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EFFECT OF VITAMIN C AND SEMEN EXTENDER ON pH , STORAGE PERIOD AND PERCENTAGE NORMAL CELLS OF CHILLED SEMEN OF FRIESIANXBUNAJI COWS

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ABSTRACT

An experiment was conducted to evaluate the effect of vitamin c levels on P^H and percentage normal cells of chilled Friesian x Bunaji bull semen in chicken and quail egg yolk extender at different storage periods. The semen was collected from three bulls between 2 – 3 years of age weekly with the aid of artificial vagina. P^H was determined using Chemo craft pH paper and pH meter. Extenders were prepared from the egg yolk of chicken and quail at three inclusion levels (0, 3 and 6mg/ml) of Vitamin C. The results shows that the inclusion levels of Vitamin C on chicken and quail egg yolk extender had significant effect ($P<0.05$) on normal cells of bull spermatozoa but no significant effect ($P>0.05$) on semen pH at 0, 24, 48 and 72 hours respectively. However, after 72 hours there was significantly higher ($P<0.05$) normal sperm cells in the quail and chicken egg yolk extenders with higher inclusion level of Vitamin C compared to their control. In conclusion inclusion of Vitamin C up to 6mg/ml in both chicken and quail egg yolk semen extenders maintained semen quality up to 72 hours.

Keywords: Semen, Friesian x Bunaji, Vitamin C, Semen Extender

INTRODUCTION

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

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, however 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) and 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) make it a popular semen extender. The phospholipids, cholesterol and low density lipoproteins of chicken egg yolk specifically have been identified as the protective components (Anton *et al*., 2006). However, the chemical composition of the egg yolk of different avian species varies particularly in terms of cholesterol, low density lipoproteins and phospholipid contents (Burris and Webb, 2009). These may influence their effectiveness when chilling, freezing and post-thawing semen extenders (Bathgate *et al*., 2006).

Sperm cells have a high content of unsaturated fatty acids in their membranes, while lacking a

significant cytoplasmic component containing antioxidants (Hu *et al*., 2010). 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 (Azawi and Hussein, 2013). Free radicals seek stability by "stealing" electrons from nucleic acids, lipids, and proteins which leads to the damage of cells (Yue *et al*., 2010). 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 (Ball *et al*., 2001). 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 (Baumber *et al*., 2000).

The objective of the study was to evaluate the effect of vitamin c levels on P^H and percentage normal cells of chilled Friesian x Bunaji bull semen in chicken and quail egg yolk extender at different storage periods.

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 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%, (IAR, 2014).

Experimental Animals and Management

Three (3) 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.

Semen Collection and Evaluation

Semen was collected weekly by means of an artificial vagina from three bulls on each collection day.

Semen pH

The hydrogen ion concentration (pH) of individual sample collected was determined using Chemo craft pH paper and pH meter. This is a pH paper calibrated from 1 to 14 with different colours. One inch of the paper was inserted into the semen while in the tube for five seconds, then it was removed and air dried properly. The colour change observed after drying were placed to the colour chart on the pH paper. The corresponding pH number for the colour on the chart was recorded as the pH of the sample.

The extenders were prepared according to procedure described by Rekwot *et al*., (1997).

Semen Dilution and Storage

A dilution rate of 1:4 v/v (semen: diluent) was used. The dilution was done in 5ml boujour bottles. 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.

Statistical Analysis

Data was analyzed using the Analysis of Variance (ANOVA) of Statistical Analysis System (SAS, 2002). Significant means were separated using Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSIONS

Table 1 shows the effect of vitamin C on pH of chilled bull semen in chicken and quail egg yolk extenders at different storage periods. There was no significant difference in pH of chicken and quail egg yolk extenders with

varying levels of vitamin C during the storage period. The pH fluctuated between 6.3 and 6.5 on the treatment that had 6mg/ml inclusion level of vitamin C, while the pH remained constant at 6.3 in all the other treatments. The observation that inclusion of different levels of Vitamin C in chicken and quail egg yolk extenders had no significant effect on pH agrees with Fazeli *et al.*(2010) who reported that Vitamin C had no significant effect on Markhoz goats seminal pH when added to a semen extender during cryopreservation. Generally pH whether in fresh semen or semen extender ranges from 6 - 7. However, report by Gernah, (2007) showed that dietary intake of Vitamin C by man had significant effect on the seminal pH when assessed.

