Research Article

Ejaculation Frequency and Extender Type Synergistically Influence Semen Quality in Vietnamese Co Ducks

Phan Nhan*

Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Cai Rang Ward, Can Tho 900000, Vietnam.

Abstract | Semen quality in avian species is strongly influenced by ejaculation frequency and storage conditions. In native Vietnamese Co ducks, limited data are available regarding optimal collection intervals and preservation strategies to support artificial insemination. This study aimed to evaluate the effects of different semen collection frequencies on sperm quality and seminal plasma characteristics, and to assess the short-term preservation efficacy of two extenders during cold storage. Thirty drakes were randomly assigned to one of three collection groups: daily, every three days, or every seven days. Semen samples were evaluated for volume, motility, concentration, morphology, and physicochemical traits. Based on optimal results from the three-day group, a second experiment tested sperm preservation in BPSE and Lorenz extenders at 5°C for 8 and 24 hours. Semen collected every three days showed superior balance across all quality parameters, with higher motility (VSL: 27.03 ± 2.24 μm/s, STR: 90.23 ± 2.19%), lower morphological defects (5.11 ± 0.84%), and favorable physicochemical traits. The BPSE extender outperformed Lorenz in preserving sperm motility and morphology over both time points, maintaining higher VCL and lower abnormality rates at 24 hours. A three-day ejaculation frequency combined with BPSE extender offers an effective protocol for semen collection and short-term preservation in Vietnamese Co ducks, enhancing the potential for reproductive success in AI programs.

Keywords | Artificial insemination, Indigenous Co duck, Sperm quality, Semen collection frequency, Ejaculation frequency, Semen extender

Received | June 05, 2025; **Accepted** | August 04, 2025; **Published** | August 26, 2025
***Correspondence** | Phan Nhan, Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Cai Rang Ward, Can Tho 900000, Vietnam; **Email:** pnhan@tdu.edu.vn
Citation | Nhan P (2025). Ejaculation frequency and extender type synergistically influence semen quality in Vietnamese Co ducks. Adv. Anim. Vet. Sci., 13(9):1941-1951.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.9.1941.1951
ISSN (Online) | 2307-8316

INTRODUCTION

Duck farming has long been a traditional agricultural sector in Vietnam, with particularly strong development in the Mekong Delta region, where it provides a primary source of income for many rural households. Among the indigenous breeds, the Vietnamese Co duck is widely raised due to its adaptability, meat quality, and economic value. The Co duck is an indigenous Vietnamese waterfowl breed valued for its high economic and reproductive potential. It plays a pivotal role in rural farming systems across the country due to its adaptability and productive traits. Artificial insemination (AI) is regarded as an effective strategy to address the inherently low maximum mating ratio in ducks, which has been reported as one male per four to eight females (Ash, 1962; Olver *et al*., 1977). In genetic improvement and breeding programs, artificial insemination is widely adopted to enhance reproductive efficiency and reduce the logistical and financial constraints associated with natural mating. An essential step in maximizing AI success is the evaluation of semen quality prior to storage, ensuring that

spermatozoa with optimal fertilizing potential are selected for preservation (Chen *et al*., 2016).

Sperm production in ducks is a highly regulated biological process that encompasses both spermatogenesis and spermiogenesis, through which undifferentiated germ cells are transformed into mature spermatozoa. Kadhem (2014) highlighted that this transformation involves critical events such as nuclear elongation and chromatin condensation, which define each developmental stage.

In practical poultry production, the frequency of semen collection is often based on anecdotal knowledge or traditional farming practices, rather than evidence-based recommendations. Studies in poultry species including chickens, geese, and ducks have shown that overly frequent semen collection can negatively affect sperm viability and concentration. Conversely, infrequent collection may underutilize the reproductive potential of breeding males. Therefore, identifying an optimal ejaculation frequency is crucial for improving sperm quality and enhancing reproductive performance in AI-based production systems.

Although this issue is well recognized, data on the impact of semen collection frequency specifically in Co ducks remains scarce. Given the increasing importance of this indigenous breed, the present study aims to evaluate the effects of varying ejaculation frequencies on seminal quality parameters. The outcomes are expected to provide practical insights for AI optimization and contribute to the effective reproductive management and conservation of Vietnamese Co ducks.

MATERIALS AND METHODS

Location and time

The experiment was conducted at Dong Loi farm in Phu Huu commune, Can Tho city, Vietnam from June to September 2024. The experimental analysis was conducted at the veterinary practice laboratory, Tay Do University.

Animals and experimental design

A total of 30 healthy, sexually mature male Co ducks, aged 7 to 8 months and weighing between 1.6 and 2.2 kg, were selected based on normal mating behavior, physical soundness, and the absence of visible abnormalities. All drakes were individually housed in separate cages, each labeled with leg tags for identification, and maintained under semi-intensive management with natural ventilation and ambient photoperiod. The birds were fed a standard pelleted diet consisting of rice bran, maize, soybean meal, broken rice, fish meal, and other plant-based ingredients, formulated to contain 17% crude protein, 3000 kcal/kg metabolizable energy, 0.8–1.2% calcium, and at least 0.62% phosphorus. Fresh water was provided ad libitum. All drakes were trained for three weeks using the abdominal massage technique as described by Kammer *et al*. (1972) to establish a conditioned response to artificial semen collection and ensure handling consistency prior to the experimental phase. All semen collections were performed under aseptic conditions. Sterile gloves and micropipettes were used, and all equipment (e.g., collection tubes, surfaces) was disinfected with 70% ethanol prior to each collection. Only visually clean ejaculates without fecal contamination were used for analysis.

Experimental design 1: Following the training period, the 30 drakes were randomly assigned to three experimental groups according to ejaculation frequency: Once daily, once every three days, and once every seven days (n = 10 per group). Semen was collected at 6:00 AM using the abdominal massage technique, consistently over a 30-day period to minimize circadian effects. Samples meeting preselection criteria were used for semen quality evaluation, aiming to assess the influence of collection frequency on semen parameters.

Based on the results of the first experiment, the group with semen collected every three days, which was determined to provide optimal quality, was selected for the second experiment. Pooled semen from this group was homogenized and equally divided into two treatment groups corresponding to two storage extenders. The first treatment used BPSE (Beltsville Poultry Semen Extender) composed of glucose (0.20 g), sodium glutamate (0.30 g), potassium acetate (0.07 g), magnesium chloride (0.02 g), sodium chloride (0.10 g), and distilled water up to 100 mL. The second treatment used the Lorenz extender, consisting of glycocoll (glycine, 0.65 g) and sodium chloride (0.56 g) in 100 mL of distilled water. After dilution, semen samples were stored at 4°C and evaluated for quality at 8 hours and 24 hours post-storage to compare the effectiveness of short-term storage extenders.

