Influence of different cryoprotocols and strains on the sperm viability of FUNAAB alpha chickens

Abstract

Semen preservation is an essential tool used for a successful application of artificial insemination in livestock industry. Studies on semen quality characteristics on poultry breeds and strains after cryopreservation have been carried out but none has been reported for Nigeria FUNAAB alpha chickens. Three studies were carried out to evaluate the different cryoprotocols on viability of cryopreserved spermatozoa of FUNAAB alpha chickens.

Thirty cocks 25-30 weeks old of normal feather, naked neck and frizzle feather of FUNAAB alpha chickens were used for this experiment. The semen samples were diluted in Tris-based extender. The semen samples were divided into 2 parts. One part washed with normal saline water by centrifuging at 500xg once for 5 minutes in order to remove seminal plasma while the second part was unwashed. Washed and unwashed semen samples were cryopreserved using slow and rapid cryoprotocols, thereafter samples were evaluated for sperm viability. The experiment was laid out in 3x2x2 factorial arrangement. Data obtained were subjected to two-way ANOVA. Higher ($P<0.05$) motility was observed in washed spermatozoa from FF subjected to SF compared to others strains either washed or unwashed, SF or RF protocols. Lower ($P<0.05$) motility of washed spermatozoa from NF, NN and FF subjected to RF were comparable with washed spermatozoa from NN and unwashed NN subjected to SF and RF. Results for livability, acrosome integrity, seminal Leukocytes and MDA concentration were comparable ($p<0.05$) for unwashed and washed spermatozoa from NN, NF and FF, subjected to slow and rapid freezing cryoprotocols.

The study concluded that slow and rapid freezing cryoprotocols had a deleterious effect on the spermatozoa of NF, NN, FF and removal of seminal plasma through centrifugation did not improve the viability of the spermatozoa.

Keywords: Sperm viability, FUNAAB alpha chicken, cryoprotocols, centrifugation

Introduction

Poultry production could be improved with the aid of assisted reproductive technologies (ARTs) like artificial insemination (AI), which allows for the most efficient use of genetically superior cockerels with high reproductive efficiency. The increased use of AI techniques in the poultry industry highlights the importance of distributing high-quality semen (Dumpala et al., 2006). As

a result, for the effective implementation of AI techniques, proper semen processing, storage, and evaluation are necessary. In poultry breeding, semen evaluation is essential for selecting breeding males and monitoring their reproductive output on a regular basis (Cheng et al., 2002). The motility, live or dead sperm, and morphological examination of semen can all be used to determine its fertilizing ability (Alkan et al., 2002). Semen selection is the first and most important stage of AI, with good collection resulting in high-quality semen and the highest number of sperms per ejaculation (Tijjani, et al., 2014).

Many experiments have been conducted in order to develop methods for preserving the reproductive capacity of bird sperm after freezing and thawing. For sperm with a very particular feature, cryopreservation is considered to be extremely stressful (fertilization). Osmotic and thermic shocks can harm the structure and metabolism of cells during the poultry semen cryopreservation process (including the cooling freezing and thawing procedures) (Long, 2006).

The qualitative evaluation of semen characteristics provides an excellent predictor of domestic bird reproductive capacity and may be a significant determinant of fertility (Peters et al., 2004). Several research on semen development and quality characteristics have been conducted on various poultry breeds and strains. Despite this, there has been little to no research on Nigeria FUNAAB chickens, especially in regards to the quality of semen obtained from this breed after cryopreservation.

The success of cryopreservation in sperm cells differs between bird species. Variations in membrane fluidity, which play a role in the restoration of the physiological state after freezing, are one of the possible biological factors responsible for such variations (Blesbois et al., 2005). Furthermore, despite its long-established importance for optimizing male genetic potential in domestic avian species, semen preservation is only occasionally used in breeding practice (Blesbois et al., 2005). One of the key reasons is that freezing procedure performance is highly variable, depending on species and line specificity, among other things (Hammerstedt 1995, Etches 1996; Massip et al., 2004). However, despite the fact that spermatozoa have identical morphological shapes and ultrastructures, the freezability of these cells tends to vary between species in domestic birds (Blesbois & Labbe, 2003; Massip et al., 2004).

