Effect of vitamin C in egg yolk extender on sperm motility of chilled stored bull semen

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Abstract

An experiment was conducted to evaluate the effect of vitamin C in chicken and quail egg yolk extender on sperm motility in chilled stored bull semen. Semen was collected from the bull with the aid of artificial vagina and six types of extenders were prepared using chicken and quail egg yolk with each extender divided into three aliquots having 0, 3 and 6 mg/mL Vitamin C levels. Bottles containing the diluted semen using the chicken and quail egg yolk extenders were stored in a refrigerator at 5°C over a period of three days and monitored or evaluated at 0, 24, 48 and 72 hours. The result showed that there was a general decline in motility of semen as storage time increases, although samples with 6mg/mL Vitamin C levels had better results. It can be concluded that adding Vitamin C in chicken and quail egg yolk extender maintained motility of chilled semen even at 72 hours of preservation at 5°C.

Keywords: Bull, Semen, Vitamin C, Egg Yolk, Motility.

Introduction

Artificial insemination (AI) is getting significant importance in modern livestock farming all over the world. Genetically superior males are being used to improve production potential of domestic animals. Livestock farmers are rapidly changing their breeding practices from traditional breeding to modern AI; however, lower pregnancy rate with cryopreserved semen is a major obstacle in getting maximum benefits from this technique (Vishwanath, 2003). Semen that has undergone processing with the principle of preservation can be stored in temperatures as low as 5°C and -196°C. However, negative changes in sperm membranes in relation to storage time and the extender have been demonstrated (Frydrychová *et al*., 2010). The use of chilled (liquid) semen has been said to be a cheap solution to the decline fertility of frozen semen and is more effective and efficient (Sri *et al*., 2012) without the need for liquid nitrogen and the incidence of fertility decline compared to frozen semen (Gadea *et al*., 2004).

Chicken egg yolk has been used as a basic component of extenders for bull semen since 1939 (Amirat *et al*., 2004) and still remain popular. Although the addition of egg yolk changes the composition of an extender, it's recommended because of the excellent protection, it offers to sperm cells (Celeghini *et al*., 2008). Also, its wide availability (Sugulle *et al*., 2006) is beneficial effects on sperm viability as a protectant of the plasma membrane and acrosome against cold shock during chilling or cryopreservation (Amirat *et al*., 2004).

Materials and methods

Study site

The study was carried out at the Artificial Insemination Unit of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika-Zaria,

Nigeria. Shika is located in the Northern Guinea Savannah between latitudes 11^0 - 12^0N and between longitudes 7^0E and 8^0E at the elevation of 650 above sea level with an annual maximum and minimum temperature of 31 and 32°C, respectively. Shika has an average annual rainfall of 1100mm usually lasting from May to October with a mean relative humidity of 72% while the dry season lasts from November to April with mean daily temperatures ranging from 15- 36°C and mean relative humidity of between 20-37% (Barje, 2006).

Experimental animals and management

Three Friesian × Bunaji bulls between 2-3 years of age were used for the experiment. The bulls were kept under intensive management system. The bulls were fed a concentrate diet at 1.5% and hay at 2.5% body weight per head per day. Water and mineral salt were provided *ad- libitum.* Animals were sprayed weekly with acaricides and any health problem was attended to regularly. Composition of experimental diet for the bulls is as represented on Table 1.

Table 1: Ingredient composition of bulls' diet

Ingredients	% (Kg)
Maize	45
Wheat offal	22
Cotton seed cake	30
Bone meal	2
Salt	1
Total	100

Experimental treatments

Six types of extenders were prepared using chicken and quail egg yolk with each extender divided into three aliquots having Vitamin C levels at 0, 3 and 6mg/ml respectively. The extenders were prepared according to procedure described by Rekwot *et al*. (1987) as follows:

Preparation of Egg yolk extender

A buffer solution was prepared by dissolving 1.45g of sodium citrate in 50ml of distilled water in a conical flask. Freshly laid eggs were sterilized by cleaning the shell with methylated spirit to avoid introduction of microorganisms. The cleaned eggs were broken in half and the albumen discarded. Any trace of the albumen was removed by rolling the egg yolk carefully on a sterile 25cm filter paper. About 40mL of the buffer solution was put in glass tube and filled up to 50mL with 10ml of the egg yolk. 0.5mL of Streptomycin Sulphate and 0.25mL of Penicillin was added to the extender to reduce contamination by microorganisms. Granulated Vitamin C was added to the extender after preparation of the extenders and mixed thoroughly.

Table 2: Composition of egg yolk sodium citrate extender with varying levels of Vitamin C

	Egg Yolk Extenders					
Components	CEYE	CEYE	CEYE	QEYE	QEYE	QEYE
SCB(mL)	40	40	40	40	40	40
Egg yolk(mL)	10	10	10	10	10	10
Streptomycin(mg/mL)	0.5	0.5	0.5	0.5	0.5	0.5
Penicillin(units/mL)	250	250	250	250	250	250
Vitamin C (mg/mL)	0	3	6	0	3	6

SCB=Sodium citrate buffer, CEYE= chicken egg yolk extender and QEYE= Quail egg yolk extender

Semen dilution and storage

A dilution rate of 1:4 v/v (semen: diluent) was used. The dilution was done in 5ml boujour bottles. The boujour bottles containing the diluted semen using chicken and quail egg yolk extenders were stored in a refrigerator at 5^0C over a period of 0, 24, 48 and 72 hours.

