JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(2): 216-222, June 25, 2023

Research Paper, PII: S2322455X2300024-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.24

Effects of Using Commercial and Homemade Extenders on Sperm Quality of Liquid Stored Semen of Horro Chicken Breed

Tarekegn Getachew[1]*, Gebeyehu Goshu[2], and Alemayehu Lemma[3]

[1]*PhD Candidate, Lecturer, Haramaya University, Haramaya, Ethiopia*
[2]*Professor of Animal Production, Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia*
[3]*Professor of Reproductive Physiology, Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia*

Corresponding author's E-mail: targech23@gmail.com

Received: 20 November 2022
Accepted: 27 February 2023

ABSTRACT

This study aimed to evaluate the suitability of homemade tris-egg yolk-based and Commercial Beltsville poultry extenders for short-term storage of semen from the Ethiopian Indigenous Horro chicken breed at refrigeration temperature. A total of 30 Horro roosters with an average age of 40 weeks were used to collect semen. The treatments (T) in the sperm quality experiment were control (semen without extender added), semen extended with homemade extender (E1), and semen extended with commercial Beltsville Poultry Semen Extender (E2). Changes in spermatozoa motility, *in vitro* viability, and morphology were evaluated in fresh semen and semen diluted as 1:4 (v/v semen to extender) and stored for 4, 8, 12, and 24 hours at 4°C. During semen storage, there was a decrease in mass motility, an increase in morphologically abnormal spermatozoa with a high incidence of the bent tail, and an increase in dead spermatozoa. The commercial Beltsville poultry extender was found to be the most suitable extender regarding mass motility and *in vitro* viability of stored spermatozoa, but there was no significant difference in sperm abnormalities across all extenders. The results showed locally prepared tris-egg yolk-based extender could be a suitable extender for short-term storage of chicken sperm regarding the sperm quality attributes.

Keywords: Horro, *In vitro* viability, Motility, Morphology, Semen, Sperm

INTRODUCTION

Growing demand for poultry products and the high rearing cost of breeder stock necessitates the development of modern solutions to increase production efficiency at reducing costs. Artificial insemination is one of the solutions that significantly lowers the cost of rearing by decreasing the number of males in the flock (Łukaszewicz et al., 2020). Artificial insemination was the first great biotechnology applied to improve the reproduction of farm animals. It has an impact worldwide on many species of farm animals and endangered species (Foote, 2002). Artificial insemination technology laid the foundation for developing other reproductive technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation, embryo harvesting, freezing, culture and transfer, and cloning. Artificial insemination in poultry grew significantly during the last few decades after the development of semen collection through abdominal massage (Siudzin´ska and Łukaszewicz, 2008). Artificial Insemination in poultry reproduction has caused investigators to become interested in studying the semen characteristics of different poultry breeds (Haunshi, et al., 2010).

One of the advantages of AI application in poultry is the efficient use of males. This, in turn, decreases the cost of breeding directly by reducing the number of roosters (Benoff et al., 1981). The increasing importance of AI in poultry reproduction has caused investigators to be interested in developing the proper conditions for liquid (short-term) semen storage (Lake, 1983). The possibility of dilution and storage of poultry semen would enable poultry breeders to use superior males and inseminate many females even on distant farms (Reddy, 1995). The most common procedure for short-term storage of semen requires suspending sperm in an extended to retain their viability *in vitro* (Reddy, 1995). A comparison of diluted and undiluted stored semen showed

that applying extenders is necessary to sustain good-quality sperm (Bilgili et al., 1987, Łukaszewicz et al., 2020). Studies have indicated that diluted poultry semen could be stored for up to 24 hours without impairing its viability and fertilizing ability (Soler et al., 2016; Silyukova et al., 2022). According to Gerzilov (2010), many factors could affect the quality of stored semen, such as the types of diluents, packaging, and cooling rates. The aim of this study was to determine the effect of two types of extenders on the qualitative characteristics of spermatozoa during short-term storage.

MATERIAL AND METHODS

Ethical approval

The present study followed institutional guidelines for humane animal treatment and complied with relevant legislation from Addis Ababa University College of Veterinary Medicine, Ethiopia.

