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Evaluation of sperm kinetic parameters and performance of artificial insemination in turkey

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ABSTRACT

The present study was conducted to investigate semen characteristics, sperm kinetics, and the comparative fertility performance of natural mating (NM) versus artificial insemination (AI) in indigenous turkeys. A total of four toms were used for semen collection and evaluation. Among them, two toms were assigned for performing NM and AI in a group of 20 hens (10 hens per group). Semen ejaculates were collected from the toms during 36 to 40 weeks of age and assessed for physical characteristics. A portion of the semen samples was analyzed to evaluate sperm kinetics using Computer-Assisted Semen Analysis (CASA), while the remaining fresh, undiluted pooled semen was deposited into the hens' vaginas within 30 minutes of collection. The results indicated significant differences ($P<0.05$) in semen volume, sperm concentration, and most sperm kinetics (e.g., total motility, progressive motility, curvilinear velocity, average path velocity, straight-line velocity, straightness, linearity, wobble, amplitude of lateral head displacement, and beat cross frequency) among the toms. Notably, two toms exhibited standard and superior semen quality and sperm kinetics. Fertility rates were significantly higher ($P<0.05$) in hens inseminated via AI (95.92%) compared to those bred through NM (74.84%). This study concluded that semen characteristics and sperm kinetics varied among individual toms. Furthermore, AI using semen from high-performing toms enhanced fertility outcomes without any adverse effects. These findings suggest that identifying toms with superior semen quality is critical for optimizing AI practices in turkey breeding.

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1.INTRODUCTION

Artificial insemination (AI) has emerged as a widely recognized technique for addressing mating challenges and improving fertility in poultry, particularly in developed countries. This method offers significant advantages over natural mating (NM). While 80–85% of eggs are typically fertile under NM, fertility rates can be enhanced by an additional 5–10% through theapplication of AI (Gee et al., 2004). In westerncountries, AI is predominantly employed in

commercial turkey production, where its role is indispensable. The reliance on AI stems from structural and behavioral challenges in turkeys: the male's extended pectoral bone, a significantly heavier body weight (18–20 kg compared to the female's 6–8 kg), and the inefficiency of toms alongside the non-receptivity of hens (Gee et al., 2004). Consequently, AI has become the sole method

for ensuring fertile egg production in commercial turkey operations (Bakst, 2008). AI has revolutionized the poultry industry by offering a precise and effective alternative to natural mating. Notably, it has transformed turkey breeding, where challenges related to physical incompatibility and inefficiencies in natural mating have necessitated its adoption on a large scale.

The quality of semen plays a critical role in determining the breeding potential of male birds, directly influencing female fertility and the reproductive success of their offspring (McGary et al., 2002). Semen evaluation involves both macroscopic and microscopic assessments. Macroscopic parameters such as volume, color, consistency, and appearance provide initial insights into semen quality, while microscopic parametersincluding sperm concentration, motility, viability, progressive motility, abnormalities, and the percentage of dead sperm offer detailed evaluations (Moce and Graham, 2008). Among these, sperm motility is widely recognized as a key indicator of fertilizing potential (Charms, 1969). Studies have demonstrated a strong correlation ($r = 0.985$, $P<0.01$) between semen quality factors and fertility, highlighting their importance in breeding programs (Liu et al., 2008).Comprehensive semen analysis serves as a cornerstone for assessing male fertility. While macroscopic traits provide an overview, microscopic parameters, particularly sperm motility, are indispensable for predicting fertilization success. The integration of these evaluations into breeding programs ensures the identification of males with superior reproductive capabilities.

In Bangladesh, turkey farming has gained traction among entrepreneurs, albeit on a limited scale and often without prior expertise. However, challenges related to low fertility rates persist. Field data reveal that the average fertility and hatchability of turkey eggs in Bangladesh stand at only 50% and 32%, respectively (Asaduzzaman et al., 2017). These figures underscore significant reproductive inefficiencies, with approximately 40% of all incubated eggs being lost due to fertility and hatchability issues. Fertility and hatchability are crucial determinants of profitability in breeding and hatchery enterprises, and two-thirds of such losses are attributed to infertility or apparent infertility (Peters et al., 2008). Despite its potential, turkey farming in Bangladesh faces critical reproductive challenges. The low fertility and hatchability rates not only hinder productivity but also affect the economic viability of the sector. Addressing these issues is imperative for the industry's growth.AI has not yet been widely adopted for turkey breeding in Bangladesh, even though its efficacy is well-documented globally. Furthermore, research on turkey semen quality and sperm kinetics remains sparse. This study was therefore designed to evaluate the semen quality and sperm kinetics of turkeys and identify superior toms for improved fertility outcomes through AI using fresh semen. In light of the limited adoption of AI in Bangladesh and the scarcity of research on turkey semen quality, this study aims to bridge the gap by evaluating key reproductive parameters. The findings are expected to pave the way for enhanced fertility management in turkey farming.

