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Animal and Plant-based Extenders for Liquid Storage of Tom Semen Preserved at 5ºC

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Abstract

The objective was to compare fertility of turkey tom semen extended in Tris coconut-water orange juice (TCWO) or Tris Egg-yolk Orange Juice (TEYO) and stored at 5ºC for 4 or 12 h. Pooled ejaculates from five toms were divided into five portions; one portion was not extended (Control; inseminated immediately); two portions were extended in TCWO and the remaining two in TEYO. Extended semen was either inseminated immediately or kept in a refrigerator (5ºC) for 4 h (Chilled) prior to insemination. Hens (n=45) were allocated into five groups and inseminated once weekly (with ~200 x 10^6 viable sperm) for 4wk with 0.05 mL of un-extended, TCWO, TEYO extended semen and 0.1 mL of 4h TCWO, TEYO Chilled semen. The experiment was subsequently repeated with 12 h chilled TCWO and TEYO semen. Eggs were collected daily, stored at room temperature and incubated weekly. The fertility rate (fertile/incubated eggs×100) was determined by candling 21 d after the start of incubation date were analyzed by one-way ANOVA and differences located with DMRT. Fertility and hatchability rate were significantly different ($P<0.05$) among Control, extended semen (TCWO vs TEYO) and chilled semen (TCWO vs TEYO) for both 4h and 12h cold storage preservation. Although 4h and 12h TCWO chilled tom semen had better values for hatchability of fertile eggs and eggs set. TCWO extender improved Fertility or hatchability for extended semen compared to TEYO extender as well as better fertility and hatchability was observed for the chilled semen compared to TEYO extender.

Keywords: Animal, Plant, Extender, Semen, Fertility, Hatchability

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1. Introduction

Turkey hens among other poultry species have been reported to have a significant reproductive physiology feature that enable her to store viable spermatozoa in her reproductive tract for long periods of time up to 70 days [1]. Despite this feature the fertility rate that could be acquired after insemination of fresh or diluted or preserved tom semen is considerably very low and do not meet the requirement for breeding for commercial production. Therefore, preservation of semen, especially liquid storage of semen outside the hen oviduct remains a sustainable option as scientist yet to achieve mimicking the hens' sperm storage tubules oviduct environment for storage of spermatozoa within the hen which could be substitute measure for storing poultry semen for extended periods of time in vitro. Dilution media is considered as one of the major components for longer *in-vitro* sperm survival, as the quality of semen used for artificial insemination is important due to its effect on the fertility rate and eventual hatchability of the eggs. Poultry semen is known to generally losses viability rapidly during *in-vitro*, most especially in absence of extenders or when kept at room temperature outside reproductive tract. Although fertility has been reported to be enhanced by constant supply of air to turkey semen during storage for about 6 to 12 h (38 to 74%) but results doesn't meet industry requirement (96 to 98%) [2].

During the last 10 years, use of plant and animal ingredients as an antioxidant together with other components has been identified as the conventional protective approach

against rapid loss in motility and sperm survival for diluted and liquid stored semen poultry semen [3-5] and cryopreservation [6-8]. Kelso et al., [9] also claimed that due to higher presence of polyunsaturated fatty acid (PUFA) in turkey sperm makes its susceptibility to lipid peroxidation, thus, an efficient antioxidant sources as well as good energy substrate for sperm survival is necessary in the semen or the hen's sperm storage tubules to protect sperm membranes from per oxidative damage. Balogun [10], also emphasized the importance of exogenous application of natural antioxidant source to poultry semen before artificial insemination or during storage. However, egg notable for its richness in vitamin E, and coconut water is also known for its presence of considerable array of minerals elements with antioxidant vitamins that also present in semen has been identified as good component of poultry semen extenders over the years [11-14]. Furthermore, majority of the extenders used in turkey reproduction and breeding experiment are of salt origin components, hence the need to evaluate and compare the Fertilizing Capacity of this plant and animal ingredients based formulated natural extender on turkey sperm short-term holding survival for fertilizing turkey hens.

2. Materials and Methods

2.1. Study Area

This study was carried out in the Department of Animal health and production technology Igboora, between latitude 110 1573, N and longitude 70 64989, 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 0C with a mean relative humidity of 72%. The dry season lasted from November to April, with mean daily temperature ranges of 14 – 36^0C and relative humidity of 20 – 30% (Anonymous, 2014).

