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Research Article

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A Comparison of Fresh Semen Characteristics and Sperm Kinematics in Etawah Grade and Anglo-Nubian Bucks (*Capra hircus*)

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ABSTRACT

Background: Artificial insemination (AI) programs in bucks depend on the availability of high-quality fresh semen to ensure reproductive efficiency and optimal genetic dissemination. Differences in breed characteristics may influence semen quality and sperm movement patterns, particularly in tropical conditions.

Aims: This study aimed to compare the fresh semen characteristics and sperm kinematics parameters in Etawah Grade and Anglo-Nubian buck groups.

Methods: Four bucks aged 3–4 years, comprising two Etawah Grade and two Anglo-Nubian bucks were used, with five ejaculates collected from each individual. Semen collection was performed twice a week using an artificial vagina under standardised management conditions. Fresh semen was evaluated macroscopically (volume, colour, consistency, and semen pH) and microscopically (motility, viability, abnormality, acrosome integrity, and sperm concentration). Sperm motility and kinematics were analysed using computer-assisted semen analysis (CASA) to obtain objective measurements of sperm movement patterns.

Results: The breed had a significant impact on slow sperm motility, sperm concentration, acrosome integrity, and certain kinematic parameters, including ALH, BCF, HAC, LIN, and STR ($p < 0.05$). However, there were no significant differences between breeds in terms of total and progressive motility, as well as fast, circle, local, and immotile motility, viability, and the kinematic parameters VCL, VSL, VAP, DCL, DSL, and DAP ($p > 0.05$). Additionally, macroscopic semen characteristics and sperm abnormalities were similar across breeds.

Conclusion: Breed-related differences were observed in specific semen quality and sperm kinematic parameters, notably sperm concentration, acrosome integrity, slow motility, and movement trajectory characteristics. In contrast, overall sperm motility was similar between Etawah Grade and Anglo-Nubian bucks.

INTRODUCTION

Semen production plays an important role in supporting the implementation of artificial insemination (AI) programs in small ruminants, including goats. The application of goat AI has been developed, including in Etawah Grade and Anglo-Nubian breeds. These breeds are widely cultivated in Indonesia because they are dual-purpose, producing both meat and milk. These breeds have different characteristics. Etawah Grade is known to be well-adapted to tropical environments (Ali et al., 2023), whereas Anglo-Nubian is larger and often associated with high genetic potential.

The success of AI programs is dependent on the quality of fresh semen, which are used as initial indicators for semen quality testing to predict male freezability and fertility. Breed-related factors in each male can affect semen quality by altering the genetic structure of sperm cells, thereby affecting the ability to

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fertilize oocytes (Khan et al., 2024), as well as seminal plasma components (Fernandez-Novo *et al.*, 2021). Xu et al. (2022) reported substantial individual variation within breeds in sperm metabolomic and lipidomic profiles associated with cryotolerance. However, in the present study, breed variation can influence semen characteristics and sperm kinematics under standardised management conditions.

According to the Indonesian National Standard (SNI) 4869-3:2023 on frozen buck semen, fresh semen must meet specific requirements for frozen semen production, including ≥ 70% progressive sperm motility and ≤ 15% sperm abnormality. The quality of semen after thawing must have ≥ 40% and concentration ≥ 50 million sperm per dose to be declared suitable for AI (BSN, 2023). Evaluating the characteristics of fresh semen and sperm kinematics is important to ensure that semen quality meets SNI and supports the success of AI applications in Etawah Grade and Anglo-Nubian bucks.

Etawah Grade and Anglo-Nubian breeds have the potential to show significant differences in fresh semen characteristics, both due to environmental adaptation factors and genetic or individual variations between animals. Previous studies have reported the influence of breeds factor variation on semen quality across several cattle breeds (Berry et al., 2019). Research on the characteristics of fresh semen and sperm kinematics between Etawah Grade and Anglo-Nubian breeds has not been widely reported. This study aimed to evaluate the characteristics of fresh semen and analyze sperm kinematic parameters in Etawah Grade and Anglo-Nubian breeds.

MATERIALS AND METHODS

The study was conducted at the Artificial Insemination Centre (AIC) in Lembang, West Bandung Regency, and at the Reproductive Rehabilitation Unit (URR) at IPB University. The study was conducted from July to September 2025 and was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University (approval number 351/KEH/SKE/VII/2025).

