Research Journal of Applied Sciences, Engineering and Technology 6(24): 4692-4696, 2013
ISSN: 2040-7459; e-ISSN: 2040-7467

Submitted: May 17, 2013 Accepted: June 11, 2013 Published: December 25, 2013

Seminal Plasma Protein as a Biomarker for Fertility and Hatchability in the Domestic Fowl *Gallus Domesticus*

Eme Efioanwan Orlu
Department of Applied and Environmental Biology, Rivers State University of Science and Technology, P.M.B.5080, Nkpolu, Rivers State, Nigeria, Tel: +234-08033169547

Abstract: This study was aimed at investigating the use of seminal plasma protein concentration to predict fertility and hatchability in the domestic fowl. Forty cocks with acceptable sperm motility and morphology were used and the seminal plasma protein concentration determined by the Biuret method. The result revealed a negative significant correlation between the seminal plasma protein concentration with sperm motility ($r = -0.5920$, $p<0.05$), $Y = -0.3144X^2+2.1578X+74.324$, $R^2 = 0.3506$ ($p<0.05$). A significant positive correlation with percentage dead spermatozoa $r = 0.9743$ ($p<0.01$), $R^2 = 0.9493$, $p<0.01$), percent abnormal spermatozoa, $r = 0.8425$, $p<0.01$), $R^2 = 0.7098$ ($p<0.01$), $Y = 0.357X^2+1.5364X+9.1886$. Egg fertility and Hatchability were negative but significantly correlated with seminal plasma protein concentration $r = -0.6302$ ($p<0.01$) vs $r = -0.8438$ ($p<0.01$) and $Y = 0.8977X^2-5.8289X+81.628$ vs $Y = -0.2391X^2+3.3871X+60.771$, respectively. There was low correlation with the quantitative semen characteristics. Prediction of Y_{max} from Seminal plasma protein concentration (X) showed that the best semen quality, highest percent fertility and hatchability would be obtained when the concentration of seminal plasma protein is >3.00 g/100 mL but <6.00 g/100 mL. It was concluded that seminal plasma protein concentration could be used as a biomarker for prediction of fertility and hatchability in the domestic fowl.

Keywords: Biomarker, domestic fowl, fertility, hatchability, seminal plasma protein

INTRODUCTION

Semen samples with optimal quality that will ultimately improve conception rates can be used as the basis of selection of high fertility sires. Routine semen quality analysis based on motility and morphology provides useful, but limited information about fertility indexes in the male. The existence of sub-fertile sires with apparent normal semen quality is an important observation showing that semen characteristics and sperm morphology measurements are not always indicative of fertility and reproductive performance in animals (Foxcroft *et al*., 2008). Thurston *et al*. (1992) reported infertility of unknown origin and poor hatchability in flocks of yearling Large White turkeys. They maintained that males with high seminal protein equal to or greater than 6 g/100 mL were responsible for significant reduction in fertility and hatchability in the flock and reported no correlation between seminal plasma protein and fertility or hatchability when the seminal plasma protein concentration was less than 6 g/100 mL.

The protein spectrum in the seminal plasma is formed mainly by the seminal vesicular fluid proteins and to a lesser extent, by the proteins in the fluids of the cauda epididymides and prostate (Dostál and Vaselský 1972). Among many components of seminal plasma, proteins and peptides play a specific role in regulation of the fertilization process, particularly through their ability to bind various types of ligands. In the bovine species as well as in other mammals, freshly ejaculated sperm are not capable of fertilizing eggs. They must first undergo capacitation and the acrosome reaction during their transit through the female reproductive tract to become fully competent to fertilize an ovum (Therien and Manjunath, 1997). Both processes, as well as, sperm motility are regulated by seminal plasma proteins (Mogielnicka and Kordan, 2011). Affinity of plasma proteins to mannans of the fallopian tube epithelium facilitates formation of spermatozoa reservoirs in the female reproductive tract (Chacur *et al*., 2010a) Interspecies analysis indicates significant structural and functional similarities of seminal plasma proteins and their relation to fertility in human, stallion (Alghamdi *et al*., 2004; Novak *et al*., 2010b) boar (Novak *et al*., 2010a) ram (Therien and Manjunath, 1997), bulls (Chacur *et al*., 2010a) *Bos taurus indicus* Chacur *et al*. (2009b) have been well documented. Proteins of the seminal plasma have an ample panorama of action and some appear responsible for establishing fertility including maintaining the stability of the membrane up to the process of capacitation as they pass through the male and female reproductive tracts. (Therien and Manjunath, 1997) In roosters, the epididymal fluid might be the source of sperm maturation proteins (Al-Aghbari, 1993) and may play a role in sperm storage or survival within the hen's reproductive tract.