Post-storage semen evaluation

At the end of each storage period, samples of the extender were taken out and warmed in a water bath at 37°C for 10 minutes. Samples of the thawed extender were taken out using a micropipette, placed in a glass slide and covered with a cover slip and analysed under a microscope at 100× magnification.

Sperm motility

A drop of thawed semen was put on a pre-warmed glass slide and was covered with cover slip under a microscope at 40X magnifications, mounted on a warm stage maintained at 37°C. A wet semen mount was made using a drop of semen placed on a microscope slide. Semen samples with 80% initial motility were used for the experiment. Gross motility was estimated as percentage score according to the procedure outlined by Zemjanis (1971).

Table 3: Descriptive and numerical scales for evaluation of gross motility of bull semen

Numerical scale	Descriptive scale	Percentage Sperm Motility	Appearance of pattern
0	Very poor	0-20%	Spermatozoa are immotile. No wave pattern
1	Poor	20-40%	Stationary bunting or weak rotary movements are exhibited by spermatozoa. No wave pattern
2	Fair	40-50%	Oscillatory or rotary movements and fewer than 50% of the spermatozoa are in motion. Barely distinguishable wave or eddies
3	Good	50-80%	Progressive rapid movement of spermatozoa with slowly moving waves and eddies. Usually 50 -80% of the spermatozoa must be progressively motile to produce wave and eddies.
4	Very good	80-90%	Vigorous, progressive movement with rapid and abruptly forming waves and eddies; indicating about 90% motile spermatozoa. Dark, distinct waves.
5	Excellent	90-100%	Very vigorous forward motion. Extremely rapid waves and eddies indicating about 100% actively motile spermatozoa

Source: Zemjanis (1971)

Results and discussion

Spermatozoal motility is the most frequently observed characteristic, however owing to acrosomal damage sperm cell could be highly motile but not fertile. Bovine sperm plasma membrane is predominantly affluent in polyunsaturated fatty acids (PUFA). This high proportion of polyunsaturated fatty acids makes spermatozoa highly prone to oxidation-reduction related cell injuries during processing of frozen semen. The results

obtained in this study emphasize that ascorbic acid can significantly improve post-thaw semen quality of cattle bulls. Ascorbic acid is well known antioxidant that protects the spermatozoa vitality by preventing oxidative damage of DNA and membranes (Padayatty *et al*., 2003). It is believed that ascorbic acid inhibits oxidative species and protects cell membrane and acrosome of sperm cells.

All steps involved in cryopreservation of semen favors ROS generation that eventually reduces fertilizing capacity of the sperms (O'Flaherty *et al*., 1999). Further, intracellular built-in antioxidant competency in spermatozoa declines after cryopreservation. Use of antioxidants has been investigated in a many species (Bilodeau *et al*., 2000; Bucak *et al*., 2010; Neagu *et al*., 2010; Tuncer *et al*., 2010b). In bovines use of antioxidants has resulted in improved semen quality (Beconi *et al.,* 1993; Foote *et al.*, 2002; Andrabi, 2008).

The sperm motility, the capability to undertake the acrosome reaction, the ability to fuse with the vitelline membrane of the egg, and the DNA integrity; all are susceptible to oxidative damage (De-Lamirande and Gagnon, 1992). Sperm motility is dependent on the integrity of the plasma and mitochondrial membranes, which are composed of phospholipids. If fatty acids in the phospholipids are oxidized by ROS, sperm may be damaged and their motility gets impaired (Alvarez and Storey, 1993; De-Lamirande and Gagnon, 1992). DNA integrity is considered more vital importance in semen evaluation. Some authors are of the opinion that genomic status is more objective indicator of sperm's functional status. Sperm cell DNA is prone to injury if its acrosomal integrity is damaged.

The result of this study shows that there was significant difference in the motility of the spermatozoa in the two egg yolk extenders each having Vitamin C included at three levels (Figures 1 and 2). However, after 72 hours it was shown that quail egg yolk extender with 6mg/ml Vitamin C and 3mg/ml had the highest rate of motility followed by chicken egg yolk extender with 6mg/ml Vitamin C inclusion. The least was the chicken egg yolk extender control with 56% motility. The result agrees with Hu *et al.* (2010) and Azawi and Hussein (2013) who reported that inclusion of Vitamin C to Awassi ram and bovine semen extender significantly increased motility at the different times of preservation at 5°C while semen extenders without Vitamin C (control) had lower motile sperm cells. Studies have shown that sperm cells are usually exposed to oxygen and visible light radiation during the process at cryopreservation leading to the formation of reactive oxidative species also known as free radicals (Aysun, 2009) such as Hydroxyl ion, super oxide, lipid peroxides, singles oxygen and excess of ROS (reactive oxidative species) impairs motility and capacity of fertilization due to the oxidative stress damage incurred on the sperm cells by free radicals (Bucak *et al.,* 2010). Anti-oxidants such as Vitamin C are agents that break the oxidative chain reaction thereby reducing oxidative stress thus a decrease in motility (Bansal and Bilaspuri, 2009). In general, anti-oxidants dispose, scavenge and suppress the formation of ROS (Free radicals) hence maintaining motility during cryopreservation (Sikka, 2001).