2.MATERIALS AND METHODS

The study comprised two experiments conducted at the Advanced Avian Research Farm and the Laboratory of Genetics and Animal Breeding, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Experimental birds and management

A total of 45 non-descriptive turkeys, including 34 hens and 11 toms aged 29 weeks, were obtained from the Advanced Avian Research Farm at HSTU. Following six weeks of intensive rearing and training of toms for semen collection, 20 hens and 4 toms were selected for the study. The research followed a Completely Randomized Design (CRD).In Experiment I, the four toms were used for semen collection to evaluate semen quality and sperm kinetics. In Experiment II, the 20 hens were randomly divided into two groups: natural mating (NM) and artificial insemination (AI), with 10 hens in each group. Toms assigned for AI were separated from hens at the initial stage of the experiment to avoid unintended mating, ensuring the integrity of the experimental design.The birds were maintained under

intensive management conditions in compliance with the guidelines and regulations of the Faculty of Veterinary and Animal Science, HSTU. All birds received a standard breeder layer feed containing maize, rice polish, soybean meal, animal protein, vitamin-mineral premix, amino acids, salt, toxin binder, and antioxidants. While the exact feed composition was not disclosed due to commercial confidentiality, the nutrient content is presented in Table 1. Feed and water were provided twice daily. The atmospheric temperature ranged between 21–35°C, with a light-to-dark ratio of 16:8 hours. During high summer temperatures, vitamin C and electrolytes were added to drinking water, which was frequently replaced with cold water.

Table 1. Nutrient composition of the feed

Nutrient (units)	Amount
Metabolizable energy (kcal/kg)	2780
Moisture (%)	12.00
Crude protein (%)	17.00
Crude fat (%)	4.50
Calcium (%)	3.50
Available phosphorus (%)	0.40

Training of toms for semen collection

Toms were trained for semen collection using the abdominal massage technique, as described by Burrows and Quinn (1937). Training began when the toms reached 29 weeks of age and was performed twice weekly. By 35 weeks of age, all toms were adequately prepared for semen collection.

Semen collection

A specialized wooden chair was designed to securely hold the toms during semen collection, ensuring their comfort while minimizing stress. The procedure involved carefully stroking and massaging the testes located at the dorsum until the cloaca protruded, enabling semen collection. Semen was collected twice weekly between 8:30 and 9:30 AM to ensure consistency in quality. Only clean, high-quality ejaculates were collected under minimal handling stress.

Semen analysis

Macroscopic analysis

Immediately after collection, semen samples were evaluated for volume, pH, and color. Samples were then incubated in a hot water bath at 37°C for five minutes to prepare them for further analysis.

Figure 1. Sequential activities for semen collection a) Locking of legs before semen collection b) Massaging of tom c) Collection of semen and d) Semen content

Microscopic analysis

Semen aliquots were diluted at a 1:20 ratio using a modified Ringer's solution (Table 2; Akcay et al., 2006). This dilution ratio was optimized for CASA analysis (Miah et al., 2020). Sperm motility and kinetics were analyzed using a CASA system (Microoptic Automatic Diagnostic System, Barcelona, Spain). For analysis, 0.5 µl of diluted semen was placed on a clean microscopic slide.Parameters evaluated included sperm concentration, total motility, progressive motility, and sperm kinetics such as curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN = VSL/VCL), straightness (STR = VSL/VAP), wobble (WOB = VAP/VCL), amplitude of lateral head displacement (ALH), and beat-cross frequency (BCF). At least 500 sperm cells per sample were counted using a phase-contrast microscope fitted with a turkey-specific condenser (ph-1) and a 10× objective.

