

# Effects of Egg Yolks from Different Avian Species on Boar Sperm Motility and Livability

Adeusi Adebolu Sunday<sup>1\*</sup> Ogunlade Jacob Taiwo<sup>1</sup> Alaba Olufemi<sup>2</sup> Adewumi Abel Adeyemi<sup>2</sup>

1. Department of Animal Production and Health Sciences, Faculty of Agricultural Sciences,  
Ekiti State University, Ado Ekiti, Ekiti State, Nigeria

2. Animal Physiology and Bioclimatology Unit, Department of Animal Science, University of Ibadan, Ibadan,  
Nigeria

## Abstract

Egg yolk is one of the most widely used components for freezing and cold storage of semen and its action on sperm motility, fertilizing ability and viability are influenced by a large number of factors. This experiment was conducted to determine the effect of egg yolk from different avian species, namely the domestic chicken (*Gallus domesticus*), the turkey (*Meleagris gallopavo*) and Japanese quail (*Cortinix japonica*) on spermatozoa quality following refrigeration of boar semen. at 8<sup>o</sup>C for 0 hour, 24 and 48 hours. Ejaculate was collected using gloved-hand technique from a mature boar and spermatological parameters were assessed. Beltsville thawing solution (BTS) was used as the experimental extender. The experimental extenders consisted of BTS only as the control, while others consisted of BTS and egg yolk from domestic chicken, turkey and quail respectively. Extended semen samples were assessed for motility and livability after refrigeration at 8<sup>o</sup> C for 0 hour, 24 and 48 hours respectively. Results showed quail yolk to have the best protective effect with respect to the highest sperm motility ( 66.67 % and 63 % ) at both 24 and 48 hours, compared to control, turkey and Japanese quail egg yolk (p < 0.05). Sperm stored in quail egg yolk had the higher livable sperm cells (83 % and 77 %) at 24 and 48 hours compared with control, turkey and quail egg yolk (p < 0.05). This study suggested that quail egg yolk can potentially replace chicken egg yolk in semen extender in refrigeration, but it warrants further evaluation in fertility trials.

**Keywords:** Egg yolk, Spermatozoa, Extender, Beltsville thawing solution, Refrigeration

## 1. Introduction

Cold shock occurs when spermatozoa are cooled below physiologic temperature (Watson, 1981). This phenomenon involves damage to the cellular membranes and alteration in metabolic function through changes in the arrangement of membrane compositions (Parks, 1997). The spermatozoa from horse, cat, dog, and humans are relatively insensitive to cold shock, whereas spermatozoa from cattle, sheep and goat exhibit intermediate sensitivity, and boar spermatozoa are extreme of sensitivity (Watson, 1985).

After cooling, membrane permeability is increased and this may be a consequence of increased membrane alteration in specific protein channels. Channel regulation of calcium uptake is affected by cooling causing a reduction in cellular function and death. The uptake of calcium during cooling affects capacitation changes and fusion events between plasma membrane and acrosomal membrane (Barbas and Mascrenhas, 2009). Cold shock decreases membrane permeability to water and solutes and damages acrosomal membranes (Purdy, 2006). Irreversible alterations in the sperm membrane which include disturbances in the protein lipid bilayer structure, decreased membrane fluidity, increased membrane permeability, acrosome damage, dehydration, enzyme and phospholipid liberation, reduced metabolic activity and diminished consumption of ATP are all consequences of cooling and freezing. These effects may compromise fertility (Hammerstedt *et al.*, 1990).

Boar semen is unique in several ways compare to semen of other domestic animals. It is produced in large volumes and is extremely sensitive to sudden cooling immediately after collection (known as “cold shock”) (Bengt, 2000). In many cases the conditions of on-farm storage temperature of semen are not appropriate with its attendant reduced sperm quality.

Boar sperm membranes possess discrete domains of differing membrane fluidity, which is significantly affected by temperature (Buhr *et al.*, 1994). Moreover, the sperm membranes appear to be the major site of damage induced by reduced temperature and alterations in their molecular constituents can significantly impair the process of fertilization (Buhr *et al.*, 1995).

In recent times, egg yolk is a popular component of most semen cryopreservation extenders for domestic animals. It has been demonstrated to have a beneficial effect on sperm cryopreservation-as a protectant of the plasma membrane and acrosome against temperature-related damage, working in synergy with the other components (Amirat *et al.*, 2004). It is believed that the phospholipids, cholesterol and low density lipoproteins in egg yolk may be factors that provide protection to sperm against cold shock during cold storage and the freeze-thaw process.

Several reports have been made that egg yolk from avian species such as the duck, quail, pigeon or chicken have different combinations or profiles of fatty acids, phospholipids and cholesterol, which could lead to

variation in cryopreservation effects on the sperm (Trimeche *et al.*, 1997; Choi *et al.*, 2001; Bathgate *et al.*, 2006; Humes and Webb, 2006; Andrabi *et al.*, 2007; Clulow *et al.*, 2007; Moreno *et al.*, 2008; Su *et al.*, 2008). There are however limited reports comparing the effect of egg yolk of different species (domestic chicken, turkey, duck and Japanese quail) in the extender on the efficiency of cold storage preservation of boar sperm.