Ethics statement

This study involved non-invasive procedures and did not require ethical approval, in accordance with institutional guidelines and Vietnamese national regulations on animal welfare. All animal handling, feeding, housing, and semen collection procedures strictly followed ethical principles and good husbandry practices as outlined in Decision No. 24/2010/QĐ-UBND issued by the People's Committee of Can Tho City regarding the management and use of livestock in agricultural research and production.

Measurements

Semen volume

Immediately after collection, semen volume was measured using a calibrated micropipette. Each ejaculate was gently

aspirated and dispensed into a clean, pre-labeled 2.0 ml Eppendorf tube. Care was taken to avoid the inclusion of air bubbles during handling. The volume of each sample was determined based on the final micropipette reading. Only complete ejaculates free from any contamination such as fecal material, urates, or water were included in the analysis to ensure accuracy and consistency across all samples.

Motility (A: 0% < A ≤ 100%)

Progressive motility was assessed under 200× magnification at 2, 3, and 5 hours post-collection. The %age of motile spermatozoa was recorded manually through visual observation of forward movement. Additionally, sperm motility characteristics were evaluated using a Computer-Assisted Sperm Analysis (CASA) system (Hamilton Thorne, USA). This automated system provided a comprehensive and objective analysis of sperm kinetics. The following CASA parameters were recorded:

- Straight Line Velocity (VSL, μm/s): Speed measured in a straight line from the start to endpoint.
- Curvilinear Velocity (VCL, μm/s): Total velocity along the actual path of the sperm.
- Straightness (STR, %): Ratio of VSL to VAP, indicating directional accuracy.
- Amplitude of Lateral Head Displacement (ALH, μm): Lateral movement of the sperm head during progression.
- Beat Cross Frequency (BCF, Hz): Frequency with which the sperm head crosses the average path.

Sperm concentration

Concentration (C, x10^9/mL): Sperm concentration was measured using an SDM1 sperm densimeter (Minitube, Germany). Each semen sample was gently mixed and measured in triplicate to ensure accuracy. The average of the three readings was used for data analysis.

Mass activity (%): was evaluated using a Computer-Assisted Sperm Analysis (CASA) system, which measured the degree of collective swirling motion in undiluted semen. The system analyzed pixel displacement under phase-contrast microscopy and generated a numerical value representing the vigor of sperm mass movement. Results were expressed as %ages, providing a consistent and objective indicator of overall motility intensity across treatment groups.

Abnormal sperm

Abnormal sperm morphology rate (K, %): Determined using methylene blue stain. A drop of semen is placed on a clean, dry glass slide, followed by a few drops of 0.85% NaCl solution. The mixture is thoroughly mixed with a glass rod, then a second slide is used to gently smear and thin the semen drop. Allow the semen layer to air dry, then pass the slide over a flame from an alcohol lamp to fix the specimen. Stain the specimen with methylene blue dye, allowing it to penetrate the sperm for about 10 minutes. Rinse the slide with clean water, then observe under an Olympus microscope at 400x magnification. Randomly count 300–500 sperm cells, including both normal and abnormal sperm, to determine the abnormal morphology rate.

$$K(\%) = \frac{n}{N} \times 100$$

Notes: K%: %age of abnormal sperm morphology. n: Number of abnormal sperm (of various types). N: Total number of sperm counted, including both abnormal and normal sperm (N= 300-500).

pH value

Semen pH: Determined using a pH/Ion meter (WINLAB, Japan). Each sample is measured three times, and the average value of the three measurements is recorded.

Osmotic pressure

Osmotic pressure (mOsm/kg): Measured using an osmometer, specifically the Osmometer BKD-30SMC BIOBASE.

Density

Density (d): Determined using a density bottle (pycnometer).

$$d = \frac{M}{Mo}$$

Notes: d: Density. M: Mass of double-distilled water. Mo: Mass of the liquid to be measured with the same volume.

Viscosity

Viscosity (η): Determined using a micropipette. The viscosity is measured at 20 ˚C.

$$\eta = \frac{d.t}{do.to}$$

Notes: η: Relative viscosity compared to double-distilled water. d: Density of the liquid to be measured. t: Flow time of the liquid through a bulb or capillary. do: Density of double-distilled water. to: Flow time of water through a bulb or capillary.

Buffering capacity

Buffering capacity (β): According to the Salisbury Method (1978) for 0.1N HCl. Use a clean, dry, neutral bottle with

a capacity of 5–10 ml. Add 0,5 ml of the liquid to be tested and measure its pH. Using a micropipette, gradually add 0.1N HCl solution (n=3,6) into the bottle until the pH reaches 4.0. Record the pH deviation (dpH).

$$\beta = \frac{a.n.1000}{dpH.v} \times 100$$

Notes: β: Buffering capacity calculated per 1000 ml of liquid, a: Volume of acid used (amount of 0.1N HCl), n: Equivalent weight of the acid, dpH: pH deviation before and after treatment, v: Volume of liquid used.

Statistical analysis

Data were recorded in Microsoft Excel 2016 and analyzed using Minitab 16.0. One-way analysis of variance (ANOVA) was used to compare treatment means. When significant differences were detected ($P < 0.05$), Tukey's Honest Significant Difference (HSD) test was applied as a post-hoc method for pairwise comparisons. Descriptive statistics are presented as mean ± standard deviation (SD). Pearson correlation coefficients (r) were computed to evaluate relationships between semen quality parameters. No adjustment for multiple comparisons (e.g., Bonferroni correction) was applied, as the correlation analysis was exploratory in nature.

RESULTS AND DISCUSSION

Evaluation of semen quality and physicochemical characteristics in Vietnamese Co ducks under different ejaculation frequencies

The data presented in Table 1 demonstrate that semen collection frequency has a significant impact on the qualitative and quantitative traits of semen in Vietnamese Co drakes. All evaluated parameters showed statistically significant differences among the three treatment groups ($P < 0.05$), indicating that ejaculation interval plays a critical role in determining both sperm productivity and functionality.

