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Optimizing liquid storage duration of two poultry species semen with plant based extender

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Abstract

Liquid storage of semen preservation for poultry species is deemed to be the only reliable and cost effective method compare to cryopreservation. This experiment was conducted to examine the preservation capacity of Tris coconut-water orange juice extender (TCWOE) on tom and cock semen for artificial insemination. TCWOE was prepared. Semen ejaculates were collected and pooled from five toms and cocks separately. The pooled semen from each species was divided into two portions and in one portion of each species the extender was added in the ratio of 1:3 (semen: extender), making a total of four treatments. Experimental design used was a complete randomized design consisting of three trials. Semen microscopic parameters like motility, viability, membrane integrity, and acrosome integrity were examined and recorded for freshly extended semen and preserved semen at 4-8^{o}C every 12 h interval till 72 h. The motility and membrane integrity of freshly extended cock semen was significantly higher ($P<0.05$) compared to motility of extended tom semen and membrane integrity of un-extended tom semen. During storage up to 24 h, extended tom and cock semen showed significantly higher ($P<0.05$) motility and membrane integrity compare to un-extended semen. Extended cock semen had significant ($P<0.05$) higher percentage motility, livability and membrane integrity compared to un-extended and extended tom semen from 36 h to 72 h of storage. However, no significant difference ($P>0.05$) was observed for acrosome integrity. Conclusively, cock semen survived longer and had better results considerable for artificial insemination than tom semen up to 72 h storage. However, the extender was beneficial for storage of both species semen.

1. Introduction

Improving the survival rate and motility of chilled poultry semen stored at 4-8 oC for a period of 48–72 h with a natural extender such as Tris Coconut-water orange juice extender (TCWOE) could be an effective way of increasing utilization of artificial insemination (AI) in the poultry breeding industry. The success and prospects of artificial insemination in poultry have been documented that they generally vary with species due to variation in semen quality in terms of functionality within and between species, males as well as ejaculates (Blanco et al. 2000; King et al. 2000; Yu et al. 2002; Blesbois et al. 2005; Chaveiro et al. 2006; Roca et al. 2006; Leahy and Gadella 2011). Ultimately, prospects of semen dilution with extender for artificial insemination varies with species, methods, and procedures for semen preservation and insemination. According to Hafez and Hafez (2000) 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. Tabatabaei et al. (2009) stated that the mechanism behind prolonged sperm storage is still a misery to scientists. However, it is assumed to be attributed to the ability of the oviduct to keep the sperm cells in a quiescent manner and state till oviposition time.

Compared to frozen-thawed semen, liquid stored semen gives better and reliable results when used for artificial insemination in poultry species. It has been reported that both chicken and duck liquid refrigerated semen resulted in better semen quality and fertilizing ability (Lemoine et al. 2011,

Kasai et al. 2000; Penfold et al. 2001). Above all, chilled semen is easier and cost-effective to handle and transport to different locations compared to frozen semen (Diaz et al. 2013). Under the standard conditions the fertility rate of artificial insemination (AI) is normally greater for chilled semen than frozen semen in most species of farm animals including poultry (Linde-Forsberg 1995). Hence, there is a need to further improve on liquid storage of poultry semen irrespective of species and breeds.

Several experiments on the use of coconut water tris based diluents supplemented with varieties of juice extracts as a natural diluent for dilution and liquid preservation of roosters semen have shown tremendous promising results both in-vitro and in-vivo over the years (Balogun et al. 2016, 2017a, 2017b, 2018, 2019). The success of the liquid preservation regime of turkey and cock semen with salt-based extenders are considerably low and varies over time. Moreover, it is also deemed that the dilution and storage capability of the extender may be species-specific. Hence, it is therefore imperative to compare and optimize the dilution and liquid preservation capability of TCWO extender on two different poultry species – domestic fowl and turkey. This experiment was therefore aimed at evaluating the effect of dilution and storage on microscopic semen quality of preserved tom and cock semen with tris coconut-water orange juice extender.

2. Materials and Methods

2.1 Tom and cock management

A total of five matured toms and cocks in their reproductive age of 30-40 weeks were used for the experiment. They were kept together in separate pens, respectively. Feed and water were provided as per turkey and poultry breeder requirements.

2.2 Training of tom and cock for semen collection

The toms and cocks were trained for semen collection for a period of two weeks by poultry semen collection procedure with suitable modification (Balogun et al. 2015). Semen was collected once a week for a period of four weeks for adequate sperm reserve dilutions.

