ISSN 1819-1878

Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2023.1.5

Research Article
Standardization of Fresh Cow Milk with Sodium Citrate Buffer for Cold Storage of Turkey Semen

Adedeji Suleimon Balogun, Akintunde Akinbola Akinosun, Idris Idowu Bello, Bankole Ademola Oluwemimo and Barakat Bishola Hammed

Department of Animal Production Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo, Nigeria

Abstract

Background and Objective: The low fertility of diluted and preserve tom semen for short periods at cold temperatures have prompted the standardization of some potential extender agents used for the dilution and preservation of other farm animal species semen for liquid preservation of turkey semen. This study was designed to standardize fresh cow milk (FCM) for liquid preservation of tom semen. **Materials and Methods:** One litter of fresh cow milk was collected from the Fulani cattle kraal and heated for 5 min. The FCM extenders were prepared using different concentrations of FCM viz: 25, 50, 75 and 100%. Five toms were ejaculated individually and pooled. The pooled semen was divided into five portions making five treatments and extenders were added to it in a ratio of 1:3 (semen extender). The experimental design used was a Completely Randomized Design. Microscopic semen parameters such as motility and viability membrane integrity were examined and recorded for freshly extended semen preserved semen for 4, 24 and 48 hrs at 4-8°C. **Results:** The result showed that no significant difference ($p>0.05$) was observed in 0 hr. However, from 4 to 48 hrs of preservation, tom semen preserved with 25, 50 and 75% FCM were statistically similar ($p>0.05$) but, significantly different ($p<0.05$) in motility and membrane integrity from un-diluted semen and 100% FCM. However, 75% FCM has the highest motility values of 75.00, 60.00 and 36.67% and membrane integrity values of 60.67, 56.33 and 42.00% at 4, 24 and 48 hrs, respectively. At 0 and 24 hrs, tom semen preserved with 50% FCM extender has the highest non-significant percentage live sperm value of 89.67 and 80.00%, respectively compared to tom semen preserved in 25 and 75% FCM extender. While at 4 and 48 hrs, no significant difference was observed among the treatments. **Conclusion:** It was therefore concluded that 75% FCM supplemented with 25% sodium citrate buffer preserved tom semen for 48 hrs better than other combinations.

Key words: Fresh cow milk, sodium citrate, turkey, semen, cold storage, extender

Citation: Balogun, A.S., A.A. Akinosun, I.I. Bello, B.A. Oluwemimo and B.B. Hammed, 2023. Standardization of fresh cow milk with sodium citrate buffer for cold storage of Turkey semen. Asian J. Anim. Sci., 17: 1-5.

Corresponding Author: Adedeji Suleimon Balogun, Department of Animal Production Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reproductive performance in the male is mostly judged by the libido and ejaculation of semen containing viable spermatozoa (quality) in adequate numbers (quantity), Male and female are notably deemed to contribute equally genetically to their offspring, however, fertility of the breeding stock is more dependent on the male reproductive performance[1,2].

Semen from high performing males, with a relatively quick reaction time[3]and better semen characteristics greatly determines the reproductive potentials of breeding sire[4], which is capable of inseminating a greater number of females and further dilution with an extended to insemination more females compared to undiluted ejaculates. However, ejaculates from toms are usually very small although highly concentrated while associated with a drastic decline in quality within a few hours after collection. Thus, the need to develop an appropriate extender for turkey semen dilution and preservation in Nigeria to enhance turkey breeding.

Good semen quality has been reported to be fertility and subsequent hatchability indicators or markers[5]. In-addition semen from high-performing toms may be further diluted and preserved for later use. Among reliable components/ingredients of a reliable extender is milk which contains casein that is capable of decreasing damages to cell membrane lipids and improves sperms motility and live-ability[6]. It is hydrophilic and cannot diffuse the cell wall of the sperm cells, which protects the cell wall and prevents freeze shock.

