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Research Article

Standardization of Home-Made Skimmed Cow Milk with Tris Buffer for Turkey Semen Dilution and Preservation

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Abstract

An experiment was design to standardize homemade skimmed cow milk (HSCM) concentration suitable for liquid preservation of tom semen. A cow was milked to harvest about one liter of milk from her udder. HSCM was prepared from FCM and different concentrations were used to formulate the extenders viz: 25%, 50%; 75%, and 100. Semen was ejaculated and pooled from five different toms. The pooled semen was divided into five parts, and four portions were diluted with different concentrations of HSCM extenders in the ratio of 1:3 (semen: extender), making five treatments. The samples were stored for 48h inside the refrigerator. Microscopic semen evaluation was done for motility, viability, and membrane integrity for both freshly diluted semen and samples preserved for 4hrs, 24h, and 48h at 4-8oC. The result revealed that the same non-significant values of 80.00% were recorded for motility in all treatments at 0h. At 4h and 24h periods of preservation, the highest value of motile sperm (55.00%, 41.67%) was recorded for 50% HSCM diluted tom semen, although was not significantly different ($p > 0.05$) from 25% and 75% HSCM. The highest percentage of live sperm of 92.67% was recorded for 25% HSCM but was not significantly different ($p > 0.05$) from 75% HSCM. No significant difference ($p < 0.05$) was observed for viability among the treatments at 4 and 24h. While at 48h, 75% HSCM has the highest significant value of 68.00% viability. Percentage membrane integrity of tom semen preserved with 25%, 50% and 75% HSCM showed no significant difference ($p > 0.05$) among the treatments from 0 to 24h of preservation. It is therefore concluded that 50% HSCM with 50% tris buffer is effective for the successful preservation of turkey semen.

Keywords: Home-Made; Skimmed Milk; Tris; Turkey; Semen; Preservation; Extender

Introduction

Fresh Cow's milk has been recently considered a highly nutritious and valuable option for semen extender preparation [1,2]. Though, highly susceptible to many contaminating organisms that are capable of reducing its shelf-life and its efficiency as an extender most especially when not collected in an hygienic conditions. However, subjecting fresh cow milk to any subsequent treatments preferably heating before use for semen extender preparation is therefore recommended to achieve better result [3]. Preservation of semen has been long successfully been achieved with fresh milk and skimmed milk diluted directly and stored at 4°C or frozen in the presence of glycerol [4-7].

Since skimmed milk has very little or no lipids and is as efficient as whole milk in protecting sperm during semen storage at 4°C or during cryopreservation [3,8], while the content of Lactose in cow milk has been estimated to be 4.8% (w/v) [9], besides lactose other protective constituent of milk, such as micelles of caseins, the major proteins of having the potential of improving the efficiency of HSCM extenders, but may not be sufficient to protect sperm during liquid storage or freezing.

In addition, It has been reported that ram sperm also benefits from the protective capability of casein micelles isolated from milk

during storage at 4-5°C [10]. Furthermore, buffer is also considered as an appropriate conjugate for any extender preparation. Tris buffer has been identified as one of the best buffer for poultry semen extender preparation over the years [11-14]. Accordingly, it is necessary to standardize and develop semen extender that will be used to preserve turkey semen effectively for AI practice and semen preservation in turkey. It is therefore, deemed that augmenting the efficiency of home-made skim cow milk (HSCM) with tris buffer for preservation of turkey semen may be a reliable approach for successful turkey semen preservation thus, this experiment therefore aim at determining the appropriate proportion or percentage of HSCM for the formulation of turkey semen extender.

Materials and Method

Experimental site

The experiment was done at the turkey unit, Department of Animal Health and Production Technology, Oyo State College of Agriculture and Technology Igboora Oyo State.

Toms management

Five (5) sexually reproductive matured toms of 30-40weeks of age were used for the experiment. They were housed in a pen. Feed and water were supplied based on turkey breeder recommendation.

Training of tom for semen collection

The toms were trained for semen collection for period of two weeks by using Balogun., *et al*. [15]. Semen collection modified procedures combining mid back stroke and abdominal massage semen collection procedure together for optimum ejaculation from the toms. The toms were ejaculated once in a week for the period of four weeks to encourage adequate sperm reserve.

Preparation of buffers

3.780g of tris hydroxymethly aminoethane and 2.110g of citric acid were dissolved in 100 ml of distilled water. The mixture was stirred and the pH was adjusted to 7.2.