Some biological and biophysical factors, such as those involved in the resistance of the plasma membrane to thermal and osmotic changes, can affect the ability of sperm cells to prevent damage caused by freezing-thawing processes (Holt, 2000). Membrane permeability, lipid

composition, and fluidity are among these variables (Holt, 2000). Under various osmotic conditions, variations in spermatozoa membrane permeability have been observed between species in birds (Blanco et al., 2000). Semen freezability in chickens has also been linked to intra-species differences in cholesterol/phospholipids content (Ansah & Buckland, 1982).

In Nigeria, there is paucity of information on the semen cryopreservation of indigenous chicken breed. This study therefore focuses on the comparative evaluation of semen quality parameters of three strains of FUNAAB Alpha chicken subjected to different cryoprotocols and the fertility of cryopreserved spermatozoa.

Materials and Methods

Experimental site

The study was carried out at the Teaching and Research farm, FUNAAB, which falls within 7°10N and 3°2E and altitude 76m above sea level. It lies in the South-West part of Nigeria with a prevailing tropical climate, a mean annual rainfall of 1037 mm and average temperature of 34.7°C. The laboratory analyses were carried out at the Animal Physiology Laboratory of the Department of Animal Physiology, Federal University of Agriculture Abeokuta, Ogun State Nigeria.

Experimental Birds and Management

A total number of thirty (30) cocks between 25-30 weeks old of three strains of FUNAAB alpha chickens consisting of 10 Normal feathers, 10 Naked-neck and10 Frizzle feather was used for this experiment. The birds were fed *ad-libitum* with commercial breeder mash containing 17.5% crude protein and 2700kcal metabolizable energy. Clean water was supplied *ad-libitum.* Medications and vaccinations were done as required.

Semen collection

Semen samples were collected from thirty cocks, ten for each strain of chicken. Semen was collected using the abdominal massage method described by Burrows & Quinn (1937). For the control group, after collection, semen was placed in a water bath at 37°C and transferred to the laboratory for semen evaluation in 15 minutes.

For the washed semen samples, the collected semen was washed with non-culture medium (normal saline) by centrifuging at 5000 revolution once for 5 minutes each in order to remove semen plasma while the control group was not washed. Following washing and removal of supernatant, the sediments were then diluted at 32^{o}C and cryopreserved using slow and rapid freezing protocols.

Cryopreservation protocols

Rapid freezing (vitrification)

The rapid freezing or vitrification involves a swift speed of temperature conduction which provides a significant increase in cooling rate (Mphaphathi *et al.*, 2012). The procedure consisting of different cryoprotectants was carried out on semen samples that were diluted with fraction 1 solution for the respective treatments as described by Srirattana *et al*. (2013) with some modifications. Holding medium (HM) for rapid freezing/vitrification was prepared by supplementing phosphate-buffered saline (PBS) with 20% bovine serum albumin (BSA) (v/v). Vitrification solution I (VS-I) consisting of 12.5% ethylene glycol (EG) and 12.5% dimethylsulfoxide (DMSO) was prepared just prior to use by mixing EG, DMSO and the HM in ratio of 1:1:6 while vitrification solution II (VS-II) consisting of 25 % EG and 25 % DMSO was prepared just prior to use by mixing EG, DMSO and the HM in ratio of 1:1:2. The diluted semen sample for each treatment was exposed to the different cryoprotectants consecutively as follows: The diluted samples (2ml) were first exposed to 50µl of HM for 10m, thereafter 50µl of VS-I was added to the mixture (diluted samples + HM) and left for 4 m. Finally, 50µl of VS-II was added to the mixture (diluted samples + HM + VS-I) and left for 1 m. During equilibration in VS-II, the samples were loaded into 2 mL straws replicated twice (2 straws per treatment) and sealed with polyvinyl. The straws containing diluted spermatozoa were placed in a canister at 4cm above liquid nitrogen in a vapour phase for approximately 10 m to avoid cold shock before rapidly plunging them directly into liquid nitrogen tank. After cryopreservation for 24 hours, the straws were thawed in a water bath (37°C) and thereafter evaluated for sperm functional attributes and oxidative stress parameters.