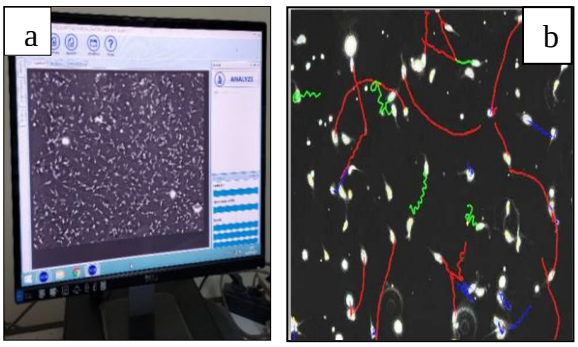

Figure 2. Analysis of semen by CASA: a) Evaluation of sperm kinetics d) Movement pathway of sperm

Natural mating (NM) and artificial insemination (AI) in hens

For the NM group, a male-to-female ratio of 1:5 was maintained. AI was conducted using freshly collected semen from two toms. The "venting" method, as described by Hafez and Hafez (2000), was employed for insemination. Briefly, 0.02 ml of undiluted semen was deposited 2–3 cm into the vent using a 1 ml syringe without a needle. Insemination was performed weekly, 25–30 minutes post-semen collection, between 8:30 and 9:30 AM. Only ejaculates with >70% mass motility and a milky appearance were utilized. Gentle handling of hens was ensured throughout the process to prevent semen regurgitation and subsequent fertility loss.

Data collection

Egg collection

Eggs were collected three times daily, marked according to hen groups and collection dates, and stored in egg crates at approximately 15°C with 75% relative humidity before incubation. Egg production rates were calculated based on these collections.

Table 2. Composition of the semen diluents

Ingredients (units)	Amount
Sodium chloride (g)	9.50
Potassium chloride (g)	0.20
Calcium chloride (g)	0.26
Sodium bicarbonate (g)	0.20
Glucose (g)	1.00
Distilled water (litre)	1.00

Figure 3. Breeding of turkey:a) Natural mating and b) Artificial insemination

Incubation, candling, and hatching of eggs

The well-shaped and healthy eggs were selected, maintained at ambient temperature, and then incubated in an automatic forced-air cabinet incubator (Ova-Easy Advance Series II, Brinsea, FL, USA). The incubator automatically controlled the temperature, humidity, and egg turning (five times daily). Eggs were candled on the 10th and 20th days of incubation to assess fertility and embryonic development. The total incubation period of 28 days was divided into the setting period (1–25 days) and the hatching period (26–28 days). Temperature and humidity were strictly monitored and maintained during the incubation period according to the parameters outlined in Table 3.

After candling and at the end of the incubation period, eggs were classified into the following categories: fertile eggs, infertile eggs, early embryonic death, late embryonic death, and dead in shell. All the dead embryos were considered as fertile. Hatched poults were collected, counted, and weighed using a calibrated electronic scale.

Table 3. Temperature and relative humidity maintained during the incubation period

Stage of incubation	Duration (Days)	Temperature (^{0}C)	Relative humidity (%)
Setting	1–25	38	60–65
Hatching	26–28	37	65–70

Reproductive performance

The fertility levels of each turkey group were calculated as described by Sotirov et al. (2002) and expressed as percentages. The hatchability percent of each group was calculated as outlined

by Hafez and Hafez (2000) and recorded in percentage:

Embryonic mortality rates were categorized into early embryonic death (0–10 days), late embryonic death (11–28 days), and dead-in-shell embryos. These were recorded and analyzed for reproductive performance evaluations.

Statistical analysis

The data for sperm concentration, motility, progressive motility, sperm kinetics (VCL, VSL, VAP, LIN, STR, WOB, ALH, and BCF), fertility, hatchability, embryonic mortality, day-old poult weight, and survivability were analyzed using a Completely Randomized Design (CRD). The analyses were conducted using the Generalized Linear Model (GLM) procedure in SPSS software version 22.0(SPSS Inc., Chicago, IL, USA).

To determine the significance of differences among the means of toms or hen groups, Duncan's Multiple Range Test (DMRT) was employed within the same software package. Results were presented as the Mean ± Standard Error of the Mean (SEM), and a significance level of $P<0.05$ was considered to indicate statistically significant differences.

3. RESULTS

Experiment I:Macroscopic and Microscopic Evaluation of Semen

The present study assessed sperm kinetics and egg fertility in turkey hens bred through Natural Mating (NM) and Artificial Insemination (AI) using fresh semen collected from toms.

