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Research Article
Standardization of Egg-Yolk with Three Different Buffers for Optimizing Turkey Semen Cold Storage at 4°C

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Abstract

Background and Objective: Identifying a suitable salt for preparing a buffer to formulate an egg-yolk extender for turkey semen dilution and preservation may be a good approach to improving fertility in turkey species. An experiment was conducted to evaluate the buffering potentials of three common salts (tris, sodium citrate and sodium phosphate) for dilution and preservation of tom semen. **Materials and Methods:** Three buffers were prepared and added to the egg yolk in the same proportion. Semen was ejaculated from five toms, pooled and divided into four and diluted at the ratio of 1:4 (semen to extender). Samples were evaluated for motility, viability and membrane integrity immediately after dilution and semen was stored for the period of 4, 24 and 48 hrs at 4-8°C. **Results:** The pH result revealed that the tris egg-yolk extender (TEY) has the highest pH of 7.3. It was observed that the percentage of sperm motility showed no significant difference ($p<0.05$) across the treatments at 0 hr. From 4 to 48 hrs of preservation, (TEY) and sodium citrate egg-yolk (SCEY) were statistically similar and had a significant ($p>0.05$) higher percentage of motile sperm compared to un-extended semen and sodium phosphate egg-yolk (SPEY). Viability values recorded for tom semen diluted with egg-yolk extender containing different buffers showed no significant ($p<0.05$) difference from 0 to 48 hrs. Membrane integrity also showed no significant difference ($p<0.05$) at 0 hr. However, from 4 to 48 hrs of preservation, TEY and SCEY extender were statistically similar but significantly higher ($p>0.05$) to undiluted semen and SPEY. **Conclusion:** Tris and sodium citrate buffer exhibited good buffering capability for the formulation of egg-yolk extender for turkey semen dilution and preservation.

Key words: Buffer, tom, semen, liquid storage, egg-yolk, extender, turkey species

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

INTRODUCTION

The idea of semen dilution coupled with the improvements in the technique of avian semen storage in cold conditions has led to increased multiplication of economic poultry species in developed parts of the world. The situation is rather different in developing countries where these techniques are not adopted and producers have to rely on natural mating and egg-hatching techniques to produce poults[1]. 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 the experimental phase[2]. 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 extender is required. Moreover, it is difficult to inseminate and require an appropriate dosage of sperm cells is required by the hen for optimum fertility to take place. Furthermore, it is deemed that extended poultry semen is more efficient than fresh unextended poultry semen given the sheer number of hens that will be inseminated.

Inseminating appropriate dosage will maximize the tom use. And this can only be achieved with the application of a semen extender in turkey breeding practices. In addition, to enhance the survival of sperm after collection, semen extenders are essential. The appropriate extender for the preservation would be to increase volume, minimize damage and maintain the viability of spermatozoa till fertilization.

Essential components of a reliable extender include substances that are capable of stabilizing the pH and controlling microorganism growth, a readily available source of energy and ingredients that stabilize membranes and maintain the metabolic function of sperm, as well as neutralizing metabolic substances[3]. Salt used as a buffer is one of the important components in semen extenders. Moreover, a buffer has been identified over the years as one of the important components of egg-yolk extenders. However, extenders should have a buffering effect on the pH of semen diluted to balance the production of metabolic substances from sperm or bacteria[4].

Furthermore, glutamic acid is said to be the most important chemical component of poultry seminal plasma and is known as a standard component of diluters[5]. Moreover, egg yolk is generally accepted to be an effective agent in semen extenders for the protection of spermatozoa against cold shock and the lipid phase transition effect[6]. However, optimizing the potentials of egg yolk through the identification of potential and appropriate buffer salts that are capable of dilution and preserving poultry sperm cells for a longer duration both *in vivo* and *in vitro* may be a significant approach to successful poultry semen preservation. This experiment, therefore, aims to determine the comparative effectiveness of three different buffers supplemented with egg-yolk orange juice extender on tom semen.

MATERIALS AND METHODS

Study area and duration: The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, of the Oyo State College of Agriculture and Technology, Igboora, Nigeria. The experiment was carried out from April, 2022 to June, 2022.

Toms management: A total number of five matured toms at their reproductive age of 30-40 weeks were used for the experiment. They were purchased from a reputable breeding farm in Ibadan, Oyo State, Nigeria. They were kept together in a pen. Feed and water were supplied based on turkey breeder requirements.

Training of toms for semen collection: The toms were trained for semen collection for 2 weeks by using Balogun *et al.*[7] modified procedures for poultry semen collection. Semen is usually collected once a week for 4 weeks for adequate sperm reserve durations. The experiment lasted for 8 weeks.

Preparation of buffers: Three different buffers were prepared with different salt components for egg-yolk extender preparation viz, tris buffer, sodium citrate and sodium phosphate.