Slow freezing

In slow freezing, the temperature is gradually reduced in a stepwise manner during cryopreservation process (Mphaphathi et al., 2012). The procedure was carried out as described

by Tarig et al. (2017). In this procedure, semen samples were diluted at room temperature in a two-step process with a Tris-based extender composed of 2 fractions containing 7% glycerol. The fraction 1 solution contained tris-hydroxymethyl-amino methane (2.42 g), citric acid (1.36 g), glucose (1 g) and penicillin (0.028 g) plus egg yolk of 20mL and distilled water added to make up 100mL. The fraction 2 solution had the same composition as fraction 1 solution with the addition of glycerol. The pooled fresh semen samples were split into five equal fractions in different test tubes and diluted at room temperature with fraction 1 solution. Fraction 2 solution was subsequently added. Following dilution, the samples were loaded into 2mL plastic straws (2 straws per treatment), sealed with polyvinyl, gradually cooled to 4ºC at 0.25 ºC/min and equilibrated at 4ºC for 10 m in a TYFSF Refrigerated Incubator (Model: SPX-7OB III, Hebei China). Subsequently, the straws were placed in a canister at 4 cm above liquid nitrogen in the vaporous phase for about 10 minutes and finally immersed vertically and gradually into liquid nitrogen tank.

Semen evaluation

Ejaculates were evaluated for volume (ml), colour, pH, density, mass movement (0-5), sperm motility (%), sperm abnormality (%) and sperm viability (%) using eosin-nigrosin staining and sperm concentration (n x 10^9 sperm/ml) by haemocytometer.

Semen was also evaluated for sperm motility, recovery rate, acrosome integrity, sperm membrane integrity, live sperm, sperm abnormality, leukocyte counts and seminal, malondialdehyde concentration.

Sperm motility was determined as described by Bearden and Fuquay (1997). The recovery rate of cryopreserved spermatozoa total motility was calculated according to Ricci *et al.* (2009) using the following formula:

$$\text{Recovery rate} = \frac{\text{Total motility after freezing}}{\text{Total motility before freezing}} \times 100$$

The percentage of spermatozoa with intact acrosomes was determined according to Khan& Ijaz, (2007) and Ahmad et al. (2014). Intactness of acrosome characterized by normal apical ridge or spermatozoa with presence of crescent shaped acrosomes was considered normal and recorded. The percentage of acrosome integrity was calculated as follows:

Number of spermatozoa with normal apical ridge x 100.

Total spermatozoa that was counted

Hypo-osmotic swelling test (HOST) assay as described by Zubair et al. (2013) was used to determine sperm membrane integrity. Eosin–nigrosin staining was used to evaluate sperm abnormality and livability (Pintado et al., 2000) according to the method of Bearden and Fuquay (1997). Spermatozoa that appeared white (unstained cells) was regarded as live (viable) spermatozoa while those that picked up the stain (cells stained by eosin-nigrosin) were regarded as dead spermatozoa. Sperm livability (%) was calculated as

= (Total number of live sperm) x 100.

(Total number of sperm observed)

The levels of malondialdehyde (MDA) as indices of lipid peroxidation in the stored semen were measured in a thiobarbituric acid reactive substance (TBARS) according to Yagi (1998).

Statistical Analysis

The experiment was laid out in 3x2x2 factorial arrangements. Data obtained was subjected to a two-way ANOVA and significantly ($P<0.05$) different means were separated by Duncan Multiple Range Test using SAS 2000.

Results and Discussion

The interactions of different cryoprotocols and strains on the sperm functional attribute of FUNAAB alpha chickens are presented in Table 1. The results showed no variations($p<0.05$) among the washed and unwashed semen samples subjected to slow and rapid freezing on NF, NN and FF for motility, recovery rates ,livability and abnormalities, hence the value ranges from 41.33-75.20% ,1.53-20.53%, 41.33-75.20% and 29.09-54.16% respectively. The results showed that motility, recovery rate, livability and abnormalities were comparable in all the strains irrespective of the protocols used.

