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Standardization of Insemination Frequency for Turkey Hens with Tom Semen Preserved at 5ºC in Plant Based Extender

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Abstract

Use of chilled semen is necessary in turkey for better application of Assisted Reproductive Biotechnology Techniques. This experiment is therefore design to identify appropriate insemination interval to achieve optimum fertility with tom semen preserved with Tris Coconut-water Orange Juice Extender (TCWOE) for 4 and 12hours at 5ºC. A completely randomized design (CRD), with a 4×2 factorial experiment involving four semen types and two insemination frequencies was used. Thirty-six (36) hens of 37 weeks age were used. Nine (9) Hens/group were inseminated once/week and twice/week with 0.05mL un-preserved semen (un-extended and extended) and 0.1mL tom semen preserved at 5ºC (4h and 12h chilled semen) containing about 200 x 10^6 viable spermatozoa for 4 weeks. 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. The result revealed that twice/week insemination interval gave better percentage fertility for 4H and 12H TCWO chilled semen compared to once per week insemination interval. It was also indicated that 4H and 12H TCWO chilled semen inseminated twice per week has higher percentage hatchability of fertile eggs values close to 80.00% compare un-extended and TCWO extended tom semen. While twice per week insemination interval has higher percentage hatchability of eggs set values compare to once per week insemination interval in both preserved, unpreserved and un-extended semen. Conclusively, better fertility and hatchability is achieved in this study with twice per week insemination interval for turkey hen using TCWOE to preserved tom semen at 5ºC.

Keywords: Artificial insemination, Chilled semen, Standardization, Extender, Insemination Interval

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1. Introduction

Artificial insemination (AI) has been a critical component of reproduction in turkeys since the 1960s, and is used almost exclusively for commercial flock production in some areas of the world [1]. The contrast in the size of the toms and hens and consequent low fertility of the males after natural mating has resulted in the complete integration of AI in commercial production. Reproductive performance is critical to efficient production in poultry and selection of males based on semen characteristics have been suggested in roosters and toms [2]. For successful AI, quality semen containing sperm cells capable to reach the site of fertilization at ovi-position, and initiate capacitation is highly paramount in poultry species [3]. The AI is used almost exclusively for commercial turkey flock production in some areas of the world while semen dilution and preservation in turkey breeding is still at experimental phase in most developing [4]. Although better fertility can be achieved in Turkey with AI using fresh ejaculates from tom immediately after collection for insemination, however, its low volume and viscous nature

with a higher concentration of sperm cells makes it difficult for the semen to be expelled nor discharged freely from the insemination gun, to inseminate the semen conveniently to the oviduct of the hen to serve more number of hens.

Thus, dilution with an efficient extender is required. According to Hafez and Hafez [5] domestic fowl sperm can spend up to 32 days in the oviduct while the turkey sperm can spend up to 72 days in the oviduct before fertilizing the egg yolk cell. An important factor that determines insemination interval is the sperm storage potential of the hen which varies with species. Turkeys are known to have a higher capacity to store sperm cells than chickens [6]. This can be attributed the higher number of sperm storage tubules they possess [7]. For this reason, insemination schedules, intervals for turkeys may be wider than that of chickens. Several reports of insemination intervals varying from 3 days to 14 days have been suggested for turkeys based on the undiluted semen dosage or number of sperm cells inseminated [8-10]. This experiment is therefore design to identify the most appropriate insemination interval ideal for turkey hen's optimal fertility and hatchability when inseminated with semen diluted and preserved with plant based extender. And may be a significant approach towards transfer of turkey genetics materials across boundaries.

2. Materials and Methods

2.1. Ethical Approval

The study was based on 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 Production Technology Igboora, Nigeria. 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 last from November to April, with mean daily temperature ranges of 14-36^{0}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 36 hens of 38 weeks weighing 3-4kg 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 1m -1.6mls per 25kg body weight.

2.4. Housing and management of the turkeys

Turkey toms were housed individually in 30x30x 40 cage and allowed to acclimatize for a period of two weeks during which they trained for semen collection. Three hens were housed per pen. They fed with hybrid commercial layer mash. Water was supplied *ad libitum* and 180g of feed were supplied per hen/day while 220g of feed fed per tom/day. The hens and toms were exposed to 12hours day length light.