Therefore this experiment was carried out to investigate the cold-shock protective effect of different avian species egg yolk on boar semen stored at refrigerated temperature.

## 2. Materials and Methods

### 2.1 Experimental Location

The study was carried out at the piggery unit of Teaching and Research farm of University of Ibadan, Ibadan, Nigeria located on the latitude 7°20'N and 3°50'E, 200m above sea level. The semen preservation and analysis were done at the Physiology Laboratory, Department of Animal Science, University of Ibadan.

### 2.2 Experimental Animal and Semen Collection

Semen collection for this study was done on mature crossbred boar of 3 years of age. Prior to semen collection, the boar, particularly its prepuce pouch was thoroughly washed to remove urine and other materials that could increase the chances of semen contamination during collection.

Semen collection was done manually using gloved-hand technique. The boar was teased by being made to mount sow on standing heat and the spiral end of penis was grabbed firmly and pressure was applied. This process was similar to the pressure of the cock screw shape of the sow's vagina and this made the boar to ejaculate. Polyvinyl glove was used because latex gloves are considered spermicidal.

The semen was collected into an insulated/ thermos semen cup (Minitube® USA) covered with gauze to filter out the gel portion of the semen.

### 2.3 Egg Yolk Extraction

Cleaned eggs of three avian species (chicken, turkey and quail) were cracked in half and albumen was discarded. The remainder of egg white, which was still attached to the yolk, was removed by slowly moving the egg yolk around a 12 cm diameter filter paper. The intact egg yolk was then punctured and the internal yolk was removed and allow to flow in a clean glass beaker.

### 2.4 Preparation of Extenders

Beltsville thawing solution (BTS) was used as the control experimental extenders. It consisted of glucose (3.91g), sodium citrate (0.63g), EDTA (0.132g), sodium bicarbonate (0.13), potassium chloride (0.079g), penicillin (0.11g), and streptomycin (0.11g) which were dissolved in 80ml of distilled water. 20ml of egg yolk from three avian species i.e. chicken egg yolk, turkey egg yolk and quail egg yolk were added respectively to the BTS. The composition of BTS for control that was devoid of egg yolk was diluted with 100ml of distilled water.

### 2.5 Semen Dilution

An aliquot of 4ml of each of the experimental extenders was prepared in a three different sterilized sample bottles, 1ml of the semen was taken and diluted with each of the extender respectively making the dilution ratio of 1:4 as described by Fiser *et al.* (1993). Each treatment was replicated three times. Each treatment with their replicates were prepared in three places to represent evaluation at 0 hour, 24 and 48 hours intervals.

### 2.6 Initial Assessment of Fresh Semen Collected for Study

The fresh semen collected from the boar was assessed for the following parameters:

pH: 7.3

Motility: 90 %

Morphological abnormality: 4 %

% Livability: 99%

Colour: milky white colour

Odour: no noticeable offensive odour

### 2.7 Experimental Treatments Layout

Treatment 1(Control)	BTS 100% (vol/vol)
Treatment 2	BTS 80% (vol/vol) + chicken egg yolk 20 % (vol/vol)
Treatment 3	BTS 80% (vol/vol) + turkey egg yolk 20 % (vol/vol)
Treatment 4	BTS 80% (vol/vol) + quail egg yolk 20 % (vol/vol)

## 2.8 Semen Evaluation

The extended semen was refrigerated at 8 °C and evaluated at 0 hour, 24 and 48 hours for visual motility and live/dead ratio.

### 2.8.1 Motility

A 5µl drop of semen was placed on a warmed (37 °C) glass slide and cover-slipped. Visual motility was recorded under phase contrast microscope at magnification of ×400. 100 sperm cells were counted per slide.

### 2.8.2 Live and Dead Spermatozoa

A drop of semen was placed on glass slide; A drop of eosin nigrosin stain was added and gently mixed; The mixture was smeared on a slide with the edge of another clean slide, air dried and viewed under light microscope at magnification of ×400.

## 2.9 Experimental Design and Statistical Analysis

The experimental design was completely randomized design in a 3 by 4 factorial arrangements. The first factor is the experimental treatments which are the BTS only (control) and BTS+egg yolk from 3 different avian species while the second factor is the time lags which range from 0hr, 24 and 48 hours.

All data obtained from this study were analysed using statistical analysis system (SAS), 2003 and means were separated using Duncan's procedure.

## 3. Results

Table 1 shows the proportion (%) of motile boar spermatozoa at 0 hour, 24 and 48 hours of storage. The results showed that there were significant differences ( $p < 0.05$ ) in percentage of motile spermatozoa among the treatments at 24 and 48 hours of storage. There was no significant difference ( $p > 0.05$ ) at 0 hour of storage among the treatments.