Macroscopic evaluation

Semen color and volume were the key parameters for macroscopic evaluation. The semen appeared milky white, indicating normal quality, with no signs of blood or foreign particles. Semen collected from four different toms was evaluated for volume, pH, and sperm concentration (Table 4). Among the toms, Tom 2 produced significantly ($P<0.05$) the highestsemen volume per ejaculation (0.60 ± 0.07 mL), whereas Tom 1 produced the lowest volume (0.40 ± 0.06 mL). While pH differences were not statistically significant, Tom 2 had a slightly higher pH (7.19 ± 0.17) than the others, and Tom 1 had the lowest pH (7.09 ± 0.14). Sperm
concentration varied significantly ($P<0.05$) among toms, with Tom 1 showing the highest concentration (5.17×10^9 /mL) and Tom 3 the lowest (3.57×10^9 /mL).

Total motility and progressive motility

Total sperm motility and progressive motility were evaluated for semen from four toms (Figure 4a, b). The highest total sperm motility was observed in Tom 3 (98.96%) and Tom 4 (99.64%), which were significantly ($P<0.05$) higher than Tom 1 (90.98%) and Tom 2 (92.47%). Progressive motility was highest in Tom 4 (50.96%), significantly outperforming Tom 1 (17.17%)**,** Tom 2 (18.77%), and Tom 3 (40.17%).

Curvilinear velocity (VCL) and average path velocity (VAP)

The average sperm velocities were measured as VCL and VAP (Figure 5a, b). The VCL values for Tom 1, Tom 2, Tom 3, and Tom 4 were 53.03, 50.75, 76.9, and 96.71 µm/sec, respectively, with Tom 4 having significantly ($P<0.05$) the highest VCL. Similarly, Tom 4 had the highest VAP (51.22 µm/sec), followed by Tom 3 (49.08 µm/sec), while Tom 1 and Tom 2 had significantly lower values (31.05 and 31.77 µm/sec, respectively).

Straight line velocity (VSL) and straightness (STR)

The mean VSL and STR were also evaluated (Figure 6a, b). Tom 3 had the highest VSL (29.4 µm/sec), significantly ($P<0.05$) greater than Tom 1 (17.03 µm/sec), Tom 2 (20.29 µm/sec), and Tom 4 (25.69 µm/sec). Conversely, Tom 2 exhibited the highest STR (62.23%), which was significantly ($P<0.05$) higher than the other toms.

Linearity (LIN) and Wobble (WOB)

The average sperm LIN and WOB percentages were analyzed (Figure 7a, b). Tom 2 had the highest LIN (44.12%) and WOB (66.24%), significantly ($P<0.05$) outperforming the other toms. Tom 4 had the lowest LIN (27.10%) and WOB (53.36%).

Table 4. Macroscopic and microscopic evaluation of turkey semen

Parameters	Tom 1	Tom 2	Tom 3	Tom 4	Level of Significance
Semen volume (ml)	0.40±0.06^{a}	0.60±0.07^{c}	0.50±0.06^{b}	0.50±0.05^{b}	*
pH of the semen	7.09±0.14	7.19±0.17	7.13±0.16	7.14±0.17	NS
Sperm concentration ($\times10^{9}$/ml)	5.17±0.14^{c}	4.80±0.16^{b}	3.57±0.15^{a}	4.76±0.13^{b}	*

Values are Means±SEM; ^{a,b,c}indicates significant differences between toms; statistically significant difference is expressed as *(P<0.05); NS = non-significant (P>0.05).