Semen collection

Semen was collected twice weekly in the morning using an artificial vagina. The study involved four mature bucks, specifically two Etawah Grade and two Anglo-Nubian bucks, aged 3–4 years. Five ejaculates were obtained from each buck, totaling 20 ejaculates. Within each breed, the two bucks served as independent biological subjects, while repeated ejaculates from the same buck were considered repeated measurements rather than independent biological replicates. This experimental design was implemented to account for within-buck variation in semen characteristics. Fresh semen was transported to the laboratory and maintained at 32°C until evaluation.

Evaluation of fresh semen characteristics

Fresh semen was evaluated for macroscopic (volume, colour, consistency, and pH) and microscopic qualities (motility, viability, sperm abnormalities, acrosome integrity, and sperm concentration). Semen volume, colour, and consistency were observed according to Rosnizar et al. (2021). Colour and consistency were assessed using a subjective scoring system, where semen colour was scored as 1 (yellow), 2 (milky), and 3 (creamy), while semen consistency was scored as 1 (watery), 2 (moderate), and 3 (thick). The degree of semen acidity was measured using a pH meter by inserting the probe into the semen.

Sperm motility and kinematic parameters were evaluated using the AndroScope CASA system (Minitube, Germany). Semen samples were placed in a chamber maintained at 37°C, with a final dilution ratio of 1:49. For each sample, four microscopic fields were analyzed, with approximately 250–350 spermatozoa assessed per field. Sperm motility subcategories were defined based on CASA operational thresholds: immotile sperm exhibited an HAC of less than 0.087 µm; local motility was characterized by a VCL of less than 48 µm/s and a VSL of less than 24 µm/s; circular motility was defined by a motility radius of 9–90 µm and a rotation greater than 0.70; slow motility was defined by a VCL of less than 120 µm/s; and fast motility included all remaining motile spermatozoa. Sperm concentration was determined using a photometer SDM 6 (Minitube, Germany) by placing semen in a cuvette containing 0.9% physiological NaCl at a ratio of 1:500. Sperm viability and abnormality were assessed using eosin-nigrosine staining, as described by Arifiantini (2012).

Statistical Analysis

The research findings are presented in tables as means ± standard error of the mean (SEM). Data were analyzed using a linear mixed-effects model, with breed designated as a fixed effect and buck identity as a random effect to account for repeated semen collections from the same individual. The five ejaculates collected from each buck were considered repeated measurements. Prior to conducting the parametric analysis, the assumptions of normality and homogeneity of variance were evaluated. For the ordinal scoring variables, specifically semen color and consistency, differences between breeds were analyzed using the

Mann–Whitney U test. Statistical significance was determined at $p < 0.05$. Statistical analyses were executed using the Statistical Package for the Social Sciences (SPSS) version 30 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Characteristics of fresh semen in Etawah Grade and Anglo-Nubian bucks

The macroscopic characteristics of fresh semen, such as volume, colour, consistency, and pH, did not exhibit significant differences between Etawah Grade and Anglo-Nubian bucks ($p > 0.05$) (Table 1). However, there was a tendency for semen volume and pH to be higher in Anglo-Nubian than in Etawah Grade bucks. Notably, the semen volume in both breeds surpassed the upper limit of 2.0 mL as reported by Herrera Vargas et al. (2023). This variation may be attributed to breed and seasonal effects (Barbas et al., 2024), as well as age-related differences in semen characteristics (Pascal et al., 2024).

The results indicated no significant differences in total and progressive sperm motility between the Etawah Grade and Anglo-Nubian bucks, as presented in Table 2. Sperm motility is affected by various cellular processes, such as energy metabolism, mitochondrial activity, and sperm membrane integrity, all of which contribute to the ability of sperm cells to sustain movement. Both glycolysis and mitochondrial oxidation are involved in adenosine triphosphate (ATP) production in buck sperm; however, mitochondrial activity is more crucial for maintaining progressive motility (Setiawan et al., 2023).