2.5. Training of tom for semen collection

Toms trained for semen collection for period of two weeks by using [11] modified procedure for poultry semen collection. Semen is usually collected once in a week for period of four weeks for adequate sperm reserve durations.

2.6. 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, TCWO diluted, 4 hours TCWO chilled semen and 12 hours TCWO chilled semen were the four types of semen that were employed. While insemination frequencies were once per week and twice per week.

2.7. Semen Collection, Dilution and Preservation

For every artificial insemination during the eight (8) weeks of insemination, Ejaculated semen were usually collected separately and pooled from two Nigeria indigenous toms at 4hours and 12hours prior to insemination. The pooled semen was usually divided into two portions and extender would be added to it in ratio 1:3 (semen: extender). The diluted semen preserved for 4h and 12h at the Physiology and Reproduction biology laboratory of Oyo State College of Agriculture and Technology Igboora, Nigeria. Semen was diluted with extender at the ratio of 1:3 (semen: extender). Immediately after dilution, one part of the diluted semen was usually refrigerated for 4h and the other part for 12h at 4^{o}C. After storage of diluted semen for 4h and 12h, The Ejaculated semen would be collected again separately and the pooled from two to three Nigeria indigenous toms for the semen dilution (1:3) and the immediate insemination of the undiluted semen.

2.8. Artificial insemination of the turkeys

Fertility trial was carried-out at the turkey unit at the poultry farm of Oyo state College of Agriculture and Technology, Igboora, Nigeria. The fertilizing ability of spermatozoa was assessed by intra vaginal insemination of 12 females per experiment group including unpreserved semen (the positive and negative control group). Hens were inseminated twice and once per week with 0.05ml undiluted semen, 0.05ml TCWO diluted semen and 0.1ml for preserved semen of dosage containing about 100 to 200 x 10^6 viable spermatozoa per 0.lml, for 8 weeks with un-extended semen, extended semen and semen preserved for 4h and 12 h at 4 °C are shown in Figure 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 of fertile eggs about 28 days after start of incubation with the aid of petersime incubator manufactured at Belgium.

Fertility and hatching rates were calculated using the following formulas:

Fertility rate = (fertile/incubated eggs×100)

Hatching rate = (hatching/fertile eggsx100)

2.9. 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 of $p < 0.05$ were considered significant. All statistical analysis was done using the SPSS Software, version 22 (2021).

3. Results and discussion

3.1. Results

3.1.1. Comparative percentage fertility and hatchability of inseminated hens

The result of comparative percentage fertility and hatchability potentials of TCWO extended and chilled turkey semen is presented in Table1.

