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Standardization of Home-Made Skimmed Cow Milk with Sodium Citrate Buffer for Dilution and Cold Storage of Turkey Semen

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Abstract

Low volume and highly viscous nature of turkey semen calls for needs to process and standardize cow milk close in appearance and less harmful to sperm cells, for effective dilution and liquid storage of turkey semen. This experiment therefore aimed at optimizing home-made skimmed cow milk (HSCM) prepared from fresh cow milk (FCM) for extension and liquid storage of tom semen. Extenders containing different concentration of HSCM via: 25%, 50%; 75% and 100 were formulated. Five toms were milked every week for semen collection and pooled together, then divided into five portions for a period of four weeks. Semen was diluted with each extender in ratio of 1:3 (semen: extender). Samples were examined under microscope and recorded for both extended and un-extended semen samples stored for 48h at 4°C. The result revealed that at 0h, un-extended semen had significantly ($p<0.05$) lowest motile sperm of compared to extended tom semen. At 4h of preservation, 75% HSCM was significantly ($p<0.05$) higher in sperm motility and membrane integrity compared to other treatments. At 24h and 48h, 75% HSCM have significantly ($p>0.05$) higher in motile sperm and intact membrane compare to other treatments. Better percentage livability of preserved tom sperm observed in 75% and 50% HSCM at 0h and 4h of storage. At 24h, tom semen preserved in 100% HSCM has highest non-significant percentage live sperms. While at 48h, semen stored in 75% HSCM has highest non-significant percentage live sperms. The extender containing 75% HSCM and 25% sodium citrate buffer maintained better sperm activities compared to the other samples.

Keywords: Skimmed milk; Home-made; Tom; Semen; Liquid Storage

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1. Introduction

To improve poultry species economic efficiency, Artificial Insemination (AI), semen dilution and preservation can impact different productivity projects, such as chicken and turkey breeding and husbandry thereby accelerating genetic gain in virtually all poultry species as it is practiced in cattle and other mammals. Therefore, AI, semen dilution and preservation has a great potential to impact on the economic in poultry breeding. Furthermore, fresh or well-preserved semen is required for AI practices. Unfortunately, fresh semen is usually used for 95% of all AI in poultry species. Thus, semen must be diluted and maintained in an effective medium to maintain its quality. Accordingly, it is essential task for poultry reproductive scientists to standardized and develop a reliable standard semen extender that will be used for diluting and preserving turkey semen (Liquid storage and freezing). Semen dilution in turkey is expected to improve the processing of semen and ease semen evaluation such as their concentration, motility, viability and pH as tom semen is usually very small in quantity and highly viscous. Diluents solutions are deemed to maintain the activities of spermatozoa and to increase the semen volume [1] and further preserve the sperm cells till use.

Lack of diluent in the Sperms storage chickens and turkey protocols is inefficient processes [2]. However, the excessive increase of dilution ratio and liquid storage period may pose negative effect on tom sperm activities. Varieties of ingredient have been evaluated for developing extender to

sustain sperm viability, among them are agent of cryoprotectants and anti-cold shock such as glycerol and egg yolk [3], lactated Ringer's glucose, glucose-Trisglucose and lactated Ringer's extenders [4], skimmed milk and Tris-citrate extenders [5] orange juice [6] and vitamins of A, C and E [7]. However, few or no reports on the successful use of home–made skimmed fresh cow milk for extension and liquid storage of turkey sperm cells. In addition, the semen pH can be improved with skim milk proteins and may also be capable of chelating any heavy metal ions, thus standardizing home-made skimmed milk for tom semen extension and cold storage is deemed necessary for improved fertility and further accelerate genetic improvement in turkey species.

2. Materials and Methods

2.1 Experimental site

The experiment was carried at the Turkey Unit of the Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igboora.

2.2 Turkey management

30-40weeks of age sexually matured toms where used for the experiment. They were kept together in a pen. Feed and water were supplied once daily to the tom.

2.3 Training of toms for semen collection

The toms were trained for semen collection for period of two weeks by using semen collection procedure by [8]. Toms ejaculated once in a week to have adequate semen reserve before another ejaculation for period of 4 weeks.

2.4 Sodium Citrate buffer Preparation

2.9g of sodium citrate was dissolved in 100ml of distilled water, and pH was finally adjusted to 7.2.