As illustrated in Table 2, there were no significant diffrences between breeds in fast, circle, local, and immotile sperm motility, whereas slow motility differed significantly between breeds ($p < 0.05$). The significant difference in slow motility, despite the lack of significant differences in total and progressive motility, implies that the distribution of sperm movement patterns may vary between breeds without affecting the overall motility. Variations in sperm motility patterns may be linked to differences in sperm membrane integrity, flagellar function, or mitochondrial activity; however, these mechanisms were not directly assessed in the present study. Sperm concentration also differed significantly

Table 1. Fresh semen macroscopic quality of Etawah Grade and Anglo-Nubian breed

Semen Variable	Etawah Grade	Anglo-Nubian	p-value
Volume	2.43 ± 0.21	2.78 ± 0.19	0.09
Colour	2.30 ± 0.21	2.80 ± 0.29	0.17
Consistency	3.00 ± 0.00	2.90 ± 0.10	0.32
pH	6.63 ± 0.02	6.65 ± 0.02	0.08

Table 2. Sperm motility and concentrations of fresh semen from Etawah Grade and Anglo-Nubian breed

Sperm Variable	Etawah Grade	Anglo-Nubian	p-value
Total Motility (%)	83.41 ± 2.03	76.36 ± 2.95	0.18
Progressive Motility (%)	80.63 ± 2.29	73.33 ± 2.86	0.23
Fast Motility (%)	25.23 ± 3.08	21.40 ± 1.74	0.12
Slow Motility (%)	2.76 ± 0.40^{b}	3.03 ± 0.34^{a}	0.02
Circle Motility (%)	52.63 ± 2.87	48.89 ± 2.30	0.44
Local Motility (%)	2.73 ± 0.42	3.03 ± 0.46	0.21
Immotile (%)	16.65 ± 2.06	23.64 ± 2.95	0.19
Concentrations (x 10^{6} sperm /mL)	2394.50 ± 34.80^{a}	2259.90 ± 42.97^{b}	0.03

Values with different superscripts in the same row indicate significant differences among breeds.

between Etawah Grade and Anglo-Nubian bucks ($p < 0.05$) (Table 2).

Sperm concentration differed significantly between Etawah Grade and Anglo-Nubian bucks ($p < 0.05$) (Table 2). The sperm concentrations observed in this study were within the normal range, indicating a satisfactory reproductive status. The average sperm concentration across several breeds was 1,936 × 10^6 mL^{-1}, with a range of 78.0–4,320 × 10^6 mL^{-1} (Herrera Vargas et al., 2023). This significant variation in sperm concentration may be attributed to differences in testicular size, spermatogenic capacity, and individual reproductive characteristics. Atawalna et al. (2022) reported similar findings, demonstrating that testicular morphometrics, including weight, length, and volume, significantly correlate positively with sperm reserves and production in small ruminants.

Spermatogenic activity is heavily influenced by testosterone, which is crucial for maintaining sperm formation and maturation in the seminiferous tubules. Testosterone acts through Leydig cells to stimulate mitosis; therefore, its stability is a critical factor in spermatogenesis. Miyaso et al. (2022) elucidated that testosterone produced by Leydig cells under LH stimulation is vital for the initiation and maintenance of spermatogenesis. In conjunction with FSH, testosterone activates Sertoli cells to produce androgen-binding protein, thereby sustaining high testosterone levels around the seminiferous tubules and supporting optimal germ cell differentiation. These endocrine mechanisms may contribute to variations in sperm production between individuals or breeds; however, reproductive hormone concentrations were not measured in the present study, precluding confirmation of their direct contribution to the observed difference in sperm concentration.

Sperm quality is also affected by nutrient intake, including proteins, minerals, and vitamins, which are essential for supporting spermatogenesis and maintaining the integrity of sperm cell membranes. Esfiokhi et al. (2023) reported that zinc supplementation significantly enhanced sperm motility and membrane integrity, as evidenced by improved antioxidant parameters. A combination of selenium and vitamin E injections in bucks can increase sperm concentration to 2.101 × 10^6 mL^{-1} (Murdiyanti et al., 2024). Additional factors, such as age, breed, physiological condition, individual variability, and the timing and frequency of semen collection, also influence the number of sperm per milliliter (Ben Moula & El Amiri, 2022). Therefore, the significant difference in sperm concentration observed in the present study may result from a combination of breed-related and individual factors rather than breed alone.