The evaluation of semen quality traits in poultry has been recognized as a reliable indicator of reproductive potential and is considered a key factor influencing fertility and the subsequent hatchability of eggs (Peters *et al.*, 2004). Semen volume increased proportionally with the length of the collection interval. The highest volume was recorded in the group collected every seven days (0.78 ± 0.07 mL), followed by the three-day group (0.64 ± 0.04 mL), while the lowest was observed in the daily collection group (0.36 ± 0.02 mL). Łukaszewicz *et al.* (2020) reported that in Muscovy drakes, semen parameters such as volume showed a wide range, varying from 0.05 mL to 2.45 mL. This pattern can be attributed to the extended time available for seminal fluid accumulation in the reproductive tract. However, increased volume does not necessarily equate to improved semen quality, as will be discussed in relation to motility and morphology. Over a 24-day experimental period, Tan (1980) reported that increasing semen collection frequency from once every three days to twice per day resulted in a significant and linear rise in total mean semen volume, from 2.15 mL to 940 mL. In contrast, the average semen volume per collection exhibited a significant and linear decline, decreasing from 1.07 mL to 0.78 mL. It is plausible that a regulatory axis involving the pituitary gland, thyroid, and testes was activated in response to the frequency of massage used for semen collection. These findings align closely with the fundamental principles of reproductive physiology described by Etches (1996). In addition, various studies have indicated that semen quality is influenced by multiple factors, with seasonality being one of the most prominent. Its effects on reproductive performance are mediated by macro- and microclimatic conditions, including temperature, humidity, rainfall, and photoperiod (Bhakat *et al.*, 2010), as well as poultry husbandry and management practices (Silyukova *et al.*, 2022).

Table 1: Effects of semen collection frequency on sperm quality parameters in indigenous ducks (Mean±SD)

Sperm quality parameters (Mean ± SD)	Every 1 days	Every 3 days	Every 7 days
Volume (V, mL)	0.36±0.02^{c}	0.64±0.04^{b}	0.78±0.07^{a}
VSL (µm/s)	28.22±3.08^{a}	27.03±2.24^{b}	23.16±4.94^{c}
VCL (µm/s)	91.65±3.26^{a}	86.07±1.05^{b}	55.92±3.18^{c}
STR (%)	80.76±1.97^{b}	90.23±2.19^{a}	51.25±5.16^{c}
ALH (µm)	2.39±1.17^{b}	2.75±0,47^{a}	1.52±0.46^{c}
BCF (Hz)	0.82±0.04^{a}	0.95±0.01^{b}	0.43±0.38^{c}
Concentration (x10^{9}/mL)	2.15±0.15^{c}	3.09±0.04^{b}	5.17±0.96^{a}
Mass Activity (%)	82.12±1.85^{b}	90.09±1.06^{a}	48.21±3.19^{c}
Abnormal sperm morphology rate (K, %)	6.24±0.96^{b}	5.11±0.84^{c}	16.02±1.48^{a}

$^{a, b, c:}$ Means with different superscripts in the same row differ significantly ($P<0.05$). Abbreviations: VCL: curvilinear velocity (µm/s); VSL: straight line velocity (µm/s); STR: straightness (%); ALH: amplitude of lateral head displacement (µm); BCF: beat cross frequency (Hz).

Sperm motility is one of the most frequently assessed parameters in semen analysis. Nevertheless, it alone is not a reliable indicator of fertilizing potential (Graham *et al.*, 1990). According to Gliozzi *et al.* (2017), total motility accounted for only 10 % of the variation observed in fertility outcomes. Other kinetic parameters, including curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), and amplitude of lateral head displacement (ALH), also contributed significantly to fertility prediction

models, particularly in the context of frozen and thawed semen. At present, the evaluation of sperm samples commonly relies on kinetic parameters measured through the Computer-Assisted Sperm Analysis (CASA) system, with particular emphasis on achieving progressive motility rates greater than 50 %. In addition, certain laboratory protocols incorporate vital dye staining along with manual microscopic assessment of sperm morphology under high magnification (Waberski *et al*., 2022; Kamphuis *et al*., 2020). Therefore, constructing effective statistical models requires the inclusion of relevant fertility indicators and, ideally, the evaluation of sperm characteristics in both fresh and cryopreserved samples. Incorporating novel functional parameters that are not based on morphology is especially important for enhancing the predictive accuracy and robustness of trained models (Abadjieva *et al*., 2023). Straight-Line Velocity, a key indicator of progressive sperm movement, was highest in the daily group (28.22 ± 3.08 µm/s). This value declined slightly in the three-day group (27.03 ± 2.24 µm/s) and dropped substantially in the seven-day group (23.16 ± 4.94 µm/s). The decline in VSL with longer collection intervals suggests that older spermatozoa stored within the excurrent ducts may undergo structural or metabolic degradation, which adversely affects their capacity for forward progression. Curvilinear Velocity followed a similar trend to VSL. The daily group exhibited the highest curvilinear velocity (91.65 ± 3.26 µm/s), followed by the three-day group (86.07 ± 1.05 µm/s), while the seven-day group had the lowest value (55.92 ± 3.18 µm/s). Reduced VCL in the seven-day group reflects a potential decline in flagellar beating efficiency and mitochondrial function, likely due to oxidative stress and aging processes affecting sperm stored for prolonged periods. Path Straightness, which reflects the linearity of sperm movement, showed the highest value in the three-day group (90.23 ± 2.19 %). The daily group recorded a moderately high STR (80.76 ± 1.97 %), while the seven-day group displayed a drastic reduction (51.25 ± 5.16 %). These findings indicate that sperm from the seven-day group exhibited less coordinated movement, which may impair their fertilization potential. The superior STR observed in the three-day group supports the hypothesis that this interval provides sufficient recovery time without inducing storage-related deterioration. Amplitude of Lateral Head Displacement was greatest in the three-day group (2.75 ± 0.47 µm), indicating optimal head oscillation associated with capacitation-like movement. The daily group showed slightly lower ALH (2.39 ± 1.17 µm), while the seven-day group had the lowest value (1.52 ± 0.46 µm). Reduced ALH in the seven-day group may reflect compromised sperm viability or stiffness of the head-neck joint due to prolonged retention in the seminal ducts. Beat Cross Frequency (BCF) values were highest in the three-day group (0.95 ± 0.01 Hz), followed by the daily group (0.82 ± 0.04 Hz), with a substantial decrease observed in the seven-day group (0.43 ± 0.38 Hz). Lower BCF suggests reduced frequency of flagellar crossing, which is linked to decreased sperm vigor and impaired progression. In a study conducted by Kasai and Izumo (2001), microscopic examination of highly diluted semen samples collected using both the artificial vagina (AV) technique and the manual massage method demonstrated that sperm motility was significantly higher in samples obtained via the AV method (73.4 ± 2.0) compared to those collected by manual massage (61.1 ± 3.5). Consistent with these findings, Chen *et al*. (2016) also reported positive associations between in vitro sperm quality parameters such as motility, viability, normal morphology, and plasma membrane integrity, and reproductive outcomes including fertility, early embryonic mortality, and embryo survival rate following artificial insemination in Muscovy drakes.

