi et al., 2022). Increasing goat productivity can be achieved through the use of reproductive technology. Artificial Insemination (AI) is one such technology that can be applied to increase livestock productivity. The success of an AI program in livestock is influenced by the quality of fresh semen (Rokana et al., 2022). Moradpour (2019) stated that AI has proven to be the most effective tool for genetic improvement in animals, particularly in the cattle, sheep, and goat industries. Therefore, accurate methods for evaluating semen quality are necessary.

Evaluation of sperm cell motility in the field is still carried out manually. Isnaini et al. (2020) reported a method for evaluating the progressive motility of individual sperm under a light microscope at 400× magnification. Semen samples showing individual motility ≥70% can be further processed. A more accurate method for assessing sperm motility is Computer-Assisted Semen Analysis (CASA). CASA provides accurate and objective measurements of sperm kinematics, providing comprehensive information on the kinematic characteristics of ejaculate based on the analysis of individual sperm cell movement (Tanga et al., 2021; Seyoum and Lemma, 2022). Hyperactivation (HA) is a significant phenomenon of sperm established through capacitation (the process of obtaining the capacity to fertilize). HA comprised of behavioural changes in the flagellar waveform such as increased amplitude of flagellar beating, reduced beat frequency, and non-progressive side-to-side. HA has been connected to fertilization in animals. HA is the only kinetic parameter of clear clinical value. The percentage hyperactivation can be recognized (>20% HA associated with potential fertility) by using a combination of kinematic parameters. Head centroid tracking of sperm and mainly flagellar movement study is done. Sperm morphology is positively correlated to predict fertilization in both natural and assisted reproduction (Agustinus and Pakpahan, 2020).

Research on spermatozoa motility patterns using the CASA method is essential for determining the individual movement of spermatozoa. This study aimed to examine differences in spermatozoa movement patterns across goat breeds using the CASA method.

MATERIALS AND METHODS

Location and Time

This research was conducted in October 2023 at the Reproduction Laboratory of the Applied Zoology Research Center, Animal Reproduction Research Group – National Research

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and Innovation Agency (BRIN), Cibinong District, Bogor Regency, West Java.

Research Material

The livestock used in this study were frozen semen from four goat breeds: Boer, Ettawa crossbred (PE), Saanen, and Anglo-Nubian, each aged 2-7 years. The semen was collected at the Lembang Artificial Insemination Center. Semen evaluation was performed on thawed frozen semen.

Research Method

The experimental design used was a non-factorial completely randomized design (CRD). Treatments were four goat breeds, with six replications of semen collection with artificial vagina method. Samples were taken from two frozen semen straws from each semen collection batch.

Spermatozoa kinematic movement parameters measured using the CASA method and SpermVision 3.7 software (Minitube–Germany®) included: average path distance (DAP), curve linear distance (DCL), straight linear distance (DSL), velocity average pathway (VAP), velocity curve linear (VCL), velocity straight linear (VSL), straightness (STR), linearity (LIN), wobble (WOB), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF) (Afriani et al., 2024).

Research Procedure

Thawing Procedure: Frozen semen straws were placed in a water bath containing warm water at 37°C for 30 seconds. The thawed semen was then transferred into a 1.5 ml microtube and placed on a heating table maintained at 37°C.

Sperm Motility Pattern Evaluation Using CASA Procedure: A 5 µl drop of semen was placed on a microscope slide and covered with a coverslip. The sample was then evaluated using a microscope equipped with a heated stage and connected to the CASA system (Mahmoud et al., 2023).

Data Analysis

The research data were analyzed using analysis of variance (ANOVA) with IBM SPSS version 26. When significant differences among treatments were detected, Duncan's Multiple Range Test (DMRT) was performed as a post hoc analysis.

