COMPARATIVE EFFECT OF CHILLING AND FREEZING ON SPERM VIABILITY AND FUNCTIONAL INTEGRITY OF FUNAAB ALPHA NORMAL FEATHER CHICKEN

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ABSTRACT

This Study Investigated the Effect of Chilling and Freezing on the Sperm Viability and Functional Integrity of FUNAAB Alpha (Normal feather) chicken semen. Pooled semen collected from ten FUNAAB Alpha (Normal feather) chicken were diluted with Tris –based extender. Diluted semen was divided into two fraction, the first fraction was subjected to chilling at 4^{o}c and the second fraction was subjected to freezing at 1^{o}C for 24hrs. Thereafter semen were evaluated for viability and functional integrity. The result showed a consistent variation in the semen viability and functional integrity. However, Sperm motility and livability preserved at 4^{o}C was significantly higher ($p<0.05$) than those preserved at 1^{o}C. Abnormal sperm (head, mid piece and tail) recorded for those preserved at 4^{o}C was significantly lower ($p<0.05$) than those preserved at 1^{o}C. The findings indicate that sperm viability, functional integrity of FUNAAB Alpha (Normal feather) chicken were better preserved by chilling compared to freezing method.

Keyword: Chicken, Fertilization, Integrity, Preservation, Sperm

INTRODUCTION

Assisted reproductive technologies such as artificial insemination has led to an advancement in poultry production. The semen quality test performed, allows only high quality semen from a superior males to be use for artificial insemination. Proper semen handling, storage, thawing and evaluation are perquisite for implementing a successful artificial insemination program (Dumpala *et al*., 2006). Preservation of the structural and functional integrity of semen over a period of time is the main essence of cryopreservation and providing a suitable technique for spermatozoa to withstand the freezing and thawing stress (Sharma, *et al.,* 2015). In poultry semen however, factors such as variation in thawing temperatures, osmotic pressure, breeds, stress, extender effects affect semen quality parameters such as viability, motility and membrane integrity during cryopreservation (Tuncer *et al.,* 2011). The capability of gametes to initiate normal embryonic development needed for fertilization is impaired due to oxidative stress caused by Reaction Oxygen Species, depending on its concentration (Aitken, 2017). To protect sperm cells from oxidative damage caused by higher concentrations of ROS, Chilling and freezing protocols as a means of cryopreservation, have helped to reduce the temperature of sperm, dehydrate sperm, and induce intra and extra cellular freezing (Lucio *et al.,* 2016; Pignataro *et al.,* 2020).

MATERIALS AND METHOD

Experimental site

The experiment was carried out at PEARL- FUNAAB poultry Breeding center, federal University of Agriculture, Abeokuta, and the laboratory analysis was conducted at the Department of Animal Physiology Laboratory, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta.

Experimental Animals

Ten (10) parent birds (Normal feathered FUNAAB Alpha) of 52 weeks old were used for the experiment. The animals were reared in an intensive battery cage management system and housed individually. Fresh clean water was given ad-*libitum* along with breeder mash concentrate feed was made available to them.

Semen collection

Semen samples were collected early in the morning from the cock according to Burrows and Quinn (1937) by abdominal massage procedure, using graduated eppendorf tube. The volume of each semen sample were recorded.

The semen samples collected from each cocks were pooled together for uniformity, the pooled semen were diluted with Tris egg yolk extender and then maintained in the dark by covering the holding tube with aluminum

foil. The pooled semen were subjected to chilling at 4ºC and freezing at 1ºC for 24 hours in a refrigerator. Thereafter semen were evaluated for viability and functional intergrity.