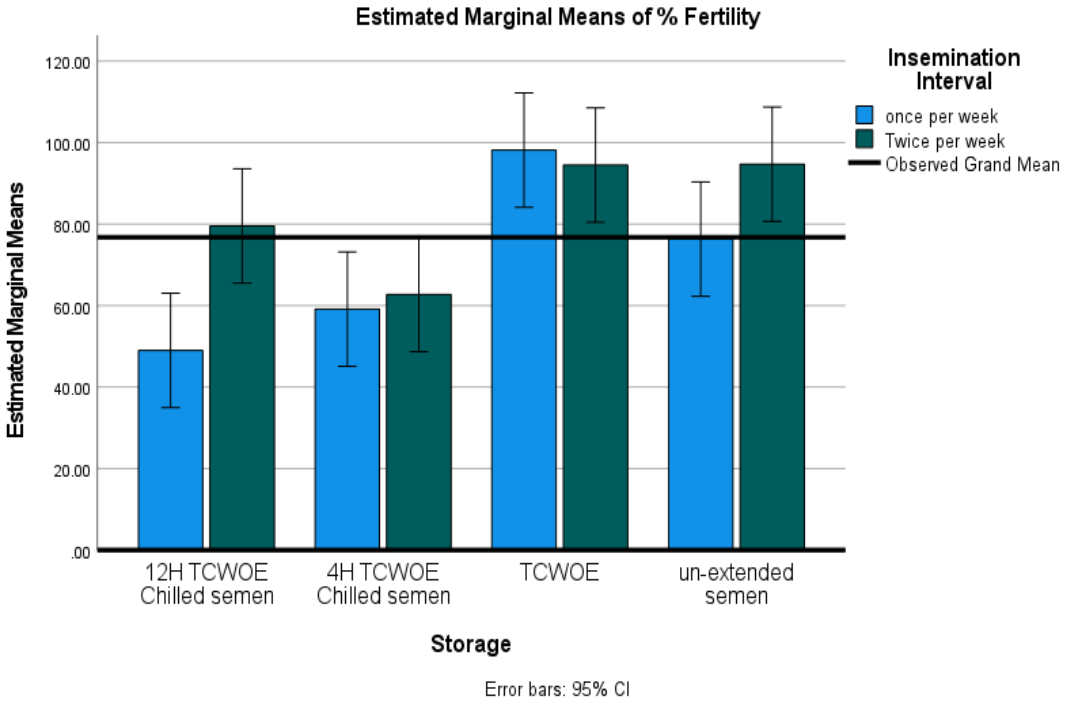

Figure 1. Percentage fertility of incubated eggs collected from 30weeks Nigeria indigenous turkey hens inseminated at different intervals

TCWO: Tris Coconut-water orange
Un-extended semen: Semen void of extender
TCWO unchilled Semen: TCWO extended tom semen without cold storage
4h TCWO chilled Semen: TCWO extended tom semen preserved for 4h at 5^{o}C
12h TCWO chilled Semen: TCWO extended tom semen preserved for 12h at 5^{o}C

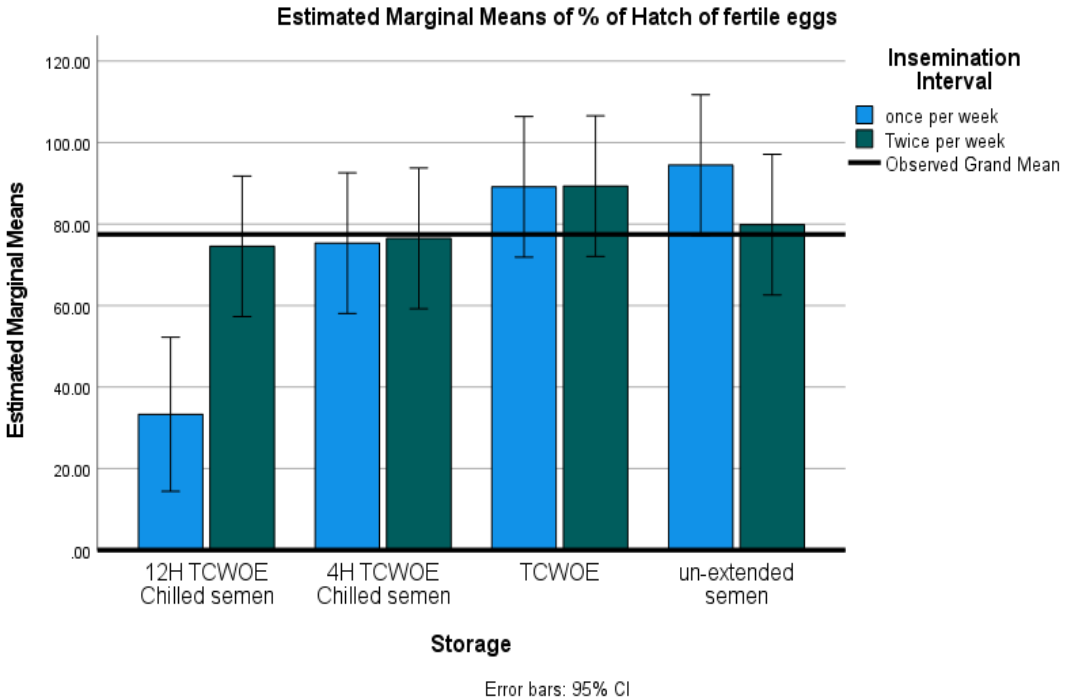

Figure 2. Percentage hatchability of fertile eggs of incubated eggs collected from 30weeks nigeria indigenous turkey hens inseminated at different intervals

TCWO: Tris Coconut-water orange
Un-extended semen: Semen void of extender
TCWO unchilled Semen: TCWO extended tom semen without cold storage
4h TCWO chilled Semen: TCWO extended tom semen preserved for 4h at 5ºC
12h TCWO chilled Semen: TCWO extended tom semen preserved for 12h at 5ºC

