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Original Article

Evaluating sperm quality characteristics obtained through the female teaser method in native chicken breeds

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Abstract

Objective: The quality and quantity of chicken sperm are critical factors influenced by the semen collection method**.** Therefore, this study aims to evaluate the sperm quality obtained from three native chicken breeds using the female teaser method.
Methods: These three native chicken breeds are Arabic, Birma, and Kampung Unggul Balitbangtan (KUB) chickens. Each breed comprised a group of three roosters, and the semen collection was performed ten times. Macroscopic and microscopic evaluations were conducted to assess the chicken sperm. The collected data were analyzed using Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT).
Result: The results of this study showed significant differences (P<0.05) in the sperm volume among the chicken breeds, with Birma (0.51 ml) and KUB chickens (0.60 ml) exhibiting distinct volumes compared to Arabic chicken (0.25 ml). Generally, the chicken sperm exhibited a milky white color and thick consistency. Microscopic evaluation yielded a mass motility rating of 3+. Meanwhile, individual motility and viability did not exhibit significant differences (P>0.05) among the three native chicken breeds. Abnormalities displayed significant variations (P<0.05), with Birma chicken showing the highest abnormality rate at 9.77%± 0.28. Sperm concentration did not exhibit significant differences (P>0.05), with the highest concentration observed in Arabic chicken at 4265.33±59.84 ($x10^6$ cells/ml). However, total sperm count and total motile sperm count exhibited significant variations (P<0.05), with the highest values recorded in KUB chicken at 2241.82±264.13 ($x10^6$ cells) and 1936.27±169.85 ($x10^6$ cells), respectively.
Conclusions: In conclusion, the semen collection method using the female teaser yielded native chicken sperm with high quality and quantity and meets the required standards for successful insemination. This method holds potential and is recommended for application in native chicken breeding programs.

Keywords: Native chicken; Semen collection; Spermatozoa; Female teaser

INTRODUCTION

Native chicken, which is a local breed widely developed in various regions of Indonesia, plays a crucial role in bolstering the nation animal protein sources [1]. However, the development of the native chicken population faces limitations due to the availability of quality breeding stock. To address this challenge, Artificial Insemination (AI) has emerged as a reproductive biotechnology to enhance these chicken

breeds. In the modern poultry breeding and production industry, AI is widely employed to achieve higher reproductive efficiency, improve genetic quality, and reduce management costs [2]. The primary goal of AI is to boost the population by efficiently producing a large number of Day-Old Chicks (DOC) within a relatively short timeframe [3].

Furthermore, the process of AI involves the semen collection from the male chicken and subsequent deposition into the female reproductive tract. The success of this procedure in producing fertile eggs relies on the expertise of skilled operators who handle the semen collection and deposition processes. Among the critical steps in AI, technology semen collection plays a crucial role. Several methods have been developed for semen collection in poultry, including massage methods, with one of the cooperative approaches being the use of a stimulation dummy or female teaser [4]. The semen collection method significantly impacts sperm quality and subsequent egg fertility [5].

The massage method is widely used for semen collection in chickens [6]. Macroscopically, sperm quality is characterized by a milky white color and thick consistency. The acidity level of the sperm ranges from 6.56 to 6.62 [7,8]. However, the massage method has its drawbacks, as it can cause injury to the cloaca area, leading to potentially contaminated semen samples [4]. Additionally, this method yields a small collection volume and low sperm concentration. Previous studies using the massage method reported an average sperm volume ranging from 0.23 to 0.45 ml, with a concentration of 1.46 to 3.25 x10^9(cell/ml) [7,8]. Successful semen collection requires a calm and patient operator, as the roosters need time to adapt to the massage method, which may cause stress when not performed skillfully.

Another semen collection method in poultry is the female teaser approach, which has been employed in quails, ostriches, emu birds, and native chickens [5], with the successful production of high-quality sperm. However, the semen collection method has not been extensively used in native chicken breeding units as an alternative to support AI technology. Previous studies on native chicken have utilized this approach but with a limited number of collections [9]. Therefore, continuous development and evaluation are necessary to ensure the success of this semen collection method.

The objective of this study is to evaluate the quantity and quality of sperm obtained in three native chicken breeds through the female teaser method. Successful implementation of this method is expected to provide an alternative semen collection approach for chickens and can be easily applied in native chicken breeding units.

