ISSN:
Print - 2277 - 0755
Online - 2315 - 7453

Journal of Agricultural Science and Environment

COMPARATIVE EFFECTS OF EGG YOLKS FROM DIFFERENT POULTRY BREEDS ON VIABILITY OF REFRIGERATED SPERMATOZOA FROM WEST AFRICAN DWARF BUCKS

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ABSTRACT

.Egg yolk from avian species is used as a common component of most semen extenders because of its wide availability, beneficial effect on sperm viability and protective effect on acrosome against temperature-related damage during semen preservation. This study was carried out to determine the comparative effects of egg yolks from different poultry breeds on viability of refrigerated spermatozoa of West African Dwarf (WAD) bucks. Pooled semen from five intact WAD bucks was diluted with extender containing egg yolks from Normal feather (NF), Nickel neck (NN), Frizzle feather (FF), Nera black (NB), Oba Marshall black (OB) and Yaffa brown (YB) chickens. Following dilution, the semen samples were assessed subjectively after *in vitro* storage at 5^{o}C for 24, 48, 72 and 96 hours as regards progressive sperm motility, acrosome status and abnormalities using a phase-contrast microscope. The results showed that the ability of these egg yolks to sustain progressive motility ranked in this order: OB > NN > NF >YB > NB > FF. Intact acrosome was comparable during the 24, 48, 72 and 96 hours of storage and followed similar trend across the egg-yolk types in the extender. Extender containing YB had the highest percentage abnormality at the end of this study. The findings of this study reveal that OB, NN and NF have better protective ability to maintain motility of refrigerated spermatozoa of WAD bucks.

Keywords: Buck, egg yolks, refrigerated spermatozoa, viability

INTRODUCTION

Goats are the most numerous of the domestic livestock species in Nigeria and present a great potential to alleviate the problem of protein malnutrition in the country (FAO, 2006). As the demand for these animals in the sub region is constantly high, the prospects for increasing the numbers and productivity of these animals need to be utilized. Genetic improvement of goats requires the selection of superior breeding stock and the application of artificial insemination (AI) technique. The successes of AI in goats generally depend on knowledge of semen preservation and insemination techniques (Bitto and Egbunike, 2006). Traditionally, chicken egg yolk has been used as a common component of most semen extenders for freeze preservation of spermatozoa because of its wide availability (Bathgate *et al*., 2006) and beneficial effect on sperm viability, as a protectant of the plasma mem-

brane and acrosome against temperature-related damage, in association with other components during semen cryopreservation (Amirat *et al*., 2004). The phospholipids, cholesterol and low density lipoproteins in egg yolk is claimed to account for the protection of sperm against cold shock during the freeze-thaw process. However, the chemical composition of the egg yolks of different avian species varies, particularly in terms of the cholesterol, fatty acid and phospholipid contents (Bair and Marion, 1978; Burris and Webb, 2009), which may influence their effectiveness during cooling, freezing, and thawing steps (Bathgate *et al*., 2006). Recently, it has been shown that egg yolk from avian species, other than the chicken, might be more beneficial for cryopreservation of sperm from stallion (Clulow *et al*., 2007; Burris and Webb, 2009), jackass (Trimeche *et al*., 1997), bull (Su *et al*., 2008), and ram (Kulaksiz *et al*., 2010). However there has not been any report comparing the effect of egg yolk from different poultry breeds in the extender on the viability of chilled West African Dwarf buck semen. This requires screening of egg yolk from different avian or poultry breeds for the storage of spermatozoa to get optimum post-thaw quality. The present study was, therefore, designed to compare the protective effect of egg yolk from different poultry breeds (Normal feather, Nickel neck, Frizzle feather, Nera black, Oba Marshall black and Yaffa brown) in the extender on the viability of refrigerated spermatozoa of West African Dwarf bucks.

MATERIALS AND METHODS

The study was carried out at the Goat Unit of the Teaching and Research Farm Division, Federal University of Agriculture, Abeokuta, located in the tropical rain forest zone of South Western Nigeria within 7^0 10'N and 3^0 2'E. Five intact WAD bucks ranged between 2.5-3 years of age and kept under semi intensive management system were used for this study. Ejaculate samples showing more than 80% progressive motility were collected with the aid of artificial vagina and pooled. Each semen sample was diluted using a Tris-egg yolk based extender consisted of (Tris [$OHCO_3$] amiozomethane (2.42g), Citric acid monohydrate (1.36g), glucose (1g), Na penicillin G (0.028g), and Egg yolk (20ml) prepared from different poultry breeds (Normal feather NF, Nickel neck NN, Frizzle feather FF, Nera black NB, Oba Marshall black OB and Yaffa brown YB). The pooled semen was diluted at ratios of 1:5 (semen: extender v/v). Following dilution, the semen samples were drawn into eppendorf tubes, sealed and chilled at 5^oC and maintained at this temperature for 96 hours in a refrigerator.

