OPTIMIZING THE LIQUID STORAGE PRESERVATION ABILITY OF NATURAL FORMULATED POULTRY SEMEN EXTENDER FOR TURKEY BREEDING

***Balogun A. S., Akinosun A. A., Lawal I. A., Hassan U. O., and Hammed B. B.**
Department of Animal Production Technology, Oyo State College of Agriculture and Technology, Igboora.Oyo State. Nigeria.
*Corresponding author e-mail: *balogunadedeji001@gmail.com*

ABSTRACT
An experiment was designed to identify appropriate insemination dosage to achieve optimum fertility with tom semen preserved for 4 and 12hours at 5oC. Five (5) healthy indigenous toms and thirty-six (36) hens between 37-38 weeks of age were used for this study. A completely randomized design (CRD), with a 4×2 factorial arrangement involving four semen types and two insemination dosage were used. Undiluted, TEYO diluted, 4 hours TEYO chilled semen and 12 hours TEYO chilled semen were the four types of semen that were employed. While insemination dosages were 0.05mL and 0.1mL. The result revealed that hens inseminated 0.1mL dosage of 4hrs TEYO chilled semen have significant ($p<0.05$) higher percentage fertility, hatchability of fertile eggs and eggs set value comparable to 0.05mL dosage of TEYOE extended tom semen and un-extended semen compared to hens inseminated 0.05mL 4hrs TEYO chilled semen and 12hrs TEYOE chilled semen. Furthermore, Hens inseminated with 0.1mL TEYOE chilled semen has higher percentage hatchability of fertile eggs and eggs set than 0.05mL dosage and was above observed grand mean comparable to un-extended and TEYO extended semen. It can therefore be concluded that 4hrs TEYO chilled turkey semen inseminated at 0.1mL had fertility and hatchability result comparable to unpreserved semen with or without extender.
Keywords: Artificial insemination, Chilled semen Standardization; insemination dosage

INTRODUCTION

Semen preservation is a powerful and unique technique use to augment Artificial Insemination (AI). The first animal was conceived using AI in 1784, and the first trial to produce straw for AI was at the beginning of the 20th century (Ombelet and Van Robays, 2015). Artificial Insemination technique was targeted to increase the number of insemination doses, while with extender the insemination doses can be further increase. AI needs fresh or well-preserved semen, and 95% of all AI is accomplished using preserved semen (Raheja *et al.,* 2018). Thus, semen must be preserved in a perfect medium to maintain its quality (Hernández-Avilés *et al.,* 2020). Accordingly, Santos *et al.,* 2018 and Balogun 2019 emphasized the needs to develop and evaluate semen extenders used to preserve semen during chilling or cryopreservation.

Semen of the domestic turkey cannot be stored longer than 6 hrs without a loss of fertilizing capability. The improvement of long-term liquid storage procedures of semen is important since the commercial production of turkey relies almost entirely on artificial insemination. Therefore, studies improving storage regimens would allow longer storage and consequently hen fertility (Iaffaldano and Meluzzi, 2003). Assessment of diluted and undiluted stored cock semen has revealed the relevant of extender is essential to sustain semen quality which could be preserved up to 24- 72 hours without significant detrimental effects on the viability and fertilizing ability of the semen (Lukaszewicz *et al.,* 2008; Balogun, 2019). Semen extenders have the ability to support the biochemical composition of semen, otherwise the semen could rapidly dry with a loss of viability.

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 is to maintains motility, fertilizing capacity 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 to the detriment of live spermatozoa (Singh, 2005). However, Balogun *et al.,* 2022; 2021; 2020 also reported that egg-yolk in combination with the right additives is also a very good medium for sperm preservation. Also several plant sources such as coconut, tomato and carrot have been screened for their semen extending potentials (Banerjee, 2011).

This experiment was therefore aimed at standardizing and identifying insemination dosage for TEYO chilled semen to achieve optimum fertility.

MATERIALS AND METHODS

Housing and Management of the Turkey

The toms were housed individually in a pen and allowed to acclimatize for a period of two weeks during which they were trained for semen collection. The hens were housed three per pen. They were fed with hybrid layer mash. Water and 180g of feed were supplied per hen/day while 220g of feed were fed per tom / day

Experimental design

To accomplish our objective, a completely randomized design (CRD), with a 4×2 factorial experiment involving four semen types and two insemination dosage was used. Undiluted, TEYO diluted, 4 hours TEYO chilled semen and 12 hours TEYO chilled semen were the four types of semen that were employed, while insemination doses of 0.05 and 0.1 were used.