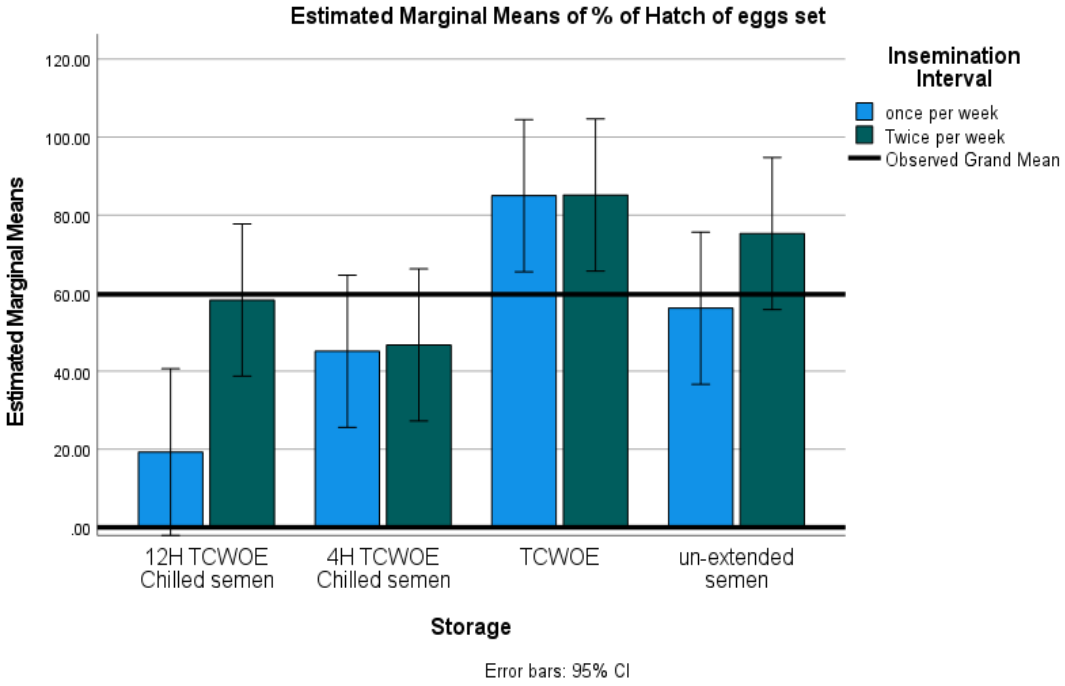

Figure 3. Percentage hatchability of fertile eggs of incubated eggs collected from 30weeks nigeria indigenous turkey hens inseminated at different intervals

TCWO: Tris Coconut-water orange
Un-extended semen: Semen void of extender
TCWO unchilled Semen: TCWO extended tom semen without cold storage
4h TCWO chilled Semen: TCWO extended tom semen preserved for 4h at 5ºC
12h TCWO chilled Semen: TCWO extended tom semen preserved for 12h at 5ºC

