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Research Article

Harnessing the Power of Honey: A Natural Antioxidant for Enhancing Aseel Sperm Motility During Preservation

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Abstract

Semen cryopreservation is essential for poultry breeding, preserving genetic diversity, and the advancement of artificial insemination techniques. However, oxidative stress remains a major limitation during semen storage, as it negatively affects sperm motility, structural integrity, and fertilizing ability. This study investigated the potential of honey, a natural antioxidant-rich substance, in improving sperm motility of Aseel roosters during short-term storage. Semen was collected from sixteen healthy and mature Aseel roosters and diluted using modified Ringer's solution supplemented with varying concentrations of honey (1%, 2%, 3%, 4%, and 5%). A control group without honey supplementation was maintained for comparison. Semen quality parameters, including sperm concentration, total motility, progressive motility, non-progressive motility, and percentage of immotile sperm, were evaluated at 0, 2, and 4 hours post-storage using a Computer-Assisted Semen Analyzer (CASA). The results indicated that honey significantly ($P<0.05$) enhanced sperm motility, with the 3% honey group showing the highest total motility (87.80%) at 2 hours of storage, compared to the control (66.23%). The improvement in motility is attributed to the antioxidant compounds in honey, particularly flavonoids and polyphenols, which help reduce lipid peroxidation and preserve cellular function. These findings demonstrate that a 3% concentration of honey is optimal for maintaining sperm motility during short-term preservation. The use of honey as a natural and non-toxic additive in semen extenders offers a promising alternative to synthetic compounds. Incorporating honey into semen preservation protocols may enhance reproductive efficiency and support the sustainable conservation of indigenous poultry breeds like the Aseel.

Keywords: Aseel rooster; honey; antioxidant; semen preservation; sperm motility.

Introduction

The preservation of poultry semen is essential for modern reproductive technologies, including artificial insemination, selective breeding, and conservation of indigenous genetic resources. Among indigenous breeds, the Aseel rooster is notable for its superior meat quality, disease resistance, and adaptability to harsh conditions. However, this breed faces genetic dilution due to the rise of commercial broilers. One major obstacle to effective semen preservation in poultry is oxidative stress, which arises during cooling and storage and leads to a reduction in sperm motility, viability, and membrane integrity. This is primarily due to the high content of polyunsaturated fatty acids (PUFAs) in avian sperm, which are highly susceptible to lipid peroxidation (Arif *et al.*, 2025).

To counteract these effects, various semen extenders have been developed. Traditional cryoprotectants such as glycerol and dimethyl sulfoxide (DMSO) have been used, but they are associated with cellular toxicity and osmotic imbalance, which may compromise sperm functionality. Recent studies have focused on incorporating antioxidants into extenders to improve preservation outcomes. These antioxidants work by neutralizing reactive oxygen species (ROS) and reducing lipid peroxidation, thereby protecting sperm cells from oxidative damage (Kargari *et al.*, 2024).

Natural antioxidants are of particular interest due to their safety, availability, and compatibility with biological systems. Among them, honey has received attention because it contains phenolic compounds, flavonoids, ascorbic acid, glucose oxidase, and other components with strong antioxidant potential. Honey also contributes sugars that may serve as energy sources for sperm metabolism and promote motility. In KUB roosters, supplementation of 0.4 percent honey in a lactated Ringer's egg-yolk extender was shown to preserve total motility, membrane integrity, and viability up to 48 hours of chilled storage at 5 degrees Celsius (Arif *et al.*, 2023).

In mammalian studies, honey has also been successfully used to enhance post-thaw motility and acrosomal integrity. For instance, Gardela *et al.* (2023) demonstrated that a combination of honey with coenzyme Q10 and vitamin E improved motility and membrane stability in rabbit sperm after freezing. Furthermore, hydroxytyrosol, another natural antioxidant derived from olives, has been reported to enhance motility and acrosome integrity in rooster sperm post-thaw at a concentration of 25 micrograms per milliliter (Kargari *et al.*, 2024).

While such results are encouraging, specific research on the use of honey in Aseel rooster semen preservation remains scarce. Aseel roosters represent a valuable genetic resource in South Asia, and preserving their reproductive potential is crucial for sustainable poultry development. Therefore, it is important to explore natural, non-toxic, and effective additives for use in extenders that can maintain semen quality over short-term storage periods.

This study aimed to investigate the effects of honey as a natural antioxidant on the motility of Aseel sperm during short-term preservation. By evaluating different concentrations of honey in semen extenders, this research sought to determine the optimal level of supplementation that maximized sperm motility and progressive movement. The findings contributed to the advancement of sustainable poultry breeding practices and provided a natural alternative to synthetic cryoprotectants in semen preservation.