Evaluation of Sperm Viability Parameters

The frozen semen was thawed in Clifton water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37°C for 30mins following 24 hours of storage. For semen evaluation, sperm motility, livability and abnormalities were evaluated as described by Bearden and Fuquay (1997), sperm membrane integrity by Zubair *et al.* (2013) and Acrosome integrity by Ahmad *et al.* (2014)

Statistical analysis

The data collected were subjected to two-way analysis of variance (ANOVA) using SPSS 2000. While Tukey HSD was used to separate significantly different means. The model is stated below:

RESULT

The viability parameters of sperm from FUNAAB Alpha Normal Feather chicken subjected to chilling and freezing are presented in Table 1. Consistent higher ($p<0.05$) sperm motility and sperm livability was observed in chilled semen compared to the frozen semen. Abnormal cells (head, mid piece and tail) were lower ($p<0.05$) in chilled semen compared to the frozen semen.

The functionality parameters are presented in Table 2. The acrosome and sperm membrane integrity for the chilled and frozen semen was observed to be in consistency with the fresh semen, but was higher ($p<0.05$) in chilled semen compared to the frozen semen.

Table 1; Effect of Freezing and Chilling on Sperm Viability of FUNAAB ALPHA (Normal Feather) Chicken

Protocol	Mortality (%)	Livability (%)	Abnormal Head (%)	Abnormal Mid-Piece (%)	Abnormal Tail (%)
Fresh	98.67 ± 0.63^{a}	99.73 ± 4.08^{c}	0.44 ± 0.11^{c}	0.52 ± 0.12^{c}	1.53 ± 0.19^{a}
Chilled (4^0C)	92.00 ± 2.11^{b}	94.00 ± 2.73^{a}	0.80 ± 0.24^{b}	0.73 ± 0.36^{ab}	0.19 ± 0.40^{c}
Freezing (1^0C)	65.33 ± 1.57^{c}	73.00 ± 12.48^{ab}	1.90 ± 0.36^{a}	2.54 ± 0.56^{a}	0.68 ± 0.24^{b}
P value	0.000	0.005	0.000	0.004	0.000

^{a,b,c} *Values within column with different superscript differs significantly ($p<0.05$)*

Table 2; Effect of Freezing and Chilling on Sperm Functionality of FUNAAB ALPHA (Normal Feather) Chicken

Protocol	Acrosome Integrity (%)	Sperm Membrane (%)
Fresh	95.67 ± 0.92	98.33 ± 0.55^{a}
Chilled (4^0C)	96.33 ± 0.86	97.33 ± 0.62^{ab}
Freezing (1^0C)	94.67 ± 1.07	96.00 ± 0.84^{c}
P value	0.465	0.054

^{a,ab,c} *Values within column with different superscript differs significantly ($p<0.05$)*

DISCUSSION

A reversible reduction of metabolic activity is strategic in extending the life of spermatozoa, therefore, hypothermia reduces metabolic activity of cells by decelerating enzymatic reactions (Viswanathan *et al.,* 2000; Freitas-Ribeiro *et al*., 2019). Improved sperm viability and functional integrity observed in spermatozoa subjected to chilling at 4°C indicated the efficacy of this protocol to maintain quality characteristics of stored spermatozoa over the frozen at 1°C. This is in line with the work of (Dumpala *et al.*, 2016) who stored semen at different temperature (4°C, 21°C and 41°C) respectively and obtained the best result at 4°C. The difference could be as a result of less production of toxic end products such as 3- carbon glycolytic intermediates which subsequently form toxic by-product (Riddle, 1998). Also in the study of Vasicek and Chrenek, (2021), no negative effect of low temperature (4-8 °C) on storage of rooster spermatozoa was observed. The improvement in the sperm quality indices revealed the preservative effect of storing spermatozoa at a lower temperature of about 4°C than freezing at about 1°C, the reason could be attributed to the changes in the osmotic rate and mitochondria activities (Kumar *et al.,* 2019). Cold shock causes structural and biochemical damages in sperm as a result from sudden reduction in temperature (Bucak *et al.,* 2009). Such damages can be forestall by cooling

semen slowly in the presence of protective agents. Slow dilution helps to reduce the metabolic rate of sperm and minimizes damage due to cold shock (Curry, 2007).

CONCLUSION AND RECOMMENDATION

In the preservation of cock semen using liquid storage cryopreservation techniques, chilling should be employed as a better means of preservation for an effective post thawed, efficient artificial insemination and a high productivity.

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