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Research

Comparative evaluation of different protocols and strains on sperm characteristics parameters of FUNAAB alpha chickens

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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. This study was carried out to evaluate the different cryoprotocols on viability of cryopreserved spermatozoa of different strains 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. Semen samples were diluted in Tris-based extender and preserved using electric freezer (refrigeration and freezing) for 24 hours. The experiment was laid out in 3x2 factorial arrangements. Results showed that semen samples subjected to refrigeration protocols were comparable to control but had higher ($p<0.05$) percentage in motility and livability when compared to freezing protocol. Sperm abnormality was reduced ($p<0.05$) in refrigeration compared with freezing. There was no significant interaction between different cryoprotocols and strains of FUNAAB Alpha chickens for leukocyte and Reduced ($p<0.05$) acrosome integrity, membrane integrity, leukocyte and malondialdehyde were observed in freezing when compared to refrigeration. Motility of the NF, NN and FF were comparable in refrigerated semen and control. The study concluded that sperm viability of refrigerated spermatozoa were better than the frozen spermatozoa of Normal Feather (NF), Naked Neck (NN) and Frizzle Feather (NF).

Keywords: Artificial Insemination, Freezing, Refrigeration, Spermatozoa, Strain

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Introduction

Assisted reproductive technologies such as artificial insemination (AI) and semen cryopreservation play an important role in preserving and transfusing valuable genes to the future generation. Artificial Insemination is a vital labour saving technique being used in animal production to improve livestock productivity. It was introduced by early workers like Philips and Lardy (1940) and Salisbury *et al.* (1947). In poultry, AI is a viable alternative for efficient and maximum production of chicks for meat and eggs.

For good results in artificial insemination, the quality of semen should be ensured (Alkan *et al*., 2002). Avian semen quality is often defined by determination of its volume, colour, concentration, motility, viability and morphology of spermatozoa (Giza, 2016; Santiago-Moreno *et al*., 2016).

The qualitative assessment of semen characteristics gives an outstanding indicator of reproductive potential of domestic birds and a possible major determinant of fertility (Peters et al., 2004). Several studies have been carried out on semen production and quality characteristics using different poultry breeds and strains (Peters et al., 2004). Nonetheless little or none has been reported on Nigeria FUNAAB chickens with particular reference to the quality of semen obtained from this breed after cryopreservation.

FUNAAB Alpha strains of chicken was developed at the Poultry Breeding units of the Directorate of the University farm, Federal University of Agriculture, Abeokuta, Ogun State in Nigeria. The selection process for the traits of interest which is the meat and the eggs started around 1997 with over 10 generations of selection for improved meat and egg production. The egg type chicken is a dual purpose; which was developed through a rigorous, systematic and selective breeding of the Nigerian indigenous chicken without eroding their tropical adaptive features and disease resistance traits. The potential variability that exists among the indigenous chickens was utilized to upgrade them. They still maintained the different plumage colors and the three feathering pattern (Normal feathered, Frizzled feathered and Naked neck) exhibited by the Nigerian indigenous chicken. The average chick weight at hatch is between 30-35g, age at first lay ranged between 16-18weeks, average body weight at first lay is between 1200g-1728g, and weight of first egg at lay is between 35-40g. The average egg lay per year ranges between 200-250 eggs (Wheto *et al.*, 2017).

The study is therefore aimed at evaluating the effect of different cryopotocols and strains on the sperm viability of FUNAAB Alpha cocks as semen preservation is an essential tool in successful artificial insemination program.

Materials and methods

Experimental site

The study was carried out at the Teaching and Research farm, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The site is located on the rainforest vegetation zone of South –West Nigeria on latitude 7^{o}13’49.46’N, longitude 3^{o}26’, 11.98’E and altitude of 76 meters above sea level. The annual mean temperature and humidity is 34.7^{o}C and 82% respectively (Google, 2020).

The laboratory analyses were carried out at the Animal Physiology Laboratory of the Department of Animal Physiology, FUNAAB.