Artificial Insemination of the Turkeys

The fertilizing ability of spermatozoa was assessed by intravaginal insemination of 12 females per experiment group and unpreserved semen control group. Hens were inseminated twice and once per week with 0.05 unpreserved semen, 0.05 and 0.1 for preserved semen of dosage containing about 200 x 10^6 viable spermatozoa, for 3 weeks with fresh semen, diluted semen and semen preserved for 4 and 12 hrs at 4 °C as shown in Fig 1. Eggs were collected daily, stored at room temperature and incubated weekly. The percentage fertility (fertile eggs /eggs set ×100) was determined by candling 21 days after the start of incubation. Percentage Hatchability of fertile eggs (hatch eggs/fertile eggs x100) of eggs set (hatch eggs/ eggs set x100) was determined by hatching of fertile eggs about 28 days after start of incubation.

Data Analyses

Data collected were expressed as means ± standard deviation. Two Way Analysis of Variance (ANOVA) was used for the analysis of the data, followed by Duncan multiple range test. Values of ($p< 0.05$) were considered significant. All statistical analysis was done using SPSS 22 Software version

Results

Percentage fertility of chilled and un-chilled turkey semen inseminated with different insemination dosage is presented in Fig 2. The figure revealed that hens inseminated with 0.1mL dosage of 4h TEYO chilled semen have significant ($p<0.05$) higher value comparable to 0.05mL dosage of TEYOE extended tom semen and un-extended semen compared to hens inseminated 0.05mL 4hrs TEYO chilled semen and 12hrs TEYOE chilled semen. Furthermore, hens inseminated with 0.1mL dosage of 4hrs TEYO chilled semen was above observed grand mean comparable with un-extended semen and TEYO extended semen.

Percentage hatchability of fertile eggs for chilled and un-chilled turkey semen inseminated with different insemination dosage is presented in Fig 2. The figure shows same trend with percentage fertility of eggs set. Hens inseminated with 0.1mL insemination dosage has significant ($p<0.05$) higher percentage hatchability of fertile eggs than 0.05mL dosage and was above observed grand mean comparable to un-extended and TEYO extended semen. but the value obtained in 12hrs chilled TEYO chilled semen was significantly lower for both dosages compared to 4hrs TEYO chilled semen and un-chilled semen (TEYO extended semen and un-extended semen).

Percentage hatchability of eggs set for chilled and un-chilled turkey semen inseminated with different insemination dosage is presented in Fig 3. The figure follows same trend with percentage hatchability of fertile eggs, although lower in value compare to it. Hens inseminated 0.1mL insemination dosage has higher percentage hatchability of eggs set than 0.05mL dosage and was above observed grand mean comparable to un-extended and TEYO extended semen. but hens inseminated with 12hrs chilled TEYO chilled semen demonstrated significantly lower for both dosages compared to 4hrs TEYO chilled semen and un-chilled semen (TEYO extended semen and un-extended semen).