Semen collection and evaluation

Following dilution, the semen samples were assessed subjectively after *in vitro* storage at 5^oC for 24, 48, 72 and 96 hours as regards progressive sperm motility using a phase-contrast microscope (400x magnification), with a warm stage maintained at 37^oC. A wet semen mount was made using a drop of semen placed on a microscopic slide. For each sample, at least five microscopic fields were examined. The mean of the five successive evaluations was recorded as the final progressive motility score. Acrosome status and spermatozoa abnormality were evaluated with eosin-nigrosin smears. At the end of every 24hours of storage, 3*u*l of semen sample was placed on a microscopic slide, 2*u*l of eosin-nigrosin was dropped on it, and a smear was made using a microscopic slide. The proportion of sperm cells with intact, damaged or missing acrosome was estimated under a phase-contrast microscope. Morpho-

logical examination of the sperm cells was carried out (Bearden and Fuquay, 1997) and primary abnormality of sperm cells located in the head, mid-piece and tail were observed under a phase-contrast microscope

Data Analysis

Data were subjected to analysis of variance (ANOVA) and means separated by Duncan Multiple Range Test (Duncan, 1955) in SPSS version 19.

RESULTS

The effects of different egg-yolk types on progressive spermatozoa motility of post chilled WAD bucks semen are presented in Table 1. The results showed that extender containing NN, OB and NB had highest ($P<0.05$) progressive motility followed by FF as compared to YB and NF in the first 24 hours of storage. Similar trend was observed after 48 hours of storage except that FF had zero progressive motility ($P<0.05$). Percentage of progressive sperm motility was higher ($P<0.05$) in extender containing NN, OB and NF in 72 and 96 h compared to YB, NB and FF that had zero progressive sperm motility at the end of the study. The ability of these egg yolks to sustain motility ranked in this order: OB > NN > NF >YB > NB > FF.

Table 1. Progressive motility of WAD buck spermatozoa extended with egg-yolks from different poultry breeds

Duration (h)	NN	NF	OB	YB	NB	FF	SEM
24.00	76a	52c	76a	58bc	74a	66b	3.419
48.00	74a	42c	74a	42c	68b	0d	3.655
72.00	52a	36c	46b	14d	0e	0e	2.948
96.00	26b	30b	42a	0c	0c	0c	3.066

[a,b,c,d] Values within rows with different superscripts differ significantly ($P<0.05$)

The effects of different egg-yolk types on intact acrosome of post-chilled WAD bucks semen are presented in Table 2. Intact acrosome was comparable during the 24, 48, 72 and 96 h of storage and followed similar trend across the egg-yolk types in the extender.

Table 2. Intact acrosome of WAD buck spermatozoa extended with egg-yolks from different poultry breeds

Duration (h)	NN	NF	OB	YB	NB	FF	SEM
24.00	91.67	95.00	88.33	88.33	91.67	85.00	1.556
48.00	87.33	79.33	88.33	79.33	86.00	72.67	2.830
72.00	87.67	87.67	91.67	89.67	88.33	96.00	1.438
96.00	72.33	80.00	78.33	75.67	88.67	87.67	3.009

The effects of different egg-yolk types on percentage abnormality of post-chilled WAD bucks semen are presented in Table 3. The results showed variations in the percentage of abnormality during 24, 48, 72 and 96 h in the extender containing the different egg-yolk types. Extender containing YB had the highest percentage abnormality at the end of the study.