2.2. Procurement of the experimental Turkey

Five healthy indigenous toms and 36 hens aged between 37-38 weeks were used for this study. The toms were sourced from local markets within Ibadan. The toms and hens were weighed, screened and treated for helminthes and blood parasites prior to the onset of the study.

2.3. Housing and Management of the Turkeys

Turkey toms were housed individually in cages and allowed to acclimatize for a period of one 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.

2.4. Training of tom for semen collection

The toms were trained for semen collection for a period of two weeks by using [15] procedure for poultry semen collection. Semen is usually collected once a week for a period of 4 weeks for adequate sperm reserve durations.

2.5. Experimental design

To accomplish our objective, a completely randomized design (CRD) is employed.

2.6. Preparation of extenders

2.6.1. Preparation of tris egg-yolk orange (TEYO) 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. After which the yolk is pierced to open the membrane covering it to collect the liquid.25ml of egg-yolk was collected and stirred vigorously in a beaker. Tris buffer of 7.0pH was prepared and added to the egg-yolk and both were mixed together. Finally, 10ml of orange juice was later supplemented to the tris egg-yolk solution to make tris egg-yolk orange juice (TEYO) extender.

2.6.2. Preparation of tris coconut-water orange juice (TCWO) extender

Tris coconut-water orange juice extender was prepared by piercing ripe coconut-water at the top. Water was collected in a beaker and filtered. Tris buffer of 7.0pH was added to coconut-water at ratio 1:1 and mixed vigorously together. Finally, 10ml of orange juice was later supplemented to the tris egg-yolk solution to make tris coconut-water orange juice (TCWO) extender.

2.6.3. Artificial Insemination of the Turkeys

The fertilizing ability of spermatozoa was assessed by intra vaginal insemination of 6 females per experiment group and unpreserved semen control group. Hens were inseminated once per week with 0.05ml for (extended and un-extended) and 0.1ml semen dosage for 4h chilled semen containing about 200 x 10^6 viable spermatozoa for 3 weeks with fresh semen and diluted semen and 4hours stored semen as shown in fig 1. Eggs were collected daily, stored at room temperature and incubated weekly. The fertility rate (fertile/incubated eggs×100) was determined by candling 21 days after the start of incubation. Hatching rates (hatching/fertile eggsx100) was determined by hatching fertile eggs about 28 days after start of incubation.

2.7. Data Analyses

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

3. Results and discussion

Comparative Percentage fertility and hatchability of hens inseminated with un-extended, extended and Four hours chilled semen at 5^{o}C is presented in Table 1. The result shows that hen inseminated with 4hours TCWO and TEYO chilled tom semen has significant ($p<0.05$) lower percentage fertility compared to those inseminated with un-extended and TCWOE extended tom semen. The 4hours chilled tom semen irrespective of extender has less fertility and hatchability values. This is an indication that fertilizing ability of tom semen subject to cold condition is far from un-extended and extended turkey semen and cannot in any case be compared. However, in a situation where stored semen is the only available option, 4hoursTCWO chilled semen can still be managed for artificial insemination and its efficiency may also be improved by doubling or increasing the insemination dosage or frequency.