MATERIALS AND METHODS

Animals sample and rearing

This study was conducted at the Poultry Division and Reproduction Laboratory of Politeknik Pembangunan Pertanian Malang using a Completely Randomized Design (CRD). Three native breeds, including Arabic chicken, Birma chicken, and Kampung Unggul Balitbangtan (KUB) chicken, aged 12 months, were included in the study. The average body weight of the chicken ranged from 2.2 to 2.5 kg. Semen collection was performed ten times from the three roosters of each breed, with a frequency of twice a week. Both the roosters and the female teaser (hens) were individually housed in cages. A hen was placed in a separate cage located approximately 2-3 meters in front of the roosters' cage to stimulate their libido. Commercial chicken feed formulated specifically for native chicken was provided as the dietary source. The nutrient content of commercial feed consisted of approximately 12-14% crude protein, 4% crude fat, 6% crude fiber, 7.5% ash, 0.9% calcium, and 0.6% phosphorus.

Semen collection equipment

The equipment used for collecting semen from the female teaser includes tissue, physiological NaCl, a collection tube, a 1 ml syringe, and a microtube. The collection tube, made of plastic or glass, has a diameter of 3 (three) cm and is adjusted to fit the estimated size of the cloaca of the roosters. Furthermore, the tube is equipped with a rubber rope that is attached to the back of the roosters.

Macroscopic evaluation

The macroscopic evaluation comprised an assessment of the sperm volume, pH, consistency, and color. Semen color and consistency were evaluated visually. The sperm volume was determined by measuring the scale on the syringe used to extract semen from the collection tube. pH measurement was conducted using pH paper with a scale ranging from 0 to 14 by reading the color indicator on the scale.

Microscopic evaluation

The microscopic evaluation included assessing mass motility, individual motility, sperm viability, sperm abnormality, sperm concentration, Total Sperm (TS), and Total Motile Sperm Count (TMSC). Mass motility was examined by placing a drop of semen on a warm slide under a light microscope (Olympus CX-23) at a magnification of 100x without using a glass cover. A drop of semen was mixed with four drops of physiological