Sperm concentration was inversely related to collection frequency. The seven-day group yielded the highest concentration (5.17 ± 0.96 x10^9/mL), followed by the three-day (3.09 ± 0.04) and daily groups (2.15 ± 0.15). According to Łukaszewicz *et al*. (2020), semen characteristics in Muscovy drakes, including sperm concentration ranging from 0.15 to 4.44×10^9/ml and the %age of live sperm varying between 51% and 99%, exhibit substantial variability. This highlights the necessity of selecting males prior to the breeding season. In addition, proper storage conditions are essential for maintaining semen quality. This reflects the expected accumulation of sperm cells over longer intervals. However, as shown in subsequent parameters, this quantitative increase came at the expense of functional and structural integrity. These results are in agreement with earlier findings by Kamar (1962), who recorded sperm concentrations of 3.63 billion/ml in Sudani ducks and 5.85 billion/ml in Pekin ducks. However, values obtained in this study remain lower than those reported by Zawadzka *et al*. (2015) for two Polish native duck breeds, which reached 6.9 and 8.5 billion/ml, respectively. Mass activity, indicative of coordinated group movement in fresh semen, was highest in the three-day group (90.09 ± 1.06 %), intermediate in the daily group (82.12 ± 1.85 %), and lowest in the seven-day group (48.21 ± 3.19 %). The sharp decline in the seven-day group supports the assertion that sperm functionality diminishes with prolonged storage *in vivo*. Simões *et al*. (2012) described duck spermatozoa as possessing a short head and acrosome with a relatively fragile architecture, which may render them more vulnerable to morphological damage under non-optimal conditions. Abnormal morphology was significantly elevated in the seven-day group (16.02 ± 1.48 %), compared to 6.24 ± 0.96 % in the daily group and 5.11 ± 0.84 % in the three-day group. Morphological defects such as bent tails and misshapen heads may result from

aging, oxidative damage, and membrane destabilization. Abnormalities in sperm morphology, whether in live or dead cells, were categorized into three main groups: defects of the head, midpiece, and tail regions (Yurchuk *et al*., 2021). The relatively low abnormality rate in the three-day group further reinforces its suitability as the optimal ejaculation interval for preserving sperm quality in Co ducks. From a practical standpoint, these results support the recommendation by Ghonim *et al*. (2009), who showed that collecting semen twice weekly in Domyati drakes improved motility and reduced sperm agglutination, particularly when combined with appropriate dilution rates. Although this figure remains below the maximum morphological threshold of 20% that still permits fertilization (Putranti *et al*., 2010), such abnormalities particularly bent-neck and misaligned-head spermatozoa have been linked to reduced fertilizing capacity (Saeki and Brown, 1962; Yamane *et al*., 1966). These findings may indicate an elevated metabolic rate required to meet the physiological demands of organs during the process of spermatocytogenesis. The connection between thyroid function and testicular development is well established, with thyroid status playing a crucial role in semen production and quality. This influence is exerted through the regulation of nutrients and nucleic acids essential for spermatogenesis and sperm metabolism. The present results are consistent with those reported by Sturkie (2000). Although this study did not directly measure oxidative stress indicators (e.g., reactive oxygen species, lipid peroxidation) or apoptosis in spermatozoa, the decline in motility and morphological normalcy observed in the 7-day group suggests underlying cellular degradation. Prolonged sperm residence in the epididymal or vasal ducts may expose cells to increased oxidative stress and metabolic byproducts, leading to mitochondrial dysfunction and membrane damage (Graham *et al*., 1990; Aire, 2003). The superior quality observed in the 3-day interval group may therefore reflect a physiological balance between recovery from ejaculation and the avoidance of intracellular degeneration. Future research should incorporate biochemical markers and apoptosis assays to confirm this mechanism.

Table 2: Effects of semen collection frequency on physicochemical properties of semen in indigenous ducks (Mean±SD).

Evaluation criteria	Every 1 days	Every 3 days	Every 7 days
Osmotic pressure (mOsm/kg)	292.07±4.14^{c}	321.16±3.82^{b}	339.21±3.95^{a}
Buffering capacity (β)	21.11±0.19^{a}	18.42±0.15^{b}	14.03±0.92^{c}
Density (g/cm^3)	1.01±0.14	1.01±0.05	1.01±0.06
Viscosity (η)	1.23±0.19^{c}	1.52±0.08^{b}	2.81±0.12^{a}
pH	7.22±0.05^{b}	7.31±0.04^{a}	6.82±0.21^{c}

$^{a, b, c:}$Means with different superscripts in the same row differ significantly (P<0.05). No significant differences were observed in density among groups (P > 0.05).

Table 2 presents the effects of semen collection frequency on several physicochemical properties of seminal plasma in Co ducks, including osmotic pressure, buffering capacity, density, viscosity, and pH. These parameters are critical for maintaining sperm viability, motility, and membrane stability, particularly in avian species where semen has a relatively small volume and is highly sensitive to biochemical fluctuations. Significant differences (P < 0.05) were found across treatment groups, highlighting the importance of ejaculation interval on the chemical environment of semen.

Osmotic pressure increased progressively with longer collection intervals. The daily collection group exhibited the lowest osmotic pressure at 292.07 ± 4.14 mOsm/kg, while the seven-day group reached the highest value at 339.21 ± 3.95 mOsm/kg. This pattern indicates that infrequent ejaculation may lead to greater accumulation of solutes and cellular debris in the seminal plasma, likely due to prolonged residence time of sperm and secretions within the male reproductive tract. Elevated osmolarity has been associated with reduced sperm membrane integrity and increased oxidative stress, potentially contributing to the decline in motility and morphological normalcy observed in the seven-day group. Buffering capacity followed a reverse trend to osmotic pressure. The highest buffering capacity was recorded in the daily group (21.11 ± 0.19), while the seven-day group exhibited the lowest (14.03 ± 0.92). This suggests that more frequent ejaculation helps maintain a stable acid–base balance in the semen, which is essential for protecting spermatozoa from pH fluctuations during storage or transit in the female reproductive tract. The decline in buffering strength with longer collection intervals may be due to reduced turnover of seminal plasma and depletion of bicarbonate and other buffering agents, as previously reported in duck and turkey semen by Sexton (1988). No significant differences in semen density were observed across all treatment groups, with all values remaining constant at 1.01 g/cm^3 (± small variations). This result suggests that semen density is a relatively stable trait not easily influenced by collection frequency in Co ducks. Unlike mammals, avian semen is less dependent on seminal vesicle secretions, and thus density may reflect a more consistent cellular content relative to fluid volume. Viscosity increased significantly with decreased ejaculation frequency. The seven-day group had the highest viscosity (2.81 ± 0.12), compared to 1.52 ± 0.08 in the three-day group and only 1.23 ± 0.19 in the daily group. High seminal viscosity is generally unfavorable for sperm movement and artificial insemination efficiency. In birds, increased viscosity may result from prolonged accumulation of proteins, glycoproteins, and desquamated epithelial cells. According to Lake and Stewart (1978), high viscosity in avian semen hinders sperm motility and

reduces the success rate of fertilization, particularly in AI protocols with diluted semen. The semen pH varied significantly between groups, peaking in the three-day group (7.31 ± 0.04), slightly lower in the daily group (7.22 ± 0.05), and dropping to acidic levels in the seven-day group (6.82 ± 0.21). Optimal semen pH in birds typically ranges between 7.0 and 7.4, with deviations impairing sperm motility and viability. The decline in pH in the seven-day group may reflect lactic acid accumulation due to cell degradation and bacterial metabolism. In contrast, the three-day group maintained pH within a favorable physiological window, which may explain the better sperm activity and morphology noted in Table 1.