- **Tris buffer:** It was prepared by dissolving 3.780 g and 2.110 g of tris hydroxymethyl aminoethane and citric acid respectively into100 mL of distilled water, pH was finally adjusted to 7.2
- **Sodium Citrate buffer:** It was prepared by dissolving 2.9 g of sodium citrate in 100 mL of distilled water, pH was finally adjusted to 7.2
- **Sodium Phosphate buffer:** It was prepared by dissolving 0.34 g of sodium phosphate in100 mL of distilled water, pH was finally adjusted to 7.2

Preparation of egg-yolk extenders with different buffers: Tris egg-yolk orange juice extender was prepared by breaking eggs to collect their yolks void of albumen. The yolk is separated from the eggs by draining the albumen and collecting the yolk on filter paper to drain the remnant albumen. The membrane covering the yolk was pierced to as collect the yolk 25 mL of egg-yolk was collected and stirred vigorously in a beaker. Tris sodium citrate and sodium phosphate buffers of 7.2 pH were mixed vigorously separately with egg-yolk, at the 1:3 ratios. It was stored in the refrigerator for further use.

Experimental design: Each tom was ejaculated and semen from five toms was pooled. The pooled semen was divided into four portions making four treatments and extenders were added to three parts at the ratios of 1:3 (semen: extender). The experimental design used was a completely randomized design. The experiment consists of four treatments and the trial was conducted thrice. Microscopic semen parameters like motility, viability and membrane 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.

Analysis of semen

Progressive motility: As 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 model CX21FS1. Manufactured by Olympus Corporation, Tokyo, Japan at 400X for their progressive movement.

Sperm livability: The mixture of eosin-nigrosin stain was prepared to examine the sperm viability. A slide was placed on the stage warmer, 10 µL of semen was placed on the slide and two drops of eosin-nigrosin stained were applied on it with the aid of a dropper and left for 2 min. A thin smear was prepared from the mixture using a clean, pre-warmed glass slide and the stained slide was examined under oil immersion (1000X) using a bright-field microscope after the slide is air-dried using Olympus light microscope model CX21FS1. Manufactured by Olympus Corporation, Tokyo, Japan. About 200 sperm were counted and recorded and the percentage was determined. Stained, partially stained and unstained sperms were considered dead and alive, respectively. The percent viability was calculated by the formula[8]:

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

Membrane integrity: Sperm membrane integrity quality was evaluated by the hypo-osmotic swelling test (HOST) procedure as described by Jeyendran *et al.*[9]. The solution was prepared and 10 µL of semen was mixed with 200 µL of hypo-osmotic solutions 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 and curled 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) at a 5% level of significance using IBM SPSS Statistics 20. Software and means were separated with Duncan's Multiple Range Test.

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

RESULTS

pH of extenders: The pH of the different extenders revealed that among the three extenders prepared with the three different buffers, tris egg yolk extender has the highest pH of 7.3 followed by sodium phosphate egg-yolk and sodium citrate egg yolk having a pH of 7.2 and 7.1, respectively.

Effect of different buffers supplemented with egg-yolk on preserved tom sperm motility: Sperm motility of tom semen preserved with egg-yolk extender supplemented with different buffers was presented in Table 1. The percentage of sperm motility showed no significant difference ($p<0.05$) observed at 0 hr across the treatments. However, from 4 to 48 hrs of preservation, tris egg-yolk and sodium citrate egg-yolk extender were statistically similar ($p<0.05$) and significantly different ($p>0.05$) from undiluted semen and sodium phosphate egg-yolk extender.

Effect of different buffers supplemented with egg-yolk on preserved tom sperm viability: The sperm viability of tom semen preserved with an egg-yolk extender supplemented with different buffers was presented in Table 2. Records on percentage sperm viability showed no significant ($p<0.05$) different across the storage period on the preserved tom sperm. However, sodium phosphate coconut water and

Table 1: Effects of different buffers on sperm motility of tom semen diluted with egg-yolk extender

	Preservation periods			
Treatments	0 hr	4 hrs	24 hrs	48 hrs
Neat-semen	85.00	46.67[b]	16.67[b]	3.33[b]
Tris egg-yolk	86.67	68.33[a]	51.67[a]	38.33[a]
Sodium citrate egg-yolk	86.67	65.0[a]	50.00[a]	25.00[a]
Sodium Phosphate egg-yolk	85.00	48.33[b]	20.00[b]	8.33[b]
Standard error of mean	1.61	4.01	5.69	4.65

[a,b]Means with different superscripts within the column differ significantly ($p<0.05$)

Table 2: Effects of different buffers on sperm viability of tom semen diluted with coconut-water extender

	Preservation periods			
Treatments	0 hr	4 hrs	24 hrs	48 hrs
Neat-semen	93.33	82.00	79.00	73.00
Tris egg-yolk	96.00	90.67	86.33	78.33
Sodium citrate egg-yolk	95.33	93.00	82.67	84.33
Sodium Phosphate egg-yolk	93.67	90.33	85.00	86.67
Standard error of mean	0.80	2.05	1.75	2.81

