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Vitamin C inclusion levels in chicken and quail egg yolk tri- sodium citrate extenders on motility and viabilty of friesian x bunaji bull semen over storage period

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Target Audience*:* Animal Reproductive Physiologist/Scientist and Semen Companies

Abstract

An experiment was conducted to evaluate the effect of vitamin c inclusion levels in chicken and quail egg yolk tri-sodium citrate extender on Friesian x Bunaji bull semen characteristics. Semen was collected weekly with the aid of an artificial vagina for five weeks. Three (3) Friesian × Bunaji bulls between 2-3 years of age were used for the study. Vitamin C was included at 0, 3 and 6mg/ml in chicken and quail egg yolk extenders. Samples were analysed for initial spermatozoa characteristics, which include colour, volume, live and dead sperm cells, motility, pH, concentration and morphology. Extended semen was then stored for 0, 24, 48 and 72 hours, respectively at a temperature of about 5^{o}C. The result of this experiment shows that the inclusion levels of Vitamin C on chicken and quail egg yolk extender had significant effect (P<0.05) on motility and viability of Friesian x Bunaji bull spermatozoa. After 72 hours there was significant difference (P<0.05) on motile and viable sperm cells in the quail and chicken egg yolk extenders with higher inclusion level of Vitamin C compared to their control. It can be concluded that inclusion of Vitamin C in chicken and quail egg yolk tri-sodium citrate extenders aid in maintaining chilled semen motility and viability up to 72 hours of storage.

Key words: Friesian x Bunaji, Semen, Vitamin C, Egg yolk, Extender.

Description of Problem

FAO (1) estimated the number of cattle in Africa during the period 2001 to 2010 to be twice the estimates for the years 1961–1970. The role of cattle in developing countries like Nigeria as a source of high–quality food, draft animals, and as source of manure and fuel cannot be over emphasized (2). Cattle represent important contribution to household incomes (3), and in drought prone areas they can serve as an insurance against weather risk (4). As the demand for these animals and their products is constantly increasing around the world, the prospects for increasing the number and productivity of these animals need to be utilized (1). Some techniques of reproductive physiology have been applied to animal breeding for the achievement of faster genetic improvement. These techniques include artificial insemination, oestrus synchronization, induction of multiple ovulation, anti-steroid immunization, long term storage of gametes and embryo transfer (5). Using Artificial insemination (AI) as the

first biotechnology widely implemented in practice is important for selection and breeding of cattle (6). The process of AI involves semen collection, evaluation, processing, preservation and final introduction into the genitalia of an oestrous female (7). Semen that has undergone processing with the principle of preservation can be stored in temperatures as low as 5^0C and -196^0C. However, negative changes in sperm membranes in relation to storage time and the extender has been demonstrated (8; 9). 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 (10) without the need for liquid nitrogen and the incidence of fertility decline compared to frozen semen (11).

Sperm cells have a high content of unsaturated fatty acids in their membranes, while lacking a significant cytoplasmic component containing antioxidants (12). Sperm cells are very susceptible to lipid peroxidation by free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl radical, which could lead to the structural damage of the sperm membranes during the storage (13). Free radicals seek stability by "stealing" electrons from nucleic acids, lipids, and proteins which leads to the damage of cells (14). Free radicals are mostly eliminated by antioxidant systems. The addition of anti-oxidants is well known method to improve viability and motility during cryopreservation of equine sperm cells (15). Ascorbic acid (Vitamin C) is a non-enzymatic antioxidant that plays an important role in scavenging free radicals which otherwise may cause lipid peroxidation of sperm plasma membranes (16). The objective of this study was to evaluate the effect of inclusion levels (0, 3 and 6mg/ml) of Vitamin C on motility and viability of chilled bull semen in chicken and quail egg yolk tri-sodium citrate extenders.

Materials and Methods

Study Area

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 ^{0}E at the elevation of 650 m above sea level with an annual maximum and minimum temperature of 31 and 32^0C, respectively. Shika has an average annual rainfall of 1100 mm 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- 36^0C and mean relative humidity of between 20-37%, as described by (17).

Experimental materials

The materials used for the experiment included:

Microscope, glass slides, cover slips, micro pipette, test tubes, conical flask, beaker (100 ml), haemocytometer, methylated spirit, pH meter, boujour bottles, detergent cotton wool, penicillin and streptomycin, artificial vagina, 25cm sterile whatman filter paper and chemo craft pH paper.

Experimental Animals and their Management

Three (3) Friesian x 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 against ectoparasites with acaricides (benzene hexachloride) and any health problem was attended to promptly.

Semen Collection and Evaluation

Semen was collected by means of an artificial vagina weekly from three bulls on each collection day. Samples from each bull were analysed for initial spermatozoa characteristics which include colour, volume, live and dead sperm cells, motility, pH, concentration and morphology before being pooled together.

