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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng
Website: http://journals.napri.gov.ng

EFFECT OF FOUR DIFFERENT AVIAN EGG YOLKS TRI- SODIUM CITRATE EXTENDER ON STORAGE TIME OF FRIESIAN X BUNAJI SEMEN CHARACTERISTIC

[1]Achi, J. N., [2]Barje, P. P., [2]Rekwot, P. I., [2]Achi, N. P. and [3]Alphonsus, C.
[1]Departemnt of Animal Science, Ahmadu Bello University, Zaria.
[2]National Animal Production Research Institute/Ahmadu Bello University, Shika-Zaria.
[3]Department of Animal Science, Kaduna State University.
Corresponding: jessicagoje@gmail.com. +2348038802646

ABSTRACT

An experiment was conducted to evaluate the effect of four different avian egg yolk tri-sodium citrate extender on Friesian x Bunaji semen characteristics at the Artificial Insemination Unit of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika-Zaria, Nigeria. Three (3) Friesian x Bunaji bulls between 2-3 years of age were used for the experiment. Semen was collected with the aid of an artificial vagina for five weeks on a weekly interval. Four (4) types of extenders were prepared using egg yolk from four avian species namely; chicken, guinea fowl, turkey and quail stored at 0, 24, 48 and 72 hours, respectively. The experiment showed that egg yolks had significant effect ($P<0.05$) on motility, viability and total abnormalities of bovine spermatozoa. Chicken egg yolk Tris-sodium citrate extender had significantly higher ($P<0.05$) motility and viability with lower ($P<0.05$) total abnormalities after 72 hours. Turkey egg yolk extender had significantly lower ($P<0.05$) motile, viable sperm cells and higher ($P<0.05$) total abnormalities. Quail egg yolk extender had significantly lower ($P<0.05$) sperm abnormalities than turkey egg yolk extender though there was no significant difference in their motility after 72 hour. It was concluded that chicken egg yolk extender is the best in cryopreservation of bull semen and is followed by quail egg yolk.

Keywords: Egg yolks, Extenders, Semen Characteristics, Cryopreservation

INTRODUCTION

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 (Taddesse *et al*., 2003). Cattle represent important contribution to household incomes (Seo and Mendelsohn, 2006), and in drought prone areas they can serve as an insurance against weather risk (Fafchamps and Gavian, 1997). 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 (FAO, 2011). 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 (Gordon, 2004). Using Artificial insemination (AI) as the first biotechnology widely implemented in practice is important for selection and breeding of cattle (Gravance *et al*., 2009). The process of AI involves semen collection, evaluation, processing, preservation and final introduction into the genitalia of an oestrous female (Thibier and Wagner, 2002). 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 (Watson, 2000[b]; 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).

Chilling semen can be prepared using extenders which are based on either ionic or non-ionic substances that prevent changes in osmolality and act as a buffer against changes in pH (Vishwanath and Shannon, 2000). Semen diluents (extenders) provide nutrients for spermatozoa during the storage period, allowing spermatozoa to be able to keep moving progressively. They are non-toxic to spermatozoa, contain buffer and are able to protect the spermatozoa from cold shock during cooling or freezing processes (Sri *et al*., 2012).

The objective of this study was to evaluate the effect of including egg yolks from different avian species egg yolk (chicken, turkey, guinea fowl and quails) in tri-sodium citrate diluents (extenders) on characteristics of chilled bull semen on freeze storage.

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 ^{0}E at the elevation of 650 above sea level with an annual maximum and minimum temperature of 31^0C and 32^0C, 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- 36^0C and mean relative humidity of between 20-37%, as described by Barje (2006).

Experimental materials

The materials used for the experiment included

Microscope, Slides and cover slips and micro pipette, Test tubes, conical flask, Beaker (100ml), 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 Management

Three (3) FriesianxBunaji 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.

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.

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

Four (4) types of extenders were prepared using egg yolk from four avian species namely chicken, guinea fowl, turkey and quail.

Table 2: Composition of Egg Yolk Tris-Sodium Citrate Extender.

