Letters in Animal Biology

Journal homepage: www.liabjournal.com

Standardizing insemination interval for turkey hens with diluted semen preserved at 5 °C for optimum fertility

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Article info
Received: 16 November 2024
Received in revised form: 19 January 2025
Accepted: 17 February 2025
Published online: 22 February 2025

Keywords
Artificial insemination
Chilled semen
Extender
Turkey
Orange juice

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Reviewed by:
Consent to publish names of both the reviewers could not be obtained

Abstract

Standardizing insemination interval with liquid stored semen is necessary for better application of assisted reproductive biotechnology for genetic improvement in turkey. This experiment was designed to identify appropriate insemination intervals to achieve optimum fertility with tom semen preserved with Tris Egg-yolk Orange (TEYO) extender for 4 and 12 hours at 5 °C. Five healthy indigenous toms and ninety six hens of 38 weeks of age were used for this study. A 4x2 factorial design with two insemination frequencies and four types of semen was employed in a completely Randomized Design. The four varieties of semen used were undiluted, TEYO diluted, 4 hours TEYO chilled, and 12 hours TEYO chilled. The second factor is once or twice weekly insemination frequencies. The results showed that turkey hens inseminated with 12 h TEYO chilled semen had significantly lower fertility rates, hatchability of viable eggs, and egg sets than those inseminated with 4 h TEYO chilled semen, TEYO extended semen, and un-extended sperm. The value for 4 hours of TEYO chilled semen, however, did not differ appreciably from the un-chilled semen value. The results showed that twice per week insemination had significantly higher percentage fertility value compared to once per week insemination for un-chilled and 12 h TEYO chilled semen. The results revealed that once per week insemination had a higher hatchability percentage of fertile eggs for 4 h TEYO chilled semen, TEYO extended semen, and un-extended semen compared to twice per-week insemination. The results revealed that once per week insemination had a higher hatchability percentage of eggs set compared to twice per week insemination for 4 h TEYO chilled tom semen. The results revealed that only in un-extended semen and 4 h TEYO chilled semen dead-in-shells were recorded. It was therefore concluded that twice per week insemination interval indicated better fertility and hatchability results for 12 h TEYO chilled turkey semen, while once per week insemination gave a better fertility and hatchability result for 4 h TEYO chilled turkey semen.

1. Introduction

Successful liquid storage of turkey semen may encourage indigenous turkey genetic improvement, as gene introgression through cross-breeding can be achieved by transfer of chilled semen from an exotic turkey breeder without purchasing the breeder sire or eggs or frozen semen which are usually expensive. However, effective, cheap, and available extender is required to achieve this task (Balogun et al. 2023a). Even though preserved semen is used in 95% of cases of artificial insemination (AI), fresh or well-preserved semen is still necessary (Raheja et al. 2018). For this reason, semen needs to be kept in the best possible condition (Hernández-Avilés et al. 2020). Among the semen extender components with good semen preservation and anti-cold shock record is egg-yolk. This component has been established as one of the most promising components available locally and eco-friendly for poultry semen dilution and preservation especially when combined in the right proportion with other components of semen extenders (Balogun et al. 2023b). Generally, an extender will facilitate semen handling by maintaining sperm viability and inhibit the pathways that are detrimental to semen survival. The addition of extender to semen maintains motility, fertility, and preserves sperm membrane integrity (Riha et al. 2006). Egg yolk is generally accepted to be an effective agent in semen extenders (Aboagla and Terada 2004), but it provides substrates for hydrogen peroxide production which is detrimental for live spermatozoa (Singh 2005) which can be prevented by addition of antioxidant agents such as orange juice rich in vitamin C and ferulic acid to the extender.