Roosters' management

For the purpose of semen collection, thirty adult Horro roosters with an average age of 40 weeks were purchased from Debrezeit Agricultural Research Center, Bishoftu, Ethiopia. All experimental animals were managed at the poultry farm of the Debre Zeit Agricultural Research Center. The roosters were kept separately from the hens and trained for semen collection by abdominal massage technique for 2 weeks. The roosters were kept in a deep litter system and fed with a breeder ration containing 17% CP and 2800 Kcal/Kg energy (Table 1). Feed was provided twice a day with an amount of 110 gm/rooster/day, and water was provided *ad libitum*. All experimental chickens were dewormed and vaccinated for major diseases, including Newcastle, Marek's, Gumboro, fowl pox, and fowl typhoid (Table 2). The roosters were acclimatized for two weeks before sample collection.

Table 1. Breeder ration formula used during the experiment

Serial number	Feed ingredient	Inclusion rate (%)
1	Corn	52
2	Soy cake	10
3	Meat and bone meal	6
4	Wheat bran	15
5	Noug cake	9
6	Limestone	6
7	Breeder premix	0.5
8	Lysine	0.1
9	Methionine	0.1
10	Molasses	1
11	Salt	0.3

Breeder premix: Industrial, well-balanced premix that ensures fertile, hatching eggs and ultimately strong chicks. It contains vitamins and minerals.

Table 2. Vaccination schedule of Horro chickens in the present experiment

Age	Vaccine	Administration route
Day-1	Marek	Subcutaneous (neck)
Day-2	Newcastle disease	Eye drop
Day-7	Gumboro	Drinking water
Day-14	Newcastle (Lasota)	Drinking water
Day-18	Gumboro	Drinking water
Week-6	Newcastle (Lasota)	Drinking water
Week-8	Fowl typhoid	Injection
Week-9	Deworming	Drinking water
Week-10	Fowl pox	Wing stab
Week-14	Fowl typhoid	Injection

The vaccines originated from the National Veterinary Institute, Bishoftu, Ethiopia

Table 3. Contents of the homemade extender

Contents	Amount
Tris (base)	2.42 gm
Citric acid	1.48 gm
Fructose	4 gm
Egg-yolk	20 % v/v
Gentamicin	25 mg
Double distilled water	100 ml

pH was adjusted to 6.8

Extender preparation

The homemade extender used in this study was tris-egg-yolk-based. Semen diluents were prepared by mixing tris (base), citric acid, fructose, and egg yolk, into which an antibiotic was added. The ingredients of the extender were purchased from a local supplier. The composition of diluents is presented in Table 1. The second extender (E2) was the Beltsville commercial extender (P2-7450, continental, Delavan, WI, USA), a standard extender for the preservation of avian semen. Its composition was sodium glutamate (8.67 g/l), dipotassium phosphate (7.59 g/l), sodium acetate (3.2 g/l), fructose (5 g/l), potassium citrate (0.64 g/l), n-tris (hydroxymethyl) methyl 1-2 amino ethane sulfonic acid (TES; 3.2 g/l), monopotassium phosphate (0.7 g/l) and magnesium chloride (0.34 g/l). Osmolarity and pH were set at 310 mOsmol/kg and 7.1, respectively.

Semen collection and initial evaluation

Semen was collected using the Quinn and Burrows abdominal massage technique developed in 1936. The semen was collected with a sterile tube. Two ejaculates were collected from each rooster. The ejaculate volume varies from rooster to rooster, which averages 0.3 ml. The roosters were trained for semen collection following the two weeks of acclimatization. After collection, the semen was maintained in a water bath at 37°C and subjected to on-site pre-freeze evaluation, including volume, color, pH, sperm concentration

(bill/ml), motility (%), morphological abnormality (percentage of abnormal sperms) and live percent. Qualifying ejaculates having > 60% motility, > 70% live percent, and < 30% morphological abnormality were pooled to get sufficient semen for replication and further processing (Getachew et al., 2015).