Preparation of home-made skimmed cow milk extender with tris buffers

Skimmed milk preparation

One of the lactating cows was milked to collect fresh cow milk at the Fulani cattle kraal. Disinfection of udder teat was done before milking the cow. The 100mL of fresh cow milk was poured into a saucepan and heated for 6minutes under medium heat with consistence stirring till is boiled. After boiling, the fat rises to the top as the milk cools down within few minutes. Gradual scraping of the cream was carried out. The saucepan was covered with a lid and the milk was refrigerated for 8 hours. **As it cools, scooping of the fat** was gently done with a spoon making sure the fat is totally separated from the milk. The skimmed milk was poured into 100ml sample bottle and was diluted with tris buffer immediately at different concentration of skimmed milk (25%, 50%, 75% and 100%) and was preserved till used.

Experimental design

Five toms were ejaculated to harvest their semen and pooled together. The semen sample was divided into five parts making five treatments and extenders were added to four parts at the ratio 1:3 (semen: extender). A total of three trials were conducted in a complete randomized design. Evaluation of microscopic semen parameters such as motility, viability and membrane integrity were done for freshly extended semen and semen stored for 48hrs at 4-8°C. The semen evaluation was done at 0h, 4h, 24h and 48h. The treatments comprises of

- Treatment 1: neat semen
- Treatment 2: 25% home-made skimmed cow milk +75% tris buffer
- Treatment 3: 50% home-made skimmed cow milk + 50% tris buffer
- Treatment 4: 75% home-made skimmed cow milk +25% tris buffer
- Treatment 5: 100% home-made skimmed cow milk

Analysis of semen

Progressive motility

A clean slide was pre-warmed and 5ul of samples were loaded on the slide, covered with a cover-slip, and observed under a light microscope at 400X for the progressive motile sperms.

Sperm livability

Eosin-nigrosine stain was prepared to differentiate the live sperms from the dead ones. 10ul of semen was place on a stage warmer, and two drops of eosin-nigrosine stain was applied on it, and thin smear was prepared on the clean slide. The slide was air-dried. Stained slide was observed under oil immersion (1000 X) using a bright-field microscope. About 200 Sperm were observed and recorded to determine the percentage live sperms. Stained, partially stained and were counted as dead while unstained sperms were counted as live sperm. The percent livability was calculated by the formula

$$\text{Sperm livability (\%)} = \frac{\text{No. of live sperm}}{\text{Total sperm}} \times 100$$

Membrane integrity

Hypo-osmotic swelling test (HOST) procedure was used to evaluate the intactness of the sperm cells membrane. 10µl of semen was placed in the 200µl HOST solution, mixed and incubated at 37° C for 30mins. A drop of sample was placed on the slide under a bright-field microscope of 400X. About 200 sperm were observed, percentage curl and uncurled spermatozoa were recorded for each samples.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data collected. Significantly different means were separated using Duncan's Multiple Range Test (DMRT) of the SPSS 22 version software.

Results

Motility of tom sperm diluted and preserved with HSCM

Percentage motile sperm of tom semen preserved with home-made skimmed cow milk is presented in table 1. At 0h period of preservation, the same non- significant values of 80.00% was recorded for all treatments including neat semen. At 4h period of preservation, highest value of motile sperm (55.00%) was recorded for tom semen diluted with 50% HSCM although was not significantly different ($p > 0.05$) from tom semen preserved with 25% and 75% HSCM. Similarly, at 24h of preservation highest value of motile sperm (41.67%) was recorded for tom semen preserved with 50% HSCM but was not significantly different from tom semen preserved with 75% HSCM. Finally, tom semen preserved with 75% HSCM has the highest non-significant value for motility compared to other treatments.

	Preservation Periods			
Treatments (%)	**0h**	**4h**	**24h**	**48h**
Neat Semen	80.00	30.00^{b}	5.00^{c}	0.00^{c}
25 HSCM	80.00	40.00^{ab}	11.67^{bc}	1.67^{c}
50 HSCM	80.00	55.00^{a}	41.67^{a}	11.67^{b}
75 HSCM	80.00	51.67^{ab}	36.67^{ab}	26.67^{a}
100 HSCM	80.00	35.00^{ab}	10.00^{c}	11.67^{b}
SEM	1.09	3.65	4.03	3.68

Table 1: Effects of different concentration of home-made skimmed cow milk tris buffer on motility of diluted tom semen.