Materials and Methods

Study Location and Duration

The study was conducted at the Advanced Avian Research Farm of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, under the project titled "Conservation and Germplasm Cryobanking of Native Chicken Genetic Resources of Bangladesh." The research spanned a period of seven months, from initial setup to the completion of data collection and analysis. This duration allowed for sufficient semen collection, management of experimental birds, and analysis of semen quality parameters over time.

Experimental Birds and General Management

Sixteen mature Aseel roosters (12–18 months of age) were selected from the Advanced Animal Research Farm. These birds were chosen based on their reproductive health, history, and genetic background to ensure consistent and high-quality semen. Prior to the start of the experiment, the roosters were checked for disease and reproductive abnormalities, ensuring that all birds were in optimal health. This selection process was essential for obtaining high-quality semen for the study.

The Aseel roosters were housed in deep-litter pens to encourage natural behaviors while minimizing stress and aggression. These pens were carefully designed to provide adequate space for the birds, allowing for freedom of movement and proper ventilation. Birds were maintained under a semi-intensive management system, following standard practices as per the regulations of the Bangladesh Veterinary Council. The temperature of the housing environment was maintained between 21°C and 35°C, and a 16-hour light/8-hour dark photoperiod was used to optimize reproductive activity.

Table 1: Nutrient composition of experimental diet

Nutrients	Amount (%)
DM (%)	88.00
Crude protein (%)	21.00
Calcium (%)	2.00
Phosphorus (%)	0.45
Crude Fat (%)	5.00
ME (Kcal/kg)	3000−3100

Source: *Aftab Bahumukhi Farms Ltd. Bangladesh*

The birds were provided with a balanced diet (Table 1) containing 21% crude protein and essential micronutrients such as vitamins and trace minerals, particularly zinc and selenium, which are vital for sperm production and overall reproductive health. Fresh water was provided ad libitum through an automated watering system, and rice husk was used as bedding material. Birds were monitored regularly for health, with weekly assessments that included body

weight, physical health checks, and reproductive evaluations.

Training and Semen Collection

At the age of 28 weeks, all the roosters were trained for semen collection using the abdominal massage technique, as described by Miah *et al.* (2024). Training was conducted twice a week to acclimate the birds to the procedure and reduce stress during the actual semen collection. By 32 weeks, all the roosters were fully trained and ready for semen collection.

Semen was collected using the abdominal massage method, where the bird was gently restrained, and the cloaca was massaged to induce semen ejaculation. This method ensured minimal stress and maximum semen quality. The procedure was carried out in the morning (8:00–9:00 AM) to avoid the heat of the day, further reducing stress on the birds.

Semen Extender, Dilution and Analysis

Ringer's solution was used as the base semen extender for this study as a control. The semen diluent was prepared according to the composition described by Akcay *et al.* (2006), as presented in Table 2.

Table 2. Composition of the modified Ringer's semen diluents

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

Source: Akcay *et al.* (2006)

Additionally, different levels of honey (1, 2, 3, 4, and 5%) were used as an alternative to glucose in the Ringer's solution to explore its potential antioxidant properties. Honey was chosen for its known role in protecting sperm cells from oxidative damage due to its flavonoid and polyphenol content (Zaid *et al.*, 2021). These honey-based extenders were tested alongside the standard Ringer's solution to assess the effect of honey on semen quality.

Immediately after semen collection, macroscopic parameters such as semen volume, color, and pH were recorded. The pH was measured using a standard pH meter, ensuring that the semen quality remained consistent with expected norms. The good quality semen was diluted using the prepared Ringer's solutions, including both the control and honey-modified extenders. A semen-to-extender ratio of 1:20 was used for all samples, as this dilution ratio had previously provided the best results in preliminary trials for analyzing sperm motility using the Computer-Assisted Semen Analyzer (CASA) system (Microoptic Automatic Diagnostic System, Barcelona, Spain).

For motility analysis, semen samples were stored in a water bath at 37.5°C to maintain optimal conditions. Motility was measured at 0, 2, and 4 hours post-collection. A small aliquot (0.5 µl) of the diluted semen was placed on a clean microscope slide. Care was taken to avoid touching the cover slip with hands. Excess semen was removed with a cotton bud tip to prevent floating cells. After resting for 5 seconds, the analysis was performed swiftly to prevent evaporation.

Total motility, progressive, motility, non-progressive motility and immotility of the sperm were analyzed using the CASA system under a negative phase contrast microscope with a 10X objective and a green filter. The microscope condenser was set for chicken (animal species), and the selection program was SCA motility. At least 500 sperm per sample were counted under this study.

Statistical Analysis

All data were analyzed using standard statistical methods. Significant differences between treatment groups were determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $P<0.05$. Results were expressed as means ± standard error of the mean (SEM).

Ethical Considerations

This study was conducted in compliance with institutional animal care and use guidelines. The research protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Hajee Mohammad Danesh Science and Technology University. All procedures, including semen collection and handling, were performed by trained personnel to ensure minimal stress to the birds. Efforts were made to maintain the highest ethical standards, including minimizing discomfort and ensuring humane treatment throughout the study.