Table 3 presents the Pearson correlation coefficients between semen collection frequency and key semen quality traits in Vietnamese Co ducks. The analysis reveals significant correlations between ejaculation interval and both quantitative and qualitative parameters, providing insight into the underlying trade-offs between sperm output, motility, and morphological integrity.

A strong negative correlation was observed between ejaculation frequency and semen volume ($r = -0.782$, $P < 0.001$), indicating that reduced collection frequency allows for greater semen accumulation. This finding is consistent with Nhan *et al.* (2025), who reported a similar inverse relationship in Muscovy ducks ($r = -0.767$, $P < 0.01$), suggesting that frequent collection can significantly reduce ejaculate volume. Physiologically, this trend may be explained by the limited time available between collections for the accessory sex glands to secrete fluids and for sperm to accumulate in the reproductive tract. When collection occurs too frequently, the male may not have sufficient time to recover and replenish seminal reserves, potentially leading to decreased volume and reproductive fatigue. However, it should be noted that while increased semen volume is generally considered beneficial, it does not necessarily correlate with improved sperm quality. Therefore, an optimal balance between collection frequency and semen quality must be established for maximum reproductive efficiency. Sperm concentration was moderately and positively correlated with collection frequency ($r = 0.366$, $P < 0.01$). This reflects the expected accumulation of sperm cells in the excurrent ducts when ejaculation is infrequent. Although a higher sperm count is generally favorable, it must be interpreted in the context of other traits, particularly morphology and motility, which may deteriorate under extended intervals. A statistically significant positive correlation was observed between collection frequency and abnormal sperm morphology ($r = 0.541$, $P < 0.05$). This indicates that as collection intervals increase, the proportion of defective sperm also rises. The mechanism behind this relationship likely involves prolonged retention of spermatozoa in the vas deferens, where they may be exposed to osmotic stress, reactive oxygen species, or cellular debris, leading to structural degradation. Moreover, abnormal morphology showed a very strong positive correlation with sperm concentration ($r = 0.798$, $P < 0.001$), suggesting that when sperm count increases under long storage conditions, defective forms tend to accumulate concurrently. This reinforces the observation that high sperm output under low-frequency collection is accompanied by a loss in quality.

Mass activity was negatively correlated with collection frequency ($r = -0.159$, $P < 0.05$) and with abnormal morphology ($r = -0.691$, $P < 0.001$). These inverse relationships suggest that as the frequency of collection decreases or as morphological defects increase, the collective motility behavior of sperm declines. This supports the conclusion that semen stored for prolonged periods in vivo exhibits reduced viability and coordination. Finally, semen pH showed a very weak negative correlation with collection frequency ($r = -0.062$, $P < 0.05$). Although this result is statistically significant, the effect size is negligible and may not carry meaningful biological implications. Nevertheless, the direction of the correlation is consistent with the observed pH decline in the 7-day group (Table 2), possibly reflecting minimal acidification due to prolonged *in vivo* storage. This finding should be interpreted with caution and confirmed in future studies. The relationship between pH and concentration ($r = 0.869$) was not statistically significant at the conventional threshold but suggests

Table 3: Correlation coefficients Pearson between semen collection frequency and semen quality parameters in indigenous ducks.

	Collection frequency	Volume	Concentration	Abnormal morphology	Mass activity	pH
Collection frequency	1	-	-	-	-	-
Volume	-0.782***	1	-	-	-	-
Concentration	0.366**	0.175	1	-	-	-
Abnormal morphology	0.541*	0.232	0.798***	1	-	-
Mass activity	-0.159*	-0.203*	-0.214***	-0.691***	1	-
pH	-0.062*	0.064	0.869	0.124*	0.318	1

Note: Values are Pearson correlation coefficients between semen collection frequency and semen quality parameters in indigenous ducks. *: $P < 0.05$ (statistically significant); **: $P < 0.01$ (highly significant); ***: $P < 0.001$ (extremely significant).

a trend in which denser samples tend to retain a more stable pH, possibly due to buffering by sperm cell membranes. These statistical associations support the trends observed in Tables 1 and 2 and provide a quantitative foundation for interpreting the physiological outcomes. Chen *et al.* (2016) reported a positive relationship between sperm membrane integrity and normal morphology in duck sperm. They concluded that the condition of the plasma membrane is strongly associated with the structural quality of spermatozoa. Moreover, mitochondrial function was also positively linked with normal morphology. This association may stem from developmental abnormalities during spermatogenesis, including disruptions in mitochondrial migration and the formation of the mitochondrial sheath, processes that occur alongside sperm development (Aire, 2003).

Overall, the correlation matrix confirms that longer ejaculation intervals increase semen volume and concentration, but at the expense of morphological integrity and coordinated motility. These findings reinforce the conclusion that a moderate collection interval, such as every three days, offers the best balance between quantity and quality in duck semen management.

Effects of storage duration on semen quality using two short-term semen extenders

Table 4 presents the semen quality of Co ducks after 8 hours of cold storage at 5°C using two extenders: BPSE and Lorenz. The results show significant differences ($P < 0.05$) between the two treatments across most motility-related parameters, suggesting that the composition of the extender strongly influences sperm preservation during short-term storage.

Table 4: Semen quality of Co ducks after 8 hours of storage in BPSE and Lorenz at 5°C.

Evaluation criteria	BPSE	Lorenz
VSL (μm/s)	26.14±1.96[a]	25.08±3.25[b]
VCL (μm/s)	62.11±2.14[a]	60.82±2.07[b]
STR (%)	76.18±3.24[a]	70.46±3.12[b]
ALH (μm)	2.54±0.08	2.52±0.04
BCF (Hz)	0.74±0.36[a]	0.67±0.14[b]
Abnormal sperm morphology rate (K, %)	8.34±0.11[b]	10.07±0.26[a]

[a, b]:Means with different superscripts in the same row differ significantly ($P<0.05$). No significant differences were observed for ALH ($P > 0.05$).