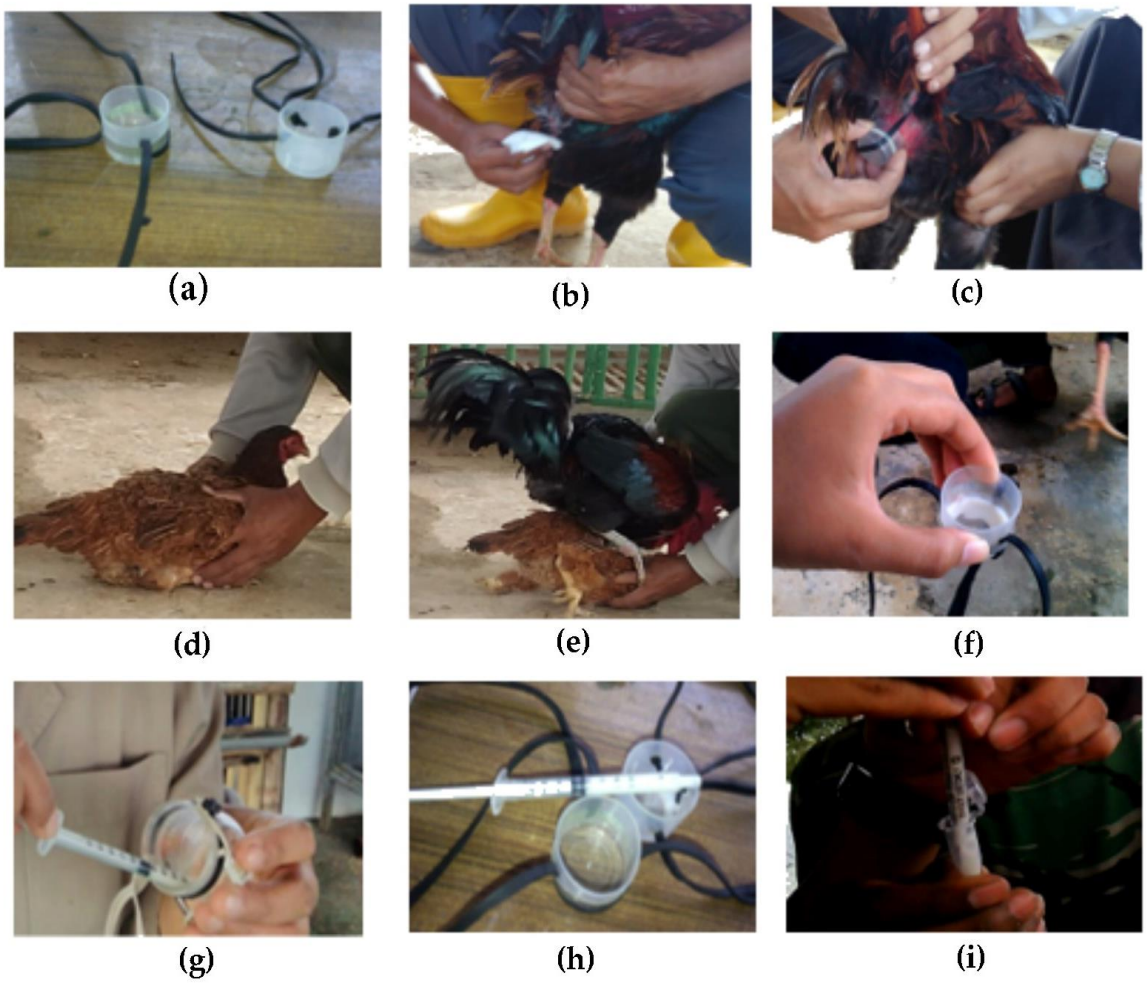

Figure 1. The steps of semen collection technique with a teaser female completted with a container tube on native chicken. **(a)** Preparation of the container tube to be installed on the rooster's cloaca, complete with the rubber strap. **(b)** The fur around the cloaca is shaved, the part around the cloaca is cleaned of dirt attached by tissue moisturized with NaCl. **(c)** Install tubes on the male cloaca tied to the rooster's back so as not to escape during ejaculation. The structure of this tube should be tight enough**. (d)** One operator holds a hen (teaser female) with a position towards the operator. **(e)** The other operator holds the rooster from a distance of 1-2 meters; after a reaction about to run, the rooster is released towards the hen, a response appears to ride the female, and within a few seconds, ejaculation will occur. The semen is collected in the tube. **(f)** Removal of the rubber strap at the tube collection as soon as possible from the male**. (g)** The semen collected is sucked into the syringe (1 ml). **(h)** Evaluation of semen volume based on the scale on syringe. **(i)** Semen is placed into a microtube for sperm examination.

NaCl and homogenized to evaluate individual motility. Furthermore, a drop of the mixture was transferred onto a clean, warm glass slide and covered with a glass cover. Sperm motility was subjectively assessed in five fields, ranging from 0% (all non-motile) to 100% (all progressive motile). The evaluation was performed using a light microscope from the euromex holland iscope series at a magnification of 400x [10].

The evaluation of live sperm was performed using the eosin-nigrosin staining procedure. Sperm viability was directly assessed under a light microscope at a magnification of 400x. Sperm displaying a pink color were considered non-viable, while unstained (transparent) cells were counted as living cells. The percentage of sperm abnormalities was calculated based on the examination of 200 sperm counts. Sperm concentrations were determined by counting in five fields of view using a Haemocytometer [10].

The total sperm count (TS) was calculated as the product of sperm volume and its concentration per ejaculation [11]. TS = volume × sperm concentration. The total motile sperm count (TMSC), representing the total value of motile sperm, was calculated by multiplying the TS by the percentage of sperm motility. TMSC = TS × motility of sperm [11].

Semen collection methods with teasers female

The roosters are trained using the following steps. The first is the roosters and hens were housed in individual cages, and daily exercise was provided by allowing the chicken to interact with the operators for at least 5 minutes each day. This initial stage of contact between the chicken and operators lasted approximately 3-7 days.

The second step is mating treatment involved using a female teaser, and one operator held the roosters while another operator held the hen as a teaser, positioning the hen towards the trainer. The distance between the hen and the roosters was approximately ±1-2 meters. The roosters were then released towards the hen, eliciting a natural mating reaction. When mating occurred (with the roosters mounting the hen), semen could be collected.