Table 2 shows the effect of vitamin C on percentage normal sperm cells of chilled bull semen in chicken and quail egg yolk extenders at different storage periods. There was a general no significant ($P>0.05$) effect on the percentage of normal cells with increase in storage time except at 0 hour of storage There was generally significant ($P<0.05$) decline in percentage of normal sperm cells with increase in Vitamin C levels in both chicken and quail egg yolk extenders across storage periods. At 0 hours, there was a range of 88-90% normal sperm cells amongst the treatments. After 24 hours, there was no significant ($P>0.05$) decline in the percentage of normal cells in the extenders. CEYE and QEYE control treatment with 3mg/ml inclusion of vitamin C had no significant ($P>0.05$) difference but there was significant difference between both extenders having 6mg/ml inclusion of vitamin C. However, QEYE with 3mg/ml vitamin C inclusion had significantly ($P<0.05$) highest percentage of viable cells (88%) and CEYE with 3 and 6mg/ml vitamin C inclusion had significantly ($P<0.05$) the least viable sperm cell.

Table 1: Effect of Vitamin C levels on pH 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	6.4	6.4	6.4
	QEYE	6.4	6.4	6.3
	SEM	0.1	0.1	0.1
	LOS	NS	NS	NS
24	CEYE	6.4	6.4	6.3
	QEYE	6.4	6.4	6.4
	SEM	0.1	0.1	0.1
	LOS	NS	NS	NS
48	CEYE	6.4	6.4	6.3
	QEYE	6.4	6.4	6.5
	SEM	0.1	0.1	0.1
	LOS	NS	NS	NS
72	CEYE	6.4	6.4	6.4
	QEYE	6.4	6.4	6.3
	SEM	0.1	0.1	0.1
	LOS	NS	NS	NS

SEM- Standard Error of Mean, LOS - Level of Significance, NS - Not significant, CEYE - Chicken Egg Yolk Extender, QEYE - Quail Egg Yolk Extender.

Table 2: Effect of Vitamin C levels on Percentage Normal Sperm Cells 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	90.0[a]	86.0[b]	88.0
	QEYE	88.0[b]	90.0[a]	90.0
	SEM	1.6	1.6	1.6
	LOS	NS	*	NS
24	CEYE	87.0	84.0	84.0
	QEYE	85.0	88.0	87.0
	SEM	1.6	1.6	1.6
	LOS	NS	NS	NS
48	CEYE	85.0	83.0	83.0
	QEYE	84.0	84.0	85.0
	SEM	1.6	1.6	1.6
	LOS	NS	NS	NS
72	CEYE	75.0	82.0	84.0
	QEYE	74.0	81.0	84.0
	SEM	1.6	1.6	1.6
	LOS	NS	NS	NS

[ab]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.

CONCLUSION

In conclusion, this experiment reveals that chicken egg yolk extender with 6mg/ml of vitamin C was the best in cryopreservation of bull semen. .

REFERENCES

Amirat, L., Tainturier, D., Jeanneau, L., Thorin, C., Gérard, O., Courtens, J. L., and Anton, M. (2004). Bull Semen In-Vitro Fertility After Cryopreservation Using Egg Yolk LDL: A Comparison With Optidyl®, A Commercial Egg Yolk Extender.*Theriogenology*, 61:895-907.

Anton, M., Nau, F., andNys, Y. (2006). Bioactive egg components and their potential uses. *World's Poultry Science Journal*, 62(4):29-38.