Post-collection handling of semen, including the application of specific extenders and antioxidants, along with careful regulation of storage duration and temperature, has been shown to enhance sperm viability and prolong their fertilizing capacity (Łukaszewicz *et al.*, 2020; Zong *et al.*, 2023). The main function of extenders is to provide sperm cells with energy, reduce mechanical and chemical stress caused by freeze and thaw cycles, and offer a suitable environment that supports short-term sperm survival (Salehi *et al.*, 2020). The restoration of sperm motility appears to be associated with a faster and more complete recovery of membrane integrity and permeability, and potentially with a more effective preservation of adenosine triphosphate (ATP) synthesis and utilization within the axoneme (Calamera *et al.*, 2010). The VSL was significantly higher in the BPSE group (26.14 ± 1.96 μm/s) than in the Lorenz group (25.08 ± 3.25 μm/s). Although both values remain within acceptable thresholds for viability, the higher velocity observed in BPSE indicates better preservation of mitochondrial activity and directional motility after 8 hours. Similarly, the VCL in BPSE-treated semen was 62.11 ± 2.14 μm/s, which was slightly but significantly higher than the 60.82 ± 2.07 μm/s recorded in Lorenz. This reflects better maintenance of flagellar motion patterns under the BPSE formulation, which may be attributed to its balanced glucose and glutamate content. STR was notably higher in BPSE (76.18 ± 3.24 %) compared to Lorenz (70.46 ± 3.12 %). Higher STR implies more linear and efficient sperm movement, which is favorable for fertilization success. Gerzilov and Andreeva (2021) provided further insight into velocity parameters, noting that values for curvilinear velocity, average path velocity, and straight-line velocity declined more rapidly between 3 and 30 hours of cold storage. After this initial period, the rate of decline became more gradual, with statistically significant changes observed ($p < 0.05$). Additionally, the proportion of morphologically abnormal sperm cells increased as storage time was prolonged. This result supports the hypothesis that BPSE provides a more stable physicochemical environment for sperm head and tail coordination. ALH values showed no significant difference between treatments, with BPSE and Lorenz reporting 2.54 ± 0.08 μm and 2.52 ± 0.04 μm, respectively. This indicates that both extenders maintained head movement amplitude at comparable levels during the early storage period. BCF was slightly but significantly higher in BPSE (0.74 ± 0.36 Hz) compared to Lorenz (0.67 ± 0.14 Hz), suggesting a better preservation of tail-beat rhythm and flagellar energy dynamics. This contributes to improved motility consistency. The %age of abnormal spermatozoa was significantly lower in BPSE (8.34 ± 0.11 %) than in Lorenz (10.07 ± 0.26 %). This result demonstrates that BPSE is more effective at protecting membrane integrity and minimizing structural defects during the initial 8-hour storage window. According to Łukaszewicz *et al.* (2020), both the duration of semen storage and the type of extender used had a significant impact on sperm morphology, with statistically meaningful differences observed ($p < 0.05$; $p < 0.01$). Atifah *et al.* (2013)

showed that semen motility and viability were significantly influenced by the type and concentration of extenders. In their study, 10% egg yolk in PBS preserved the highest motility after 4 days (46.67 ± 32.15%), while 25% yolk in PBS provided the best viability (50.00 ± 36.05%). More recently, Abadjieva *et al.* (2023) applied machine learning to Muscovy duck semen evaluation and identified ALH, VCL, and LDH as key predictors of semen quality when integrated with enzymatic and methylation data. These findings underscore the relevance of combining structural, kinetic, and molecular traits in modern reproductive biotechnology. Cryoinjury is not confined to the freezing phase; it may also arise during the thawing process as ice melts or recrystallizes (Said *et al.*, 2010). This can result in the formation of both intracellular and extracellular ice crystals, cellular dehydration, and osmotic shock (Oberoi *et al.*, 2014).

Table 5 summarizes the impact of 24-hour cold storage in BPSE and Lorenz on sperm quality. Compared to the 8-hour values, a general decline was observed across all motility traits, but differences between extenders remained evident, reinforcing the superiority of BPSE in prolonged preservation.

Table 5: Semen quality of Co ducks after 24 hours of storage in BPSE and Lorenz at 5°C.

Evaluation criteria	BPSE	Lorenz
VSL (µm/s)	23.07±2.18[a]	20.62±2.07[b]
VCL (µm/s)	42.14±2.14[a]	28.93±4.16[b]
STR (%)	61.27±2.03	61.25±2.14
ALH (µm)	2.08±0.47	2.07±0.39
BCF (Hz)	0.49±0.36[b]	0.52±0.14[a]
Abnormal sperm morphology rate (K, %)	12.07±2.09[b]	15.64±2.17[a]

[a, b]:Means with different superscripts in the same row differ significantly (P<0.05). No significant differences were observed for STR and ALH (P > 0.05).

After 24 hours, VSL declined in both groups, yet BPSE maintained a significantly higher value (23.07 ± 2.18 µm/s) compared to Lorenz (20.62 ± 2.07 µm/s). The difference indicates better preservation of energy-dependent movement in the BPSE environment. VCL showed a pronounced drop in the Lorenz group, reaching 28.93 ± 4.16 µm/s, while BPSE-treated samples retained higher curvilinear velocity (42.14 ± 2.14 µm/s). The stark difference suggests that Lorenz may lack sufficient buffering or osmotic components to maintain sperm flagellar dynamics over extended time. STR values remained nearly identical between groups at 61.27 ± 2.03 % (BPSE) and 61.25 ± 2.14 % (Lorenz), indicating that linearity of movement was preserved equally despite differences in speed and rhythm. ALH values after 24 hours were comparable between BPSE (2.08 ± 0.47 µm) and Lorenz (2.07 ± 0.39 µm), reflecting consistent head movement amplitude and suggesting that storage time affected tail mechanics more than head displacement. Interestingly, BCF was slightly higher in Lorenz (0.52 ± 0.14 Hz) than in BPSE (0.49 ± 0.36 Hz), although this reversal was minimal and possibly random, given the reduced motility seen in other parameters. It may reflect non-progressive or compensatory tail beating rather than effective locomotion. The incidence of abnormal spermatozoa increased in both treatments over 24 hours, but was significantly lower in BPSE (12.07 ± 2.09 %) compared to Lorenz (15.64 ± 2.17 %). This finding confirms that BPSE offers superior protection against morphological degradation during longer periods of cold storage. The superior performance of BPSE may be partly attributed to the presence of sodium glutamate, which has known antioxidant properties that can stabilize sperm membranes and reduce oxidative damage. In contrast, the glycine-based Lorenz extender may offer limited protection in this regard. Although antioxidant capacity was not directly compared between extenders in this study, this hypothesis warrants further investigation. Regarding storage temperature, 5°C was selected based on internal pilot observations (data not shown) where sperm motility and morphology were better maintained compared to 15–20°C during short-term storage. This choice also aimed to reduce microbial proliferation. Nevertheless, further controlled studies are needed to determine optimal temperature thresholds for Co duck semen. The occurrence of such abnormalities is attributed to osmotic pressure changes induced by cold shock during the cryopreservation process, particularly after thawing. Post-thaw sperm tail coiling may arise as a result of osmotic stress (Raad *et al.*, 2017; Gómez-Torres *et al.*, 2017). What is mentioning is that Gerzilov and Andreeva (2021) reported an improvement in total sperm motility, exceeding 80 % in samples diluted with IMV Canadyl and AU extenders, and surpassing 68 % in those diluted with HIA-1 extender. As far as I know, Penfold *et al.* (2001) found that semen from Northern pintail ducks stored at 4°C retained acceptable fertilizing capacity for up to 72 hours. Fertilization rates decreased from 51.6 % in fresh samples to 22.3 % after 72 hours, although hatchability was not significantly affected. Efforts to cryopreserve semen using DMSO or glycerol resulted in inadequate post-thaw motility for successful insemination. These findings underscore the critical role of preserving the initial semen quality prior to storage.