The interactions of different cryoprotocols and strains on the sperm functional integrities of FUNAAB alpha chickens are presented in Table 2. The results showed similarities ($p>0.05$) in acrosome and membrane integrities among the strains subjected SF and RF cryoprotocols. Higher ($p<0.05$) acrosome integrity was obtained when FF strains was subjected to slow freezing

compared to FF strains sperm cryopreserved using rapid freezing protocols. NN strains sperm subjected to slow freezing cryoprotocols had a reduced ($p<0.05$) acrosome integrity compared to NN strains sperm cryopreserved using rapid freezing protocol. Reduced ($p<0.05$) acrosome integrity was observed when NF strains was subjected to rapid freezing protocols compared to NF strain subjected to slow freezing, though the result was comparable to NN strains subjected to rapid freezing. Acrosome integrity of FF strains was higher ($p<0.05$) compared to other strains (NN and FF) when subjected to slow and rapid freezing cryoprotocols. Membrane integrity of NF strains subjected to slow freezing cryoprotocols was comparable to NF, NN and FF strains subjected to rapid freezing and NN strains subjected to slow freezing cryoprotocols. However NF strains subjected to slow freezing had a reduced ($p<0.05$) membrane integrity compared to FF strains subjected to slow freezing cryoprotocols.

The Interactions of different cryoprotocols and strains on the seminal oxidative stress parameters of FUNAAB alpha chickens is presented in Table 3. The results showed no variations ($p<0.05$) in leukocyte and MDA concentration among the strains (NF, NN and FF) subjected to slow and rapid freezing cryoprotocols, hence the value ranges from 0.73-1.14x10^3/mL and 0.29-1.22x10^6 respectively. The results showed that leukocytes and MDA concentrations were comparable in all the strains irrespective of the protocols used. The result of the present study showed that the seminal leukocyte and MDA concentration of the strains subjected to both slow and rapid freezing cryoprotocols were comparable. This means that the strains of funaab alpha chicken did not affect the leukocyte nor MDA concentration of the spermatozoa.

Table 1: Interactions of different cryoprotocols and strains on the sperm functional attribute of FUNAAB alpha chickens

Cryoprotocol	Strain	MOT	RR	LIV	ABN
SF	NF	66.87±7.04	1.53±3.24	66.87±7.04	54.16±4.79
	NN	41.33±7.04	9.83±3.24	41.33±7.04	43.51±4.79
	FF	75.20±7.04	20.53±3.24	75.20±7.04	29.09±4.79
RF	NF	58.00±7.04	5.90±3.24	58.00±7.04	42.96±4.79
	NN	62.53±7.04	11.87±3.24	62.53±7.04	48.78±4.79
	FF	70.13±7.04	18.30±3.24	70.13±7.04	40.51±4.79

SF= slow freezing, RF= rapid freezing, NF= normal feather, NN=Naked neck, FF=frizzled feather, MOT= motility, RR=recovery rate, LIV= livability, ABN=abnormalities

Table 2: Interactions of different cryoprotocols and strains on the sperm functional integrities of FUNAAB alpha chickens

Cryoprotocol	Strain	ACI	MI
SF	NF	78.00±2.79^{b}	44.67±2.45^{bc}
	NN	64.67±2.79^{d}	43.33±2.45^{c}
	FF	91.67±2.79^{a}	53.67±2.45^{a}
RF	NF	68.67±2.79^{cd}	48.67±2.45^{ab}
	NN	71.33±2.79^{c}	48.33±2.45^{ab}
	FF	83.48±3.18^{b}	45.65±2.79^{b}

$^{a, b,. c, d.}$ Values within columns with different superscripts differ (P<0.05); SF= slow freezing, RF= rapid freezing, NF= normal feather, NN=Naked neck, FF=frizzled feather, ACI= acrosome integrity, MI= membrane integrity

Table 3: Interactions of different cryoprotocols and strains on the seminal oxidative stress parameters of FUNAAB alpha chickens

Cryoprotocol	Strain	Leukocytes(x10^{3}/mL)	MDA (×10^{6})
SF	NF	1.00±0.19	0.29±0.14
	NN	1.40±0.19	0.79±0.14
	FF	1.33±0.19	0.41±0.14
RF	NF	1.20±0.19	0.87±0.14
	NN	0.73±0.19	0.97±0.14
	FF	1.47±0.19	1.22±0.14

SF= slow freezing, RF= rapid freezing, NF= normal feather, NN=Naked neck, FF=frizzled feather MDA= Malondialdehyde concentration LEU= seminal leukocyte

The interactions of different cryoprotocols, protocols and strains on the sperm functional attribute of FUNAAB alpha chickens are presented in Table 4. The result showed variations ($P<0.05$) for strains subjected to washing and cryoprotocols for sperm motility. However no variations ($p>0.05$) shown in livability and abnormality for strains spermatozoa subjected to slow and rapid freezing cryoprotocols.