Semen Dilution and Storage

A dilution rate of 1:4 v/v (semen: diluent) was used. The dilution was done in 5ml boujour bottles. Sixteen (16) boujour bottles each containing the diluted semen using the different egg yolk extenders were stored in a refrigerator at 5^0C over a period of 3 days and monitored or evaluated at 0, 24, 48 and 72 hours.

Post-Storage Semen Evaluation

At the end of each storage period, four samples, one from each extender group was taken out and thawed at 37^0C for 10 minutes. Samples of the thawed extended semen were taken out using a micropipette, placed in a glass slide, covered with a cover slip and observed under an electronic microscope at x 100 magnifications. The post storage semen characteristics determined were spermatozoa abnormalities (normal sperm, mid piece droplet, detached head, free tail, and coiled tail), motility, viability, and pH. Sperm concentration was determined by means of a spectrophotometer/Beckman model C- 400l calibrated against haemocytometer counts at 600mM wavelength.

Experimental Treatments

Six (6) types of extenders were prepared using chicken and quail egg yolk with each extender divided into three aliquots having 0, 3 and 6mg/ml Vitamin C levels.

Table 1a. Composition of chicken egg yolk Sodium citrate extender with varying levels of Vitamin C

Components	T1	T2	T3
SCB (ml)	40	40	40
CEY	10	10	10
Streptomycin(mg/ml)	0.5	0.5	0.5
Penicillin(units/ml)	250	250	250
Vitamin C (mg/ml)	0	3	6

SCB=Sodium citrate buffer, CEYE=Chicken egg yolk

Table 1b. Composition of Quail egg yolk Sodium citrate extender with varying levels of Vitamin C
SCB=Sodium citrate buffer, QEY=Quail egg yolk

Data Analysis

Data generated was analyzed using the general linear model of SAS (18). Significant means were separated using Duncan Multiple Range Test in the SAS package.

Results and Discussion

Table 2 shows the average values of the characteristics of pooled fresh semen used in the second study. Gross motility ranged between 80 and 85% average 89±1.3% (Table 2). Sperm concentration ranged between 294 and 398 × 10^6 cells per ml (average 357±9.3). Average spermatozoa viability was 88±1.14% ranged from 80-95%, while pH ranged from 6 to 7 with average 6.8±0.1 (Table 2). The effects of inclusion levels of Vitamin C in the Tris-egg yolk extenders on the motility of

chilled bull spermatozoa is shown in Table 3. There was significant increase in sperm ($P<0.05$) with increase in the level of Vitamin C in the extender at different storage periods. Although there were no significant ($P>0.05$) differences in sperm motility between semen extenders across and after 24 hours storage, motility was slightly higher in QEYE than in CEYE. The same trend was maintained over storage period, with motility becoming significantly ($P<0.05$) higher in the QEYE than CEYE across extenders. However, motility values for extenders with Vitamin C were significantly ($P<0.05$) higher than in the extender without Vitamin C.

The result of the present 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. However after 72 hours it was shown that quail egg yolk extender with 3mg/ml and 6mg/ml Vitamin C had significantly higher (70%) rate of motility followed by chicken egg yolk extender (60%) with 6mg/ml Vitamin C inclusion. The least motility (65%) was observed in chicken egg yolk extender in the control. The result agrees with (12) and (13) who reported that inclusion of Vitamin C to Awassi ram and bovine semen extender significantly increased motility at the different times of preservation at $5^{o}C$ while semen extenders without Vitamin C (control) had lower motile sperm cells respectively. 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 (ROS) also known as free radicals (19) such as Hydroxyl ion, super oxide, lipid peroxides, singles oxygen and excess of ROS impairs motility and capacity of fertilization due to the oxidative stress damage incurred on the sperm cells by free radicals (20). Anti-oxidants such as Vitamin C are agents that break the oxidative chain reaction thereby reducing oxidative stress which leads to the decrease in motility (21). In general anti-oxidants dispose, scavenge and supress the formation of ROS (Free radicals) hence maintaining motility during cryopreservation (22).

Table 4 shows viability of chilled bull spermatozoa stored in semen extenders at various levels of Vitamin C. The result shows a general, though not significant ($P>0.05$) increase in percentage of viable spermatozoa with increase in the levels of Vitamin C across the period. Although, a non significant ($p>0.05$) difference was observed in sperm viability with increase in storage periods, viability declined numerically from 82 to 61 % (Table 4) and 82 to 59 % in samples stored CEYE and QEYE without Vitamin C respectively. After 72 hours of storage, similarly sperm viability decline from 80 to 64% and 80 to 67% in CEYE and QEYE with 3mg/ml Vitamin C respectively and from 80 to 70% in CEYE and QEYE extenders with 6mg/ml respectively after 72 hours of storage. Within storage period, there was no significant ($P>0.05$) difference in viability between CEYE and QEYE diluents regardless of the level of Vitamin C.