However, liquid preservation of turkey semen without extender after few hours has been associated with lower fertility (AX et al. 2000). Although continuous introduction of air during storage, has been reported to improve fertility rates of turkey semen stored for longer than 6 to 12 h (38 to 74%) the results are still far below the industry requirement (96 to 98%) (Long and Kramer 2003). In order to improve the fertility of hens inseminated with liquid stored tom semen, it is required to test if the frequency of insemination in turkey hens might reduce and offset the effects of liquid storage duration on turkey semen. Therefore, the purpose of this experiment was to evaluate, compare, and suggest the ideal number and frequency of inseminations for maximum fertility in turkey hens inseminated with semen stored for 4 hours and 12 hours under refrigeration at 4 oC with Tris egg-yolk Orange juice extender (TEYO). It may be a significant approach towards accelerating genetic gain in Nigeria's Indigenous turkey species.

2. Materials and methods

2.1 Ethical Approval

The study was based on the ethical rules of Oyo State College of Agriculture and Technology Igboora, Nigeria.

2.2 Study area

This study was carried out in the Department of Animal Health and

 https://doi.org/10.62310/liab.v5i2.186

Production Technology Igboora, Nigeria between latitude 7.4368° N and longitude 3.2885° E at an elevation of 646 m above sea level. The mean annual rainfall in this area is 1,100 mm lasting from May to October. Mean daily temperature during season is 25 °C with a mean relative humidity of 72%. The dry season lasts from November to April, with mean daily temperature range of 14-36 °C and relative humidity of 20-30%

2.3 Procurement of the experimental turkey

This study was carried from September 2023 to November 2023 during the summer period. Five healthy Nigeria Indigenous toms and 96 hens of 38 weeks weighing 3-4 kg were used for this study. The toms were sourced from local markets within Ibadan. The toms and hens were weighed, screened, and treated for helminths and blood parasites prior to the onset of the study with the aid of Ivanov antibiotics at the dosage of 1ml per 1.6 L water.

2.4 Preparation of tris egg-yolk orange juice extender

Tris egg-yolk orange juice extender was prepared by breaking eggs to collect their yolks void of albumen. The yolk is separated from the eggs by draining the albumen and collecting the yolk on a filter paper to drain the remnant albumen. The vitelline membrane is then broken to collect the liquid egg yolk. About 25 ml of egg-yolk was collected and stirred vigorously in a beaker. Tris buffers of 7.2 pH were mixed vigorously with egg-yolk in the ratio of 1:3. And finally orange juice was added to it at 10% level. It was stored in the refrigerator for further use.

2.5 Housing and management of the turkeys

Turkey toms were housed individually in 30 × 30 × 40 cm^3 cage and allowed to acclimatize for a period of two weeks during which they were trained for semen collection. Three hens were housed per pen. They were fed with hybrid commercial layer mash. Water was supplied *ad libitum* and 180 g of feed was supplied per hen/day while 220 g of feed was fed per tom/day. The hens and toms were exposed to 12 hours day length light.

2.6 Training of tom for semen collection

The toms were trained for semen collection for a period of two weeks by using the method of Balogun et al. (2015). Semen was usually collected once in a week for a period of four weeks for adequate sperm reserve durations.

2.7 Experimental design

To accomplish this objective, a completely randomized design (CRD), with a 4×2 factorial experiment involving four semen types and two insemination frequencies was used. Undiluted, TEYO diluted, 4 hour TEYO diluted chilled semen, and 12 hour TEYO diluted chilled semen were the four types of semen employed for inseminating hens. While insemination frequencies were once per week and twice per week. Ninety six turkey hens were distributed at random among eight experimental groups with 12 hens in each.

2.8 Semen collection, dilution, and preservation

For every artificial insemination during the eight weeks period, semen ejaculates were usually collected separately and pooled from two Nigeria indigenous toms for preparation of semen doses for insemination of turkey hens. The pooled semen was divided into two portions and TEYO extender was added to them in the ratio of 1:3 (semen: extender). Immediately after dilution one portion was preserved at refrigerated temperature of 5 °C for 4 h and the other for 12 h at the Physiology and Reproduction Biology Laboratory of Oyo State College of Agriculture and Technology Igboora, Nigeria. The ejaculates from other three toms were pooled and divided into two fractions, one fraction was diluted by the extender in the ratio of 1:3 and the other was kept undiluted for immediate insemination of hens.