	Egg Yolk Extenders			
Components	CEYE	TEYE	QEYE	GEYE
SCB(ml)	40	40	40	40
Egg yolk(ml)	10	10	10	10
Streptomycin(mg/ml)	0.5	0.5	0.5	0.5
Penicillin(units/ml)	250	250	250	250

SCB=Sodium citrate buffer, CEYE=Chicken Egg yolk Extender, TEYE= Turkey Egg yolk Extender, QEYE= Quail Egg yolk Extender, GEYE= Guinea fowl 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. 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 extender were taken out using a micropipette, placed in a glass slide, covered with a cover slip and observed under an electronic microscope at 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. The experiment was replicated at weekly interval over a period of 5 weeks.

Experimental design and Data analysis

The experimental design used was a 4×4 Factorial arrangement in a Completely Randomized Design, with 4 egg yolk extenders (chicken, guinea fowl, quail and turkey) at 4 storage periods (0, 24, 48 and 72 hours). The model used for the analysis is as follows:

$Y_{ijk} = \mu + A_i + B_j + (A \times B)_{ij} + e_{ijk}$

Where:

Y_{ijk} = Observations of the semen characteristics.

μ = Overall mean

A_i = Effect of i^{th} egg yolk extender (i = 1, 2, 3, 4)

B_j = Effect of j^{th} Storage Period (j= 0, 24, 48 and 72 hours)

$(A \times B)_{ij}$ = Interaction between Egg yolk extenders and storage periods.

e_{ijk}= Random error

Data was analysed using the Analysis of Variance (ANOVA) of Statistical Analysis System (SAS, 2002). Significant means were separated using Duncan Multiple Range Test in the SAS package.

RESULTS AND DISCUSSION

The result of this study (Table 3) shows that chicken egg yolk had the highest motility while turkey egg yolk had the least after 72 hours of storage. This result agrees with Kalaksiz *et al*. (2010) and Rauch (2013) who reported that bull and ram sperm motility was poorest in turkey egg yolk extenders compared to other egg yolks used. This may be due to its dense egg yolk particles (Nagy *et al*., 2003). However, it disagrees with Burris and Webb (2009) who reported that inclusion of turkey egg yolk in semen extender produced the highest post thaw sperm motility than three other egg yolks which include chicken egg yolk, while Akhter *et al*. (2010) reported that chicken egg yolk had least sperm motility in Sawhili bulls when compared to turkey, guinea fowl and pigeon egg yolks post thawing. Motility is a strong indicator of spermatozoa quality during semen evaluation that is why it is widely used in fertility laboratories. Studies show that reduction in semen cholesterol was observed in spermatozoa after the cryopreservation cycle, which correlated with an increased number of acrosome-reacted cells and changes in the parameters of motility in boar spermatozoa (Maldjian *et al*., 2005). In general, studies have shown that phospholipids, cholesterol and low density lipoproteins in egg yolk may be the factors that provide protection to sperm against cold shock during cooling- thaw process of cryopreservation (Anton *et al*., 2006; Kalaksiz *et al*., 2010). It have been suggested that avian species and even breeds of specie have different combination of these factors (Burris and Webb, 2009).This may be the reason behind the discrepancies in motility of the spermatozoa in the different avian egg yolks.

After 72 hours, chicken egg yolk extender significantly increased the number of viable sperm cells followed by quail egg yolk. However, guinea fowl and turkey egg yolk extenders had significantly the same sperm viability. This result is similar to the findings of Su *et al.* (2008) and Kalaksiz *et al.* (2010) who reported that turkey egg yolk extender had the least viable sperm cells when evaluating ram and bovine semen characteristics stored in different avian specie egg yolk extender. However, Ibrahim *et al*. (2012) reported that duck egg yolk had more viable sperm cells than chicken egg yolk when used to preserve buffalo bull semen and Gholami *et al*. (2012) when evaluating the effect of egg yolk of four avian species on cryopreserved ram spermatozoa.

Table 3: Summary of initial bull semen characteristics.

Characteristics	Mean±SE	Coefficient of Variation (%)	Minimum	Maximum
Pooled semen volume(ml)	7.8±1.6	38.9	2.4	14.5
Gross motility (%)	92±0.5	2.7	90	95
Semen pH	6.2	6.58	6	7
Sperm Concentration($\times 10^6$)	462.3±30.6	33.3	314	649
Viability (%)	81±2.7	16.6	60	95
Sperm Morphology (%)				
MPD	0±0.1	223.6	0	1
DH	9±0.7	36.9	4	13
FT	3±0.6	120.4	0	7
CT	4±0.4	53.0	2	7
BT	6±1.1	92.1	0	15
NS	78±1.8	11.6	64	88

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

Table 4: Effect of Egg yolk extender on Sperm Motility of Chilled Bull semen at different Storage Periods.