Sperm viability did not differ significantly between the breeds (Table 3). This parameter, which indicates the integrity and functional condition of sperm cells, can be influenced by environmental and seasonal factors. Previous studies in goats have shown that these factors affect sperm motility, viability, and other semen characteristics (Isnaini et al., 2020; El-Sherbiny et al., 2023). Given the absence of significant differences, the observed variation in sperm viability should not be construed as evidence of a breed effect on sperm viability. Both breeds were assessed under identical management conditions at the Lembang AIC, which is characterized by a relatively cool highland environment. This observed variation may be attributed to individual variability and other physiological factors. Further research involving a larger number of independent bucks and diverse environmental conditions is necessary to elucidate the contributions of breed and environment to the sperm viability.

However, acrosome integrity differed significantly between breeds ($p < 0.05$) (Table 3). This parameter is a crucial indicator of sperm functional competence, as the acrosome contains enzymes essential for interaction with and penetration of the oocyte during

Table 3. Sperm viability, acrosome integrity, and abnormalities in fresh semen from Etawah Grade and Anglo-Nubian breeds

Sperm Variable	Etawah Grade	Anglo-Nubian	p-value
Viability (%)	88.91 ± 1.48	82.56 ± 2.16	0.08
Acrosome Integrity (%)	98.1 ± 0.27[a]	95.93 ± 1.04[b]	0.01
Abnormality (%)	7.68 ± 1.00	6.96 ± 0.50	0.97

Values with different superscripts in the same row indicate significant differences among breeds.

fertilization. The significant difference in acrosome integrity suggests an association with breed under the conditions of this study. The acrosome integrity observed in this study exceeded the range reported by Keller et al. (2024), who documented acrosome integrity in fresh semen from Malya and Boer bucks ranging from 85% to 88%. The relatively high acrosome integrity in both breeds indicates that the ejaculates generally maintained well-preserved acrosomal structures. Nonetheless, the biological mechanisms underlying the observed breed differences remain undetermined, as membrane composition, antioxidant status, and other molecular indicators were not directly assessed.

Sperm abnormalities did not differ significantly between the breeds (Table 3). The percentage of sperm abnormalities in this study conformed to the SNI standards (≤ 15%) (BSN, 2023). Herrera Vargas et al. (2023) reported an average sperm abnormality rate of 7.75% (2.0–32.50%) in several breeds. The lack of significant differences suggests that sperm morphological quality was comparable between the two breeds under the conditions of this study. Chenoweth (2005) noted that primary sperm abnormalities are generally genetic and related to disturbances in spermatogenesis in the testes, whereas secondary and tertiary abnormalities are often attributed to environmental factors or semen handling management.

Sperm kinematics of fresh semen of Etawah Grade and Anglo-Nubian bucks

The kinematic analysis of fresh semen demonstrated significant breed differences in ALH, BCF, HAC, LIN, and STR ($p < 0.05$), while VCL, VSL, VAP, DCL, DSL, and DAP did not exhibit significant differences between Etawah Grade and Anglo-Nubian bucks ($p > 0.05$) (Table 4). The lack of variation in the primary velocity parameters (VCL, VSL, and VAP) suggests that sperm from both breeds displayed similar overall movement velocities. Conversely, the differences in ALH, BCF, HAC, LIN, and STR indicate that breed-related variation was more pronounced in the pattern and trajectory of sperm movement rather than in movement speed.

ALH differed significantly between breeds ($p < 0.05$) (Table 4). The ALH values observed in this study were consistent with the range reported for fresh buck semen. Anand et al. (2016) documented an ALH of 5.15 µm in fresh Sirohi buck semen, which decreased to 3.57 µm after freezing and thawing, suggesting that sperm trajectory can be affected by cryopreservation-related membrane and osmotic stress. The breed difference in ALH observed here may therefore reflect variation in lateral head displacement and flagellar movement patterns, although the underlying cellular mechanisms were not directly assessed.