The second step is the roosters were trained to accept the insertion of a semen collection tube into their cloaca. Initially, the roosters exhibited a rejection reaction, requiring time for the male to adapt to the collection tube. This stage of training lasted for a minimum of three days. The trainer ensured that the tube was securely attached to the male. When the rope was too tight, the roosters would resist, and when the rubber strap was too loose, the tube could become tilted or detached from the cloaca during mating, causing semen spillage. A successful semen collection was indicated by the presence of milky-white semen liquid coming out of the cloaca and being captured in the collection tube.

The steps of semen collection are presented in Figure 1.

RESULTS

Characteristics of chicken semen

The analysis of the collected semen showed satisfactory quality and quantity, meeting the established standards [12]. The evaluation of the sperm quality obtained through the female teaser method is presented in Table 1.

Table 1 provides an overview of the macroscopic and microscopic quality of sperm across three breeds, including Arabic chicken, Birma chicken, and KUB chicken. The sperm volume of Birma and KUB chickens was

Table 1. Characteristics of semen quality from native chicken using teaser female stimulation (Average±SEM)

Chicken Breeds	Macroscopic				Microscopic			
	Volume (ml)	pH	Colour	Consistency	Mass movement	Motility (%)	Viability (%)	Abnormality (%)
Arabic	0.25±0.01^{a}	7.67±0.78^{b}	MW	thick	3+	84.73±2.60^{a}	90.23±0.64^{a}	8.57±0.35^{a}
Birma	0.51±0.02^{b}	7.76±0.12^{b}	MW	thick	3+	83.67±2.92^{a}	90.19±0.87^{a}	9.77±0.28^{b}
KUB	0.60±0.05^{b}	7.20± 0.88^{a}	MW	thick	3+	86.78±3.02^{a}	89.35±0.52^{a}	8.56±0.27^{a}

^{a,b} different superscripts within columns indicate significant differences (P<0.05); MW is milky white.

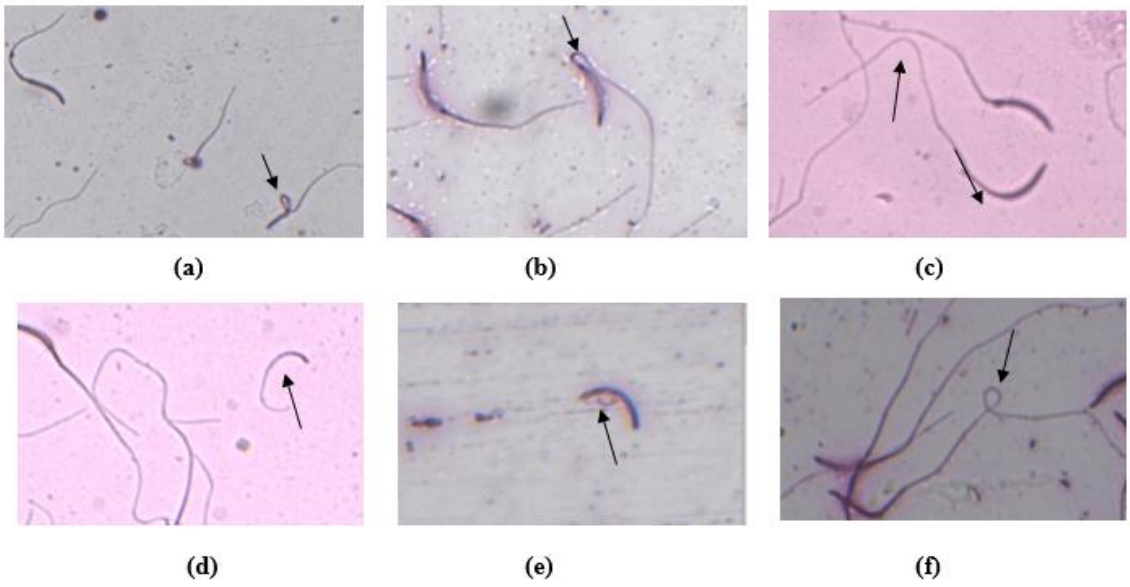

Figure 2. Representative of sperm abnormal morphology of three native chicken breeds. **(a).** knotted head **(b)** bent neck **(c)** bent tail **(d)** broken and lost head **(e)** broken and lost tail **(f)** knotted tail (1000x magnification of the euromex binocular microscope with iscope series)

significantly higher compared to that of Arabic ($P<0.05$). The pH level of KUB chicken semen was significantly lower compared to Arabic and Birma chickens. However, no significant differences were observed in mass motility, individual motility, and viability ($P>0.05$) among the three breeds. Notably, the Birma chicken exhibited a significantly higher rate of sperm abnormalities compared to both Arabic and KUB chickens ($P<0.05$).