Semen processing for liquid storage assessment

After pre-freeze evaluation for semen quality attributes, qualifying semen was pooled to get 15 ml of semen aliquots. The semen aliquots were divided into three groups with 5ml each and diluted at 37°C within 10 minutes with two pre-warmed extenders (homemade extender (E1), and commercial Beltsville extender (E2) at 1:4 ratio (v/v)). A 5 ml third un-extended semen portion was set as a control. Each treatment had 5 replications. Semen was diluted immediately after initial evaluation and stored at 4°C for 4, 8, 12, and 24 hours. All the semen quality assays were performed at 4, 8, 12, and 24 hours of storage (Silyukova et al., 2022).

Semen quality assays for liquid-stored semen

Semen was first evaluated for volume (ml), color, texture, and pH. The concentration (mil/ml and billion) was measured using a hemocytometer (Counting chamber, Muhwa, China), while motility (%), viability (%), and morphology (%) were evaluated under the light microscope (MSC-P200). An eosin-nigrosin stain was used to evaluate morphology at X1000 magnification under oil immersion. A total of 200 spermatozoa were counted to determine the percentage of abnormal sperms (Siudzin´ska and Łukaszewicz, 2008).

Statistical analysis

The data collected during the study period were subjected to Analysis of Variance (ANOVA) using STATA software (version 12). Means values were compared using LSD. A significance level of 5% was used to determine statistical significance when F-test was found significant ($p < 0.05$). Factorial 3*4 completely randomized design was utilized to evaluate the effect of storage time and types of extenders.

Table 4. Treatments and experimental layout for fresh semen quality assessment of Horro Breed

Type of extender \ Time of storage	4 hours	8 hours	12 hours	24 hours
0 (Control)	0 (H4)	0 (H8)	0 (H12)	0 (H24)
E1	E1 (H4)	E1 (H8)	E1 (H12)	E1 (H24)
E2	E2 (H4)	E2 (H8)	E2 (H12)	E2 (H24)

E1: Extender 1; E2: Extender 2; H: Hour

RESULTS

Fresh semen characteristics

A summary of the results of semen characteristics addressed in this study is presented in Table 5. The effect of semen extenders and storage time on sperm quality is presented in Table 6.

Effect of semen extenders and storage time on sperm quality

There were significant differences in sperm motility and *in vitro* viability across the interactions of storage time and a group of extenders ($p < 0.05$). No significant difference was observed in sperm morphological abnormalities across the interactions of storage time and extenders ($p < 0.05$). Significantly highest sperm motility and *in vitro* viability rate was observed in semen extended using a commercial extender at 4 hours of storage ($p < 0.05$). The percentages of live sperms in treatments were observed at 83.6% and 82.6% for commercial extender extended semen and locally prepared extender extended semen, respectively. Semen extended with E2 and E1 extenders was observed consistently higher motility, compared to the control extender, irrespective of storage time.

Effect of semen extenders on sperm quality

There were significant differences ($p < 0.05$) in sperm motility, morphological abnormalities, and *in vitro* viability between the control and the two extenders. However, there was no significant difference ($p < 0.05$) in all semen quality parameters between the commercial and locally prepared extenders. Significantly highest sperm motility, morphological abnormalities, and *in vitro* viability rates were observed in semen extended samples using commercial and locally prepared extenders when compared to the control treatment ($p < 0.05$).

Effect of interaction of semen extenders and storage time on sperm quality

There were significant differences in progressive sperm motility across all groups of treatments ($p < 0.05$). There were significant differences observed in sperm morphological abnormalities between all groups except the 12 and 24 hours of storage ($p < 0.05$). There were also significant differences ($p < 0.05$) in *in vitro* sperm viability across the durations of storage except for between 8 and 12 hours of storage. Significantly highest sperm motility, lower morphological abnormalities, and higher *in vitro* viability rates were observed in semen stored for 4 hours compared to other groups ($p < 0.05$).