2.9 Artificial insemination of the turkeys

Fertility trial was carried-out at the turkey unit of the poultry farm of Oyo state College of Agriculture and Technology, Igboora, Nigeria. The fertilizing ability of spermatozoa was assessed by intravaginal insemination of all the females in each experiment group with the respective semen type and insemination frequency for a period of eight weeks. Hens were inseminated with 0.05 ml undiluted semen, 0.05 ml TEYO diluted semen, and 0.1 ml preserved semen containing about 100-200 × 10^6 viable spermatozoa per 0.1 ml (Fig. 1). Eggs were collected daily, stored at room temperature, and incubated weekly. The fertility rate was determined by candling at 21 days of incubation and hatching rate was determined by hatching of fertile eggs at 28 days of incubation with the aid of Petersime incubator manufactured at Belgium.

Fertility and hatching rates were calculated using the formulas as follows:

Fertility rate = (No. of fertile eggs /No. of eggs set ×100)

Hatchability based on fertile eggs set = (No. of eggs hatched /No. of fertile eggs set × 100)

Hatchability based on total eggs set = (No. of eggs hatched /No. of eggs set × 100)

Dead-in-shell percentage = (No. of dead in shell chicks /No. of eggs set × 100)

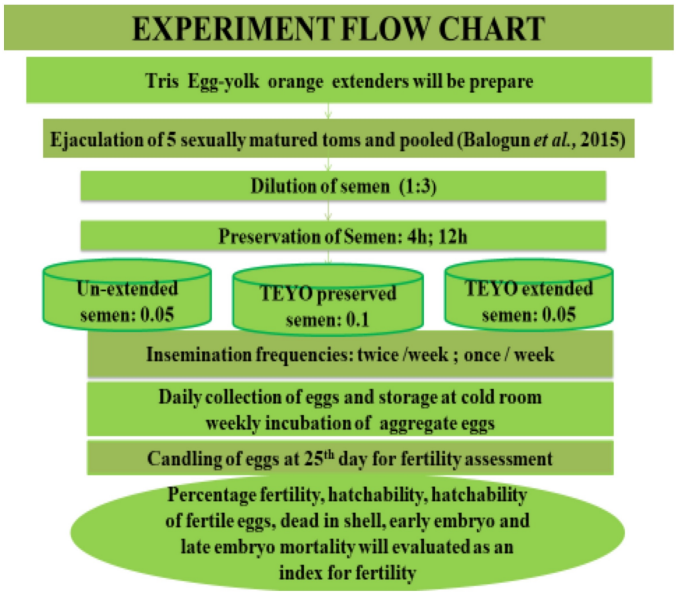

***Fig. 1**: Flowchart of the experiment*

2.10 Data Analyses

Data collected from this study were expressed as means ± standard errors of means (SEM). Two Way Analysis of Variance (ANOVA) was used for the analysis of the data, means were separated with Ducan multiple range test comparison tests. Values with $p < 0.05$ were considered significant. All statistical analysis was done using the SPSS Software, version 22 (2021).

3. Results

3.1 Comparative percentage fertility and hatchability of eggs

Comparative percentage fertility and hatchability of eggs from inseminated hens with TEYO extended and chilled turkey semen are

Table 1 Comparative percentage fertility and hatchability of eggs from turkey hens inseminated with unextended, TEYO unchilled, and chilled semen

Treatments	Unextended semen	TEYO unchilled semen	4 h TEYO chilled semen	12 h TEYO chilled semen	P value
Fertility (%)	85.53 ± 13.71^{a}	79.33 ± 28.11^{a}	67.35 ± 16.31^{a}	33.66 ± 32.48^{b}	< 0.001
Hatchability of fertile eggs (%)	87.19 ± 18.25^{a}	74.12 ± 24.49^{ab}	58.33 ± 23.71^{b}	17.50 ± 37.36^{c}	< 0.001
Hatchability of eggs set (%)	65.73 ± 29.09^{a}	60.00 ± 25.62^{a}	45.00 ± 18.96^{a}	15.00 ± 33.00^{b}	0.003
Dead-in-Shell (%)	9.40 ± 11.19^{a}	0.00 ± 0.00^{b}	5.17 ± 9.50^{ab}	0.00 ± 0.00^{b}	0.017