Live sperm of tom sperm diluted and preserved with HSCM

Percentage live sperm of tom semen preserved with home-made skimmed cow milk is presented in table 2. Highest percentage live sperm of 92.67% was recorded for tom semen preserved with 25% HSCM, but was not significantly different ($p > 0.05$) from tom semen preserved with 50 and 75% HSCM. At 4 and 24h of storage all the treatments were statistically similar ($p < 0.05$). While at 48h, tom semen preserved with 75% HSCM has highest significant value of 68.00% compared to other treatments.

	Preservation Periods			
Treatments (%)	**0h**	**4h**	**24h**	**48h**
Neat Semen	87.67^{b}	82.67	65.00	50.00^{c}
25 HSCM	92.67^{a}	82.67	65.67	60.00^{b}
50 HSCM	89.67^{ab}	81.33	70.00	62.33^{b}
75 HSCM	89.00^{ab}	83.67	66.67	68.00^{a}
100 HSCM	87.00^{b}	86.67	68.00	60.00^{b}
SEM	0.75	0.79	1.53	1.72

Table 2: Effects of different concentration of home-made skimmed cow milk tris buffer on viability of diluted tom semen.

Membrane integrity of tom sperm diluted and preserved with HSCM

Home-made skimmed cow milk preserved tom sperm membrane integrity is presented in table 3. Percentage membrane integrity of tom semen preserved with 25%, 50% and 75% HSCM showed no significant different ($p > 0.05$) among the treatments from 0 to 24h of preservation. Although, at 0h, and 24h tom semen preserved with 50% has the highest percentage of sperm membrane integrity (78.67% and 36.33%) while at 4h, tom semen preserved with 75% HSCM has the highest value of membrane integrity (55.67%). Finally at 48h, tom semen preserved with 75% HSCM has the highest non- significant ($p > 0.05$) membrane integrity value of 28.67%.

	Preservation Periods			
Treatments (%)	0h	4h	24h	48h
Neat Semen	67.67^{b}	20.00^{c}	11.33^{b}	8.67^{c}
25 HSCM	73.00^{ab}	46.67^{ab}	21.00^{ab}	6.67^{bc}
50 HSCM	78.67^{a}	52.33a	36.33^{a}	12.33^{b}
75 HSCM	78.00^{a}	55.67^{a}	33.00^{a}	28.67^{a}
100 HSCM	71.67^{ab}	33.33^{bc}	15.00^{b}	9.33^{bc}
SEM	1.41	3.96	3.38	2.63

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

Discussion

Our approach to identify the most appropriate percentage of home-made skim milk effective for formulation of turkey semen extender deemed to be successful, as best percentage of home-made skimmed milk effective for turkey semen preservation was identified in this study. It was evident in this study that tom semen diluted with 50% and 75% HSCM showed higher sperm activities such as motility, viability and membrane integrity compared to undiluted semen, 25% and 100% HSCM extended tom semen.

Although at 48h, 75% HSCM extender seems to performed better compared to 50% HSCM extender. This performance may be attributed to higher casein content present in 75% HSCM as compared to 50% HSCM. Casein has been identified in milk as a protective agent of sperm cells with great potentials to reduce damage to cell membrane lipids thereby improving sperms motility and viability [16,17].

However, the quality of stored semen is generally affected by the preservation procedures irrespective of the media composition or percentage of HSCM in which they are preserved, which may be traceable to probably increased generation of Reactive oxygen species (ROS) during storage of both diluted and undiluted semen, which has been reported by various researchers that after ejaculation with or without extenders, spermatozoa are usually affected with the generation of reactive oxygen species during handling and storage protocols [18-20]. Nevertheless, it was evident in this study that rate of lipid peroxidation was step down in semen samples containing 75% HSCM dilution media compared to other media. As it is revealed by better sperm activities such as motility, viability and membrane integrity exhibited by this samples during storage.

Conclusions

From this present study, it can be concluded that 75% HSCM with 25% tris buffer, is an effective media for preservation tom semen compared to other combinations. For effective liquid preservation of tom at 4-8°C, 75% HSCM with 25% tris buffer is recommended for formulation of tom semen extender. However, fertility trial should be done to ascertain is fertilizing capability.

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