2.5 Preparation of home-made skimmed cow milk extender with sodium citrate buffer

A lactating cow was identified; the teat was sterilized and milked for collection of fresh cow milk. About 1litre of milk was harvested. The 100ml of fresh cow milk was poured into a pot. The milk was boiled for 6 minutes at medium heat. During boiling it was constantly stirred. After boiling the milk was cooled for 2minutes. The cream, or the fat, rises to the top as the milk cools down. The cream was gently scrape off the top with a large spoon. The pot was covered with a lid and the milk was refrigerated for 8 hours. As it cools, the milk separated even further and the fat rises to the top. A spoon was gently use to skim rest of cream off of milk, making sure mixing it back into saucepan is was avoided. The skimmed milk was transfer from the saucepan into 100ml sample bottle and was diluted with Sodium citrate buffer (SCB) immediately. Citrate buffer mixed vigorously with different concentration of skimmed milk (25%, 50%, 75% and 100%), and kept inside refrigerator for further use.

2.6 Experimental design

Five toms ejaculated and pooled together in a single container. The pooled semen divided into 5 parts and four parts diluted with extenders in ratio 1:3 (semen: extender), making 5 treatments. Experimental design used completely randomized design. The experiment consists of 5 treatments and trials conducted thrice. Microscopic semen parameters like motility, viability membrane and acrosome integrity examined and recorded for freshly extended semen and semen stored for 72hrs at 4-8ºC. Semen evaluation carried out every at 4h, 24h and 48h. Treatments comprises of:

Treatment 1: neat semen

Treatment 2: 25% home-made skimmed cow milk +75% SCB

Treatment 3: 50% home-made skimmed cow milk + 50% SCB

Treatment 4: 75% home-made skimmed cow milk +25% SCB

Treatment 5: 100% home-made skimmed cow milk

2.7 Semen Evaluation

Microscopic semen parameters such as motility, viability, and membrane and acrosome integrity were examined and recorded for freshly extended semen and semen samples preserved for 4h, 24h and 48h.

2.8. 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 and discussion

3.1 Results

Motile sperm evaluation of stored turkey semen diluted with different concentration of HSCM is presented in table 1. At 0h, tom semen diluted with 100% HSCM had highest sperm motility value of 90.00%, but was statistically similar ($p>0.05$) to 75% and 50% HSCM. At 4h of preservation, un-diluted had significantly ($p<0.05$) lower motile sperm cells (38.33%) compared to other treatments with different concentrations of HSCM. Moreover, at 24h and48h of preservation75% HSCM had significant ($p>0.05$) higher in motile sperm cells compare to the other treatments (undiluted semen, 25%, 50% and 100% HSCM), but the motility value of 75% HSCM was also drops below average starting from 24h of preservation period. Percentage livability evaluation of tom semen diluted and preserved in different concentration of HSCM is presented in table 2. Turkey semen diluted with 75% HSCM has the highest percentage of life sperms of 93.33%, but was not statistically similar ($p>0.05$) to 25, 50 and 100% HSCM. At 4h of storage, highest life percentage of sperm (87.00%) was observed in tom semen preserved in 50% HSCM, although was not significantly different from undiluted semen, 75% and 100% HSCM.

At 24h of storage, turkey semen preserved in 100% HSCM had the highest percentage of live sperm (78.67%) but was not significantly different from 50% and 75% HSCM. At 48h of storage, tom semen preserved in 75% HSCM had the highest percentage of live sperm (68.33%) but was not significantly different from 25, 50 and 100% HSCM. Percentage membrane integrity evaluation of turkey semen preserved with different concentration of HSCM is presented in table 3. Turkey semen diluted with 50% HSCM had the highest sperm membrane integrity of 77.67%, although was statistically similar ($p>0.05$) to 25, 75% HSCM including neat semen. At 4h of storage, highest sperm membrane integrity (72.00%) was observed in tom semen preserved in 75% HSCM, although was not significantly different from 25% and 50% HSCM.

Table 1: Effects of different concentration of home-made skimmed cow milk with sodium citrate buffer on motility of diluted tom semen

	Preservation Periods			
Treatments (%)	**0h**	**4h**	**24h**	**48h**
Undiluted Semen	76.67^{c}	38.33^{c}	5.00^{c}	0.00^{c}
25 HSCM	80.00^{bc}	48.33^{bc}	13.33^{c}	10.00^{b}
50 HSCM	85.00^{abc}	58.33^{b}	36.67^{b}	11.67^{b}
75 HSCM	86.67^{ab}	75.00^{a}	46.67^{a}	31.67^{a}
100 HSCM	90.00^{a}	43.33^{bc}	41.67^{ab}	11.67^{b}
SEM	1.65	3.96	4.51	2.87

Means with different superscripts a, b, c are significantly different from each other.