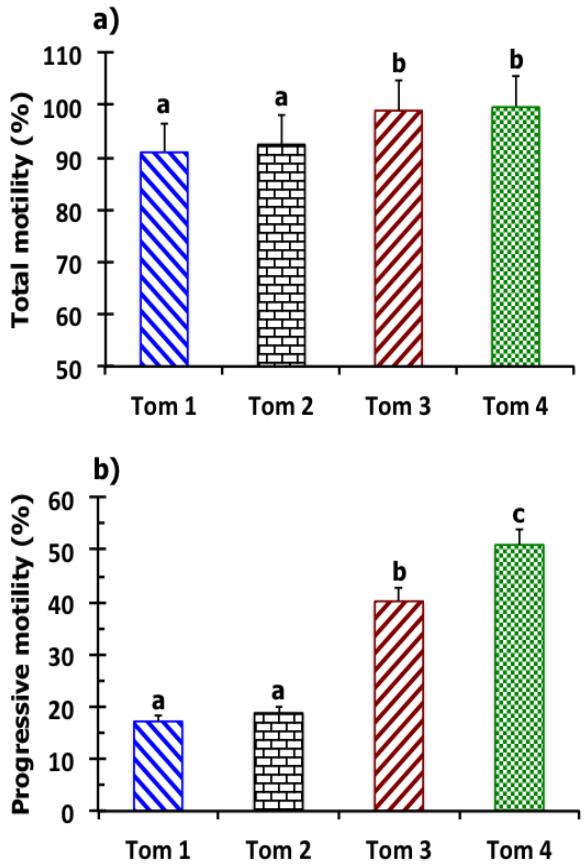

Figure 4. a) Total sperm motility and b) Progressive sperm motility of turkey semen obtained from 4 different toms. Each bar with an error bar represents the mean±SEMvalue. Different letters on the error bar indicate significant differences (P<0.05) among the toms

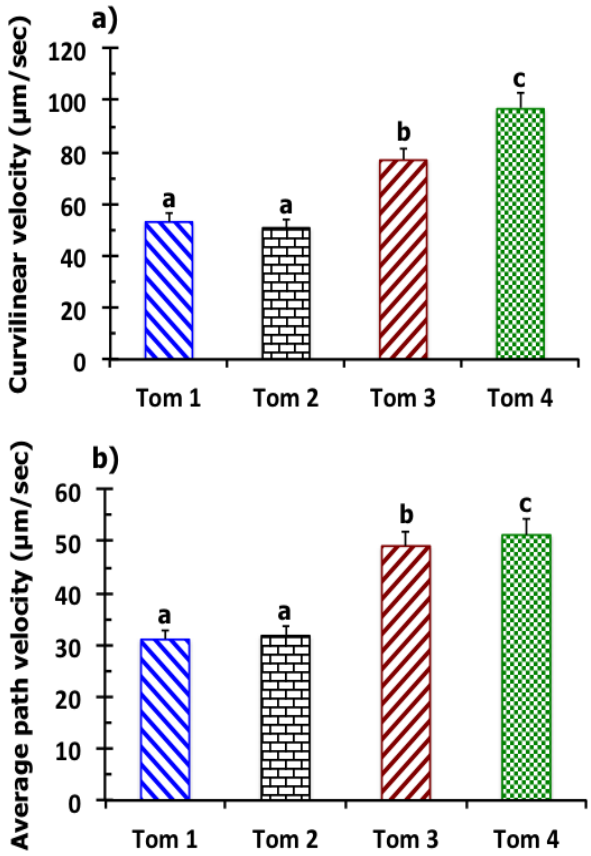

Figure 5. a) Curvilinear velocity and b) Average path velocity of turkey sperms were obtained from 4 different toms. Each bar with an error bar represents the mean±SEMvalue. Different letters on the error bar indicate significant differences (P<0.05) among the toms

Amplitude lateral head displacement (ALH) and beat cross frequency (BCF)

The ALH and BCF parameters were recorded for sperm from the four toms (Figure 8a, b). The highest ALH was observed in Tom 4 (5.04 µm), which was significantly (P<0.05) higher than the other toms. On the other hand, Tom 3 exhibitedthe highest BCF (5.66 Hz), which wassignificantly (P<0.05) greater than the values for Tom 1, Tom 2, and Tom 4.

Experiment II: Comparative Performance Between NM and AI in Turkey

The performance of AI using fresh semen from the toms was compared with NM for key reproductive traits, including fertility, embryonic mortality, hatchability, survivability, and day-old poult weight (Table 5).

Hens bred using AI with fresh semen from Tom 3 and Tom 4 showed significantly (P<0.05) higher fertility rates than hens bred by NM. However, other parameters such as early and late embryonic mortality, dead-in-shell rates, hatchability, survivability, and day-old poult weight did not differ significantly (P>0.05) between hens bred by NM and those bred using AI.