RESULTS AND DISCUSSION

The analysis of variance on spermatozoa motility patterns among different goat breeds in this study showed that DSL and STR values in PE were not significantly different ($P>0.05$) from those in Saanen. Similarly, the DSL and STR values in Saanen did not differ significantly ($P>0.05$) from those in Anglo-Nubian and Boer. However, DSL and STR values in PE were significantly different ($P<0.05$) from those in Anglo-Nubian and Boer. LIN values in PE, Saanen, and Anglo-Nubian showed no significant differences ($P>0.05$), and LIN values in Anglo-Nubian and Boer were also not significantly different ($P>0.05$). LIN values in PE and Saanen differed significantly ($P<0.05$). ALH values showed no significant differences ($P>0.05$) among PE, Saanen, and Anglo-Nubian. Additionally, Saanen did not differ significantly ($P>0.05$) from Boer. However, significant differences ($P<0.05$) in ALH were observed between PE and Anglo-Nubian. The analysis of variance for BCF revealed that PE and Anglo-Nubian differed significantly ($P<0.05$) from Saanen and Boer. In Boer, PE, Saanen, and Anglo-Nubian, the values of VAP, VCL, VSL, DAP, DCL, and WOB showed no significant differences ($P>0.05$). The results of spermatozoa motility pattern analysis using CASA in Boer, PE, Saanen, and Anglo-Nubian goats are presented in Table 1. The study by Islam et al. (2020) on Black Bengal, Boer, and Jamunapari goat breeds demonstrated highly significant differences ($P<0.01$) in DSL, DAP, VCL, VSL, and VAP parameters. However, no significant differences were observed in DCL, BCF, LIN, STR, and WOB. Genetic variation among breeds within the same species can influence not only productivity traits, such as milk yield and quality but also animal health, including fertility (Bogdaniuk et al., 2023).

The lowest mean values of VAP, VCL, and VSL were observed in PE, with sequential values of 71.92±5.71; 109.68±11.27; and 47.31±3.19 µm/sec, respectively. The highest mean VAP and VCL values were recorded in Saanen goats at 75.51±7.84 and 118.91±16.17 µm/sec, followed by Boer and Anglo-Nubian goats with VAP values of 74.91±6.41 and 73.12±7.71 µm/sec, and VCL values of 114.02±13.09 and 113.36±15.74 µm/sec, respectively. Boer exhibited the highest mean VSL value at 52.01±5.55 µm/sec, followed by Saanen and Anglo-Nubian goats at 51.95±6.07 and 49.29±6.05 µm/sec, respectively. According to Lukusa and Kabuba (2020), a minimum average trajectory speed of 50 µm/sec and progressive motility of >50% are considered acceptable. Motility parameters are classified as static, slow (>40 µm/sec), medium (>70 µm/sec), and rapid (>100 µm/sec). Kinematic thresholds were defined for VCL (>80 µm/sec), VSL (>50 µm/sec), and VAP (>25 µm/sec). VCL, VAP, and VSL are considered the most critical parameters for evaluating sperm kinematic characteristics (Islam et al., 2020). Susilawati et al. (2018) further stated that VCL is a good indicator of spermatozoa 'vigor', although it does not reflect the directionality or progression of movement. In contrast, VSL describes the velocity of spermatozoa in a single direction and serves as a primary indicator of forward movement.