Means with different superscripts a,b,c indicated significant difference ($p < 0.05$)
TEYO: Tris Egg-Yolk orange
Un-extended semen: Semen void of extender
TEYO un-chilled Semen: TEYO extended tom semen without cold storage
4 h TEYO chilled Semen: TEYO extended tom semen preserved for 4 h at 5 ^{o}C
12 h TEYO chilled Semen: TEYO extended tom semen preserved for 12 h at 5 ^{o}C

presented in Table 1. Percentage fertility, hatchability of fertile eggs set, and total eggs set among Turkey hens inseminated with 12 h TEYO chilled semen was significantly lower ($p < 0.05$) than 4 h TEYO chilled semen, TEYO extended semen, and Un-extended semen. However, the value recorded for 4 h TEYO chilled semen was not significantly different from un-extended and TEYO extended un-chilled semen ($p > 0.05$). Also, un-extended semen had significantly higher percentage dead-in-shells compared to both chilled and extended semen ($p < 0.05$).

3.2 Percentage fertility of hens

Percentage fertility of hens inseminated with TEYO chilled and un-chilled turkey semen at different insemination intervals is presented in Fig. 2. The results indicated that twice per week insemination had higher percentage fertility rate compared to once per week insemination for 12 h TEYO chilled semen, un-extended semen, and TEYO extended semen ($p < 0.05$). However, for 4 h TEYO chilled semen, the reverse was the case, as once per week insemination had a better percentage fertility value compared to twice per week insemination of turkey hens.

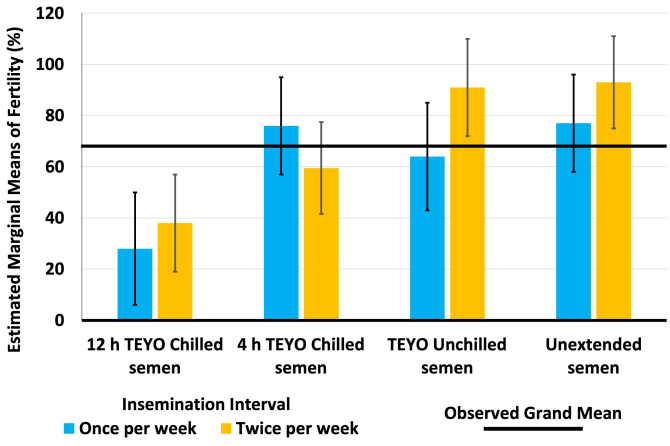

***Fig. 2**: Percentage fertility of incubated eggs collected from turkey hens inseminated at different intervals*
TEYO: Tris Egg-Yolk orange

3.3 Percentage hatchability of fertile eggs

Percentage hatchability of fertile eggs from hens inseminated with chilled and un-chilled turkey semen at different insemination intervals is presented in Fig. 3. The result revealed that once per week insemination had higher percentage hatchability of fertile eggs for 4 h TEYO chilled semen, TEYO extended semen, and un-extended semen compared to twice per week insemination ($p < 0.05$). However, 12 h TEYO chilled semen revealed zero percentage hatchability of fertile eggs for single insemination per week.

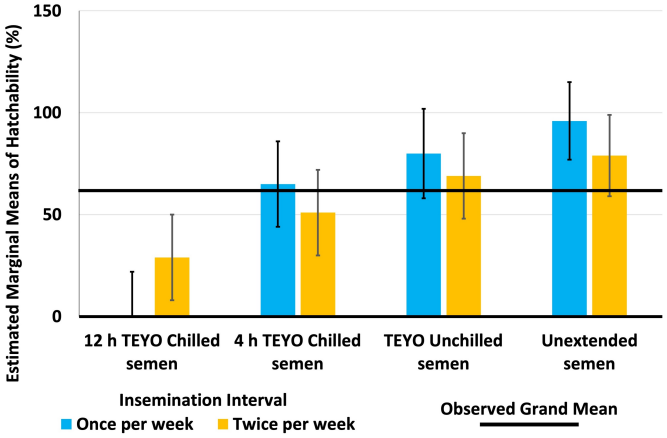

***Fig. 3**: Percentage hatchability of fertile eggs of turkey hens inseminated at different intervals*
TEYO: Tris Egg-Yolk orange