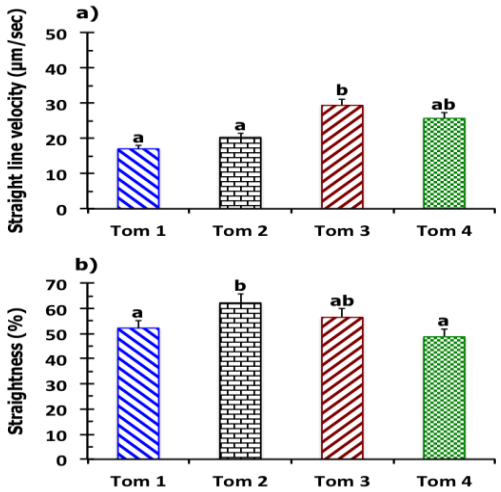

Figure 6. a) Straight line velocity and b) Straightness of turkey sperms were obtained from 4 different toms. Each bar with an error bar represents the mean±SEMvalue. Different letters on the error bar indicate significant differences (P<0.05) among the toms

4. DISCUSSION

The present study evaluates the sperm kinetic parameters of toms and egg fertility of turkey hens bred by AI using fresh semen collected from the toms. Sabra et al. (2017) reported that the average semen volume of toms ranged from 0.32 to 0.41 ml, and the average sperm concentration was 3.18–4.86 × 10^9/ml, with results similar to those found in the present study. Iorio et al. (2020) observed that the total motility, progressive motility, VCL, VSL, VAP, LIN, STR, WOB, ALH, and BCF of tom semen were 82.2 ± 1.2%, 26.2 ± 2.2%, 60.1 ± 3.9 µm/sec, 41.4 ± 3.6 µm/sec, 27.8 ± 2.2 µm/sec, 56.1 ± 3.5%, 35.1 ± 2.4%, 55.3 ± 2.4%, 2.8 ± 0.2 µm, and 4.6 ± 0.4 Hz, respectively. The WOB, ALH, and BCF values were comparable to those in the present study, although the total motility, VAP, and STR were lower, while VSLand LIN were higher than those observed in this study. On the other hand, the progressive motility and VCL of Tom 1 and Tom 2 were lower, while Tom 3 and Tom 4 showed higher values compared to Iorio et al. (2020).

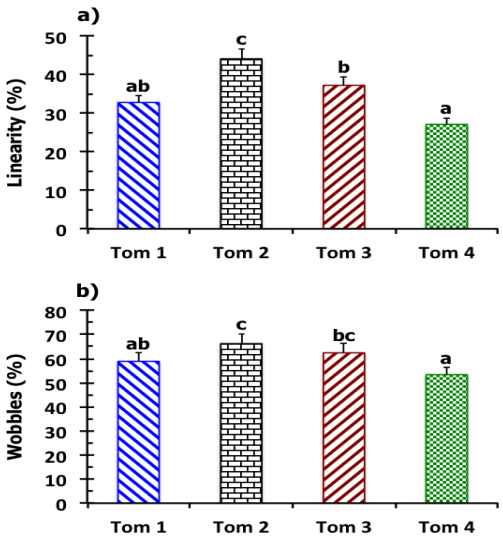

Figure 7. a) Linearity and b) Wobbles of turkey sperms were obtained from 4 different toms. Each bar with an error bar represents the mean±SEMvalue. Different letters on the error bar indicate significant differences (P<0.05) among the toms.

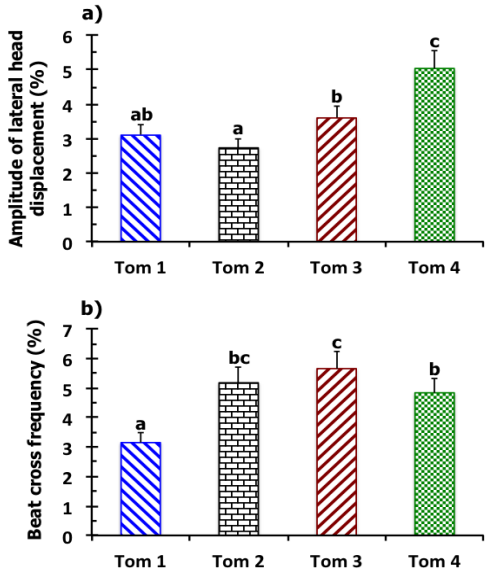

Figure 8. a) Amplitude of lateral head displacement and b) Beat cross frequency of turkey sperms were obtained from 4 different toms. Each bar with an error bar represents the mean±SEMvalue. Different letters on the error bar indicate significant differences (P<0.05) among the toms.