The major proteins in milk extenders such as casein micelles, α-lactalbumin and β-lactoglobulin, have been identified to also interact with BSP proteins present in the seminal plasma. Furthermore, the sperm membrane does not suffer much lipid loss as a result of casein micelles present in milk and thereby effectively maintained sperm function during stoarage[3,6]. With milk having all these qualities, it is therefore deemed that suitability of fresh cow milk (FCM) may be harness as a reliable extender for tom semen preservation. This study was therefore design to standardize of fresh cow milk with sodium citrate buffer for tom semen dilution and preservation.

MATERIALS AND METHODS

Experimental site: The experiment was carried out at the Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igboora, Oyo State. The study was conducted June, 2022 and lasted for 8 weeks.

Toms management: A total number of five matured toms at their reproductive age of 30-40 weeks were used for the experiment. They were kept together in a pen. Feed and water were supplied based on tom breeder requirements.

Training of tom for semen collection: The toms were trained for semen collection for a period of 2 weeks by using Balogun *et al.*[7] modified procedures for poultry semen collection. Semen is usually collected once a week for a period of 4 weeks for adequate sperm reserve durations.

Preparation of buffers

Sodium citrate buffer: Sodium citrate buffer was prepared by dissolving 2.9 g of sodium citrate in 100 mL of distilled water. The pH was finally adjusted to 7.2.

Preparation of fresh cow milk extender with sodium citrate buffers: Fresh cow milk was collected from the Fulani cattle kraal. The udder teat was disinfected before milking the lactating cow and about 1 L of fresh cow milk was collected. The 100 mL of fresh cow milk was poured into a saucepan and bring it to a boil. The milk was boiled for 6 min over medium heat. It was stirred slightly constantly so that the bottom of the milk does not burn. The saucepan was taken off the heat and the milk was cooled for 2 min. The cream, or the fat, rises to the top as the milk cools down. The cream was gently scraped off the top with a large spoon. Sodium citrate buffer of 7.2 pH was mixed vigorously with different concentrations of fresh cow milk (25, 50, 75 and 100%). It was stored in the refrigerator for further use.

Experimental design: Ejaculate semen was collected and pooled from five toms. The pooled semen was divided into five portions making five treatments and extenders were added to it in a ratio of 1:3 (semen extender). The experimental design used was a completely randomized design. The experiment consists of five treatments and the trial was conducted thrice. Microscopic semen parameters like motility, viability membrane and acrosome integrity were examined and recorded for freshly extended semen and semen stored for 48 hrs at 4-8°C. The semen evaluation was done at 4, 24 and 48 hrs. The treatments comprise of:

- **Treatment 1:** Neat semen
- **Treatment 2:** 25% fresh cow milk+75% sodium citrate buffer
- **Treatment 3:** 50% fresh cow milk+50% sodium citrate buffer
- **Treatment 4:** 75% fresh cow milk+25% sodium citrate buffer
- **Treatment 5:** 100% fresh cow milk

Ethical consideration: This study was exempted from approval from the Institution Animal Ethics because the semen collection using abdominal massage and mid back stroke procedure does not affect the normal physiology of the animal.

Analysis of semen

Progressive motility: The 5 µL of both un-extended and extended semen samples were placed on a pre-warmed slide, covered with a cover-slip and observed under an Olympus light microscope at 400X for their progressive movement.

Sperm livability: It was assessed by preparing Eosin-nigrosin stain as described by Balogun *et al.*[5], 10 µL of semen was place on a stage warmer and dropper was used to apply two drops of eosin-nigrosin stain on it and left for 2 min. A thin smear was prepared on a clean, pre-warmed glass slide. The stained glass was left to air dried for few minutes and the stained slide was examined under oil immersion (1000X) using a bright-field. A minimum of 200 sperm were counted and recorded and the percentage live sperms were determined. Stained, partially stained and unstained sperms were considered as dead and live respectively. The percent viability was calculated by the formula:

$$\text{Sperm livability (\%)} = \frac{\text{Number of live sperm}}{\text{Total sperm}} \times 100$$