3.4 Hatchability percentage of total eggs set

Percentage hatchability of eggs set from hens inseminated with chilled and un-chilled turkey semen at different insemination intervals is presented in Fig. 4. The result revealed that once per week insemination had higher percentage hatchability of eggs set compare to twice per week insemination for 4 h TEYO chilled semen ($p < 0.05$). However, for un-extended semen, TEYO extended semen, and 12 h TEYO chilled semen twice per week insemination had higher percentage hatchability of eggs set compared to once per week insemination.

3.5 Percentage dead-in-shells

The percentage dead-in-shell from hens inseminated with chilled and un-chilled turkey semen at different insemination intervals is presented in Fig. 5. The results revealed that only in un-extended semen and 4 h TEYO chilled semen, dead-in-shells were observed. Furthermore, twice per week insemination recorded significantly higher dead-in-shells compared to once-per-week insemination in both semen types.

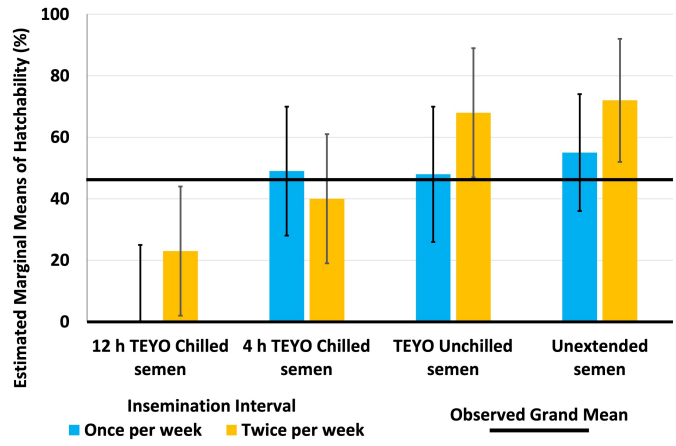

***Fig. 4**: Percentage hatchability of incubated eggs collected from turkey hens inseminated at different intervals*
TEYO: Tris Egg-Yolk orange

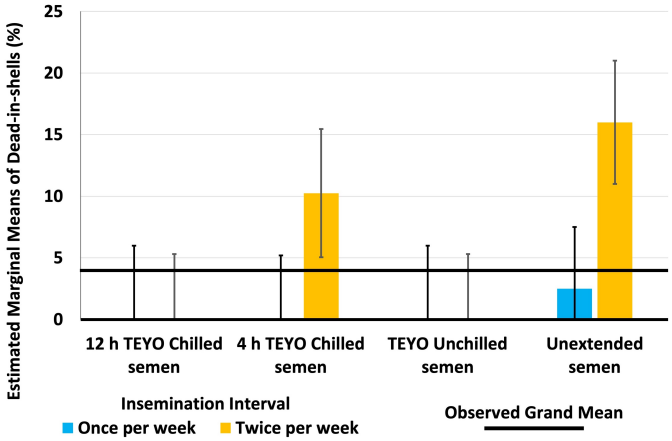

***Fig. 5**: Percentage dead in shells of incubated eggs from turkey hens inseminated at different intervals*
TEYO: Tris Egg-Yolk orange