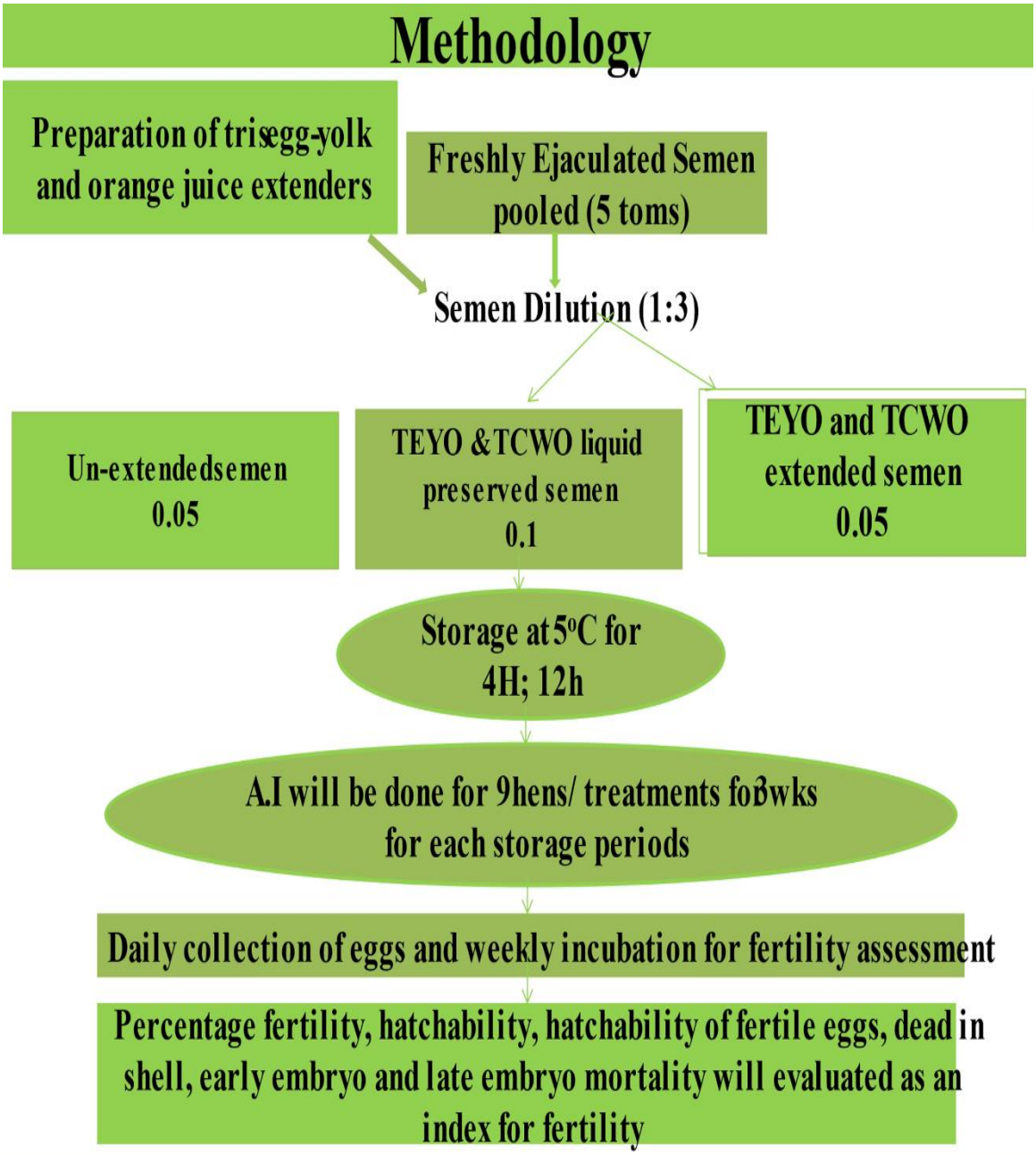

Figure 1: Shows the flow chart of the experiment methodology.

Table 1: Comparative Percentage fertility and hatchability of hens inseminated with un-extended, extended and Four hours chilled semen at 5°C

Treatments	Un-extended semen	Extended semen		4h Chilled Semen	
	Control	TCWO	TEYO	TCWO	TEYO
Fertility (%)	85.53±13.72^{ab}	97.00±9.95^{a}	79.33±28.11^{bcd}	60.95±14.70^{d}	67.35±16.31^{cd}
Hatchability of fertile eggs (%)	87.19±18.25^{a}	88.27±14.69^{a}	74.12±24.29^{ab}	75.93±18.10^{ab}	58.33±23.71^{b}
Hatchability of eggs Set (%)	65.73±29.09^{b}	86.00±18.41^{a}	59.57±25.62^{bc}	45.93±15.24^{c}	44.85±18.96^{c}
Dead in shell (%)	9.36±11.20^{a}	0.00±0.00^{b}	0.00±0.00^{b}	2.64±4.98^{b}	5.17±9.50^{ab}

Means with different superscripts a, b, c, d revealed significant difference (P>0.05)

Table 2: Comparative Percentage fertility and hatchability of un-extended, extended and Twelve (12) hours chilled semen at 5°C

Treatments	Un-extended semen	Extended Semen		12h Chilled Semen	
	Control	TCWO	TEYO	TCWO	TEYO
Fertility (%)	85.53±13.72^{ab}	97.00±9.95^{a}	80.56 ±29.31^{ab}	64.28±30.12^{b}	33.66±32.47^{c}
Hatchability of fertile eggs (%)	87.19±18.25^{a}	88.27±14.69^{a}	76.53±24.40^{ab}	55.93±34.07^{b}	17.50±37.36^{c}
Hatchability of eggs Set (%)	65.73±29.09^{a}	86.00±18.41^{a}	62.20±25.40^{ab}	39.94±32.58^{b}	14.30±33.00^{c}
Dead in shell (%)	9.36±11.20^{a}	0.00±0.00^{b}	0.00±0.00^{b}	1.42±4.91^{b}	0.00±0.00^{b}