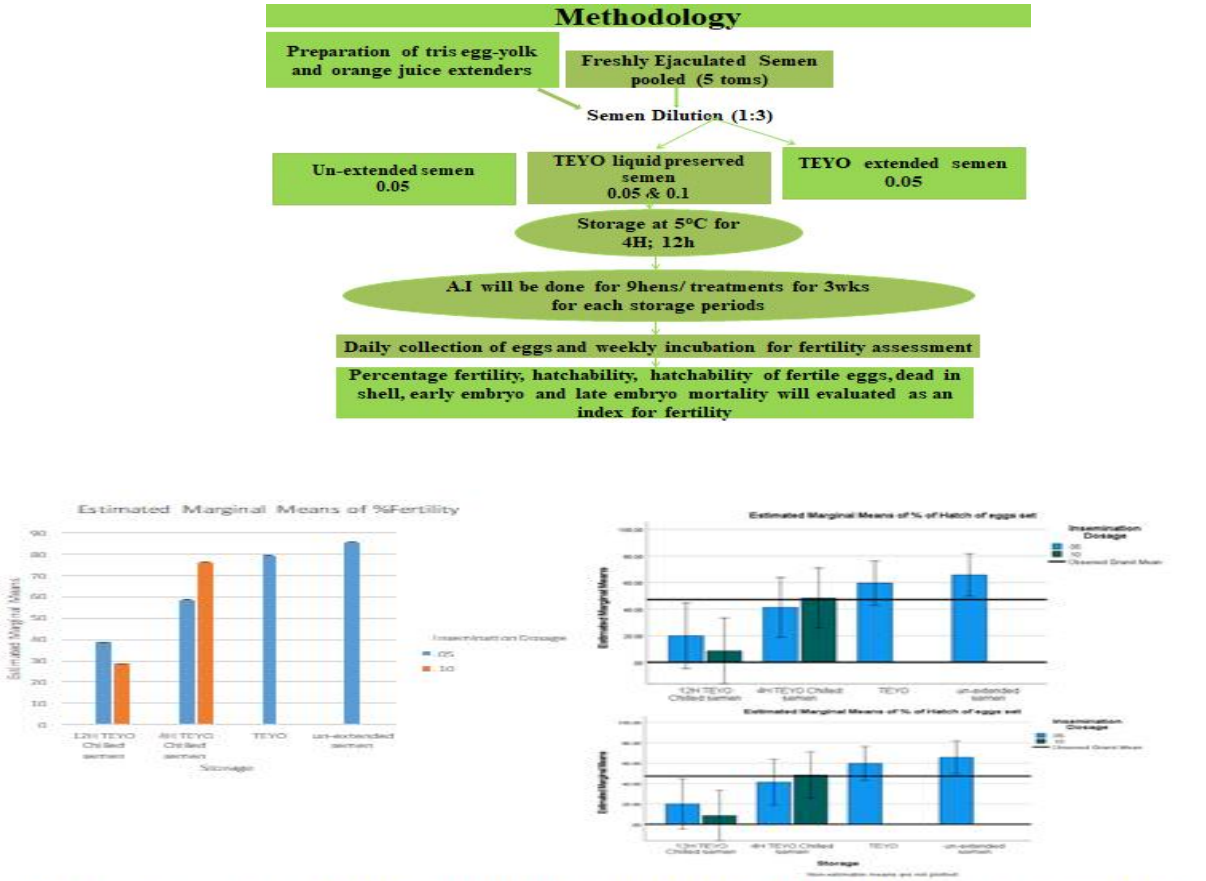

Fig 2: Percentage Fertility and Hatchability of eggs set from hens inseminated with with 0.05ml and 0.1ml dosage of chilled and un-chilled semen

DISCUSSION

The result from this study obviously revealed that insemination dosage significantly influenced fertilizing ability and hatchability of preserved tom semen. Higher insemination dosage of 0.1mL resulted into better fertility and hatchability rate for 4hrs TEYO chilled turkey semen. This clearly revealed the needs for insemination require number of sperm cells into the hen oviducts that is capable of encouraging better fertility and hatchability in poultry birds irrespective of the storage conditions of the inseminated semen. Similarly Balogun *et al.,* 2020 reported a close fertility rate of 79.00% for comparable to the one obtained in the present study when standardizing dilution rate for TEYO extenders for chicken semen.

Furthermore, it was evident that despite the 4hrs length of cold storage of turkey semen, the fertility, hatchability of fertile eggs and eggs set were above observed margin mean and fairly comparable to that of un-preserved turkey semen (un-extended turkey semen and TEYO extended turkey semen). This implies that turkey semen can effectively store under cold storage condition in TEYO extender for 4hrs without necessarily losing it fertilizing ability as compared to un-preserved semen provided sufficient numbers of sperm cells is inseminated to compensate for the losses during storage. Similarly, Harper 1995, held turkey semen at 55° and 60° F for periods up to four hours and observed no detrimental effects on fertility for hens inseminated during a 3-week period. However, parameters that determine the fertilizing ability of semen are the sperm characteristics which includes viability, plasma membrane integrity, and mitochondria activity are important factors contributing to sperm fertilization potential (Blesbois and Brillard, 2007; Shabani *et al.,*2021)

It was also noteworthy that 12h liquid storage of tom semen with TEYO extender is not desirable and does not encourage better fertility and hatchability rate in turkey hen irrespective of the insemination dosage used in this study. It is however, deemed that higher insemination dosage above 0.1mL with 12hrs chilled turkey semen could encourage better fertility and hatchability. Contrarily, Giesen and

Sexton (1983) also reported that holding turkey semen for 18 hrs at 5ºC did not affect fertility except when preserved at higher temperature like15ºC, 25ºC and room temperature.