4. Discussion

The results presented in this study, on identifying appropriate insemination interval for turkey hens with TEYO tom semen preserved at 5 °C to achieve optimum fertility, convincingly depict that frequency of insemination and storage period are positively correlated with and significantly influence the fertility and hatchability potential of turkey eggs. It was revealed that once per week insemination is desirable and sufficient for TEYO chilled turkey semen preserved for 4 h, as it was observed in percentage fertility and hatchability results. This finding is an indication that sufficient concentration of sperm cells required to fertilize eggs in the oviduct of the hen for a week can be inseminated once in a week instead of exposing the birds to insemination stress twice per week which may cause side effects on production traits. In agreement with present study, Balogun et al. (2023a) reported that once per week insemination has encouraging fertility value compared to twice per week insemination and furthermore buttress that the percentage fertility of eggs collected for 15 days after insemination were not affected. Furthermore, Donoghue and Donoghue (1997) confirmed that providing sperm numbers in excess of 50 to 200 million will surely give consistently high fertility levels irrespective of the frequency of insemination. Contrary to the current findings, McCartney (1976) reported the best fertility was obtained in eggs produced by broiler chicken breeder pullets inseminated two times in a week.

Furthermore, the result also revealed that preservation of tom semen with TEYO extender beyond 4 h is greatly detrimental to the sperm cells and resulted in too low fertility and hatchability irrespective of the frequency of insemination. It is therefore perceived that TEYO doesn't have sufficient available nutrients, energy, and antioxidant capacity to maintained sperm cells for longer duration beyond 4 hours at 5 °C to achieve satisfactory optimum fertility desirable by commercial turkey breeders compared to un-extended and TEYO extended semen. Apparently, TEYO extended tom semen performed comparable to un-extended tom semen which depict that TEYO extender doesn't have deleterious effects on diluted tom semen (Balogun 2023b). This is an indication that fertilizing ability of tom semen subject to cold condition is much lower compared to un-extended and extended turkey semen. However, in a situation where storage of semen is the only available option for the insemination of turkey hens, 4 hours TEYO chilled semen can still be managed for artificial insemination and also fertility and hatchability rates may be improved by doubling or increasing the insemination dosage. Donoghue and Wishart (2000) advocated that, for better fertility of *in-vitro* stored semen, samples have to be preserved at 2-8 °C. Slanina et al. (2015) also found that turkey semen can be stored at 4-8 °C. Mohan et al. (2017a; 2017b) stored chicken semen for 24 h at 7-8 °C with very good fertility. Despite observing the storage temperature conditions prescribed by all these scientists in the past, the fertility and hatchability percentage of 4 h and 12 h storage of TEYO chilled turkey semen still remain lower compared to un-chilled semen.

5. Conclusion

It was therefore concluded that turkey hens inseminated twice per week demonstrated better fertility and hatchability result for 12 h TEYO chilled turkey semen while hens inseminated once per week by 4 h TEYO chilled turkey semen gave better fertility and hatchability results. Furthermore 4 h TEYO chilled turkey semen gave better fertility and hatchability results than 12 h TEYO chilled turkey semen irrespective of the insemination intervals. Further studies may be conducted by increasing the insemination intervals with chilled semen on turkey hens.

Declarations

Acknowledgements: Authors appreciate the Oyo State College of Agriculture and Technology for their support and providing facilities for the research

Funding: This research was supported and funded by 2021 Tetfund Institutional Based Research grant OYSCATECH/RG/2021/14

Availability of data and materials: The datasets generated and collated during this research are available from the corresponding author upon request

Conflict of interests: There is no conflict of interest to declare

References

Aboagla EM, Terada T (2004). Effects of egg yolk during the freezing step preservation on the viability of goat spermatozoa. Theriogenology 62: 1160-1172. https://www.doi.org/10.1016/j.theriogenology.2004.01.013

AX RL, Dally MR, Didon BA, Lenz RW, Love CC, Varner DD, Hafez B, Belline ME. (2000). Artificial insemination. In: Hafez B, Hafez ESE, editors, Reproduction in farm animals, 7th edition.

Philadelphia: Lea and Febinger, USA. Pp 381-394. https://www.doi.org/10.1002/9781119265306.ch26

Balogun AS, Jimoh O A, Ojo JA, Oke TD, Akinosun AA. (2015). Assessment of selected of selected fresh garlic extracts antioxidant potentials for mitigating reactive oxygen species (ROS) in diluted rooster semen used for artificial insemination. Book of abstract of Humboldt Conference, Alexander Von Humboldt Foundation 2 – 7 August, Osun State University, Osogbo, Osun State, Nigeria, Pp. 28.