Higher ($P<0.05$) motility was observed in washed spermatozoa from FF subjected to SF compared to others strains either washed or unwashed, SF or RF protocols. Lower ($P<0.05$) motility of washed spermatozoa from NF, NN and FF subjected to RF were comparable with washed spermatozoa from NN and unwashed NN subjected to SF and RF, respectively. The results showed that livability and sperm abnormality were comparable in all the strains irrespective of the protocols used.

The interactions of different cryoprotocols, protocols and strains on the sperm functional integrities of FUNAAB alpha chickens are presented in Table 5. No variations ($p>0.05$) observed in acrosome integrity of washed and unwashed spermatozoa (NN, NF and FF) subjected to slow and rapid freezing. However variations ($p<0.05$) were observed for membrane integrity of washed and unwashed spermatozoa from NF, NN and FF subjected to washing using slow and rapid freezing cryoprotocols.

The results showed that acrosome integrity for washed and unwashed spermatozoa (NF, NN and FF) subjected to slow and rapid freezing cryoprotocols were comparable. Higher ($p<0.05$) membrane integrity was observed in unwashed FF spermatozoa subjected to rapid freezing compared to unwashed slow freezing and washed slow and rapid freezing cryoprotocols. However, reduced ($p<0.05$) membrane integrity was observed in washed NF and FF spermatozoa subjected to rapid Freezing compared to others.

The interactions of different cryoprotocols, protocols and strains on the seminal oxidative stress parameters of FUNAAB alpha chickens are presented in Table 6. No variations ($p>0.05$) observed in leukocyte and MDA concentration for unwashed and washed spermatozoa subjected to slow and rapid freezing cryoprotocols.

The results for seminal Leukocytes and MDA concentration were comparable ($p<0.05$) for unwashed and washed spermatozoa from NN, NF and FF, subjected to slow and rapid freezing cryoprotocols.

The present study showed that the strains of funaab alpha chicken did not affect the seminal leukocyte and MDA concentration of unwashed and washed semen cryopreserved using slow and rapid freezing cryoprotocols.

Table 4: Interactions of different cryoprotocols, protocols and strains on the sperm functional attribute of FUNAAB alpha chickens

Centrifugation	Cryoprotocol	Strain	MOT	LIV	ABN
Unwashed	SF	NF	50.33±3.26^{b}	58.70±6.49	53.77±4.63
		NN	36.00±3.26^{bc}	73.10±6.49	51.97±4.63
		FF	48.33±3.26^{b}	69.60±9.18	51.67±6.55
	RF	NF	23.67±3.26^{c}	69.60±5.30	44.24±3.78
		NN	20.00±3.26^{cd}	67.73±5.30	52.80±3.78
		FF	24.00±3.26^{c}	69.33±5.30	43.58±3.78
Washed	SF	NF	28.64±3.26^{c}	72.80±5.30	70.89±3.78
		NN	8.33±3.26^{d}	75.13±5.30	69.22±3.78
		FF	76.67±3.26^{a}	81.20±5.30	63.20±3.78
	RF	NF	1.00±3.26^{d}	58.50±6.49	76.47±4.63
		NN	3.67±3.26^{d}	57.30±6.49	61.00±4.63
		FF	2.67±3.26^{d}	51.10±6.49	63.90±4.63

$^{a, b,. c, d.}$ Values within columns with different superscripts differ (P<0.05); SF= slow freezing, RF= rapid freezing, MOT= motility, LIV= livability, ABN=abnormalities, NF=normal feather, NN=naked neck, FF=frizzled feather.

Table 5: Interactions of different cryoprotocols, protocols and strains on the sperm functional integrities of FUNAAB alpha chickens

Centrifugation	Cryoprotocol	Strain	ACI	MI

Unwashed	SF	NF	46.00±2.25	68.00±2.91^{bc}
		NN	39.67±2.25	76.33±2.91^{b}
		FF	49.67±2.25	63.33±2.91^{c}
	RF	NF	42.33±2.25	65.67±2.91^{c}
		NN	49.00±2.25	69.00±2.91^{bc}
		FF	47.00±2.25	92.67±2.91^{a}
Washed	SF	NF	43.33±2.25	70.00±2.91^{bc}
		NN	34.33±2.25	63.00±2.91^{c}
		FF	45.00±2.25	64.67±2.91^{c}
	RF	NF	36.00±2.25	30.33±2.91^{e}
		NN	43.33±2.25	45.33±2.91^{d}
		FF	48.33±2.25	36.33±2.91^{e}

a, b,. c, d, e Values within columns with different superscripts differ (P<0.05); SF= slow freezing, RF= rapid freezing , ACI= acrosome integrity, MI= membrane integrity, NF=normal feather, NN=naked neck, FF=frizzled feather.