Azawi, O. S. and Hussein, E. K. (2013). Effect of Vitamin C or E Supplementation to Tris Diluent on Semen Quality of Awassi Rams Preserved at 5^0C. *Veterinary Research Forum*, 4(3): 157-160.

Ball, B. A., Medina, V., Gravance, C. G., and Baumber, J. (2001). Effects of Oxidants on Preservation of Motility, Viability and Acrosome Integrity of Equine Spermatozoa during Storage at 5^0C. *Theriogenology*, 56: 577-587.

Bathgate, R., Maxwell, W. M.C., and Evans, G. (2006). Studies on the Effect Of Supplementing Boar Semen Cryopreservation Media With Different Avian Egg Yolk Types on In-Vitro Post-Thaw Sperm Quality. *Reproduction of Domestic Animals*, 41: 68-73.

Baumber, J., Ball, B.A. and Gravance, C. G. (2000).The Effect of Reactive Oxygen Species on Equine Sperm Motility, Viability, Acrosomal Integrity, Mitochondrial Membrane Potential and Membrane Lipid Peroxidation.*Journal of Andrology*, 21: 895-902.

Burris, C. And Webb,G.(2009). Effects of Egg Yolk Source on the Cryopreservation of Stallion Semen. *Journal of Equine Veterinary Science*, 29(33): 6-7.

Celeghini, E.C.C., De Arruda, R.P., De Andrade, A.F.C., Nascimento, J., Raphael, C.F., and Rodrigues, P.H.M. (2008). Effects that Bovine Sperm Cryopreservation Using Two Different Extenders have on Sperm Membranes and Chromatin. *Animal Reproduction Science*, 104: 119-131.

Duncan, D. B. (1955).Multiple Ranges and Multiples F-tests.*Biometrics*, 11: 1-42.

Fazeli, P., Zamiri, M. J., Farshad, A., and Khalili, B. (2010). Effects of Vitamin C on Testicular and Seminal Characteristics of Markhoz Goats. *Iranian Journal of Veterinary Research*, 11(3): 1-6.

Gernah, D. I., Atolagbe, M.O., and Echegwo, C.C. (2007). Nutritional Composition of the African Locust Bean (*ParkiaBiglobosa*) Fruit Pulp. *Nigerian Food Journal*, 25 (1):190-196.

Hu, J., Tan, W., and ZhoaX. (2010). The Cryoprotective Effect of Ascorbic Acid Supplementation on Bovine semen Quality. *Animal Reproductive Science*, 12(1):71-72.

I.A.R. (2014) Institute for Agricultural Research. Meteorological station report.

Rekwot, P. I.,Oyedipe, E. O., Dawuda, P.M., and Sekoni, V. O.(1997). Age And Hourly Related Changes Of Serum Testosterone and Spermiogramof Pre- Pubertal Bulls Fed Two Levels of Nutrition. *The Veterinary Journal*, 153: 341-347.

Sri, W., Hermanto,N., Agus, B., and Panji, B. (2012). Effects of Sperm Concentration and Length of Storage at 5^0C on Motility of Goat Spermatozoa. *World Academy of Science, Engineering and Technology*, 66:1099-1101.

Statistical Analysis System (2002). SAS User's Guide. SAS institute. Inc. Cary NC.

Sugulle, A. H., Bhuiyanm, M. U., and Shamsuddin, M. (2006). Breeding

Soundness of Bulls and Quality of their Frozen Semen used in Cattle Artificial Insemination in Bangladesh. *Livestock Research for Rural Development*, 18(54): 14-18.

Vishwanath, R. and Shannon, P. (2000).Storage of Bovine Semen in Liquid and Frozen State.*Animal Reproduction Science*, 62: 23-53.

Yue, D., Yan, L. and Luo, H. (2010).Effect of Vitamin E Supplementation on Semen Quality and Testicular Cell Membrane and Mitochondrial Antioxidant Abilities in AohanFinewool Sheep. *Animal Reproduction Science*, 118: 217-222.