CONCLUSIONS

A three-day semen collection interval optimally balanced sperm quantity and quality in Vietnamese Co ducks. For short-term storage, the BPSE extender outperformed

Lorenz in preserving sperm motility and morphology. This combination is therefore recommended for routine artificial insemination protocols in Co duck breeding programs. To further support practical applications, future studies should investigate long-term storage methods, post-thaw viability, and field fertility outcomes such as egg fertilization and hatchability rates.

ACKNOWLEDGEMENT

We acknowledge the support of time and facilities from Tay Do University (TDU) for this study.

NOVELTY STATEMENT

This study is the first to evaluate the combined effects of semen collection frequency and cold storage using different extenders on the semen quality of Vietnamese Co ducks. It identifies a three-day collection interval and BPSE extender as optimal for maintaining sperm viability, offering practical implications for improving fertility outcomes in native duck breeding programs.

AUTHOR'S CONTRIBUTION

The author was responsible for the study conception and design, experimental execution, data acquisition and analysis, and drafting and revising the manuscript. All aspects of this work were carried out independently by the Phan Nhan.

Funding

This research received no external funding.

Conflict of interest

The authors have declared no conflict of interest.

REFFERENCES

Abadjieva D, Georgiev B, Gerzilov V, Tsvetkova I, Taushanova P, Todorova K, Hayrabedyan S (2023). Machine learning approach for Muscovy duck (*Cairina moschata*) semen quality assessment. Animals, 13(10): 1596. https://doi.org/10.3390/ani13101596

Aire TA (2003). Ultrastructural study of spermiogenesis in the turkey, *Meleagris gallopavo*. Br. Poult. Sci., 44(5): 674–682. https://doi.org/10.1080/00071660310001643651

Ash WJ (1962). Studies of reproduction in ducks. 1. The duration of fertility and hatchability of White Pekin duck eggs. Poult. Sci., 41: 1123–1126. https://doi.org/10.3382/ps.0411123

Atifah Y, Saleh DM, Pramono H, Sistina Y (2013). Motilitas dan viabilitas spermatozoa itik lokal (*Anas platyrhyncos*) setelah penyimpanan dalam medium berbeda dikombinasi krioprotektan kuning telur berbagai konsentrasi. Inflamm. Bowel Dis., 30(1): 8–14.

Bhakat M, Mohanty TK, Gupta AK, Raina VS (2010). Effect of season and management on semen quality of breeding bulls. A review. J. Agric. Rev., 30(2): 79–93.

Calamera JC, Buffone MG, Doncel GF, Brugo-Olmedo S, de Vincentiis S, Calamera MM, Storey BT, Alvarez JG (2010). Effect of thawing temperature on the motility recovery of cryopreserved human spermatozoa. Fertil. Steril., 93(3): 789–794. https://doi.org/10.1016/j.fertnstert.2008.10.021

Chen YC, Liu HC, Wei LY, Huang JF, Lin CC, Blesbois E, Chen MC (2016). Sperm quality parameters and reproductive efficiency in Muscovy duck (*Cairina moschata*). J. Poult. Sci., 53(3): 223–232. https://doi.org/10.2141/jpsa.0150162

Etches RJ (1996). Reproduction in poultry in CAB International (Ed), pp 1-307 Wallingford, UK

Gerzilov V, Andreeva M (2021). Effect of three extenders on the motility and morphological characteristics of spermatozoa in diluted Muscovy semen stored at 4 °C up to 120 hours. Bulgar. J. Agric. Sci., 27: 1187–1193.

Ghonim AIA, Awad AL, El-Sawy MA, Fatouh MH, Zenat AI (2009). Effect of frequency of semen collection, dilution rate and insemination dose on semen characteristics and fertility of Domyati ducks. Egypt. Poult. Sci. J., 29(4): 1023–1045.

Gliozzi TM, Turri F, Manes S, Cassinelli C, Pizzi F (2017). The combination of kinetic and flow cytometric semen parameters as a tool to predict fertility in cryopreserved bull semen. Animal, 11: 1975–1982. https://doi.org/10.1017/S1751731117000684

Gomez-Torres MJ, Medrano L, Romero A, Fernandez-Colom PJ, Aizpurua J (2017). Effectiveness of human spermatozoa biomarkers as indicators of structural damage during cryopreservation. Cryobiology, 78: 90–94. https://doi.org/10.1016/j.cryobiol.2017.06.008

Graham JK, Kunze E, Hammerstedt RH (1990). Analysis of sperm cell viability, acrosomal integrity, and mitochondrial function using flow cytometry. Biol. Reprod., 43(1): 55–64. https://doi.org/10.1095/biolreprod43.1.55

Kadhem AZ (2014). The cycle event of spermatogenesis and spermiogenesis in the testes of indigenous duck (*Anas platyrhynchos*). Bashkirian J. Vet. Res., 1(2): 16–22. https://doi.org/10.33762/bvetr.2014.98787

Kamar GAR (1962). Semen characteristics of various breeds of drakes in the subtropics. J. Reprod. Fertil., 3: 405–409. https://doi.org/10.1530/jrf.0.0030405