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

Tris coconut-water orange juice extender was prepared by piercing ripe coconut at the top and the water was collected in a beaker followed by filtration. Tris buffer of pH 7.4 was added to coconut-water in the ratio of 1:1 and mixed vigorously. Finally, 10 ml of orange juice was added to the tris coconut-water solution to make tris coconut-water orange juice (TCWO) extender.

2.4 Experimental design

The semen ejaculates were collected from five toms and cocks, and pooled separately. The pooled semen from each species were divided into two portions and in one portion of each species the extender was added in the ratio of 1:3 (semen: extender), making a total of four treatments. Following a completely randomized design the experiment consisted of four treatments and the trials were conducted thrice. Microscopic semen parameters like motility, viability, membrane, and acrosome integrity were examined and recorded for freshly extended semen and semen stored for 72 h at 4-8 ^{o}C. The evaluation of stored semen was done at every 12 h interval till 72 h.

2.5 Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA) using SPSS software and means were separated with Ducan multiple range test.

3. Results

Microscopic semen analysis result of tom and cock semen preserved with TCWO extender from 0 to 72 h preserved at 4-8 oC is presented in Table 1. Motility (95.0 %) of freshly extended cock semen diluted with TCWO extender was significantly higher ($P<0.05$) compared to extended tom semen, but was not significantly ($P>0.05$) different from un-extended cock and tom semen. Similar observation was recorded for membrane integrity (93.0 %). Percentage live sperm and acrosome integrity of freshly extended semen were not significantly different, with acrosome integrity having the same value of 100% in both extended and un-extended cock and tom semen.

At 12 h to 24 h of storage the results of motility, acrosome integrity, live sperm, and membrane integrity follow the similar trend. The cock semen extended with TCWO extender had significantly higher ($P<0.05$) motility and membrane integrity compared to un-extended cock and tom semen, although the differences were not significant ($P>0.05$) compared to extended tom semen. Percentage live sperm and acrosome integrity showed no significant differences across the treatments except at 12 h where extended tom semen had significantly higher live sperm percentage (94.75%) compared to un-extended tom semen (89.50%). All sperm acrosomes were also observed to be intact irrespective of species or dilutions. Motility and membrane integrity of extended cock semen were still above 75.00% and significantly higher ($P<0.05$) compared to extended tom semen (61.25% and 62.25%), un-extended tom (25.00% and 30.25%) and cock semen (46.25% and 47.50%) at 36 h of liquid storage period. But, the percentage live sperm of extended tom and cock semen were not significantly different ($P>0.05$) from each other, while acrosome integrity had same value of 100.00% in all the treatments.

At 48 h to 60 h all observations followed the similar trend. Motility and membrane integrity were still above 50.00% for extended cock semen and significantly higher ($P<0.05$) from extended tom, un-extended cock, and tom semen. However,

Table 1 Effects of TCWO extender on Tom and Cock semen preserved for 72 h at 4-8 °C