Table 6: Interactions of different cryoprotocols, protocols and strains on the seminal oxidative stress parameters of FUNAAB alpha chickens

Centrifugation	Cryoprotocol	Strain	LEU(x10^3/mL)	MDA (×10^6)

Unwashed	SF	NF	0.90±0.18	1.35±0.31
		NN	0.80±0.18	1.49±0.31
		FF	0.80±0.25	0.88±0.31
	RF	NF	0.53±0.14	0.53±0.31
		NN	0.80±0.14	0.33±0.31
		FF	0.53±0.14	0.55±0.31
Washed	SF	NF	0.80±0.14	0.09±0.31
		NN	0.53±0.14	0.35±0.31
		FF	0.13±0.14	0.94±0.31
	RF	NF	0.30±0.18	0.82±0.31
		NN	0.20±0.18	1.69±0.31
		FF	0.40±0.18	1.36±0.31

SF= slow freezing, RF= rapid freezing, MDA= Malondialdehyde concentration LEU= seminal leukocyte, NF=normal feather, NN=naked neck, FF=frizzled feather.

Sperm Recovery rates of FUNAAB alpha chickens subjected to preservation protocols are presented in Table 7. The results showed variations ($p<0.05$) in recovery rate of semen samples subjected to refrigeration and freezing protocols. The results showed higher ($P<0.05$) recovery rates for refrigerated spermatozoa compared to frozen spermatozoa.

Sperm recovery rates of FUNAAB alpha chickens subjected to cryopreservation protocols are presented in Table 8. The results showed no variations ($p>0.05$) in recovery rate of semen samples subjected to slow and rapid freezing cryoprotocols. The results of recovery rate for slow and rapid freezing cryoprotocols are comparable.

Table 7: Means (SEM) sperm Recovery rates of FUNAAB alpha chickens subjected to preservation protocols

Protocol	**Recovery rates**

Refrigeration	90.93±1.21[a]
Freezing	33.97±1.05[b]

[a, b,.] Values within columns with different superscripts differ (P<0.05), SEM= standard error mean

TABLE 8: Means (SEM) sperm Recovery rates of FUNAAB alpha chickens subjected to different cryoprotocols

Cryoprotocol	Recovery rates
SF	10.63±2.09
RF	11.49±1.91

SF= slow freezing, RF= rapid freezing, SEM= standard error mean

Discussion

The present study indicated that sperm motility, recovery rate, livability and sperm abnormality of funaab alpha chicken strains (NF, NN, FF) subjected to slow and rapid freezing cryopreservation protocols were comparable regardless of strains. These results agree with the findings of Makhafola et al. (2009) that White Leghorn, Ovambo, and Potchefstroom breeds had no major impact on motility and livability. Although Siudziska and Ukaszewicz (2008) found that the White Crested Black Polish, Green Leg Partridge, Italian Partridge, and Black Minorca breeds have major variations in terms of fresh semen consistency and freezability. Han et al. (2005) and Tabatabaci et al. (2009), on the other hand, found that freezing substantially decreased sperm viability and motility, regardless of breed. The differences between the present study and previous findings might be attributed to different extenders or cryoprotectants and different concentration of the cryoprotectant used.

The present study showed that high acrosome integrity was obtained in frizzled feather strain of FUNAAB alpha chicken subjected to slow freezing compared with other strains subjected to

slow freezing also, frizzled feather strain was also found to be better in rapid freezing compared with other strains (NN and NF), this could be attributed to their genetic background and natural tendencies. Significant genotype variations and season affect cock semen characteristics, according to Omeje & Marine (1990). The findings of this study appear to corroborate those of Peters et al. (2008) and Ajayi et al. (2014), who found differences in semen length, concentration, motility, and acrosome integrity among Nigerian indigenous cocks strains. Furthermore, frizzled feather semen had the highest membrane integrity when subjected to slow freezing, despite the fact that rapid freezing had lower membrane integrity in the current sample. The membrane integrity of frizzled feather semen exposed to slow freezing was comparable to that of naked neck and standard feather semen exposed to fast freezing. According to Ibe (1988), the Frizzled and Naked neck genes are heat tolerant, disease resistant, and have increased semen output ability. The evaluation of a poultry bird's semen quality characteristics provides an excellent indicator of their reproductive capacity and has been stated to be a major determinant of fertility and subsequent egg hatchability.