Table 5. General semen characteristics of the Horro chicken breed

Semen characteristics	Mean semen characteristics
Ejaculate volume (ml)	0.36
Color	Milky white
Texture	Moderate viscous
Sperm total concentration/ml	$5.5X10^9$
Sperm count/ejaculate	$1.98X10^9$
Ph	7.2

Table 6. Effect of interaction of semen extenders and storage time on sperm quality of Horro chicken

Mean ± SE sperm parameters / Factor	Progressive motility (%)	Abnormality (%)	Viability (%)
Extender (storage time Significance) at $p < 0.05$	***	NS	***
Control (4 hours)	77 ± 2.54^a	15.4 ± 1.81	73.2 ± 1.39^b
Control (8 hours)	59 ± 1.87^b	16.2 ± 1.80	55.8 ± 1.66^c
Control (12 hours)	42 ± 1.22^c	25.4 ± 2.78	49 ± 1.22^c
Control (24 hours)	21 ± 1.00^d	27.2 ± 1.59	11.4 ± 1.21^d
E2 (4 hours)	87 ± 1.22^a	10.4 ± 0.51	83.6 ± 1.63^a
E2 (8 hours)	79 ± 1.00^a	15 ± 1.82	77.8 ± 1.28^a
E2 (12 hours)	50 ± 2.74^b	17.8 ± 1.62	68.4 ± 1.50^b
E2 (24 hours)	46 ± 1.87c	23 ± 2.30	51 ± 1.14^c
E1 (4 hours)	84 ± 1.00^a	12.2 ± 1.39	82.6 ± 1.36^a
E1 (8 hours)	72 ± 1.22^a	16.6 ± 1.57	73.8 ± 1.93^b
E1 (12 hours)	49 ± 1.87^b	19.8 ± 2.08	66.4 ± 1.50^b
E1 (24 hours)	45 ± 1.58^c	25.4 ± 1.21	46.4 ± 1.44^c

E1: Extender 1; E2: Extender 2; NS: Non-significant; SE: standard error; [abcd] Different letters within the same row show significant differences among the groups ($p < 0.05$).

Table 7. Effect of semen extenders on sperm quality of Horro chicken

Mean ± SE sperm parameters / Factor	Progressive motility (%)	Abnormality (%)	Viability (%)
Control	49.75 ± 4.82^b	21.05 ± 1.54^b	47.35 ± 5.21^b
E1	65.25 ± 4.22^a	16.55 ± 1.30^a	70.2 ± 2.90^a
E2	62.75 ± 3.71^a	18.5 ± 1.32^a	67.3 ± 3.15^a

E1: Extender-1; E2: Extender-2; SE: Standard error; [abc] Different letters within the same row show significant differences among the groups ($p < 0.05$).

Table 8. Effect of storage time on sperm quality of Horro chickens

Mean ± SE sperm parameters / Factor	Progressive motility (%)	Abnormality (%)	Viability (%)
4 hours	82.67 ± 1.45^a	12.67 ± 0.91^a	79.8 ± 1.48^a
8 hours	70 ± 2.34^b	15.93 ± 0.95^b	69.13 ± 2.70^b
12 hours	47 ± 1.44^c	21 ± 1.46^c	61.26 ± 2.45^b
24 hours	37.33 ± 3.19^d	25.2 ± 1.05^c	36.26 ± 4.77^c

SE: Standard error; [abcd] Different letters within the same row show significant differences among the groups ($p < 0.05$).

DISCUSSION

Semen color depend on the species of chicken used; however, generally, present findings of milky white semen were in agreement with previous reports by Peters et al. (2008) and Mussa et al. (2023). The color of domestic fowl semen varies from a dense opaque suspension to a watery fluid secreted by various reproductive glands, from a relatively high sperm density or degrees of clear to milky white, with declining sperm numbers (Hafez and Hafez, 2000). According to Yadav et al. (2019), the color of semen may depend on the species of chicken used, but generally semen should be creamy which indicates a high sperm concentration which is in agreement with the current study. Color could also serve as an indicator of contamination (Yadav et al., 2019).

According to Peters et al. (2008), the average ejaculate volume of semen from chicken using the

abdominal massage technique was 0.01 ml to 0.35 ml in Giriraja, Frizzled feathered chicken. Bah et al. (2001) also reported an ejaculate volume of 0.28 ml in Nigerian local cocks. Cole and Cupps (1977) reported ejaculate volume within the range of 0.1 ml to 1.5 ml per ejaculation. On the other hand, Hafez and Hafez (2000) indicated that the average sperm volume collected from white leghorn varies from 0.2 to 0.5 ml. These studies are in agreement with the result found in this study on Ethipian Horro Chicken which is 0.36 ml/ejaculate.