Source of varation			Motility (%)	Live sperm (%)	Membrane integrity	Acrosome integrity
Preservation periods	Species	Dilution				
0 h	Turkey	Un-extended	90.00^{ab}	96.00	84.25^{b}	100.00
		Extended	88.75^{b}	96.00	90.00^{ab}	100.00
	Cock	Un-extended	93.75^{ab}	96.50	91.00^{a}	100.00
		Extended	95.00^{a}	98.25	93.00^{a}	100.00
	SEM		1.00	0.51	1.19	0.00
12 h	Turkey	Un-extended	40.00^{c}	89.50^{b}	41.00^{c}	100.00
		Extended	75.00^{ab}	94.75^{a}	74.00^{ab}	100.00
	Cock	Un-extended	66.25^{b}	92.00^{ab}	64.75^{b}	100.00
		Extended	85.00^{a}	93.75^{ab}	87.75^{a}	100.00
	SEM		4.85	0.84	5.27	0.00
24 h	Turkey	Un-extended	26.75^{c}	89.25	29.25^{c}	100.00
		Extended	70.00^{a}	92.25	71.25^{a}	100.00
	Cock	Un-extended	48.75^{b}	90.00	51.50^{b}	100.00
		Extended	77.50^{a}	92.75	76.50^{a}	100.00
	SEM		5.50	0.73	5.50	0.00
36 h	Turkey	Un-extended	25.00^{d}	84.75^{c}	30.25^{d}	100.00
		Extended	61.25^{b}	90.50^{ab}	62.25^{b}	100.00
	Cock	Un-extended	46.25^{c}	88.00^{b}	47.50^{c}	100.00
		Extended	77.50^{a}	92.75^{a}	78.75^{a}	100.00
	SEM		5.48	0.89	4.95	0.00
48 h	Turkey	Un-extended	15.00^{d}	80.50^{c}	21.75^{c}	100.00
		Extended	51.25^{b}	87.75^{ab}	52.25^{b}	100.00
	Cock	Un-extended	27.50^{c}	85.50^{bc}	29.25^{c}	100.00
		Extended	63.75^{a}	91.25^{a}	66.75^{a}	100.00
	SEM		5.18	1.27	4.87	0.00
60 h	Turkey	Un-extended	2.50^{c}	74.00^{c}	7.25^{c}	100.00
		Extended	12.50^{bc}	81.50^{b}	11.75^{bc}	100.00
	Cock	Un-extended	21.25^{b}	83.00^{ab}	20.00^{b}	100.00
		Extended	53.75^{a}	89.50^{a}	54.50^{a}	100.00
	SEM		5.40	1.77	5.07	0.00
72 h	Turkey	Un-extended	0.00^{b}	69.50^{b}	4.75^{b}	100.00
		Extended	2.50^{b}	79.50^{a}	9.25^{b}	100.00
	Cock	Un-extended	3.75^{b}	75.50^{ab}	8.75^{b}	100.00
		Extended	48.75^{a}	83.25^{a}	46.50^{a}	100.00
	SEM		5.29	1.89	4.67	0.00

Means with different superscript abc within the column differ significantly (P<0.05); SEM: Standard error of mean

lives sperm percentage of un-extended tom semen was significantly lower among the treatments at 48h (80.50%) and 60h (74.00%) period of storage. Finally at 72 h of liquid storage, motility and membrane integrity were below average in both species and dilution. The extended cock semen still showed the significantly higher values for motility (48.75%), live sperm (83.25%), and membrane integrity (46.50%) compared to extended tom semen, un-extended cock, and tom semen. However, live sperm percentage of extended cock semen was statistically similar (P>0.05) to both extended tom and un-extended cock semen. Similarly to the previous storage periods, zero percent loss in acrosome integrity was recorded for all treatments

The effect of species on the rate of decline in percentage motility, viability, and membrane integrity of fresh and liquid stored semen was presented in Fig 1, 2, and 3. The rate of decrease in percentage motility of extended cock was gradual

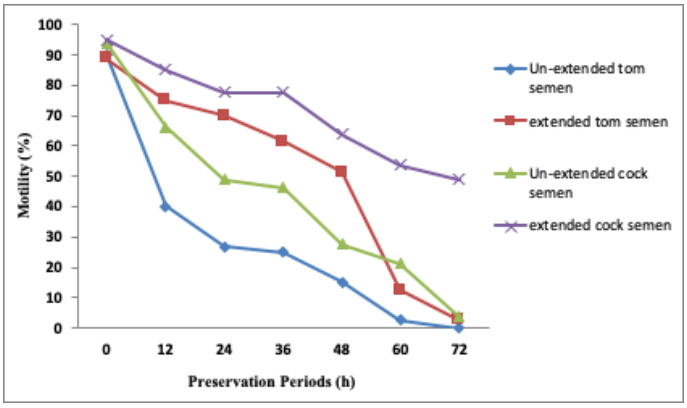

Fig 1. Decline in rate of motility of extended tom and cock semen preserved for 72 h

and above 50.00 % till 72 h of storage, while the extender could maintain the motility of tom semen above 50.00% for only 48 h. Un-extended cock semen had motility above 50.00% till 24 h, will motility of un-extended tom semen drastically dropped below 40.00% after 12 h of storage. Percentage live sperm for both species (extended and un-extended) were all above 70.00% till 72 h period of storage. However, extended cock and tom semen exhibited higher live sperm percentage of above 80.00% till 72 h of storage compare to un-extended semen. Percentage membrane integrity of extended cock semen was higher and above 50.00% till 72 h storage compare to extended tom semen which had membrane integrity above 50.00% till 48 h storage. Membrane integrity of un-extended cock semen was above 50.00% till 24 h storage while un-extended tom semen was below 40.00% at 12 h storage. All acrosomes were intact through the storage periods.