Results and Discussion

Total Motility

The effect of honey supplementation on total sperm motility at various storage intervals is presented in Table 3. Sperm motility, especially total motility, is a key determinant of fertilization capacity, as only motile sperm can reach and penetrate the ovum. In the present study, honey supplementation significantly improved total motility, with the 3% honey-treated group achieving the highest motility (87.80 ± 6.20%) at 2 hours post-storage, in comparison to the control group (66.23 ± 4.78%).

This enhancement in motility is likely due to honey's bioactive constituents, including flavonoids, phenolic acids, and ascorbic acid, which function as potent antioxidants that neutralize ROS and reduce lipid peroxidation. These

compounds help protect mitochondrial structure and function, maintaining ATP production necessary for sperm movement. A study by Arif *et al.* (2023) demonstrated similar findings in KUB roosters, showing enhanced motility and viability with 0.4% honey supplementation in chilled storage conditions.

Breque *et al.* (2003) further highlighted that antioxidant-enriched extenders significantly improve sperm quality by preventing oxidative damage to lipids and proteins. Consistent with our findings, Khan (2011) confirmed that antioxidant supplementation positively influenced rooster sperm motility during storage.

At 4 hours post-storage, a natural decline in motility was observed in all groups, likely due to progressive energy depletion and cumulative oxidative stress. Nevertheless, honey-treated groups maintained significantly higher motility levels than the control, suggesting that honey prolongs sperm viability through continuous antioxidant action (Kargari *et al.*, 2024).

Progressive and Non-Progressive Motility

Data on progressive and non-progressive motility are presented in Table 4. Progressive motility, defined as forward, linear sperm movement, is essential for successful fertilization. In this study, progressive motility was significantly improved in honey-treated samples, with the 3% honey group showing the highest progressive motility (65.78 ± 4.78%) at 2 hours, while the control exhibited only 42.34 ± 3.78%.

The improvement is likely due to honey's ability to preserve the integrity of the plasma membrane and acrosome, allowing sustained motility. Natural antioxidants in honey stabilize cell membranes and prevent mitochondrial disruption (Arif *et al.*, 2025). These observations are supported by Li *et al.* (2025), who noted enhanced sperm progression following supplementation with natural cryoprotectants, including honey-based compounds.

Non-progressive motility, which reflects uncoordinated or circular sperm movement, was significantly reduced in honey-treated groups. The control group showed a higher proportion of non-progressive motility, likely caused by oxidative damage impairing flagellar motion. Saxena *et al.* (2010) suggested that high levels of ROS damage axonemal proteins and reduce mitochondrial efficiency, leading to non-progressive movement. Honey, through its antioxidant and osmoprotective properties, helps maintain flagellar functionality, thereby reducing non-progressive motility (Arif *et al.*, 2023).

Table 3. Effect of honey supplementation on total motility (%) at different storage times (hour)

Honey	0 hr	2 hr	4 hr
Control (0%)	90.12 ± 3.45^{c}	66.23 ± 4.78^{d}	45.12 ± 3.80^{e}
1%	91.23 ± 2.98^{bc}	74.11 ± 5.01^{c}	50.78 ± 4.55^{d}
2%	92.34 ± 3.12^{b}	79.65 ± 4.85^{b}	58.45 ± 3.95^{c}
3%	94.11 ± 2.89^{a}	87.80 ± 6.20^{a}	66.34 ± 5.12^{b}
4%	93.45 ± 3.01^{a}	84.12 ± 5.43^{ab}	62.45 ± 4.67^{b}
5%	91.87 ± 3.24^{b}	78.32 ± 4.77^{b}	55.67 ± 3.89^{c}

Values represent mean ± SEM. Different superscripts within the same column indicate significant differences (P<0.05).

Table 4. Effect of honey supplementation on progressive motility (PM) and non-progressive motility (NPM) (%) at different storage times (hour)

Honey	0 hr		2 hr		4 hr	
	PM	**NPM**	**PM**	**NPM**	**PM**	**NPM**
Control (0%)	45.12 ± 3.78^{d}	24.89 ± 2.55^{a}	42.34 ± 3.78^{d}	23.89 ± 2.55^{a}	38.45 ± 3.21^{e}	22.12 ± 2.32^{b}
1%	52.23 ± 3.92^{c}	25.02 ± 3.01^{a}	50.12 ± 3.92^{c}	24.02 ± 3.01^{a}	46.11 ± 3.88^{d}	22.78 ± 2.95^{b}
2%	59.34 ± 4.34^{b}	23.42 ± 2.89^{b}	57.23 ± 4.34^{b}	22.42 ± 2.89^{b}	53.78 ± 3.65^{c}	21.55 ± 2.76^{c}
3%	68.12 ± 4.78^{a}	20.34 ± 2.54^{c}	65.78 ± 4.78^{a}	19.34 ± 2.54^{c}	60.23 ± 4.32^{b}	18.23 ± 2.45^{d}
4%	64.45 ± 3.98^{a}	21.12 ± 3.11^{c}	62.45 ± 3.98^{a}	20.12 ± 3.11^{c}	58.34 ± 3.76^{b}	19.34 ± 3.01^{d}
5%	58.32 ± 4.01^{b}	22.78 ± 2.76^{b}	55.32 ± 4.01^{b}	21.78 ± 2.76^{b}	50.12 ± 3.88^{c}	20.11 ± 2.89^{c}