	Storage Period (hours)			
Extenders	0	24	48	72
CEYE	88.0[a]	78.0[a]	72.0[a]	66.0[a]
GEYE	82.0[c]	72.0[b]	59.0[c]	55.0[c]
QEYE	85.0[b]	71.0[b]	66.0[b]	60.0[b]
TEYE	82.0[c]	70.0[c]	50.0[d]	46.0[d]
SEM	2.97	2.97	2.97	2.97
LOS	*	*	*	*

[abcd]Means within the same column with different superscript are significantly different (P<0.05). SEM= Standard Error of Mean, LOS= Level of Significance., EYE=Chicken Egg Yolk Extender, TEYE= Turkey Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, GEYE= Guinea fowl Egg Yolk Extender,

During cryopreservation, the sperm cell gets nourishment and protection of acrosome and plasma membrane from the cholesterol phospholipids and low density lipoprotein components of the egg yolk hence increasing their viability (Foote, 2002). Egg yolk phospholipid detoxifies the seminal plasma by grabbing the harmful proteins in it thereby increasing the viability of sperm cell (Bathgate *et al*., 2006). These factors could be the responsible for improved viability in sperm cells observed.

The result effect of different egg yolk extender on pH of chilled bull semen at different storage periods on table 6 shows that there was no significant difference. This result agrees with Foote *et al*. (2002) and Vishwanath (2003) who reported that pH of bull semen extenders ranges from 6-7. pH of the semen is the hydrogen ion concentration of the semen which indicates the degree of acidity and alkalinity of the semen and it ranges from 1-14 respectively.

Table 5: Effect of different Egg yolk extenders on Viability of sperm in of Chilled Bull semen at different Storage Periods.

	Storage Period (hours)			
Extenders	0	24	48	72
CEYE	79.0^{bc}	77.0^{ab}	72.0^{a}	71.0^{a}
GEYE	76.0^{c}	73.0^{abc}	72.0^{a}	54.0^{c}
QEYE	87.0^{a}	73.0^{abc}	72.0^{a}	63.0^{b}
TEYE	76.0^{c}	71.0^{c}	68.0^{b}	54.0^{c}
SEM	2.13	2.13	2.13	2.13
LOS	*	*	*	*

^{abcd}Means within the same column with different superscript are significantly different (P<0.05). SEM= Standard Error of Mean, LOS= Level of Significance, CEYE=Chicken Egg Yolk Extender, TEYE= Turkey Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, GEYE= Guinea fowl Egg Yolk Extender.

Table 6: Effect of different Egg yolk extender on pH of Chilled Bull semen at different Storage Periods.

	Storage Period (hours)			
Extenders	0	24	48	72
CEYE	6.4^{b}	6.2	6.4	6.4
GEYE	6.8^{a}	6.0	6.2	6.2
QEYE	6.2^{b}	6.0	6.2	6.2
TEYE	6.4^{b}	6.0	6.4	6.4
SEM	0.1	0.1	0.1	0.1
LOS	*	NS	NS	NS

^{ab}Means within the same column with different superscript are significantly different (P<0.05). SEM= Standard Error of Mean, LOS= Level of Significance CEYE=Chicken Egg yolk Extender, TEYE= Turkey Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, GEYE= Guinea fowl Egg Yolk Extender.

Although there was no significantly difference in the effects of the four egg yolk extenders on percent normal sperm cells across storage time, after 72 hours. Chicken egg yolk extender recorded the highest number of normal sperm cells, followed by quail egg yolk, turkey egg yolk and then guinea fowl egg yolk extender recorded the least.

There was significant difference in DH, FT, CT and BT after 72 hour of storage with more sperm abnormalities observed in turkey egg yolk and guinea fowl egg yolk than chicken egg yolk and quail egg yolk respectively. There was no significant difference between the egg yolk on the MPD of the spermatozoa. This result disagrees with that of Su *et al.* (2008), Akhter *et al*. (2012) and Gholami *et al*. (2012) who reported that chicken among other avian specie egg yolk during cryopreservation of bull and ram had more abnormalities and less normal cells.