Figure 2 presents the morphological abnormalities observed in fresh spermatozoa from the three native chicken breeds.

The sperm concentration, Total Sperm (TS), and Total Motile Sperm Count (TMSC) are presented in Table 2. This table provides the values for sperm concentration, Total sperm (TS), and Total Motile Sperm Count (TMSC) of three native chicken breeds, including Arabic chicken, Birma chicken, and KUB chicken. The sperm concentration in KUB chicken was significantly lower ($P>0.05$) compared to that of Arabic and Birma, while the Total sperm (TS) and Total Motile Sperm Count (TMSC) were significantly higher ($P<0.05$) in Birma and KUB chickens compared to Arabic chicken.

DISCUSSION

The trained chicken exhibited positive responses during semen collection, with a training duration of two weeks. When the roosters mounted the female, ejaculation occurred promptly, and the semen was successfully collected in the container tube.

Arabic and KUB chickens are well-known for their egg-producing capabilities, whereas Birma chicken are predominantly bred for meat production [13]. Previous studies commonly used the massage method for semen collection in chickens, which has been a long-standing and popular approach. However, the success of stimulation using this

Table 2. Sperm concentration, Total sperm (TS), and Total Motile Sperm Count (TMSC) of Native Chicken (Average±SEM)

Chicken breeds	Sperm concentration ($x10^6$ cell/ml)	Total sperm ($x10^6$ cell)	Total sperm motility ($x10^6$ cell)
Arabic	4265.33±59.84^b	1260.73^a±80.40^a	1067.93±67.37^a
Birma	4133.20±91.66^b	2098.27±124.90^b	1745.06±110.67^b
KUB	3739.33±26.41^a	2241.82±264.13^b	1936.27±169.85^b

$^{a, b}$ different superscripts within columns indicate significant differences ($P<0.05$).

method relies heavily on the skill and expertise of operators, as an inadequate technique can lead to trauma and stress in roosters.

In contrast, the female teaser method utilized in this study was found to be relatively less stressful compared to other methods. The effectiveness of this method may stem from its ability to evoke the male natural sexual reflex through the presence of a female teaser. Physiologically, sexual stimulation leads to rapid erection and ejaculation within a few seconds, whereas the massage method typically requires around one minute of stimulation [5]. However, it is essential to emphasize that the success of both semen collection methods depends on the selection of suitable males and their acclimation to the procedure. Minimizing the presence of unfamiliar individuals during collection is crucial, as it can cause the roosters to alter their mating behavior and negatively impact semen quality.

The female teaser method offers several advantages over massage techniques, including lower contamination levels, improved ejaculate volume, higher sperm concentration, and better fertility duration. During this method, it is crucial for the collector performing the procedure to maintain patience and a calm demeanor. Effective stimulation of the roosters is facilitated when they are intensively housed in the same flock as the hen, even when they are housed in separate individual cages. Therefore, a comprehensive understanding of chicken behavior is crucial for the success of this experiment [5]. The training process for the roosters is highly individualized and depends on the specific characteristics of each male. Typically, it takes approximately two weeks for the male to become accustomed to the semen collection procedure.

The collection of chicken semen using the female teaser method has yielded a reliable quantity and quality of semen from native chicken breeds. Macroscopic evaluations showed semen volumes ranging from 0.25 ml to 0.6 ml, which aligns with the established standards [12]. The sperm volume is within the range of 0.2 ml to 0.5 ml, typically observed in chicken semen collection. This semen collection method can be classified as a cooperative approach for chickens and has produced comparable semen volumes to the massage method. For instance, previous studies on Birma chicken using the massage method reported semen volumes of 0.4 ml [13]. In this study, the volume of Arabic chicken semen was measured at 0.25ml ±0.01, indicating a higher volume compared to a previous study reporting a volume of 0.04 ml for Arabic chicken [15]. The difference in semen volume between Birma and Arabic chickens was found to be statistically significant ($P<0.05$). This variation can be attributed to factors such as male performance, body size, and body weight. In this experiment, the Arabic chicken had an average weight of approximately 2.2 kg, while the Birma and KUB chickens had a body weight of around 2.8 kg.