Table 3. Percentage abnormality of WAD buck spermatozoa extended with egg-yolks from different poultry breeds

Duration (h)	NN	NF	OB	YB	NB	FF	SEM
24.00	1.08b	1.47b	1.99b	3.51a	1.08b	2.69ab	0.495
48.00	1.24c	3.96b	2.15bc	5.17b	2.08bc	14.15a	0.377
72.00	5.12b	2.52bc	2.52bc	12.32a	5.83b	1.93c	0.448
96.00	11.04b	5.83c	5.56c	18.58a	5.42c	4.83c	0.872

[a,b,c] Values within rows with different superscripts differ significantly ($P<0.05$)

DISCUSSION

Egg yolk is one of the most commonly used components of cryproctectants utilized during the freeze –thawing process. The beneficial effect of egg yolk in the cryopreservation of sperm can be attributed to a resistance factor, which helps to protect the sperm against cold shock; and storage factor that helps to maintain viability. The phospholipid, cholesterol and low density lipoprotein contents of the chicken egg yolk specially have been identified as the protective components (Pace and Graham, 1974; Watson, 1976; Foulkes, 1977). Egg yolk of other bird species have successfully been used as an additives for the cryopreservation of sperm in certain species especially equine (Clulow *et al*., 2007). The most important finding of the present study was that semen chilled in the extender containing NN, NF and OB egg yolks recorded higher sperm quality than semen chilled in the other egg yolks. Components such as levels of protein, lipid and cholesterol present in the eggs have been demonstrated to actively protect sperm during the various stages of cryopreservation process (Prasard *et al*., 1988; Maurice *et al*., 1994) suggesting the ability of these components present in egg yolks of these breeds of poultry to improve the protection of the sperm during the chilling processes, resulting in higher progressive sperm motility after post-chilling.

The results on improved progressive sperm motility of the present study are in agreement with other studies that egg yolks have a beneficial effect on sperm viability (Amirat *et al*., 2004; Burris and Webb 2009; Recai *et al*., 2010; Kulaksiz *et al*., 2010). Semen stored in the extender containing the different egg yolks in the present study had the ability to sustain motility and are ranked in this order: OB > NN > NF >YB > NB > FF. Similar results have been reported by Humes and Webb (2006), who found that egg yolks from different sources had variations in their ability to improve the percentage of motile stal-

lion sperm after the freezed-thawed process. The ability to improve the percentage of progressive sperm motility by the different egg yolks may be attributed to the higher levels of protein, lipid and cholesterol present in the different egg yolks. These components have been demonstrated to actively protect sperm during various stages of cryopreservation process (Prasard *et al*., 1988; Maurice *et al*., 1994). The higher levels of these components present in the different egg yolks could improve the protection of the sperm during the chilling, resulting in higher progressive sperm motility after chilling. The lower ratio of phosphatidylcholine and phosphatidylethanolamine and higher ratio of polyunsaturated fatty acids could probably be implicated for the lower progressive motility of spermatozoa chilled with YB, NB and FF egg yolk extenders (Trimeche *et al*. 1998; Choi *et al*. 2001). Moreover, a specific problem in the preservation of goat semen is the detrimental effect of seminal plasma on the viability of the spermatozoa in diluents containing egg yolk-based media and this is attributed to an enzyme originating from the bulbourethral gland secretion in seminal plasma, named egg yolk coagulating enzyme (Iritani, and Nishikawa, 1961). The toxicity of egg yolk coagulating enzyme differs with the quantity of the hydrolysates which vary with pH, temperature and seminal plasma concentration, season of semen production and breed of fowl providing the egg yolk. Therefore, the improvement or decline in post-chilled motility/quality of spermatozoa with egg yolk of different poultry breeds in the refrigerated extender might probably be attributed to the differences in the biochemical composition of the egg yolks (Trimeche *et al*., 1997; Surai *et al*., 1999; Bathgate *et al*., 2006)

Acrosomal intactness is mandatory for the acrosome reaction required at a proper time to facilitate fertilization (Thomas *et al*. 1997). The change in acrosomal cap is mainly due to sperm aging or cryoinjury (Ansari *et al*. 2010). Some studies on different livestock species suggest that different egg yolk sources (Choi *et al*. 2001) affect the percentage of spermatozoa with intact acrosomes after freezing (Andrabi *et al*., 2008). Intact acrosome in this study was comparable during the 24, 48, 72 and 96 hours of storage and followed similar trend across the egg-yolk types in the extender. The present trial shows that egg yolks from different poultry breeds do not have different protective actions on acrosome of WAD buck spermatozoa during chilling. This is in accordance with the study of Su *et al*. (2008), who showed no differences between different avian egg yolks. The findings are however in contrast to those of Akhter *et al*. (2010) who reported significant differences in spermatozoa with intact acrosome after freezing of Holstein bull semen in extender containing different avian egg yolks. In this study, appreciable percentage of spermatozoa with normal acrosome observed was comparable and indicated reduced damage to the sperm cells during chilling. The results indicated the potential effect of egg yolks that seemed to delayed onset of the physiological acrosome reaction. This delay is essential for maintaining functional properties, since the acrosome must remain undamaged to enable binding to the zona pellucida and to respond to the appropriate signals from oocytes (Wassarman, 1988).