The average sperm concentration in the present study was $5.5X10^9$/ml. Results from Antalan et al. (2015); AL-Saeedi et al. (2019) showed that, the concentration of ranging 3.4 to $6.8X10^9$/ml in Lohmann Brown cocks. According to Gordon (2005) reported the average sperm concentration of poultry semen was $5000X10^6$ sperm/ml. On the other hand, the sperm concentration recorded from the present study was within the range of a report by Hafez and Hafez (2000), which is $3000\text{-}7000X10^6$ spermatozoa/ml. The average pH of the semen collected was slightly alkaline and ranges from 7.2-7.5. Alkalinity of the poultry semen is due to the accessory sex gland fluid is generally alkaline as reported by Bah et al. (2001) and Peters et al. (2008). Results from Hafez and Hafez (2000), Gordon (2005), Antalan et al. (2015), and AL-Saeedi et al. (2019) are all within the range of the current study.

The results from the present study demonstrated the effect of a Glycerolized tris-egg-yolk-based extender on the Ethiopian indigenous Horro chicken breed's semen sperm motility, morphology, and *in vitro* viability. Results in this study showed that semen stored in a Glycerolized tris-egg-yolk-based extender has sperm motility that is fit for insemination. The current result of sperm motility agrees with a similar study by AL- Saeedi et al. (2019) which utilized a Tris-based extender for short-term storage of Lohmann brown breeders. As reported by Ponglowhapan et al. (2004) motility is an important indicator of sugar utilization by spermatozoa as sugars serve as an external energy source essential for maintaining motility. This study demonstrated that semen extended with a commercial extender and stored at 4 hours produced higher sperm motility (87 ± 1.22 %). In this study, the overall average sperm motility was 59.25%, which was in general agreement with 42-80% reported by Hafez and Hafez (2000).

In this study, extending semen with a commercial extender and storing it for 4 hours yielded the least sperm abnormalities (10.4 ± 0.51%). Whereas, the average sperm morphological abnormality semen stored using a Glycerolized tris-egg-yolk-based extender was 18.5%. The number of live sperm with abnormalities in fresh cockerel semen varied from 6 to 9 percent (Tselutin et al., 1999), which was lower than the results of this study. However, Tuncer et al. (2006) reported that the number of abnormal sperm in cockerel semen varied from 9.2 to 11.23%, which is in agreement with sperm abnormalities recorded in semen extended using a commercial extender.

A commercial extender at 4 hours of storage was the best combination (83.6 ± 1.63%) for better *in vitro* sperm viability as compared to other treatments. Sperm *in vitro* viability recorded using LPE at 4 hours of storage was slightly lower than that of commercial extenders (82.6 ± 1.36%). The LPE improved the longevity of sperm in this study as Bearden et al. (2004) reported "presence of fructose will not greatly change the metabolic rate, however, will extend the life span of the sperm". According to the report by Gebriel et al. (2009), 81.79% of sperm *in vitro* viability was recorded at 6 hours of storage, which was a similar to results of the present study. In this study, the percentage of dead sperm increased by 36.2% over 24 hours of storage for semen extended with LPE and which was positively correlated with the storage time. In general, the results of sperm quality attributes observed in this study are comparable to several studies (Lukaszewicz et al., 2008; AL- Saeedi et al., 2019).

CONCLUSION

In this study, tris-egg-yolk-based LPE yielded comparable results in all sperm quality attributes when compared with commercial Beltsville Poultry Semen extender. Semen stored for more than 12 hours at refrigeration temperature showed significantly lower sperm quality. Semen stored using a commercial extender for 4 hours was recorded with a higher level of sperm quality. Further studies are recommended to explore the possible ways to store poultry semen for 24 hours at refrigeration temperature without decreased sperm quality significantly.

DECLARATION

Acknowledgments

The authors are grateful to Addis Ababa University and Debrezeit Agricultural Research Institute, Bishoftu, Ethiopia for arranging experimental area. This study was funded by Ministry of Education, Ethiopia.