Maldjian *et al*. (2005) reported that spermatozoa were damaged significantly by the cryopreservation cycle hence increasing percentage of head, neck and tail abnormalities. Low density protein of egg yolk is proposed to be the main factor for protecting spermatozoa against abnormalities, and it does this by stabilizing the membrane phospholipid losses and engulfing any harmful seminal plasma protein (Manjunath *et al*., 2002).

Table 7: Effect of different Egg yolk extender on Percentage Normal Sperm in Chilled Bull semen at different Storage Periods.

	Storage Period (hours)			
Extenders	0	24	48	72
CEYE	84.0	84.0	84.3	82.0[a]
GEYE	85.0	83.0	82.0	78.0[c]
QEYE	86.0	83.0	81.0	81.0[abc]
TEYE	86.0	84.0	82.0	81.0[abc]
SEM	1.9	1.9	1.9	1.9
LOS	NS	NS	NS	*

[abcd]Means within the same column with different superscript are significantly different (P<0.05). SEM= Standard Error of Mean, LOS= Level of Significance. CEYE=Chicken Egg Yolk Extender, TEYE= Turkey Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, GEYE= Guinea fowl Egg Yolk Extender.

Table 8: Effect of different Egg yolk extender on Percentage Sperm abnormalities of Chilled Bull semen at different Storage Times.

		Storage Time (hours)				
Abnormalities	Extenders	0	24	48	72	SEM
MPD	CEYE	0.0	0.0	0.0	0.0	0.04
	GEYE	0.0	0.0	0.0	0.0	
	QEYE	0.0	0.0	0.0	0.0	
	TEYE	0.0	0.0	0.0	0.0	
DH	CEYE	6.0^{a}	6.0^{b}	9.0^{a}	8.0^{b}	1.18
	GEYE	5.0^{b}	6.0^{b}	9.0^{a}	9.0^{a}	
	QEYE	6.0^{a}	7.0^{a}	7.0^{b}	8.0^{b}	
	TEYE	5.0^{b}	7.0^{a}	7.0^{b}	10.0^{a}	
FT	CEYE	2.0^{c}	3.0^{b}	4.0^{b}	6.0^{b}	0.66
	GEYE	3.0^{b}	3.0^{b}	5.0^{a}	8.0^{a}	
	QEYE	3.0^{b}	3.0^{b}	4.0^{b}	5.0^{c}	
	TEYE	4.0^{a}	4.0^{a}	4.0^{b}	5.0^{c}	
CT	CEYE	1.0^{a}	1.0^{b}	1.0^{b}	2.0^{b}	0.42
	GEYE	0.0^{b}	1.0^{b}	1.0^{b}	3.0^{a}	
	QEYE	1.0^{a}	2.0^{a}	2.0^{a}	2.0^{b}	
	TEYE	1.0^{a}	1.0^{b}	2.0^{a}	2.0^{b}	
BT	CEYE	4.0^{a}	4.0^{a}	4.0^{ab}	5.0^{b}	0.77
	GEYE	3.0^{ab}	4.0^{a}	5.0^{a}	6.0^{ab}	
	QEYE	3.0^{ab}	4.0^{a}	5.0^{a}	5.0^{b}	
	TEYE	3.0^{ab}	3.0^{ab}	4.0^{ab}	8.0^{a}	

^{abcd}Means within the same column with different superscript are significantly different (P<0.05). CEYE=Chicken Egg Yolk Extender, TEYE= Turkey Egg Yolk Extender, QEYE= Quail Egg Yolk Extender, GEYE= Guinea fowl Egg Yolk Extender, SEM= Standard Error of Mean, LOS= Level of Significance. Normal Sperm =NS, Free Tail=FT, Mid piece Droplet =MPD, Detached Head=DH, Coiled Tail=CT and Bent Tail

CONCLUSION

From the result of this study, it was concluded that chicken egg yolk extender did best in cryopreservation of bull semen and quail egg yolk can be used as an alternative to conventional use of chicken egg yolk due to lower abnormalities of sperm cells stored in it after 72 hours.

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