Membrane integrity: The Hypo-Osmotic Swelling test (HOST) procedure as described by Jeyendran *et al.*[8], was used to assess the rate of intactness of the membrane integrity of the sperm cells. The solution was prepared, 200 µL of the solution was dispense in the sample tubes and left for 1 min incubation and 10 µL of semen was mixed with 200 µL of hypo-osmotic solution and incubated at 37°C for 30 min. A drop of the sample was examined under a bright-field microscope of 400X magnifications for curled and uncurled tail spermatozoa. About 200 sperm were counted, curl and uncurled spermatozoa were recorded for each sample. The percentage number of curled tail spermatozoa was determined and recorded.

Statistical analysis: Data collected were subjected to One-way Analysis of Variance (ANOVA) using at a 5% level of significance using IBM SPSS statistics 20. Software and means were separated with Duncan's Multiple Range test.

RESULTS

The percentage of motile sperm of tom preserved with different concentrations of fresh cow milk (FCM) sodium-citrate was presented in Table 1. The result showed that no significant difference ($p>0.05$) was observed in 0 hr. However, from 4 to 48 hrs of preservation, tom semen preserved with 25, 50 and 75% FCM were statistically similar ($p>0.05$) but significantly different from ($p<0.05$) from undiluted semen and 100% FCM. However, 75% FCM has the highest motility values of 75.00, 60.00 and 36.67% at 4, 24 and 48 hrs, respectively. In addition, at 4 hrs, tom semen preserved with 100% FCM has the lowest significant value of 19.00%, while at 24 and 48 hrs, neat semen has the lowest significant values of 11.67 and 5.00%.

The percentage of live sperm of tom preserved with different concentrations of fresh cow milk (FCM) sodium-citrate was presented in Table 2. At 0 and 24 hrs, tom semen preserved with 50% FCM extender has the highest non-significant percentage live sperm value of 89.67 and 80.00%, respectively compared to tom semen preserved in 25 and 75% FCM extender. While at 4 and 48 hrs, no significant difference was observed among the treatments. However, tom preserved in 75% FCM has the highest sperm percentage values of 78.67.00 and 70.33% at 24 and 48 hrs.

The percentage of sperm membrane integrity of tom preserved with different concentrations of fresh cow milk (FCM) sodium-citrate was presented in Table 3. The result showed that no significant difference($p>0.05$) was observed in 0 hr. However, at 4 and 24 hrs of preservation, no significant

Table 1: Effects of different concentration of fresh cow milk (FCM) sodium citrate buffer on motility of diluted tom semen

	Preservation periods			
Treatment (%)	0 hr	4 hrs	24 hrs	48 hrs
Neat semen	80.00	36.67^{b}	11.67^{b}	5.00^{c}
25 FCM	81.67	60.00^{a}	51.67^{a}	15.00^{abc}
50 FCM	85.00	68.33^{a}	58.33^{a}	28.33^{ab}
75 FCM	83.33	75.00^{a}	60.00^{a}	36.67^{a}
100 FCM	70.00	19.00^{c}	18.33^{b}	13.33^{bc}
SEM	2.40	6.19	5.77	3.95

^{a,b,c}Means with different superscript letters within the column differ significantly $p<0.05$

Table 2: Effects of different concentration of fresh cow milk with sodium citrate buffer on viability of diluted tom semen

	Preservation periods			
Treatment (%)	0 hr	4 hrs	24 hrs	48 hrs
Neat semen	82.67^{bc}	82.00	70.00^{bc}	61.00
25 FCM	87.67^{ab}	78.00	75.00^{ab}	65.33
50 FCM	89.67^{a}	77.33	80.00^{a}	68.33
75 FCM	88.33^{ab}	85.00	78.67^{a}	70.33
100 FCM	79.33^{c}	76.33	69.33^{c}	62.00
SEM	1.24	1.63	1.32	1.80

^{a,b,c}Means with different superscript letters within the column differ significantly p<0.05

Table 3: Effects of different concentration of fresh cow milk with sodium citrate buffer on membrane integrity of diluted tom semen