Table 2: Effects of different concentration of home-made skimmed cow milk with sodium citrate buffer on viability of diluted tom semen

	Preservation Periods			
Treatments (%)	**0h**	**4h**	**24h**	**48h**
Undiluted Semen	89.33^{b}	81.33^{ab}	67.67^{b}	58.00^{b}
25 HSCM	93.00^{ab}	76.67^{b}	69.00^{b}	61.67^{ab}
50 HSCM	91.33^{ab}	87.00^{a}	74.00^{ab}	67.33^{a}
75 HSCM	93.33^{a}	83.67^{a}	73.00^{ab}	68.33^{a}
100 HSCM	90.67^{ab}	87.33^{a}	78.67^{a}	64.00^{ab}
SEM	0.59	1.52	1.29	1.31

Means with different superscripts a, b, are significantly different from each other.

Table 3: Effects of different concentration of home-made skimmed cow milk with sodium citrate buffer on membrane integrity of diluted tom semen

	Preservation Periods			
Treatments (%)	**0h**	**4h**	**24h**	**48h**
Undiluted Semen	70.67^{ab}	18.33^{c}	10.00^{c}	7.00^{c}
25 HSCM	75.00^{ab}	55.67^{ab}	21.00^{b}	12.33^{bc}
50 HSCM	77.67^{a}	60.33^{ab}	34.00^{a}	17.33^{b}
75 HSCM	75.00^{ab}	72.00^{a}	40.00^{a}	27.33^{a}
100 HSCM	68.00^{b}	43.33^{b}	34.00^{a}	11.00^{bc}
SEM	1.39	5.39	3.18	2.06

Means with different superscripts a, b, c are significantly different from each other.

At 24h of storage, tom semen stored in 75% HSCM had the highest percentage of live sperm (40.00%) but was not significantly different from 50 and 100% HSCM. At 48h of storage, tom semen preserved in 75% HSCM has highest percentage of live sperm (68.33%) and was significantly different from 25, 50 and 100% HSCM.

3.2 Discussion

Dilution of tom semen with extender expected to improve their sperm activities during storage, although may reduce viability of sperm cells as storage period increases and ultimately resulted in low fertilizing ability of sperm cells.

Our observation in this present trial agrees with the results of [9], who opined that the length of preservation of semen under 5^{o}C storage condition has a great impact on sperm motility of guinea fowls. Similarly, Rahman, et al., [5] reported that Sperm motility and viability of sperm cells usually gradually declines after collection. However, compare to un-diluted tom semen, use of home-made skim cow milk-based extender (HSCM) irrespective of percentage, provides an efficient successful liquid storage condition for tom sperm preserved at 4-8^{o}C for the period of 48h yet it has some negative effects on the sperm cells activities as storage period progresses. In present study, the HSCM based diluents with 75% HSCM and 25% sodium citrate preserved tom sperm better for longer period than 25%, 50% and 100% HSCM which clearly evident in percentage of motile, viable sperm cells and membrane intactness of extended semen.

This was evident at 24 and 48h of storage respectively. However, viability value was not significantly difference to 50% HSCM but was higher in value compared to it. These results agrees with the findings of Rahman et al., [5] that milk as component for extending semen significantly has positive effects on percentage motile sperms and livability when compared to the solely Tris based extender tested at different periods on ram semen. Also, Jones and Foote, [9] reported that Skim milk contained all essential nutrients needed for maintaining live-ability of Buffalo spermatozoa at (4-7°C) liquid storage. Furthermore, was found superior to Tris and EYC considering linearity, lateral head displacement (ALH), average path velocity (VAP), and motility [10]. All these previous findings justify HSCM efficiency observed in our present study on tom semen preservation, though best efficiency observed in 75% HSCM.

4. Conclusions

The extender containing 75% HSCM supplemented with 25% sodium citrate buffer proved to be best among the HSCM formulated extenders considering its highest percentage of motile sperm, and optimum percentage

viability and membrane integrity compared to other extender and undiluted semen. It therefore recommended that for liquid storage of turkey semen at 4-8ºC, 75% HSCM with SCB is effective for successful tom semen dilution and short term storage.

Declarations

- Ethics approval and consent participate: Not applicable
- Availability of data and materials: All data generated or analyzed during this study are included in this published article [and its Supplementary information files]
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- Authors' contributions: ASB design, carried out the research and wrote first and final draft of the manuscript. AAA and MNB took part in the laboratory procedure AJA, ITR and BBH did the data collection and Management of the birds. All authors have read and approved the manuscript.

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