The present study indicated improved frizzled feather sperm motility compared with others when seminal plasma was removed through centrifugation or washing and cryopreserved using slow freezing cryoprotocol. However, the strains (NF, NN and FF) showed very low sperm motility on washed semen subjected to rapid freezing. This is not in agreement with the Santiago-Moreno et al. (2019) who reported that the elimination of seminal plasma had no effect on sperm motility in frozen thawed semen from various chicken breeds. The findings revealed that livability and abnormality were on par. This means that the livability and abnormality of unwashed and washed semen subjected to slow and fast freezing cryoprotocols is unaffected by different strains.

Seminal plasma's function in bird semen in vitro storage is largely unknown, as both inhibitory and stimulating effects have been observed. Seminal plasma can contain factors that protect sperm from cryoinjury, as well as components that harm sperm preservation. Blesbois & de Reviers (1992) found contrasting effects of seminal plasma fractions on chilled rooster sperm and a global deleterious effect in chickens and turkeys (Douard et al., 2005). Low molecular weight seminal plasma fractions appeared to reduce sperm fertilization capacity during storage, while high molecular weight fractions appeared to improve fertilization ability (Blesbois & de

Reviers, 1992). Blesbois & de Reviers found that seminal plasma is involved in the degradation of sperm phospholipids, probably via phospholipase activity, speeding up the sperm damage of turkey sperm during in vitro storage (1992).

The present study showed that acrosome integrity of unwashed and washed semen of funaab alpha chickens subjected to slow and rapid freezing were comparable. This means that the strains of funaab alpha chickens did not have effect on acrosomal integrity of the unwashed and washed semen using slow or rapid freezing cryoprotocols. This study is in agreement with Santiago-Moreno, (2019) who reported that after centrifugation and cryopreservation of semen, there were no differences in acrosome integrity of seven chicken breeds.

The membrane integrity of unwashed and washed semen subjected to slow and rapid freezing varied among the strains in this sample. After rapid freezing, washed semen of normal feather naked neck and frizzled feather strains of Funaab alpha chicken showed reduced membrane integrity. The reduced membrane integrity observed may be due to membrane cryodamage, which could result in a loss of fertilizing capability. Damage to the sperm membrane can occur in a variety of ways. The glycocalyx, a sugar-rich zone on sperm, has been shown to be lost during the cryopreservation process, and the sperm lipids are altered during the freeze-thawing process (Long, 2013). The susceptibility of sperm to cold shock is determined by the ratio of polyunsaturated to saturated fatty acids (Ejaz et al., 2014). Owing to the existence of several double bonds that render them vulnerable to peroxidation, a higher ratio of polyunsaturated fatty acids inside the plasma membrane is thought to impart greater fluidity and low resistance to cold shock (Giraud et al., 2000).

Recovery rate is associated with the recovery capabilities of sperm after chilling or freezing. The present study showed that spermatozoa subjected to refrigeration had a better ability to protect the sperm morphology and a reduced sperm damages compared to the frozen spermatozoa. This is in agreement with Gomes et al. (2020) who reported that at 5^{o}C, the recovery of undamaged membrane, motile, and gradually motile sperm was increased. Apart from sperm motility and viability, the recovery rate can be used to estimate the ability of the semen extender and cryoprotectant concentration to keep sperm cells passing the crucial steps during cryopreservation (Rosato and Iaffaldano, 2013).

Conclusion

It can be concluded from this study that low oxidative stress on semen samples subjected to slow and rapid freezing was not maintained irrespective of the strains compared to the control which could be the cause of low sperm viability observed due to deleterious cryo-damages that could have occurred during the process of freezing. The study also revealed that sperm viability were maintained in washed spermatozoa using slow freezing cryprotocol for the strains although frizzle feather showed a better fertilizing capacity compared with other strains(Normal feather and naked neck)

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