Balogun AS, Akinosun AA, Fatola OSG, Shobanke IA, Adebayo TO, Akanji BM, Yusuf RA. (2023a). Inseminating turkey once in two weeks is sufficient for optimum fertility. Clinical Research in Animal Science 2(5): 1-2. https://www.doi.org/10.31031/CRAS.2023.02.000547

Balogun AS, Akinosun AA, Shafi TA, Olabamiji SGF, Abdulhamid RR, Adebayo AA. (2023b). Standardization of egg-yolk with three different buffers for optimizing turkey semen cold storage at 4^{o}C. Asian Journal of Animal Science 17(1): 15-20. https://www.doi.org/10.3923/ajas.2023.15.20

Donoghue A, Donoghue D. (1997). Effects of water- and lipid-soluble antioxidants on turkey sperm viability membrane integrity and motility during liquid storage. Poultry Science 76: 1440-1445. https://www.doi.org/10.1093/ps/76.10.1440

Donoghue AM, Wishart GJ. (2000). Storage of poultry semen. Animal Reproduction Science 62(1-3): 213-232. https://www.doi.org/10.1016/S0378-4320(00)00160-3

Hernández-Avilés C, Love CC, Serafini R, Ramírez-Agámez L, Friedrich M, Ghosh S, Varner DD. (2020). Effects of glucose concentration in semen extender and storage temperature on stallion sperm quality following long-term cooled storage. Theriogenology 147: 1-9. https://www.doi.org/10.1016/j.theriogenology.2020.02.007

Long JA, Kramer M. (2003). Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored turkey semen. Poultry Science 82(11): 1802-1807. https://www.doi.org/10.1093/ps/82.11.1802

McCartney MG. (1976). The effects of semen dosage and insemination frequency on the fertility of broiler breeder hens. Poultry Science 55: 669-671. https://www.doi.org/10.3382/ps.0550669

Mohan J, Sharma SK, Kolluri G, Gopi M, Tyagi JS, Kataria JS. (2017a). Effect of different semen dilutors on fertilizing capacity of chicken semen stored for 24th Proceedings of XXVIII Annual Australian Poultry Science Symposium, Sydney, Australia, pp. 260-263.

Mohan J, Sharma SK, Kolluri G, Gopi M, Tyagi JS, Singh RP. (2017b). Effect of various dilution rates on fertilizing ability of chicken spermatozoa using CARI diluent. Proceedings of 34th Indian Poultry Science Association Conference, Bengaluru, India. pp. 61

Raheja N, Choudhary S, Grewal S, Sharma N, and Kumar N (2018). A review on semen extenders and additives used in cattle and buffalo bull semen preservation. Journal of Entomology and Zoology 6(3): 239-245. https://www.entomoljournal.com/archives/2018/vol6issue3/PartD/6-2-211-347.pdf

Riha L, Apolen D, Pivko J, Grafenau P, and Kubovicova E. (2006). Influence of implementors on sheep fertility out of season. Slovak Journal of Animal Science 39: 180-182. https://agris.fao.org/search/en/providers/122616/records/6472492853aa8c896304fa15

Slanina T, Lenka P, Michal M, Lubomír K, Juraj M, Norbert L, Jozef T, Ida P, Peter M. (2015). The effect of diluent, temperature and age on turkey spermatozoa motility *in vitro*. Journal of Applied Animal Research 43: 131-136. https://www.doi.org/10.1080/09712119.2014.928627

Singh BK. (2005). Textbook of andrology and artificial insemination in farm animals, Jaypee Brothers Medical Publisher LTD, New Delhi, pp. 440-514.

Citation

Balogun AS, Akinosun AA, Ajibade AJ, Hammed BB, Maruf NB. (2025). Standardizing insemination interval for turkey hens with diluted semen preserved at 5 °C for optimum fertility. Letters in Animal Biology 05(2): 01 – 05.