Even though there was no significant effect on the viability of the sperm cell in the two egg yolk extenders with the three inclusion levels of Vitamin C quail egg yolk extender with 3 and 6mg/ml of Vitamin C after 72 hours had relatively higher percentage of viable sperm cells followed by chicken with 3 and 6mg/ml of Vitamin C with both controls having relatively lower percentage of viable sperm cells. This result agrees with reports by (23, 12 and 24) which showed that Vitamin C supplementation during cryopreservation of bull and ram semen had significant effect on the percentage of viable sperm cells improving percentage viability of sperm cell after preservation.

Sperm cells have a high content of

unsaturated fatty acids in their membranes, but lack significant cytoplasmic component containing antioxidants (25). Lipid peroxides are spontaneously generated in the sperm plasma membrane and are released by the action of phospholipase A2 (26). They are capable of inducing DNA damage, decrease in percentage of viable sperm cells and fertility during and after cryopreservation (20). The addition of Vitamin C in extenders can bring about an optimal sperm performance by increasing the percentage of viable cells after cryopreservation by scavenging this lipid peroxides (12).

Conclusion and Application

1. Inclusion of Vitamin C up to 6mg/ml in both chicken and quail egg yolk semen extenders maintained semen quality by protecting spermatozoa against harmful effect of lipid peroxidation by free radicals during liquid storage of bull semen up to 72 hours.
2. On the overall, both chicken and quail egg yolk can be used as extenders for chilled semen.

Table 2: Summary statistics of initial bull semen characteristics.

Characteristics	Mean±SE	Coefficient of Variation (%)	Minimum	Maximum
Pooled semen volume(ml)	7.5±0.2	4.9	5	14
Gross motility (%)	89±1.3	7.3	95	80
Semen pH	6.8±0.1	6.6	6	7
Sperm Concentration($\times 10^6$)	357±9.3	13	294	398
Viability (%)	88±1.14	6.5	80	95
Sperm Morphology (%)				
MPD	0±0.1	223	0	1
DH	1±0.2	104	0	1
FT	1±0.2	81.4	0	3
CT	1±0.1	39.1	1	2
BT	2±0.2	46.5	1	3
NS	95±0.4	1.9	92	96

Standard Error = SE, Normal Sperm (NS), Free Tail (FT), Mid Piece Droplet (MPD), Detached Head (DH), Coiled Tail (CT) and Bent Tail (BT)

Table 3: Effect of Vitamin C levels on Sperm Motility of Chilled Bull semen in Chicken and Quail Egg yolk extender at different Storage Periods.

		Vitamin C Level (mg/ml)		
Storage period(hours)	Extenders	0	3	6
0	CEYE	82.0	81.0	80.0
	QEYE	82.0	82.0	80.0
	SEM	1.6	1.6	1.6
	LOS	NS	NS	NS
24	CEYE	71.0[b]	74.0	75.0
	QEYE	76.0[a]	77.0	77.0
	SEM	1.6	1.6	1.6
	LOS	*	NS	NS
48	CEYE	65.0[b]	67.0[b]	69.0[b]
	QEYE	70.0[a]	72.0[a]	75.0[a]
	SEM	1.6	1.6	1.6
	LOS	*	*	*
72	CEYE	56.0[b]	56.0[b]	65.0[b]
	QEYE	60.0[a]	70.0[a]	70.0[a]
	SEM	1.6	1.6	1.6
	LOS	*	*	*

[ab]Means within the same columns in the same storage period with different superscripts are significantly different (P<0.05). SEM= Standard Error of Mean, LOS= Level of Significance. CEYE=Chicken Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, V/C= Vitamin C.

Table 4: Effect of Vitamin C levels on Sperm Viability of Chilled Bull semen in Chicken and Quail Egg yolk extender at different Storage Periods.

		Vitamin C Level(mg/ml)		
Storage period(hours)	Extenders	0	3	6
0	CEYE	82.0	80.0	80.0
	QEYE	82.0	80.0	85.0
	SEM	2.3	2.3	2.3
	LOS	NS	NS	NS
24	CEYE	72.0	74.0	76.0
	QEYE	72.0	74.0	73.0
	SEM	2.3	2.3	2.3
	LOS	NS	NS	NS
48	CEYE	68.0	68.0	69.0
	QEYE	68.0	72.0	72.0
	SEM	2.3	2.3	2.3
	LOS	NS	NS	NS
72	CEYE	61.0	64.0	66.0
	QEYE	59.0	67.0	70.0
	SEM	2.3	2.3	2.3
	LOS	NS	NS	*

[abcd]Means within the same column with different superscripts are significantly different (P<0.05). SEM= Standard Error of Mean, LOS= Level of Significance. CEYE=Chicken Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, V/C= Vitamin C

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