Authors' contributions

Tarekegn Getachew, Gebeyehu Goshu and Alemayehu Lemma designed the experiments and

Tarekegn Getachew performed the experiments. Tarekegn Getachew derived the models and analyzed the data. Gebeyehu Goshu and Alemayehu Lemma assisted with standardizing data collection and data analysis. Tarekegn Getachew wrote the manuscript in consultation with Gebeyehu Goshu and Alemayehu Lemma. All authors read and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors have declared that no competing interest exists.

Ethical consideration

All ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all authors.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, T. Getachew, upon reasonable request.

REFERENCES

AL- Saeedi TA, AL-Juaifari AAI, and Al-mahmoudi AHJ (2019). The effect of different extenders on some fertility properties of rooster semen. International Journal of Poultry Science, 18(11): 504-507. DOI: https://www.doi.org/10.3923/ijps.2019.504.507

Antalan DG, Aquino FP, Leoveras EDC, Ocampo LC, Atabay EP, and De Leon AM (2015). The effect of semen extender and storage time on the quality of spermatozoa collected from the excurrent duct of Philippine local chicken. Journal of Biological Engineering Research and Review, 2(2): 18-20. Available at: https://www.biologicalengineering.in/gallery/15-2-2-05.pdf

Bah GS, Chaughari SUR, and Al-Amin JD (2001). Semen characteristics of local breeder cocks in the Sahel region of Nigeria. Revue d'élevage et de médecine vétérinaire des pays tropicaux, 54(2): 153-158. DOI: https://www.doi.org/10.19182/remvt.9794

Bearden HJ, Fuquay JW, and Willard ST (2004). Applied animal reproduction, 6th Edition. Pearson Education, Inc., New Jersey, pp. 109-127. Available at: https://www.worldcat.org/title/applied-animal-reproduction/oclc/469432992

Benoff FH, Rowe K, Fuguay JI, Renden JA, and Scott AR (1981). Effect of semen collector on semen volume and sperm concentration in broiler breeder males. Poultry Science, 60(5): 1062-1065. DOI: https://www.doi.org/10.3382/ps.0601062

Bilgili, SF, Sexton KJ, and Renden JA (1987). Fluorometry of poultry semen: Influence of dilution and storage on chicken spermatozoa viability and fertility. Poultry Science, 66(12): 2032-2035. DOI: https://www.doi.org/10.3382/ps.0662032

Cole HH and Cupps PT (1977). Reproduction in domestic animals, 4th Edition. Academic press., New York, p. 195. Available at: https://www.elsevier.com/books/reproduction-in-domestic-animals/cupps/978-0-08-057109-6

Foote RH (2002). The history of artificial insemination: Selected notes and notables. American Society of Animal Science, Cornell University, Ithaca, NY, USA, pp. 1-10. https://www.asas.org/docs/default-source/midwest/mw2020/publications/footehist.pdf?sfvrsn=59da6c07_0

Gebriel GM, Kalamah MA, El-Fiky AA, and Ali AFA (2009). Some factors affecting semen quality traits in Norfa cocks. Egyptian Poultry Science Journal, 29(2): 677-693. Available at: https://www.cabdirect.org/cabdirect/abstract/20113110365

Gerzilov P (2010). Influence of various cryoprotectants on the sperm mobility of Muscovy semen before and after cryopreservation. Agricultural Science and Technology, 2(2): 57-60. Available at: https://www.cabdirect.org/cabdirect/abstract/20113272102

Getachew T, Ameha N, and Tefera M (2015). Fructose addition in skim milk based extender and its effect on semen quality in White Leg-Horn chicken. Ethiopian Veterinary Journal, 19(1): 23-34. DOI: https://www.doi.org/10.4314/evj.v19i1.2

Gordon I (2005). Reproductive technologies in farm animals. CABI Publishing., UK, Chapter 1, pp. 16-28. Available at: https://www.cabi.org/animalscience/ebook/20173185376