Means with different superscripts a, b, c, d revealed significant difference (P>0.05)

Donoghue and Wishart [16] stressed the importance of storing semen in-vitro and why semen samples need to be preserved within 2-8°C. However, the record on percentage hatchability of fertile eggs shows that 4hours TCWOE chilled semen is not significantly (p>0.05) different from un-extended semen, un-chilled semen (TCWOE and TEYOE extended semen). Also, the percentage hatchability of fertile eggs value for 4 hours TEYO chilled semen is not significantly different from TEYO extended semen. Similarly, Slanina et al. [17] found that turkey sperm can be stored at 4-8°C. Mohan et al. [18-19] stored chicken semen for 24 h at 7-8°C with very good fertility. The percentage hatchability of egg set revealed that both 4hours chilled semen are significantly (p>0.05) different from the un-extended semen and un-chilled semen. Finally, the percentage dead in shell was significantly (p>0.05) higher in un-extended semen, but statistically (p<0.05) similar to 4hours TEYO chilled semen. Comparative Percentage fertility and hatchability of hens inseminated with un-extended, extended and twelve (12) hours chilled semen at 5°C is presented in Table 2. The results demonstrated that hens inseminated with both 12h TEYO and TCWO chilled semen has the lowest percentage fertility value of 33.66±32.47 and 64.28±30.12 respectively, Although, 12h the TCWO chilled percentage fertility value is not significantly (p<0.05) different from un-chilled semen (un-extended semen and the TEYO extended semen. Likewise, the percentage hatchability of the fertile eggs and eggs set also revealed that the hens inseminated with both chilled tom semen has a lower significant (p>0.05) value compared to the un-chilled semen.

Though the percentage hatchability of fertile eggs and eggs set of hens inseminated with 12h TCWO chilled semen is not significantly (p<0.05) different from TEYO extended semen. However, a fairly considerable level of fertility and hatchability which can compete favorably with un-chilled and un-extended tom semen and probably be able to produce level of fertility and hatchability that will be equivalent to the one recorded for un-chilled semen in the present study was exhibited by 12hours TCWO chilled semen compare to 12hours TEYOE chilled semen probably if the insemination dosage of chilled semen is double or increase. This evidently revealed that between both natural extenders, TCWO extenders is the extender of choice to be considered for effective liquid storage of tom semen to achieve desirable fertility and hatchability rate. Contrarily to result obtained in this present study, Mohan et al. [20] reported achieving higher fertility (91.07±1.91%) after 24 h storage of chicken semen by inseminating 89.10×106 sperm per AI dose. So, augmenting the insemination dosage will definitely increase number of available viable sperm cells needed for optimum fertility with chilled turkey semen. Slanina et al. [17] also confirmed that turkey sperm can be stored at 4-8°C and still acquire desirable fertility level. However, Significant (p<0.05) percentage dead in shell was only recorded for un-extended semen compared to chilled and un-chilled tom semen.

4. Conclusions

It can therefore be concluded that both extenders cannot adequately preserve tom semen for 12h to give optimum fertility and hatchability results like un-extended and unpreserved turkey semen. However, fertility and hatchability rate may be enhanced by increasing insemination dosage and frequencies.

Authors' contributions

ASB design, carried out the research and first draft of the manuscript AAA and AJA contribute to the resources and review of final manuscript draft. THO and BBH took part in the methodology.

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Ethical Consideration

The authors confirm that all authors reviewed and submitted the manuscript to this journal for the first time.

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

[1] E.S.E. Hafez, B. Hafez. (2013). Reproduction in farm animals. 7th ed. New York. Lippincott Williams and Wilkins. USA.

[2] J. Long, M. Kramer. (2003). Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored turkey semen. Poultry Science. 82(11): 1802-1807.

[3] A.S. Balogun, A. Shivkumar, A. Mustapha, O.S.G. Fatola and N. Imtiyaz. (2017). Spermogram and fertility assessment of cocks' semen, extended with coconut-water extender supplemented with garlic extracts. Pre-Conference on Organic Animal Husbandry, 7-8 November, 2017linked to the 19th Organic World Congress, organized by IAHA, New Delhi, India. 190-195.