CONCLUSION AND RECOMMENDATION

Convincingly, 4hrs TEYO chilled turkey semen inseminated at 0.1mL has fertility and hatchability Results comparable to unpreserved semen with or without extender, therefore recommended for liquid storage of turkey semen.

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REFERENCES

Aboagla, E. M. and Terada, T. (2004). Effects of egg yolk during the freezing step preservation on the viability of goat spermatozoa. *Theriogenology*, 62, 1160-1172.

Balogun, A.S. (2019). Evaluation of antioxidant potentials of tomato & orange juices on extended poultry semenpreserved with egg-yolk & coconut water diluents to improve selection intensity.Ph.D. Dissertation.

Balogun, A.S., Akinosun, A.A., Boladuro, B.A., Tiamiyu, A.K andOluwemimo, B.A (2020).Optimizing Dilution Rate of Cock Semen with Natural Based Extender for Optimum Fertility. *Archive of Science & Technology* 1 (2), 175 – 182

Balogun A.S. 2021. Optimizing liquid storage duration of two poultry species semen with plant-based extender. *Letters in Animal Biology* 01(2):01 – 06.

Balogun A.S. (2022). Reproductive Biotechnology; An Under-utilized Tool in Poultry Breeding. *Clinical Research in Animal Science.* 2(2). 000534.

Banerjee, C. C. (2011). A Textbook of Animal Husbandry. Eight edition. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.

Blesbois, E., and J. P. Brillard. 2007. Specific features of in vivo and in vitro sperm storage in birds. Animal 1:1472–1481.

Gİesen, A. and Sexton, T. (1983) Beltsville Poultry Semen Extender. 9. Effect of storage temperature on turkey semen held eighteen hours. *Poult. Sci.* 62: 1305-1311.

Harper, J. A. 1955. on fertilizing capacity. The effect of holding time of turkey semen. *Poultry Sci*. 34: 1289-1291.

Hernández-Avilés, C., Love, C. C., Serafini, R., Ramírez-Agámez, L., Friedrich, M., Ghosh, S. and Varner, D. D. (2020) Effects of glucose concentration in semen extender and storage temperature on stallion sperm quality following long-term cooled storage. *Theriogenology.* 147(7):1–9.

Laffaldano, N., Manchia, A., Gambacorta, M., Miachele, D. and Maria, P. R. (2010). Effect of Different Sperm Concentration of Post-thaw viability and motility of Turkey Spermatozoa Cryopreserved by pellet method. *Italian Journal of Animal Sci*. 70: 219–228

Lukaszewicz, E., Jersey, A. and Partyka, A. (2008). Efficacy of evaluation of rooster sperm morphology using different staining methods. *Research Veterinary Science*, 85, 583-588.

Ombelet, W. and Van Robays, J. (2015) Artificial insemination history. Hurdles and milestones. Facts Views Vis. Obgyn. 7(2):137–143.

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. *J. Entomol. Zool. Stud*. 6(3):239–245.

Riha, L., Apolen, D., Pivko, J., Grafenau, P. and Kubovicova, E. (2006). Influence of implememtors on sheep fertility out of season. *Slovak Journal of Animal Science,* 4, 180-182.

Santos M, Soares F, Moreira M, Beirão J. (2018) Evaluation of different extenders for the cold storage of meagre (Argyrosomus regius) semen. *Aquac. Res.* 49(8):2723–2731.

Sexton, T.J. 1980. Optimal rate for cooling semen from +5oC to -196□. *Poultry Science* 59: 2765–2770.

Shabani, S., M. Ehri, F. Shirmohammad, and M. Sharafi. 2021. Enhancement of sperm quality and fertility-related parameters in Hubbard grandparent rooster fed diets supplemented with soybean lecithin and vitamin E. *Poult. Sci*. 10:1–10.

Singh, B.K. (2005). Textbook of Andrology and Artificial Insemination in Farm Animals, (pp. 440-514) New Delhi: Jaypee Brothers Medical Publisher (P) LTD.