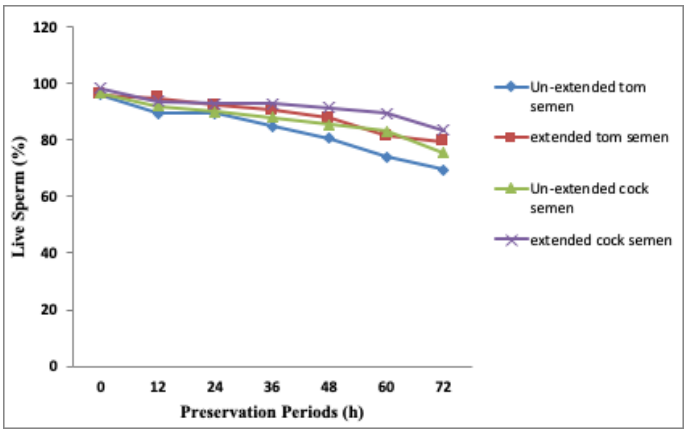

Fig 2. Decline in live sperm percentage of extended tom and cock semen preserved for 72 h

4. Discussion

This was the first study in Nigeria on simultaneously preserving cock and tom semen with plant based natural extender under cold storage temperature of 4-8 oC for 72 h. Since poultry species react differently to preservation techniques 'liquid storage and cryopreservation' (Blanco et al. 2000, 2008), evaluating extender storage capacity and its specificity for two different poultry species in Nigeria is important for optimum preservation and fertilizing ability of preserved poultry semen for successful artificial insemination. Moreover fertility rate similar to that of inseminated fresh semen has been reported for chicken semen stored for 24 h and turkey semen stored up to 6 h at 4 oC only (Donoghue and Wishart 2000).

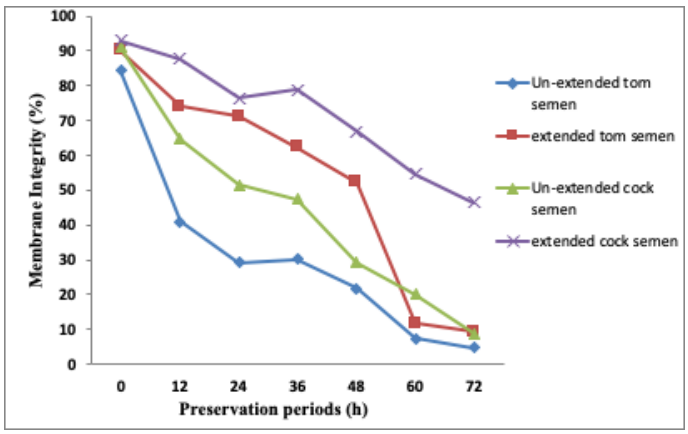

Fig 3. Decline in rate of membrane integrity of extended tom and cock semen preserved for 72 h

In this study on liquid preservation of two poultry species with plant based natural extender revealed results similar to the previous findings on refrigerated liquid cock and tom semen, where feasible storage of semen for up to 24 or 48 h at 5 °C was observed (Lemoine et al. 2011; Wishart 2009). However, contrary to the previous reports this study successfully stored cock semen for 72 h and tom semen for 48 h with better post storage semen quality. This is an indication that the plant based natural extender used in this study possessed better preservation capacity by supporting sperm metabolism even without oxygenation compared to the existing salt component extenders previously experimented on tom semen.

Although, it has been reported that turkey spermatozoa are mainly active in aerobic conditions and chicken spermatozoa are active in both aerobic and anaerobic conditions (Iaffaldano et al. 2016) the turkey spermatozoa are considered more efficient in aerobic conditions because of the low lactic acid accumulation and high oxidation rate (Sexton 1974). In contrast, the results of the present study revealed that both species can survive in anaerobic condition but only differs in rate and period of survival. However, both the extended and un-extended cock sperms survived better and longer compared to extended and un-extended tom semen, respectively. Although, age and strain are the important factors contributing to turkey sperm survival under fresh and liquid storage conditions (Iaffaldano et al. 2008) all the parameters revealed that extended semen of both the species performed better than their un-extended counterparts.

5. Conclusion

In conclusion, survivability of preserved sperm cells in extended semen of both poultry were obviously favorably elongated. The preserved cock sperm survived longer and had better results favorable for artificial insemination in poultry species compared to the preserved tom sperm cells.

Declarations

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