Values represent mean ± SEM. Different superscripts within the same column indicate significant differences (P<0.05).

Table 5: Effect of honey supplementation on immotile sperm percentage (%) at different storage times (hour)

Honey	0 hr	2 hr	4 hr
Control (0%)	9.88 ± 2.12^{a}	33.77 ± 4.78^{a}	54.88 ± 5.12^{a}
1%	8.77 ± 1.98^{ab}	25.89 ± 4.12^{b}	49.22 ± 4.67^{b}
2%	7.66 ± 2.10^{b}	20.35 ± 3.78^{c}	41.55 ± 4.34^{c}
3%	5.89 ± 1.76^{c}	12.20 ± 6.20^{d}	33.66 ± 4.89^{d}
4%	6.55 ± 2.01^{c}	15.88 ± 4.43^{d}	37.55 ± 3.78^{d}
5%	8.13 ± 2.23^{b}	21.68 ± 3.99^{c}	44.33 ± 4.22^{c}

Values represent mean ± SEM. Different superscripts within the same column indicate significant differences (P<0.05).

Immotile Sperm Percentage

The percentage of immotile sperm is presented in Table 5. At 2 hours post-storage, the control group exhibited the highest immotility (33.77 ± 4.78%), while the 3% honey-treated group recorded the lowest (12.20 ± 6.20%). These findings further affirm the antioxidant function of honey in preserving sperm vitality.

Honey is known to contain a complex mixture of bioactives, including polyphenols, vitamin C, and glucose oxidase, which work synergistically to scavenge free radicals and protect lipid bilayers from oxidation. This helps maintain sperm membrane permeability and structural stability (Gardela *et al.*, 2023). Additionally, the natural sugars in honey may serve as an energy source, prolonging sperm motility over time (Kargari *et al.*, 2024).

In contrast to synthetic cryoprotectants like glycerol and DMSO, which can induce osmotic stress and compromise membrane potential, honey offers a biocompatible, non-toxic alternative with cryoprotective benefits (Li *et al.*, 2025). This dual role as both an energy source and an antioxidant makes honey a uniquely advantageous additive for semen extenders.

These results align with the objectives of genetic conservation and artificial insemination programs, particularly for native breeds such as Aseel. According to Arif *et al.* (2025), the use of safe, natural substances in semen preservation is essential for maintaining the reproductive efficiency of indigenous poultry breeds. The findings of this study support honey's utility as an effective, low-cost, and sustainable additive in avian semen preservation protocols.

Conclusion

This study conclusively demonstrated that the supplementation of honey, particularly at a 3% concentration, significantly enhanced the quality of rooster semen during short-term preservation. Notably, honey-treated samples exhibited improved sperm motility, a marked reduction in the percentage of immotile sperm, and superior progressive movement. These improvements can be attributed to the rich natural antioxidant composition of honey, which plays a critical role in mitigating oxidative stress and cellular damage are the key factors responsible for sperm quality deterioration during storage.

The incorporation of honey as a natural additive in semen extenders presents a promising, eco-friendly, and cost-effective alternative to synthetic cryoprotectants, many of which may pose cytotoxic risks or contribute to environmental concerns. Moreover, the ease of availability, affordability, and multifunctional bioactivity of honey further enhance its practicality for application in the poultry industry, especially in developing regions where access to advanced preservation materials may be limited.

These encouraging results provide a strong foundation for future research aimed at exploring the long-term cryopreservation potential of honey-based extenders. Furthermore, *in vivo* fertility trials are recommended to validate the actual reproductive outcomes associated with honey-treated semen. Such investigations would contribute significantly to optimizing artificial insemination protocols, thereby improving reproductive efficiency, genetic conservation, and overall productivity in poultry breeding programs.

In summary, the findings of this study reinforce the potential of honey as a natural, safe, and effective bio-extender component and open new avenues for sustainable and enhanced poultry reproduction technologies.

Authors' Contribution

All authors contributed equally at all stages of research and manuscript preparation. Final form of manuscript was approved by all authors.

Conflict of Interest

Authors declare no conflict of interest with the present publication.

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