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 and 10 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 three cocks randomly from ten cocks per strain (NF, NN, and FF). Semen was collected using the abdominal massage method described by Burrows and Quinn (1937). The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes. The male responds with tumescence erection of the phallus, at which time the handler gently squeezes the cloaca extracting semen through the external papillae of the duct us deferens (vas deferens) collecting the semen into a container. As stated by Burrows and Quinn (1937), the techniques of AI actually begin prior to the procedure. It includes housing the male poultry away from the hens maximizes the amount of available semen. Because the bird's phallus is located in the same duct as his anus, removing feed 12 hours prior to collection will help prevent faecal contamination of the semen. Breeder cocks need to be routinely primed for semen collection for several days prior to the actual AI procedure to guarantee that each bird is fertile with a microscopic examination of the sperm. According to Burrows and Quinn (1937), as with semen collection of other farm animals, one must stimulate the bird's sexual organ to extend outside of his body. Preliminary evaluation of semen samples were carried out. semen was placed in a water bath at 37°C and transferred to the laboratory for semen evaluation in 15 min. 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 samples showing >80 % sperm motility were pooled for each strain to minimize individual differences. The semen sample was diluted at room temperature with a Tris-based extender containing tris-hydroxymethyl-aminomethane (2.42 g), citric acid (1.35 g), glucose (1 g) and penicillin (0.028 g), egg yolk 20mL and distilled water added to make up 100mL.

Each diluted semen sample was split into two equal parts in different test tubes for each of the strain (one part for refrigeration while the other part is for freezing) then each semen sample was further be divided into four (4) aliquot with three (3) replicates for each strain.

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The first aliquot for each strain (NN, NF, FF) served as the control (fresh semen) while the remaining three aliquots were incubated in the refrigerator for chilling in Haier thermocool Refrigerator (Model: HR-170T) with four compartments of varying temperatures of 20^{o}C, 15^{o}C, 10^{o}C and 5^{o}C for 20 minutes each. After the last compartment with 5^{o}C temperature, the semen samples for freezing was equilibrated for 10 minutes and thereafter kept in the freezer for 24hours while the samples for chilling was left at 5^{o}C in the refrigerator for 24 hours. After which the samples were evaluated.

Results and discussion

Interactions of different protocols and strains on the sperm functional attribute of FUNAAB alpha chickens are presented in Table 1. The results

showed variations (P<0.05) among the semen samples subjected to refrigeration and freezing for NF, NN and FF motility. However, no variations (P<0.05) among the semen samples subjected to refrigeration and freezing cryoprotocols for NF, NN and FF livability and abnormality, hence the values range from 58.73-96.00% and 17.04-67.64% respectively.

Interactions of different protocols and strains on the sperm functional integrities of FUNAAB alpha chickens are presented in Table 2. The results showed no variation(P<0.05) among the semen samples subjected to refrigeration and freezing cryoprotocols for NF, NN and FF for acrosome and membrane integrities, the values however ranges from 94.67-97.50% and 93.33-98.33% respectively.

The interactions of different protocols and strains on the seminal oxidative stress parameters of FUNAAB alpha chickens are presented Table 3. The results showed no variation (P<0.05) among the semen samples subjected to fresh, refrigeration and freezing cryoprotocols for NF, NN and FF for leukocyte and MDA concentration, the values obtained ranges from 0.20-0.73x10^3/mL and 0.94-2.14x10^6 respectively.

TABLE 1: Interactions of different protocols and strains on the sperm functional attribute of FUNAAB alpha chickens

Protocol	Strain	MOTILITY	LIVABILITY	ABNORMALITIES
Fresh	NF	98.67±1.48^{a}	65.73±5.69	21.09±4.72^{c}
	NN	100.00±1.48^{a}	58.73±5.69	25.53±4.72^{c}
	FF	93.00±1.48^{ab}	64.80±5.69	17.04±4.72^{d}
Refrigeration	NF	92.00±1.48^{ab}	96.00±5.69	32.67±4.72^{b}
	NN	95.00±1.48^{ab}	94.13±5.69	34.36±4.72^{b}
	FF	82.00±1.48^{b}	91.33±5.69	37.71±4.72^{b}
Freezing	NF	35.33±1.48^{c}	75.46±6.54	63.09±5.52^{a}
	NN	28.33±11.48^{d}	61.00±5.69	63.49±4.72^{a}
	FF	34.67±1.48^{c}	76.48±5.69	67.64±4.72a

$^{a, b,.c,d.}$;values within columns with different superscripts differ (P<0.05); NF= normal feather, NN=Naked neck, FF=frizzled feather

TABLE 2: Interactions of different protocols and strains on the sperm functional integrities of FUNAAB alpha chickens

Protocol	Strain	Acrosome Integrity	Membrane Integrity
Fresh	NF	95.67±0.89	98.33±0.82
	NN	97.14±0.89	97.83±0.82
	FF	97.50±0.89	96.67±0.82
Refrigeration	NF	96.33±0.89	97.33±0.82
	NN	97.00±0.89	98.33±0.82
	FF	95.83±0.89	98.00±0.82
Freezing	NF	94.67±0.89	96.00±0.82
	NN	97.00±0.89	93.33±0.82
	FF	96.83±0.89	94.50±0.82

NF= normal feather, NN=Naked neck, FF=frizzled feather.