[4] A.S. Balogun, A.A. Akinosun, B.A. Boladuro, A.K. Tiamiyu, B.A. Oluwemimo. (2020). Optimizing dilution rate of cock semen with natural based extender for optimum fertility. Archive of Science and Technology. 1(2): 175-82.

[5] A.S. Balogun, A.A. Akinosun, I.A. Shobanke, B.A. Oluwemimo, A.S. Owolabi, and T.A. Eletu. (2022a). Comparative effects of three different buffers on cock semen diluted with egg-yolk extender. Proceedings of 27th Annual Conference of ASAN 2022, Bauchi, 23rd – 27th October. Nigeria.

[6] A. Balogun, O. Jimoh, T. Olayiwola, Z. Abubakar. (2017). Semen quality and fertilizing ability of roosters semen diluted with quail egg-yolk supplemented with polar and non-polar dried garlic extracts. Journal of Advances in Biology and Biotechnology. 13(2): 1-12.

[7] M. Forouzanfar, M. Sharafi, S. Hosseini, S. Ostadhosseini, M. Hajian, L. Hosseini, P. Abedi, N. Nili, H. Rahmani, M. Nasr-Esfahani. (2010). In vitro comparison of egg yolk–based and soybean lecithin–based extenders for cryopreservation of ram semen. Theriogenology. 73(4): 480-487.

[8] M. Emamverdi, M. Zhandi, A. Zare Shahneh, M. Sharafi, A. Akbari-Sharif. (2013). Optimization of Ram semen cryopreservation using a chemically defined soybean lecithin-based extender. Reproduction in Domestic Animals. 48(6): 899-904.

[9] K. Kelso, S. Cerolini, R. Noble, N.C. Sparks, B. Speake. (1996). Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. Reproduction. 106(2): 201-206.

[10] A.S. Balogun, (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.

[11] A.S. Balogun, A.A. Akinosun, Shafi T.A. Shafi, S.G.F. Olabamiji, R.R. Olabamiji, A.A. Adebayo. (2023). Standardization of egg-yolk with three different buffers for optimizing turkey semen cold storage at 4ºC. Asian Journal of Animal Sciences. 17: 15-20.

[12] A.S. Balogun**.** (2022). Reproductive Biotechnology; An under-utilized tool in poultry breeding**.** Clinical Research in Animal Science. 2(2): 000534.

[13] A.S. Balogun. (2021). Optimizing liquid storage duration of two poultry species semen with plant based extender: Semen preservation in poultry. Letters in Animal Biology. 1(2): 01-06.

[14] A.S. Balogun, A.A. Akinosun, I.A Lawal, S.A Olawoyin and B.B. Hammed. (2024). Standardization of home-made skimmed cow milk with tris buffer for turkey semen dilution and preservation. Acta Scientific Veterinary Sciences 6.2 (2024): 15-19.

[15] A. Balogun, O. Jimoh, J. Ojo, T. Oke, A. Akinosun. (2015). In 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 held at Osun State University, Osogbo, Osun State, Nigeria. 28.

[16] A.M. Donoghue, G. Wishart. (2000). Storage of poultry semen. Animal reproduction science. 62(1-3): 213-232.

[17] T. Slanina, L. Petrovičová, M. Miškeje, L. Kňížat, J. Mirda, N. Lukáč, J. Trandžík, I. Petrovičová, P. Massányi. (2015). The effect of diluent, temperature and age on turkey spermatozoa motility in vitro. Journal of Applied Animal Research. 43(2): 131-136.

[18] J. Mohan, S. Sharma, G. Kolluri, M. Gopi, J. Tyagi, J. Kataria. (2017). In *Effect of different semen dilutors on fertilizing capacity of chicken semen stored for 24h*, Proceedings of XXVIII Annual Australian Poultry Science Symposium. Sydney, Australia. 260-263.

[19] J. Mohan, S. Sharma. (2017). In Recent advances in poultry semen diluents, Proceedings of 26th Annual Conference of Society of Animal Physiologists of India. 103-108.

[20] J. Mohan, S. Sharma, G. Kolluri, M. Gopi, J. Tyagi, J. Kataria. (2017). In Effect of various dilution rates on fertilizing ability of chicken spermatozoa using CARI diluent, Proceedings of 34th Indian Poultry Science Association Conference. Bengaluru, India. 61.