Evaluation of sperm abnormalities is one of the commonest methods to assess functional status of the semen (Rocha *et al*., 2006). Su *et al*. (2008) also reported a decrease in percentage of morphological abnormalities of Hol-

stein bull ejaculated spermatozoa frozen in extender containing pigeon egg yolk. Extender containing YB had the highest percentage abnormality at the end of this study. The low percentage of sperm abnormalities in extender containing other egg yolks could probably be due to low density lipoprotein of egg yolk in the egg yolks. It is well recognized that low density lipoprotein of egg yolk is one of the main factors responsible for the protection of spermatozoa during freezing through different mechanisms. Different proposed mechanisms through which egg yolk protects the spermatozoa include stabilizing the membrane, reducing the spermatozoon membrane phospholipids losses and grabbing the toxic seminal plasma protein (Manjunath *et al*..,2002; Bergeon and manjunath 2006). Moreover, the percentage spermatozoa abnormalities observed were within the range for post-thawed goat semen as per the Brazilian College of Animal Reproduction (Henry and Neves, 1998) in the extender.

CONCLUSION

The findings of this study reveal that OB, NN and NF have better protective ability to maintain motility of refrigerated spermatozoa of WAD bucks. The improvement or decline in post-chilled viability of spermatozoa with egg yolk of different poultry breeds in the refrigerated extender in this study could be attributed to the differences in the biochemical composition of the egg yolks.

ACKNOWLEDGEMENTS

The contribution of the Head of Department, Animal Physiology is acknowledged for granting access to facilities in the Laboratory. The authors wish to thank the Laboratory Technologists in the Department of Animal Physiology for technical assistance.

REFERENCES

Akhter, S ., Allah, B., Rakha, Andrabi, S.M.H., Ansari, M.S. 2010**.** Comparison of egg yolks from three avian species in extender for cryopreservation of Sahiwal bull epididymal spermatozoa. *Animal Science Papers and Reports*, 29 (2): 131-138

Amirat, L., Tainturier, D., Jeanneau, L., Thorin, C., Gerard, O., Courtens, J.L., 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.

Andrabi S.M.H., Ansari M.S., Ullah N., Anwar M., Mehmood A., Akhter S. 2008 – Duck egg yolk in extender improves the freezability of buffalo bull spermatozoa. *Animal Reproduction Science,* 104, 427-433.

Ansari, M.S., Rakha, B.A., Andrabi, S.M.H., Ullah, N., Akhter, S. 2010. Cryopreservation of Sahiwal bull epididymal spermatozoa. *Pakistan Journal of Zoology,* 42, 741-743.

Bair, C.W., Marion, **W.W.** 1978. Yolk cholesterol in eggs from various avian species. *Poultry Science*, 57: 1260-1265.

Bathgate R., Maxwell W.M.C., Evans G. 2006 – Studies on the effects of supplementing boar semen cryopreservation media with different avian egg yolk types on *in vitro* post-thaw sperm quality. *Reproduction in Domestic Animals,* 41, 68-73.

Bearden, H.J., Fuquay, J.W. 1997. Semen evaluation. In: *Applied animal reproduction*. 4th ed. New Jersey: Prentice Hall; pp. 158-169.

Bergeron, A., Manjunath, P. 2006 – New insights towards understanding the mecha-

nisms of sperm protection by egg yolk and milk. *Molecular Reproduction and Development,* 73, 1338-1344.

Bitto, I. I., Egbunike, G. N. 2006. Seasonal variations in sperm production, gonadal and extragonadal sperm reserves in pubertal West African dwarf bucks in their native tropical environment. *Livestock Research for Rural Development*, 18 (9), *www.lrrd.org/lrrd18/9/bitt18134.htm*

Burris, C., Webb, G. 2009. Effects of egg yolk source on the cryopreservation of stallion semen. *Journal Equine Veterinary Science*, 29:336–337.

Choi, S.H., Song, K.T., Oh, H.R. 2001. Cholesterol contents and fatty acid composition of chukar, pheasant, guinea fowl and quail egg yolk. *Asian-Australasian Journal of Animal Sciences*, 14:831–836.