BCF also showed significant differences between breeds ($p < 0.05$) (Table 4). The values obtained in this

Table 4. Fresh semen kinematics in Etawah Grade and Anglo-Nubian breeds

Variable	Etawah Grade	Anglo-Nubian	p-value
VCL (µm/s)	236.65 ± 4.26	229.62 ± 7.32	0.28
VSL (µm/s)	97.59 ± 2.61	105.12 ± 3.76	0.10
VAP (µm/s)	117.87 ± 3.04	122.56 ± 3.79	0.08
DCL (µm)	48.92 ± 1.45	49.80 ± 1.62	0.55
DSL (µm)	18.50 ± 1.07	21.84 ± 1.51	0.18
DAP (µm)	23.94 ± 1.15	26.71 ± 1.53	0.25
ALH (µm)	4.37 ± 0.10^{a}	4.09 ± 0.13^{b}	0.01
BCF (Hz)	14.69 ± 0.73^{b}	15.96 ± 0.62^{a}	0.01
HAC (rad)	0.64 ± 0.03^{a}	0.60 ± 0.02^{b}	0.02
LIN (VSL/VCL)	0.43 ± 0.01^{b}	0.48 ± 0.02^{a}	0.01
STR (VSL/VAP)	0.81 ± 0.01^{b}	0.84 ± 0.01^{a}	0.01

VCL= Velocity Curvilinear, VSL=Velocity Straight Line, VAP= Velocity Average Path. ALH = Amplitude of Lateral Head displacement, DCL = Distance Curved Line, DSL = Distance Straight Line, DAP =Distance Average Path, BCF = Beat Cross-Frequency, HAC =Head Activity, LIN = Linearity, and STR = Straightness.

study were lower than the 19.00 Hz reported for fresh Sirohi buck semen by Anand et al. (2016). Since BCF reflects the frequency of sperm head oscillation along its trajectory, the observed difference may indicate variation in the coordination or frequency of flagellar beating between breeds. However, direct assessment of flagellar activity or mitochondrial function was beyond the scope of the present study.

HAC exhibited significant variation among breeds ($p < 0.05$) (Table 4), indicating differences in the curvature of sperm trajectories. This observation, along with the significant variations in LIN and STR, implies that the breeds differed in the maintenance of spermatozoa movement direction rather than in overall swimming velocity. Yousif et al. (2023) documented LIN and STR values of 0.49 and 0.64, respectively, in buck sperm. Variations in sperm trajectory may be attributed to flagellar activity, sperm morphology, and the interaction between sperm movement and the surrounding fluid environment. Karimi et al. (2025) additionally reported that reproductive tract morphology and fluid shear stress can affect sperm movement patterns.

Overall, the kinematic profile suggests that breed-related differences were primarily associated with sperm trajectory and movement characteristics, while the main velocity parameters remained similar. These differences should be interpreted with caution, as the study did not directly assess the cellular mechanisms underlying sperm kinematics, such as mitochondrial activity, membrane properties, or flagellar function.

Study limitations

This study is subject to several limitations. The sample size was restricted to two bucks per breed, with five repeated ejaculates collected from each buck. Consequently, these repeated ejaculates reflected intra-individual variation rather than independent biological replication, which constrains the generalisability of the results to the broader breed populations. Furthermore, the research was conducted at a single AIC under standardized management and environmental conditions. As a result, the breed-related differences observed in specific semen quality and kinematic parameters should be regarded as preliminary findings within the context of this study. Additional research involving larger numbers of independent bucks and varied management and environmental conditions is necessary to validate the observed differences and assess their consistency across populations.

CONCLUSION

Under standardized management conditions at the Lembang AIC, breed-related differences were observed in slow sperm motility, sperm concentration, acrosome integrity, and selected kinematic parameters (ALH, BCF, HAC, LIN, and STR), whereas several other semen characteristics did not differ significantly between breeds. Given the limited number of independent bucks and single study setting, these findings provide a preliminary comparative assessment and require validation in larger populations and diverse environmental and management conditions.

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AUTHORS CONTRIBUTION

C. conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, and writing. MFU. formal analysis, investigation, supervision, writing review, and editing. S.S. conceptualization, methodology, validation, writing-review, and editing. T.P.N. formal analysis, methodology, software, validation, visualization, writing review, and editing. R.I.A. conceptualization, data curation, investigation, methodology, resources, supervision, formal analysis, software, validation, visualization, writing review, and editing.

"The author declares that there is no conflict of interest with the parties involved in this research".

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