The semen collected in this study exhibited a milky white color, which conforms to the established standards for chicken semen [15]. No discoloration, fecal matter, or blood contamination was observed in semen samples. In contrast, contamination can sometimes occur during the massage method, emphasizing the need for careful handling by the operator. The use of the female teaser for semen collection resulted in relatively pure samples with minimal contamination, as the collector hands were subjected to minimal manipulation during the collection process. Additionally, the natural stimulation provided by the female teaser method allows for semen ejaculation without exerting pressure on the abdominal and back areas.

The consistency of the semen indicated a normal viscosity, which is directly associated with sperm concentration [16]. The acidity (pH) of the semen exhibited significant differences among the three breeds ($P<0.05$), with KUB chicken semen showing significantly lower acidity compared to Arabic and Birma chickens. However, it is noteworthy that the pH values of all three breeds fell within the normal range defined by the standard for chicken semen [12]. Chicken semen is typically slightly alkaline, with pH values ranging from 6.0 to 8.0 [17,18].

Mass motility, which serves as an important microscopic indicator of semen quality, was rapidly examined and showed

excellent results with a score of 3+ (+++), falling within the range of scores from 1+ to 3+ [12]. This particular motility refers to the coordinated movement of sperm in groups [18]. The assessment of mass motility ranges from minimal mass movement (very slow) to wind-like movement or highly active sperm movement [18,19]. In this study, mass motility of 3+ was observed during examination using a 100x magnification microscope, characterized by the presence of thick waves resembling clouds [9].

The average individual motility of sperm ranged from 83.67 to 86.78%. Evaluating sperm motility is essential as it indicates the progressive movement of sperm toward the oocyte, a key factor for successful fertilization [20]. The average viability of sperm was observed to be between 89.35 and 90.23%. When comparing sperm motility and viability among the three breeds of chicken, no significant differences were observed ($P>0.05$) based on the standard criteria for semen quality. Regarding the evaluation of sperm abnormalities, the average abnormality rate across all samples was 8.56 to 9.77%. Notably, the percentage of sperm abnormality in Birma chicken was significantly higher than in Arabic and KUB chickens ($P<0.05$), although it remained below the maximum limit for sperm abnormality in chickens, which is set at 15% [12].

Sperm concentration is an important microscopic parameter that indicates the number of sperm cells in each ejaculate (ml). When combined with the sperm volume, it allows for the determination of the total sperm collected per ejaculation by multiplying the volume and concentration. Consequently, it can be used to calculate the number of insemination doses. The effectiveness of insemination also depends on sperm quality, which is assessed by the total motile sperm count (TMSC).

The microscopic examination of chicken semen yielded favorable results, with the sperm concentration falling within the normal range of 3739.33 to 4265.33 (10^6 cells/ml). These results indicate that the sperm concentration of chicken typically ranges from 3000 to 7000 (10^6/ml) [12]. Interestingly, this study observed a higher sperm concentration in Birma compared to previous results, specifically 3470 x 10^6 cells/ml [13]. Additionally, the use of the massage method for semen collection in Arabic chicken yielded a sperm concentration of 2200 x 10^6 cells/ml [21]. There was a significant difference ($P<0.05$) in sperm concentration between Birma and Arabic chickens, with both breeds exhibiting higher concentrations compared to KUB chicken.

The total motile sperm count (TMSC) in ejaculation is obtained by multiplying the semen volume by the sperm concentration and the percentage of sperm exhibiting progressive movement. Optimal results are achieved when the TMSC exceeds the threshold of approximately 10 million [22]. Previous studies have suggested that the total number of motile sperm serves as a criterion for artificial insemination applications. In this study, total sperm count and total motile sperm count exhibited significant differences ($P<0.05$), with the highest values observed in KUB chicken at 2241.82 ±264.13 (x10^6 cells) and 1936.27 ± 169.85 (x10^6 cells), respectively. The total motile sperm count exceeded 10 million in all samples collected, indicating good semen quality assessment.

CONCLUSION

In conclusion, the use of the female teaser for semen collection in native chicken has proven to be a cooperative method that leverages natural male stimulation and consistently yields reliable semen. The successful outcomes in terms of sperm quality and quantity show the efficacy of this collection approach, making it a highly recommended method for future studies focusing on reproductive biology and its application in native chicken breeding.

CONFLICT OF INTEREST

The authors declare no conflicts of interest with any financial organization regarding the material discussed in this manuscript.

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