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

Protocol	Strain	LEU ($x10^3$/mL)	MDA ($\times10^6$)
Fresh	NF	0.40±0.17	0.90±0.26
	NN	0.73±0.17	1.38±0.26
	FF	0.27±0.17	1.61±0.26
Refrigeration	NF	0.20±0.17	1.84±0.26
	NN	0.67±0.17	1.90±0.26
	FF	0.60±0.17	2.14±0.26
Freezing	NF	0.27±0.19	0.94±0.26
	NN	0.47±0.17	0.98±0.26
	FF	0.40±0.17	0.98±0.26

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

Sperm motility of NF, NN and FF was comparable in semen refrigerated and the control compared to semen from the same strains subjected to freezing that had reduced ($P<0.05$) motility except the higher motility in NF and NN observed in fresh semen compared to refrigerated semen from FF. The results showed lower ($P<0.05$) motility in NN subjected to freezing. Livability and sperm abnormality were similar in semen from all the strains refrigerated, frozen and the control.

The present study showed that the strains of FUNAAB alpha chicken sperm motility values were high for all the strains (NF,NN, FF) for refrigeration and control (fresh) but declined for all the strains in freezing protocol, which could be attributed to cold shock due to reduction in temperature. Livability in this study was comparable among the strain with different preservation protocol. Increased abnormal sperm for strains (NF, NN, and FF) subjected to freezing protocol compared with refrigeration and control. The increased sperm abnormality was not because of differences in the strains rather it could be attributed to mechanical injury from freezing which might have led to intracellular or extracellular ice crystal formation and signs of osmotic damage (Watson, 1995).

The results showed that acrosome and membrane integrities were comparable in all the strains irrespective of the protocols used and the control. The present study showed that the acrosome and membrane integrities of the strains semen subjected to different preservation protocols (refrigeration and freezing) were intact and they are comparable to the control.

This was in agreement with the report of Thelie *et al.* (2018) who reported that the storage of semen did not significantly affect sperm functional integrities and in turn did not affect the fertilizing ability of the stored chicken semen. The results from the present studies also ascertained the report of Polge *et al*. (1949) that chicken sperm could stay motile after freezing. The result from this study was however in contrast with the report of Abouelezz *et al*. (2017) and Rakha *et al.* (2017a and 2017b). They reported that the freezing/storage protocols and procedures had significant effect on sperm functional integrities thus there was significant reduction in the fertility potentials of the chickens. The variation could be as a result of different freezing conditions (N_2vapors compared with the use of refrigeration and freezers with programmed temperatures used in the present study). Also, their studies were on wild subspecies of chicken, the Indian red jungle fowl in extensive conditions of use and still without a programmable freezer.

The results showed that leukocyte and MDA concentrations were comparable in all the strains among strains irrespective of the protocols used and the control. The seminal leukocyte and MDA concentration of different strains of FUNAAB alpha chickens strains subjected to different chilling protocols (refrigeration and freezing) were comparable to the control (fresh semen).Seminal oxidative stress decreases the membrane fluidity and impairs its function (Saleh and Agarwal, 2002). Indeed, this decrease in fluidity could affect the membrane transport activity and thereby affecting the survivability of sperm. A number of studies have shown that lipid peroxidation affects the sperm concentration, motility, morphology and related with poor sperm quality (Huang *et al.* 2000; Hsieh *et al*, 2006; Gomez *et al.*, 1998). Kobayashi *et al.* (1991) demonstrated that MDA level in spermatozoa was significantly related to the number of immotile sperm. Suleiman *et al*. (1996) demonstrated that the MDA concentration in the seminal plasma was not correlated with the sperm concentration and motility.

However, the values obtained in this study for all seminal oxidative stress parameters were less than or equal to 2 and the different cryoprotocols were not significantly different from the control, this however suggests that the different cryoprotocols did not have any negative effect on sperm motility, concentration and sperm quality.

Conclusion

The findings revealed that refrigerated semen samples of the three FUNAAB alpha chicken strains maintained sperm viability and fertilizing capacity compared with the frozen semen samples.

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