Clulow, J.R., Maxwell, W.M.C., Evans, G., Morris, L.H.A. 2007. A comparision of duck and chicken egg yolk fort he cryopreservation of stallion sperm. *Australian Veterinary Journal,* 85:232–235.

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

Food and Agriculture Organization (FAO). 2006. A system of integrated agricultural censuses and surveys, volume 1, World Programme for the Census of Agriculture 2010. *Statistical Development Series* No 11.

Foulkes, J.A. 1977. The separation of lipoproteins from egg yolk and their effect on the motility and the integrity of bovine spermatozoa. *Journal of Reproduction and Fertility*, 49:277–284.

Henry, M., P. Neves. 1998. *Manual para exame andrológico e avaliação de sêmen animal.* 2th ed. Colégio Brasileiro de Reprodução Animal (CBRA), Belo Horizonte, MG

Humes, R., Webb, G. 2006. Use of chicken or chukar egg yolk with two cryoprotectants for preservation of stallion semen. *Animal Reproduction Science*, 94, 62–63.

Iritani, A., Nishikawa, Y. 1961. Studies on the egg-yolk coagulating factors in goat semen: II Properties of the coagulating factor and influential conditions for coagulation. *In*: Proc. Silver Jubilee Lab. Anim. Husbandry, Kyoto University, pp. 97–104.

Kulaksiz, R., Cebi, C., Akcay, E., Daskin, A. 2010. The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen. *Small Ruminant Research*, 88: 12-15.

Manjunath P., Nauc V., Bergeron A., Menard M. 2002 – Major proteins of bovine seminal plasma bind to the low-density lipoproteins fraction of hen's eggs yolk. *Biology of Reproduction,* 67:1250-1258.

Maurice, D.V., Lightsey, S.F., Hsu, K.T., Gaylord, T.G., Reedy, R.V. 1994. Cholesterol in eggs from different species of poultry determined by capillary GLC. *Food Chemistry*, 50:367–372.

Pace, M.M., Graham, E.F. 1974. The components in egg yolk which protect bovine spermatozoa during freezing. *Journal of Animal Science*, 39: 1144–1149.

Prasard, R.V., Sreenivas Reedy, M., Dhananjaya Reedy, B., Rao, P.V.A. 1988. A comparative study on the quality of fresh chicken and duck eggs. *Indian Journal of Ani-*

mal Science, 8: 978–981.

Recai, K., Cigdem, C. , Ergun, A. , Ali, .D. 2010.The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen. *Small Ruminant Research,* 88:12–15

Rocha, A., Oliveira E., Diaz J.K., Sousa M. 2006 – A novel apical midpiece defect in the spermatozoa of a bull without an apparent decrease in motility and fertility: a case study. *Theriogenology,* 66: 913-922.

Su, L., Li X., Quan J., Yang S., Li Y., He X., Tang X. 2008 – A comparison of the protective action of added egg yolks from five avian species to the cryopreservation of bull sperm. *Animal Reproduction Science,* 104: 212-219.

Surai, P.F., Speake, B.K., Noble, R.C., Mezes, M. 1999. Species-specific differences in the fatty acid profiles of the lipids of the yolk and of the liver of the chick. *Journal of the Science of Food and Agriculture*., 79: 733-736.

Thomas C.A., Garner D.L., Jarnette J.M., Marshall C.E. 1997 – Fluorometric assessment of acrosomal integrity and viability in cryopreserved bovine spermatozoa. *Biology of Reproduction,* 45:880-887.

Trimeche A., Anton M., Renard P., Gandemer G., Tainturier D. 1997 – Quail egg yolk: A novel cryoprotectants for the freeze preservation of Poitou jackass sperm. *Cryobiology,* 34: 385-393.

Trimeche A., Renard P., Tainturier D. 1998 – A procedure for Poitou jackass sperm cryopreservation, *Theriogenology,* 50: 793-806.

Wassarman, P.M. 1988. Zonal pellucid glycoproteins. *Journal Biological Chemistry*. 283: 24285-24289.

Watson, P.F. 1976. The protection of ram and bull spermatozoa by the low density lipoprotein fraction of egg yolk during storage at 5 ◦C and deep freezing. *Journal of Thermal Biology*. 1: 137–141.

(Manuscript received: 15th December, 2013; accepted: 10th May, 2014).