Table 1. Sperm kinematic characteristics in different goat breeds

Parameters	PE	Saanen	Anglo-Nubian	Boer
Progressive Motility (%) ^{ns}	48.78±6.49	49.17±6.73	46.15±7.30	45.68±7.48
VAP (µm/sec) ^{ns}	71.92±5.71	75.51±7.84	73.12±7.71	74.91±6.41
VCL (µm/sec) ^{ns}	109.68±11.27	118.91±16.17	113.36±15.74	114.02±13.09
VSL (µm/sec) ^{ns}	47.31±3.19	51.95±6.07	49.29±6.05	52.01±5.55
DAP (µm/sec) ^{ns}	29.79±2.31	32.09±3.76	30.51±3.51	31.57±2.94
DCL (µm/sec) ^{ns}	45.70±4.69	50.85±7.74	47.55±7.10	48.31±5.83
DSL (µm/sec)	19.48±1.27^{a}	21.99±3.01^{ab}	20.46±2.73^{b}	21.85±2.71^{b}
STR (%)	0.65±0.02^{a}	0.68±0.02^{ab}	0.67±0.02^{b}	0.69±0.04^{b}
LIN (%)	0.43±0.02^{a}	0.43±0.02^{a}	0.43±0.02^{ab}	0.45±0.03^{b}
WOB (%) ^{ns}	0.65±0.02	0.64±0.03	0.64±0.03	0.66±0.02
ALH (µm)	6.10±0.55^{b}	5.78±0.54^{ab}	6.19±0.39^{b}	5.45±0.73^{a}
BCF (Hz)	25.53±1.37^{a}	26.21±2.11^{b}	23.75±1.68^{a}	25.56±1,87^{b}

Note: ^{a,b} Different lowercase letters in the same column indicate a significant difference between treatments (P<0.05); ns = not significant. dance average path distance (DAP), curve linear distance (DCL), straight linear distance (DSL), velocity average pathway (VAP), velocity curve linear (VCL), velocity straight linear (VSL), straightness (STR), linearity (LIN), wobble (WOB), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF).

The highest mean values of DAP, DCL, and DSL were observed in Saanen, with respective values of 32.09±3.76; 50.85±7.74; and 21.99±3.01 µm/sec, while the lowest values were found in PE at 29.79±2.31; 45.70±4.69; and 19.48±1.27 µm/sec, respectively. Boer and Anglo-Nubian followed with mean DAP values of 31.57±2.94 and 30.51±3.51 µm/sec, DCL values of 48.31±5.83 and 47.55±7.10 µm/sec, and DSL values of 21.85±2.71 and 20.46±2.73 µm/sec, respectively. The DCL, DAP, and DSL parameters measure the distance travelled by spermatozoa per second, which may indicate differences in motility patterns between X and Y spermatozoa (Afriani et al., 2024).

The highest mean STR values, in descending order, were found in Boer 0.69±0.04%, Saanen 0.68±0.02%, Anglo-Nubian 0.67±0.02%, and PE 0.65±0.02%. The highest mean LIN value was also observed in Boer at 0.45±0.03%, while PE, Saanen, and Anglo-Nubian shared similar mean LIN values of 0.43±0.02%. WOB values were highest in Boer at 0.66 ± 0.02%, followed by PE at 0.65±0.02%, with Anglo-Nubian and Saanen goats both at 0.64±0.03%. The highest mean BCF values were recorded in Saanen 26.21±2.11 Hz, followed by Boer 25.56±1.87 Hz, PE 25.53 ± 1.37 Hz, and Anglo-Nubian goats 23.75 ± 1.68 Hz. According to Diansyah et al. (2022), sperm motility and progressive movement are determined by LIN, STR, and WOB values. Meanwhile, BCF is used to measure the frequency of spermatozoa movement and serves as an indicator of flagellum (tail) activity (Afriani et al., 2024). The highest mean ALH values were observed in Anglo-Nubian 6.19±0.39 µm, followed by PE 6.10±0.55 µm, Saanen 5.78±0.54 µm, and Boer 5.45±0.73 µm. According to Hariono et al. (2022), ALH reflects the spermatozoa's ability to penetrate cervical mucus and the cells surrounding the oocyte by indicating the strength of tail oscillation along with the frequency of cell rotation, both of which contribute to the progressive movement of spermatozoa.

CONCLUSION

It is concluded that different goat breeds influence spermatozoa motility patterns as measured by CASA, particularly in the parameters of straight linear distance (DSL), straightness (STR), linearity (LIN), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF). The motility patterns of male goat sperm cells can be used to predict fertilization during natural mating and artificial insemination.

CONFLICT OF INTEREST

The authors declared that all the material presenting in this manuscript are no conflict of interest regarding any financial issues.

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