Motility in BTS (control) extender was significantly lower at both 24 and 48 hours of storage. However, the proportion of motile spermatozoa in QEY + BTS extender appeared statistically superior to all other extending media at 24 and 48 hours of storage. The motility values in CEY + BTS and TEY + BTS extender were consistently statistically similar in both 24 and 48 hour of storage.

Table 2 shows the effect of BTS and BTS + Egg yolk from chicken, quail and turkey on proportion of live spermatozoa at different storage time 0 hour, 24 and 48 hours. There were significant differences among the diluting media at 24 and 48 hours of storage. At both 24 and 48 hours of storage, the lowest live proportion of spermatozoa was observed in the control extending medium (BTS). The proportion of live spermatozoa was consistently higher in the BTS + QEY extender at both 24 and 48 hours of storage. The values observed in BTS + CEY and BTS + TEY were statistically similar at 24 and 48 hours of storage.

Table 1. Effect of BTS + Egg yolk from different avian species and Time on Sperm Motility.

EXPERIMENTAL EXTENDERS	LENGTH OF STORAGE (HOURS)			
	0	24	48	SEM
BTS	85.00	53.33 <sup>c</sup>	45.00 <sup>c</sup>	2.27
CEY + BTS	86.67	61.67 <sup>b</sup>	58.33 <sup>b</sup>	2.32
QET + BTS	83.67	66.67 <sup>a</sup>	60.00 <sup>a</sup>	2.37
TEY + BTS	83.33	60.67 <sup>b</sup>	56.67 <sup>b</sup>	2.31

Group with different letters (a, b, c) in the same column are significantly different ( $p < 0.05$ ).

BTS: Beltsville Thawing Solution, CEY: Chicken Egg Yolk, QEY: Quail Egg Yolk, TEY: Turkey Egg Yolk, SEM: Square Mean Error.

Table 2. Effect of BTS + Egg yolk from different avian species and Time on live sperm proportion (%)

EXPERIMENTAL EXTENDERS	LENGTH OF STORAGE (HOURS)			
	0	24	48	SEM
BTS	97.33	71.00 <sup>c</sup>	58.33 <sup>c</sup>	2.21
CEYN + BTS	98.67	80.00 <sup>ab</sup>	67.00 <sup>b</sup>	2.31
QET + BTS	98.67	83.67 <sup>a</sup>	77.67 <sup>a</sup>	2.36
TEY + BTS	100	75.00 <sup>bc</sup>	68.67 <sup>b</sup>	2.30

Group with different letters (a, b, c) in the same column are significantly different ( $p < 0.5$ ).

BTS: Beltsville Thawing Solution, CEY: Chicken Egg Yolk, QEY: Quail Egg Yolk, TEY: Turkey Egg Yolk, SEM: Square Mean Error

## 4. Discussion

Cold shock is a phenomenon which occurs when spermatozoa are cooled below physiologic temperature. Boar semen has been particularly known to be extremely sensitive to sudden cooling immediately following collection (Bengt, 2000). Egg yolk is a popular component of most semen cryopreservation extenders for domestic animals.

It is believed that the phospholipids, cholesterol and low density lipoproteins in egg yolk may be factors that provide protection to sperm against cold shock during cold storage and the freeze-thaw process.

Motility of spermatozoa has always been considered as an important factor or condition required for fertilization of eggs. Even though the spermatozoa are transported to the fertilization of site mainly by uterine contractions (Langendijk *et al.*, 2002), however, sperm motility is necessary for penetration of the zona pellucida. Motility is well known to be an imperative index in predicting the fertilizing potential of sperm (Gadea, 2005). In this study spermatozoa motility was highest in the extender containing quail egg yolk. This is in concordance with Trimeche *et al.*, (1997) who reported higher percentage of motile and progressively undulating spermatozoa using 10 % of quail egg yolk compared with 10% chicken egg yolk extender. It is suggested that the higher motility of spermatozoa with quail egg yolk extender may be due to higher content of phosphatidylcholine, less phosphatidylethanolamine and a smaller ratio of polyunsaturated to saturated fatty acids compared to chicken and turkey egg yolk (Choi *et al.*, 2001).

In this work, highest proportion of live spermatozoa was observed in BTS extender containing quail egg yolk. This may also be due to composition of quail egg yolk which has smaller ratio of polyunsaturated to saturated fatty acids compared to chicken and turkey egg yolk (Choi *et al.*, 2001). Saturated fatty acids are more stable than their unsaturated fatty acids counterparts and this may be responsible for better protection capability recorded in this study.

## 5. Conclusion

This study has shown that greater proportion of live and motile boar spermatozoa were obtained using BTS extender containing quail egg yolk at both 24 and 48 hours of refrigeration. BTS extender containing quail egg yolk is a superior protective agent against deleterious effect of cold shock during refrigeration providing satisfactory results with respect to motility and livability of sperm cells.

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