Table 3: Effects of different buffers on sperm membrane integrity of tom semen diluted with egg-yolk extender

	Preservation periods			
Treatments	0 hr	4 hrs	24 hrs	48 hrs
Neat-semen	85.00	38.67[b]	16.00[b]	12.33[c]
Tris coconut-water	88.33	68.00[a]	59.00[a]	44.33[a]
Sodium citrate egg-yolk	86.33	69.67[a]	57.00[a]	42.33[ab]
Sodium Phosphate egg-yolk	86.33	48.33[b]	20.67[b]	25.67[bc]
Standard error of mean	0.77	4.55	6.64	4.58

[a,b,c]Means with different superscript within the column differ significantly ($p<0.05$)

sodium citrate egg-yolk had the highest viability of 86.67% and 84.33% compared to their counterpart at 48 hrs of storage periods.

Effect of different buffers supplemented with egg-yolk on preserved tom sperm membrane integrity: Sperm membrane integrity of tom semen preserved with egg-yolk extender supplemented with different buffers was presented in Table 3. Tom semen diluted with egg-yolk extender supplemented with different buffers showed a significant difference at 0 hr. However, from 4 to 48 hrs of preservation, tris egg-yolk and sodium citrate egg-yolk extender was statistically similar ($p<0.05$) and significantly different ($p>0.05$) from undiluted semen and sodium phosphatase coconut water extender.

DISCUSSION

Notably, the addition of three different buffers to the egg-yolk led to changes in the pH value of the three different extenders formulated in this study. When assessing the pH of the extender immediately after preparation. Extenders should have a buffering effect on the pH of semen diluted to balance the production of metabolic substances from sperm or bacteria[4].

Furthermore, an ideal buffer for spermatozoa should provide a wide choice of ionic strengths for preservation, be stable and resist enzymatic and non-enzymatic degradation without resembling enzyme substrates. The general superiority for maintenance of spermatozoa motility and membrane integrity of sodium citrate and tris with egg-yolk tested over those of undiluted semen and sodium phosphate egg yolk extended semen may be suspected to the ability of tris (hydroxy methyl amino methane) and sodium phosphate tends to precipitate or bind most polyvalent cations and either maintained metabolic processes or act as inhibitors during the sperm storage. Contrarily, Good *et al.*[10] stated that tris (hydroxymethyl amino methane) has poor buffering capacity below pH 7.5, is often inhibitory and alone is unstable to temperature change. However, essential components of semen extenders should be a substance that stabilizes the pH and controls microorganism growth[4]. Moreover, the addition of buffers to the extender has been reported to lead to favourable changes in the pH value of the extender[11].

Ideally, extender components should not pose any deleterious effects on sperm cells during semen handling, dilution and storage. Though length or period of storage and temperature may reduce the efficacy of the extender for preserving semen quality as they are contributing factors for the survival and activities of sperm that determine the rate of metabolic activities expected to be exhibited by sperm cells during storage. Moreover, Yaniz *et al.*[12], reported that during storage, sperm and contaminating bacteria generally produce metabolites that may reduce the pH of the extender, reducing both sperm metabolism and motility. These alterations can cause sperm death due to drastic changes in pH, justifying the addition of buffers to the extender[11,13].

Evidently, both extender containing tris and sodium citrate buffers semen exhibited their capability in their various medium by stepping down the rate of metabolic activities of sperm during storage and probably subjecting the sperm cells in them to a quiescent phase till they were exposed to room temperature. Evidently in our studies, both diluted and undiluted tom semen quality reduces with increasing storage length, corroborating the work of Balogun *et al.*[14], who also stated that irrespective of the extender quality extended preserved semen quality decreases as the storage period increases.

This study undoubtedly revealed that diluted turkey semen could be stored for more than 24 hrs with good sperm quality required for successful artificial insemination, it is therefore recommended that tris and sodium citrate buffer should be used as the buffer of choice with egg-yolk for dilution and liquid storage of turkey semen although the semen quality decreases drastically after 24 hrs of storage.

CONCLUSION

The result of the present study showed that the tris and sodium citrate buffer prove to be the best buffering agents most compatible with egg yolk for the formulation of turkey semen extender. It is therefore recommended that tris and sodium citrate salts may be used for the preparation of buffer for egg-yolk turkey semen preparation.

SIGNIFICANCE STATEMENT

This study is important to turkey breeding industries to effectively use a limited number of outstanding sires among the flock, there by reducing the cost of production in turkey breeding for artificial insemination practices and further encouraging the transfer of turkey germplasm across boundaries. Dilution of turkey semen at a ratio of 1:3 and its Storage at 4°C for 24 hrs with egg-yolk containing tris or sodium citrate buffer were effective for the semen storage because they gave better microscopic semen results after evaluation. However, fertility trials should be carried out with the cold preserved turkey semen to ascertain the fertilizing ability of the preserved sperm cells. Also supplementing with natural antioxidants rich in vitamin E may be explored for better results.

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