Table 5. Reproductive performance of hens bred by NM and AI

Parameters	Breeding system		Level of significance
	NM	AI	
Fertility (%)	74.84±0.62[a]	95.92±0.20[b]	*
Early embryonic mortality (%)	1.52±0.76	1.15±0.58	NS
Late embryonic mortality (%)	3.48±0.89	4.25±0.20	NS
Dead in shell (%)	3.67±0.93	3.88±1.01	NS
Hatchability (%)	91.09±0.85	90.71±1.35	NS
Survivability (%)	94.03±0.30	95.37±0.58	NS
Live weight of day-old poult (g)	46.12±0.02	45.97±0.09	NS

Values are Means±SEM; [a,b]indicates significant differences between toms; statistically significant difference is expressed as *(P<0.05); NS= non-significant (P>0.05)

Kammerer et al. (1972) found that hens inseminated with an average semen volume of 0.52 ml, progressive motility of 56%, and sperm concentration of 7.5 million/ml resulted in 80% fertility, a lower fertility rate than observed in the present study. This suggests that egg fertility is closely correlated with semen quality, with higher fertility observed in hens inseminated with fresh semen collected from the toms. This result is in line with the findings of Mohan et al. (2013) and Donoghue (1999), who reported that AI in turkeys is more effective than natural mating (NM).

AI using fresh semen collected from the toms resulted in the highest fertility, which is consistent with the observations of Emilia et al. (2010), who reported fertility rates up to 98%. The study was conducted with turkeys aged 35 to 46 weeks, during which good fertility results were obtained. Sexton (1977) noted that fertility increases to a peak before gradually declining as the hen's age increases, likely due to changes in the sperm storage tubule (SST), which can result in fewer sperm at the fertilization site. It can be inferred from this study that infertility in hens may arise from improper mating between toms and hens, as well as from improper application of the AI technique and insufficient semen retention capacity in hens.

The hatchability results indicated that AI did not affect turkey egg hatching. The hatching rate in this study (90–91%) was lower than the 95–100% range reported by Keith (2009), but higher than the 22–51% range reported by Machebe et al. (2013). Similarly, Ngu et al. (2013) and Anandh et al. (2012) reported hatchability rates of 56.25% and 52.85%, respectively, both of which were lower than those observed in this study. The non-significant difference in early embryonic mortality (EEM) among turkey groups suggests that the breeding method did not influence EEM. EEM can result from the rupture of the air sac and blood vessels due to poor handling of eggs during transportation and setting (Keith, 2009; Bramwell et al., 1996). The EEM observed in this study (lower than 13.0–23.0% as reported by Emilia et al., 2010) was also lower than the early, mid, and late embryonic mortalities (7.5%, 13.2%, and 19.3%, respectively) reported by Khan et al. (2013).

Additionally, thick egg shells can limit oxygen supply to the embryo, leading to asphyxiation and retarded development, which can cause late embryonic mortality (LEM) (Christensen and McCorkle, 1982). French (1997) suggested that metabolic heat production by the embryo can raise the egg temperature by 2°C above the surrounding air temperature, potentially causing embryonic death due to hyperthermia, a factor also observed by Hassan et al. (2004).

The present study showed that dead-in-shell increased with egg weight. Previous studies, such as that of Ngu et al. (2013), also found higher dead-in-shell percentages, with 42.75% in local and 35.16% in exotic breeds of turkey. This may result from difficulties in achieving adequate embryonic temperature during the initial stages and from the loss of metabolic heat in the later stages of incubation.

The non-significant results for the weight and survivability rates of poults indicated that the insemination procedure did not influence these factors. The average weight of poults obtained in this study was consistent with Anandh et al. (2012). The hatching weight of poults constitutes 63.5% of egg weight (Shanawany, 1987) and 7.0% of the initial egg mass for

turkey eggs (Rahn et al., 1981), and these findings agree with those reported in this study. The average survivability rate (94.0%) was also in line with the results of Anandh et al. (2012). However, mortality after hatching was observed due to improper brooding and piling.These fertility results suggest that AI using fresh semen collected from toms could be an effective and sustainable solution for addressing infertility in turkey hens in Bangladesh.

5. CONCLUSION

This study highlights AI as a sustainable solution for addressing infertility in turkey hens, especially in Bangladesh. By ensuring a higher fertility rate without compromising hatchability or poult survivability, AI offers significant potential for enhancing turkey farming. Future studies should explore the performance of AI with cryopreserved semen in commercial hybrid turkeys.

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