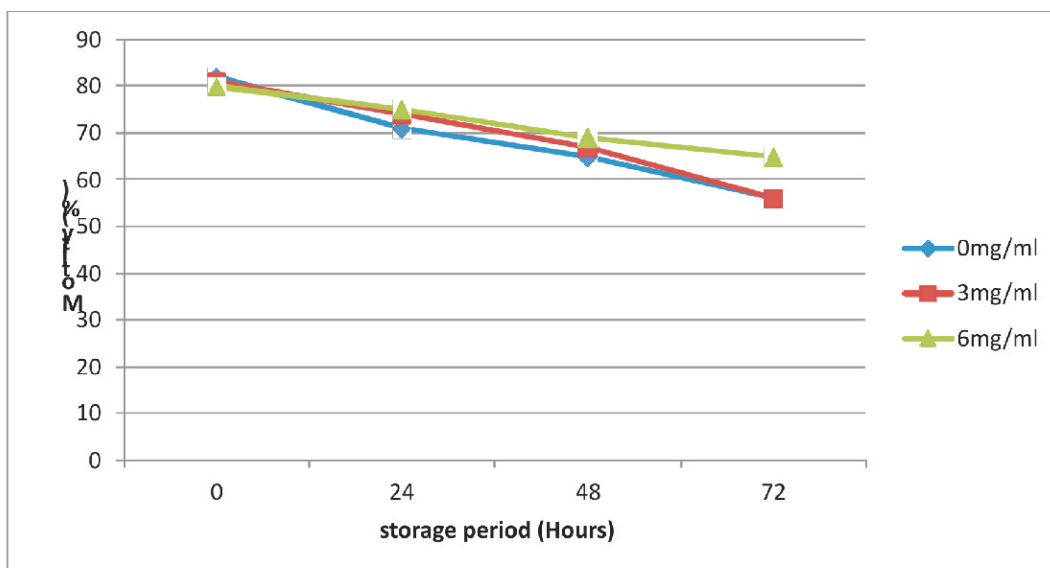

Figure 1: Effect of Vitamin C levels in chicken egg yolk extender on sperm motility in chilled bull semen

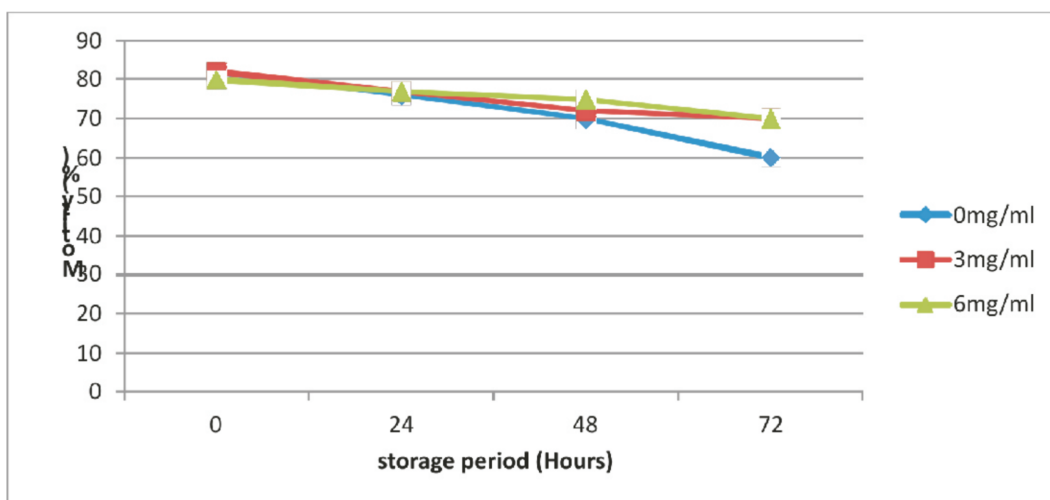

Figure 2: Effect of Vitamin C levels in quail egg yolk extender on sperm motility in chilled bull semen

Conclusion

The study showed that chicken egg yolk extender is effective in cryopreservation of semen bull. Inclusion of vitamin C up to 6mg/ml in chicken egg yolk semen extenders maintained semen quality especially semen motility by protecting spermatozoa against harmful effect of lipid peroxidation by free radicals during liquid storage of bull semen up to 72 hours at 5°C.

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