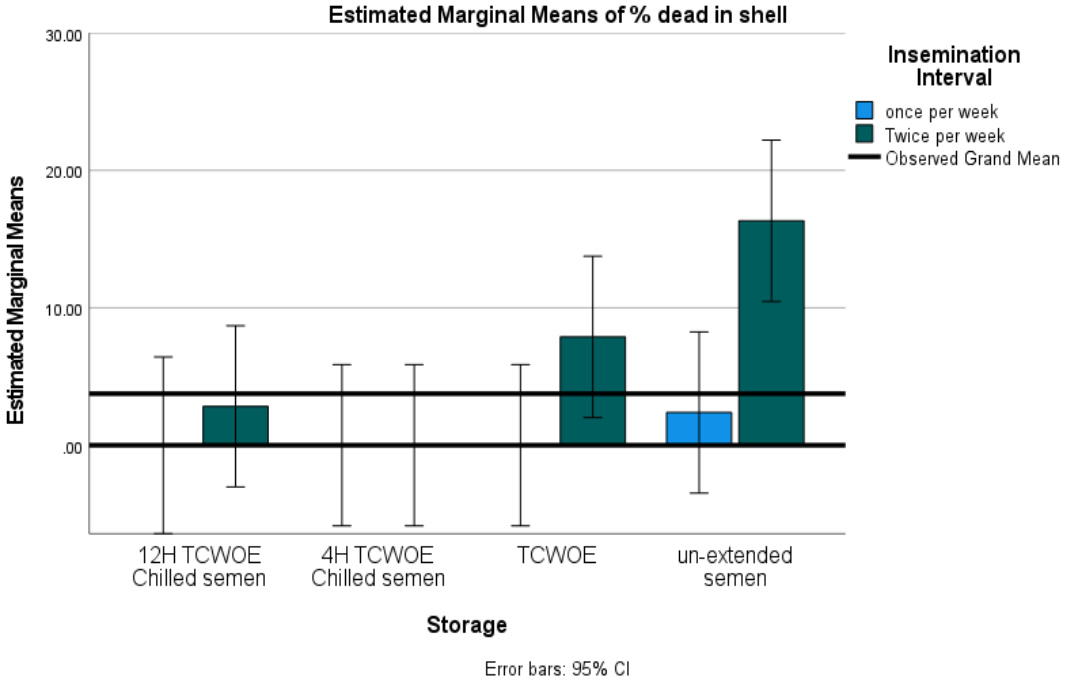

Figure 4. Percentage dead in shell of incubated eggs from 30weeks Nigeria Indigenous turkey hens inseminated at different intervals

TCWO: Tris Coconut-water orange
Un-extended semen: Semen void of extender
TCWO unchilled Semen: TCWO extended tom semen without cold storage
4h TCWO chilled Semen: TCWO extended tom semen preserved for 4h at 5^{o}C
12h TCWO chilled Semen: TCWO extended tom semen preserved for 12h at 5^{o}C

Table 1: Comparative Percentage fertility and hatchability of TCWO extended and chilled semen

Treatments	Un-extended Semen	TCWO extended semen	4h TCWO chilled semen	12h TCWO chilled
Fertility (%)	85.53±13.71^{a}	96.33±9.76^{a}	61.00± 14.70^{b}	64.28± 30.12^{b}
Infertility (%)	14.50±13.72^{b}	3.67±9.76^{b}	38.30±15.09^{a}	34.58±31.32^{a}
Bangers (%)	0.00±0.00	0.00±0.00	1.17±4.04	0.75±2.60
Hatchability of fertile eggs (%)	87.20±18.25 ^{a}	89.30±14.41 ^{a}	75.92±18.10 $^{a\ b}$	55.81±35.74 ^{b}
Hatchability of eggs Set (%)	65.70±29.09^{ab}	85.08±17.83^{a}	45.93±15.24^{bc}	40.53±34.10 ^{c}
Dead in shell (%)	9.35±11.19 ^{a}	3.94±10.09^{ab}	0.00±0.00 ^{b}	1.55±5.13 ^{b}

Means with different superscripts a,b revealed significant difference (P>0.05)
TCWO: Tris Coconut-water orange
Un-extended semen: Semen void of extender
TCWO unchilled Semen: TCWO extended tom semen without cold storage
4h TCWO chilled Semen: TCWO extended tom semen preserved for 4h at 5^{o}C
12h TCWO chilled Semen: TCWO extended tom semen preserved for 12h at 5^{o}C

Percentage fertility of TCWO extended and Chilled turkey semen revealed that both un-extended and TCWO extended tom semen had significant (p>0.05) higher percentage fertility of 85.53±13.71 and 96.33±9.76 compared to 4h and 12h TCWO preserved tom semen (61.00± 14.70, 64.28± 30.12). Percentage infertility is significantly (p>0.05) higher in 4h and 12h TCWO chilled semen compared to un-extended and TCWO extended turkey semen. No significant (p<0.05) different was observed for the percentage of bangers among the treatments. The result of percentage hatchability of fertile eggs revealed that 4h TCWO chilled semen was not significantly (p>0.05) different in percentage hatchability of fertile eggs compare to un-extended semen & TCWO extended turkey semen, however, 12h TCWOE showed significant lower value compared to un-extended and TCWOE extended. Percentage hatchability of egg sets revealed that 4h TCWOE chilled turkey semen was not significantly (p>0.05) different from un-extended semen, while 12h TCWOE has the significantly (p>0.05) lower percentage hatchability of fertile eggs compared to TCWOE extended tom semen and un-extended semen. Percentage dead in shell is significantly higher in un-extended and TCWOE extended tom semen compare to preserved semen.