Kammer DM, Moreng RE, Muller HD, Hobbs HW (1972). Turkey semen evaluation for fertility prediction. Poult. Sci., 51: 77–82. https://doi.org/10.3382/ps.0510077

Kamphuis C, Duenk P, Veerkamp RF, Visser B, Singh G, Nigsch A, Mol RMD, Broekhuijse MLWJ (2020). Machine learning to further improve the decision which boar ejaculates to process into artificial insemination doses. Theriogenology, 144: 112–121. https://doi.org/10.1016/j.theriogenology.2019.12.017

Kasai K, Izumo A (2001). Efficiency of artificial vagina method in semen collection from Osaka drakes. J. Appl. Poult. Res., 10: 206–210. https://doi.org/10.1093/japr/10.3.206

Łukaszewicz E, Jerysz A, Chełmońska B (2020). Effect of semen extenders and storage time on quality of Muscovy duck (*Cairina moschata*) drake semen during the entire reproductive season. Reprod. Domest. Anim., 55: 943–950. https://doi.org/10.1111/rda.13731

Łukaszewicz E, Jerysz A, Kowalczyk A (2020). Reproductive season and male effect on quantitative and qualitative traits of individually collected Muscovy duck (*Cairina moschata*) semen. Reprod. Domest. Anim., 55(12): 1735–1746. https://

doi.org/10.1111/rda.13833

Nhan P, Chuc NT, Tri NM, Phuong NTM, Mo TTH (2025). Effects of Collection Frequency on Semen Quality in Muscovy Ducks *(Cairina moschata domesticus)*. J. Anim. Sci. Technol., 149: 70-78. https://doi.org/10.70408/nias.i149-y2025-595

Oberoi B, Kumar S, Talwar P (2014). Study of human sperm motility post cryopreservation. Med. J. Armed Forces India, 70(4): 349–353. https://doi.org/10.1016/j.mjafi.2014.09.006

Olver M.D, Kuyper MA, Mould DJ (1977). Artificial insemination and duration of fertility in Pekin ducks. Agroanimaria, 9: 27–30.

Penfold LM, Harnal VK, Lynch W, Bird DM, Derrickson SR, Wildt DE (2001). Characterization of northern pintail (*Anas acuta*) ejaculate and the effect of sperm preservation on fertility. Reproduction, 121(2): 267–275. https://doi.org/10.1530/rep.0.1210267

Peters SO, Omidiji EA, Ikeobi CON, Ozoje MO, Adebambo OA (2004). Effect of naked neck and frizzled genes on egg traits, fertility and hatchability in local chicken. In: Self sufficiency of animal protein in Nigeria. Proceedings of the 9th annual conference of animal science association of Nigeria, Ebonyi State University, Abakaliki, Nigeria, September 13–16, pp. 262–264.

Putranti OD, Kustono, Ismaya (2010). Pengaruh penambahan crude tanin pada sperma cair kambing Peranakan Etawa yang disimpan selama 14 hari terhadap viabilitas spermatozoa. Bullet. Petern., 34(1): 1–7. https://doi.org/10.21059/buletinpeternak.v34i1.100

Raad G, Lteif L, Lahoud R, Azoury J, Azoury J, Tanios J, Hazzouri M, Azoury J (2017). Cryopreservation media differentially affect sperm motility, morphology, and DNA integrity. Andrology, 6(6): 836–845. https://doi.org/10.1111/andr.12531

Saeki Y, Brown KI (1962). Fertilizing ability and laboratory evaluation of ejaculated and vasa deferentia semen in turkeys. Poult. Sci., 41: 905–909. https://doi.org/10.3382/ps.0410905

Said TM, Gaglani A, Agarwal A (2010). Implication of apoptosis in sperm cryoinjury. Reprod. Biomed. Online, 21(4): 456–462. https://doi.org/10.1016/j.rbmo.2010.05.011

Salehi M, Mahdavi AH, Sharafi M, Shahverdi A (2020). Cryopreservation of rooster semen: Evidence for the epigenetic modifications of thawed sperm. Theriogenology, 142: 15–25. https://doi.org/10.1016/j.theriogenology.2019.09.030

Salisbury, G.W., Van Demark, N.L. and Lodge, J.R. (1978) Physiology of Reproduction and Artificial Insemination of Cattle. 2nd Edition, W. H. Freeman, San Francisco, 385-479.

Sexton T (1988). Comparison of commercial diluents for holding turkey semen 24 hours at 4 °C. Poult. Sci., 67: 131-134. https://doi.org/10.3382/ps.0670131

Silyukova Y, Fedorova E, Stanishevskaya O (2022). Influence of technological stages of preparation of rooster semen for short-term and long-term storage on its quality characteristics. Curr. Issues Mol. Biol., 44(11): 5531–5542. https://doi.org/10.3390/cimb44110374

Simões K, Orsi AM, Baraldi Artoni SM (2012). Ultrastructure of the spermatozoa of the domestic duck (*Anas platyrhynchos* sp.). Anat. Histol. Embryol., 41(3): 202–208. https://doi.org/10.1111/j.1439-0264.2011.01124.x

Sturkie PD (2000). Avian physiology (5th ed., G.C. Whittow, Ed.). Academic Press, San Diego, CA.

Tan NS (1980). The frequency of collection and semen production in Muscovy ducks. Br. Poult. Sci., 21(4): 265–272. https://doi.org/10.1080/00071668008416668

Waberski D, Suarez SS, Henning H (2022). Assessment of sperm motility in livestock: Perspectives based on sperm swimming conditions *in vivo*. Anim. Reprod. Sci., 246: 106849. https://doi.org/10.1016/j.anireprosci.2021.106849

Yamane JS, Tsukunaga, Takahashi T (1966). Hiroshima method of artificial insemination of the domestic fowl. J. Fac. Fish. Anim. Husb. Hiroshima Univ., 6: 395–429.

Yurchuk T, Petrushko M, Gapon A, Piniaiev V, Kuleshova L (2021). The impact of cryopreservation on the morphology of spermatozoa in men with oligoasthenoteratozoospermia. Cryobiology, 100: 117–124. https://doi.org/10.1016/j.cryobiol.2021.02.009

Zawadzka J, Łukaszewicz E, Kowalczyk A (2015). Comparative semen analysis of two Polish duck strains from a conservation programme. Eur. Poult. Sci., 79: 1–9. https://doi.org/10.1399/eps.2015.106

Zong Y, Li Y, Sun Y, Mehaisen GMK, Ma T, Chen J (2023). Chicken sperm cryopreservation: Review of techniques, freezing damage, and freezability mechanisms. Agriculture, 13: 445. https://doi.org/10.3390/agriculture13020445