Hafez B and Hafez ESE (2000). Reproduction in farm animals, 7th Edition. Lippincott Williams and Wilkins., New York, USA, pp. 267-292. Available at: https://b2n.ir/g36564

Haunshi S, Doley S, Kadirvel G (2010). Comparative studies on egg, meat, and semen qualities of native and improved chicken varieties developed for backyard poultry production. Tropical Animal Health and Production, 42: 1013-1019. Available at: https://link.springer.com/article/10.1007/s11250-009-9524-3

Lake PE (1983). Factors affecting the fertility level in poultry, with special reference to artificial insemination. Agricultural Research Council, 39(2): 106-117. DOI: https://www.doi.org/10.1079/WPS19830011

Lukaszewicz E, Jersey A, Partyka A, and Siudzin´ska A (2008). Efficacy of evaluation of rooster sperm morphology using different staining methods. Research in Veterinary Science, 85(3): 583-588. DOI: https://www.doi.org/10.1016/j.rvsc.2008.03.010

Łukaszewicz E, Jerysz A, and Kowalczyk A (2020). Effect of semen extenders on viability of ISA Brown and Hubbard Flex roosters' sperm stored for 24 h. Poultry Science, 99(5): 2766-2774. DIO: https://www.doi.org/10.1016/j.psj.2019.12.055

Mussa NJ, Boonkum W, and Chankitisakul V (2023). Semen quality traits of two Thai native chickens producing a high and a low of semen volumes. Veterinary Sciences, 73(10): 1-9. DOI: https://www.doi.org/10.3390/vetsci10020073

Peters SO, Shoyebo OD, Ilori BM, zoje MOO, Ikeobi CON, and Adebambo OA (2008). Semen quality traits of seven strain

of chickens raised in humid tropics. International Journal of Poultry Science, 7(10): 949-953. DOI: https://www.doi.org/10.3923/ijps.2008.949.953

Ponglowhapan S, Birgitta E, and Catharina LF (2004). Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. Theriogenology, 62(8): 1498-1517. DOI: https://www.doi.org/10.1016/j.theriogenology.2004.02.014

Quinn JP and Burrows WH (1936). Artificial insemination in fowls. Journal of Hered, 27(1): 31-38.DOI: https://www.doi.org/10.1093/oxfordjournals.jhered.a104138

Reddy RP (1995). Artificial insemination of broilers: Economic and management implications. In: M. R. Bakst (Editor), Poultry Science Association, pp. 73-89.

Silyukova M, Galimov R, Kuzmina T, Kulikova K, Nizhnikov A, and Chernyshova O (2022). Proteomic characterization of chicken spermatozoa reveals potential biomarkers of semen quality. Reproduction, Fertility and Development, 34(1): 153-165. DOI: https://www.doi.org/10.1071/RD20300

Siudzin´ska A and Łukaszewicz E (2008). Effect of semen extenders and storage time on sperm morphology of four chicken breeds. Journal of Applied Poultry Research, 17(1): 101-108. DOI: https://www.doi.org/10.3382/japr.2007-00048

Soler C, García-Molina A, Valverde A, Núñez-Martínez I, Fernández-Santos MR, Esteso MC, ... and Pérez-Gutiérrez JF (2016). Seminal plasma proteins as markers of sperm fertility. Theriogenology, 85(8): 1466-1473. DOI: https://www.doi.org/10.1016/j.theriogenology.2016.01.025

Tselutin K, Seigneurin F, and Blesbois E (1999). Comparison of cryoprotectants and methods of cryopreservation of fowl spermatozoa. Poultry Science, 78(4): 586-590. DOI: https://www.doi.org/10.1093/ps/78.4.586

Tuncer PB, Kinet H, Ozdogan N, and Demiral O (2006). Evaluation of some spermatological characteristics in Denizli cocks. Journal of The Faculty of Veterinary Medicine Erciyes University, 3(1): 37-42. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=b597d4efb95049f0d30d199ae9d2d8e774945eb0

Yadav S, Kumar R, Gupta AK, and Yadav SK (2019). Advances in poultry semen preservation: conventional and emerging technologies. Journal of Animal Science and Technology, 61(6): 331-340. https://doi.org/10.5187/jast.2019.61.6.331