	Preservation periods			
Treatment (%)	0 hr	4 hrs	24 hrs	48 hrs
Neat Semen	69.33	31.33^{b}	15.00^{b}	18.00^{cd}
25 FCM	62.67	57.33^{a}	47.67^{a}	28.33^{bc}
50 FCM	65.67	58.67^{a}	52.67^{a}	33.00^{ab}
75 FCM	66.33	60.67^{a}	56.33^{a}	42.00^{a}
100 FCM	61.67	28.00^{b}	21.67^{b}	14.00^{d}
SEM	1.61	4.13	5.13	0.58

^{a,b,c,d}Means with different superscript letters within the column differ significantly p<0.05

difference (p>0.05) was observed among tom semen preserved with 25, 50 and 75% FCM, although 75% FCM has the highest values of 60.67 and 56.33% among them. At 48 hrs, tom semen preserved with 75% has the highest significant membrane integrity value of 42.00%. In addition, at 4 hrs, tom semen preserved with 100% FCM has the lowest significant value of 19.00%, while at 24 and 48 hrs, neat semen has the lowest significant values of 11.67 and 5.00%.

DISCUSSION

Fresh cow milk has been identified as one of the important components of semen extenders. However, its precise composition is yet to be established for successful turkey semen preservation. Convincingly this study revealed that 75% FCM with 25% sodium citrate buffer outperformed the other combinations. This was evident in semen quality parameters like motility, viability and membrane integrity. The highest sperm motility recorded for tom semen preserved with 75% FCM is an indication that sufficient energy in terms of lactose is adequately present in 75% FCM and 25% sodium citrate. This result corroborated the findings of Manjunath[9], who identified phosphocaseinate and β-lactoglobulin as constituents of milk-based extender responsible for better sperm motility.

The highest membrane integrity observed for tom semen preserved with 75% FCM throughout the storage period reflected the presence of sufficient antioxidants in extenders with 75% FCM which aided motility. Florez-rodriguez *et al.*[10] reported a similar result when skim milk was used to preserve equine semen at 5°C played a crucial role in sperm preservation. Similarly, Kankofer *et al.*[11] reported that dilution of semen with milk based-diluent resulted in a significant increase in antioxidant activities of the extended semen. Similarly, Alkan *et al.*[2] and Bergeron *et al.*[6] reported that milk casein decreases damage to cell membrane lipids and improves sperm motility and viability and maintained sperm function during storage[6]. In addition, fresh bovine milk has been reported to present, antioxidant activity for the protection of its high lipid content[12]. Also, the report of Al-Saeedi *et al.*[13] corroborated the results in this present study that, at 4 or 8 hrs after collection, the full cream milk extender outperformed the other extenders compared with.

This study revealed the potential and suitability of fresh cow milk for dilution and preservation of tom semen for 48 hrs. With proper dilution and storage, genetics may be accelerated in turkey species. It is therefore recommended that, 75% FCM should be further supplemented with natural antioxidants to achieve better sperm activities during storage.

CONCLUSION

It is therefore concluded that 75% FCM with 25% sodium citrate buffer is capable of preserving turkey semen for 48 hrs better than other combinations. For effective cold storage of tom semen, 75% FCM with 25% sodium citrate buffer is recommended for formulation of an extender for tom semen dilution and preservation.

SIGNIFICANCE STATEMENT

Cow milk has been identified as one of the semen extender agents for most farm animal species except poultry. However, there is a dart of information regarding it use as an extender for poultry species. Notably, the majority of the constituent of seminal fluid is present in fresh cow milk. Since poultry species are void of seminal fluid. Dilution storage of tom ejaculate with different percentages of fresh cow milk augmented with sodium citrate buffer may be a reasonable approach to successful tom semen dilution and preservation. The 75% fresh cow milk concentration with sodium citrate buffer seems to be sufficient for tom semen dilution and preservation at 5°C for 24 hrs. Further improvement with natural antioxidants is suggested for better results during storage.

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