3.1.2. Percentage fertility of hens inseminated at different Intervals

Frequency of insemination required for optimum percentage fertility for un-extended, TCWOE extended and TCWOE chilled turkey semen is presented in Fig 1. Twice per week insemination interval resulted in better percentage fertility for 12h and 4h TCWO chilled tom semen compared to once per week insemination interval. Same trend was also observed for percentage fertility of un-extended tom semen, while in TCWOE extended tom semen once per week insemination interval gave a better percentage fertility result compare to twice per week insemination interval. Finally twice per week inseminated 12h TCWOE chilled semen revealed 80.00% fertility above observed grand mean comparable with twice and once per week TCWOE extended semen and Un-extended semen insemination interval.

3.1.3. Percentage hatchability of fertile eggs from hens inseminated at different intervals

The result of insemination interval standardization for the TCWOE diluted and Preserved tom semen on hatchability of fertile eggs is presented in Fig 2. Result clearly indicated that twice per week insemination interval has higher values close to the 80.00% compare to twice per week insemination interval in both 4h and 12h chilled turkey semen. For the TCWOE extended turkey semen both insemination intervals has similar values of above the 85.00%. While once per week the insemination interval has higher value compare to twice per week the insemination interval for the un-extended semen.

3.1.4. Hatchability percentage of eggs set from hens inseminated at different intervals

The result of insemination interval standardization for TCWOE diluted and Preserved tom semen on hatchability of eggs set is presented in Fig 3. Similar trend observed for percentage hatchability of fertile eggs was also experience in percentage hatchability of egg set in both TCWOE preserved tom semen and TCWOE extended turkey semen. Except for un-extended tom semen where twice per week insemination interval was higher in value compare to once per week insemination interval.

3.1.5. Dead percentage in shell from inseminated hens at different intervals

The dead percentage in shell from hens inseminated with chilled and un-chilled turkey semen inseminated at

different insemination interval was presented in Figure 4. The results revealed that no deed in shell was recorded for 4h TCWO chilled semen, dead in shell recorded. Furthermore, twice per week insemination recorded higher dead in shell compared to once-per-week the insemination in both samples.

3.2. Discussion

The result from this study clearly revealed that liquid stored TCWOE extended turkey semen has lower fertility, hatchability of fertile eggs and hatchability of eggs set compared to unpreserved semen. This is an indication that turkey semen stored at 4ºC rapidly declined in sperm activities such as sperm motility and viability irrespective of storage duration and insemination intervals compared to unpreserved semen. In agreement with the findings from this present study, Balogun [12-3-13] reported that percentage fertility and hatchability were found to be higher in eggs produced by chicken hens inseminated using TCWO diluted semen than those inseminated using undiluted semen which clearly revealed capability of TCWOE extenders. Furthermore, It is evident from this study that inseminating twice per week gave a better percentage fertility and hatchability of fertile eggs and eggs set compare to once per week insemination irrespective of semen condition, owing to fact that adequate sperms reserves were sufficient for optimum fertility to be achieved. Similarly, Also, Saleh *et al.*, [14] and Pym [15], found that fertility was higher in 3 days insemination interval compared with 6 days interval. Convincingly potential of TCWOE extender was evident in both diluted and preserved semen as it contributes significantly to fertilizing ability of both chilled and un-chilled the TCWOE extended turkey semen, also revealed in twice and once per week insemination interval inseminated to hens. However, twice per week insemination interval gave a better result due to abundance of sperm in the insemination practices per week.

4. Conclusion

It can therefore be concluded better fertility and hatchability is achieved in our present study with twice per week insemination interval for turkey hen using TCWOE extended preserved tom semen at 5ºC. Furthermore, 12h chilled TCWOE tom semen exhibited better percentage fertility close in value to TCWOE extended and un-extended tom semen.

Authors' contributions

BAS design, carried out the research and first draft of the manuscript AAA and AJA contribute to the resources and review of final manuscript draft. HUO and MNB took part in the methodology.

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Ethical Consideration

The authors confirm that all the 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.

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