Due to the paucity of information on the role of seminal plasma protein concentration in fertility and

hatchability in the domestic fowl, this study was aimed at evaluating the possibility of the use of seminal plasma protein concentration as a biomarker for fertility and hatchability in the barred Plymouth Rock and the Nigerian indigenous breeds of the domestic fowl.

MATERIALS AND METHODS

Experimental location: This investigation was carried out in the Department of Applied and Environmental Biology, Rivers State university of Science and Technology, Port Harcourt, Rivers State, Nigeria, (Coordinates 4°48'14"N 6°59'12"E).

Experimental birds and management: Twenty eight month old Barred Plymouth Rock and Twenty non-descript Nigeria breed of cocks were used for this investigation. They were housed individually in cages and fed standard breeders mash with cool clean water *ad libitum* for a period of twelve months. The experiment was conducted according to the institutional animal care protocols at the Rivers State University of Science and Technology and followed guidelines and ethics for the treatment of experimental animals.

Semen sample collection: Semen samples were collected from both breeds by the massage method at 48-hourly intervals between 0900-1000 h (Nkanga and Egbunike, 1988; Egbunike and Nkanga, 1999). Soon after collection, semen color and consistency were assessed by direct visual examination, semen volume was measured with 2 mL graduated vials and the volume determined to the nearest 0.01 mL. Sperm progressive motility, mass activity were evaluated on plain slides at low power(x10 objective) (Nkanga and Egbunike, 1988; Egbunike and Nkanga, 1999). Sperm concentration and sperm output determined using a Neubaeur Haemocytometer (Orlu and Ogbalu, 2011) with a Digital Microscope Model DB1-180M (China) connected to a computer through a CCD Camera system with a USB 1.0 output. The remaining semen volume was centrifuged at 3,000 g for 2 min. The seminal plasma (supernatant) was separated and diluted at a ratio of 1 in 10 with deionized distilled water and stored at -20°C until required for analysis.

Determination of total protein: Protein forms a colored complex with cupric ions in alkaline medium. This is the principle used in the Biuret method. 5.00 mL of Biuret reagent was added to a clean glass tube followed by 0.10 mL of seminal plasma. The content was mixed and incubated for 30 min at 25°C or room temperature. The Biuret reagent was used as a blank and absorbance of the test sample read at 540 nm. The protein content of the seminal plasma was determined by multiplying the absorbance by a constant and expressed as g/100 mL.

Statistical analysis: Data obtained from this investigation were subjected to ANOVA and the Students' t-test using the software XLSTAT 2011 for the means and standard Deviation. The data were further subjected to Pearson's Correlation analysis to determine any relationships and Quadratic Regression analysis to generate predictive equations.

RESULTS

The result of this investigation revealed a negative significant correlative relationship between the seminal plasma total protein concentration with sperm motility ($r = -0.5920$, $p<0.05$) with a predictive regression equation $Y = -0.3144X^2+2.1578X+74.324$ and a Coefficient of Determination $R^2 = 0.3506$ ($p<0.05$). The predictive equations for other semen characteristics from seminal protein concentration in the domestic fowl (*Gallus domesticus*) are shown in Table 1. There was a significant positive correlative relationship between seminal plasma protein concentration and percentage dead spermatozoa $r = 0.9743$ ($p<0.01$), $R^2 = 0.9493$, $p<0.01$), predictive equation $Y = 0.0197X^2-0.5233X+4.1564$). A similar trend was observed with percent abnormal spermatozoa, $r = 0.8425$, $p<0.01$), $R^2 = 0.7098$ ($p<0.01$), predictive equation $Y = 0.357X^2+1.5364X+9.1886$. However, Egg fertility and Hatchability indicated a negative correlative relationship with seminal protein concentration $r = -0.6302$ ($p<0.01$) vs $r = -0.8438$ ($p<0.01$), $R^2 = 0.3971$ vs 0.713 and predictive equations of $Y = 0.8977X^2-5.8289X+81.628$ vs $Y = -0.2391X^2+3.3871X+60.771$ for egg fertility and hatchability, respectively. There appears to be low correlative relationship between seminal protein concentration and the quantitative semen characteristics, thus, sperm concentration showed a coefficient of determination $R^2 = 0.2312$, $r = 0.4808$, ($p<0.05$), sperm output $R^2 = 0.0256$, $r = 0.16$ ($p>0,05$), Daily Sperm Production (DSP) $R^2 = 0.1757$, $r = -0.4192$ ($p>0.05$) and the efficiency of sperm production (DSP/g) $R^2 = 0.1628$, $r = 0.4035$ (>0.05), their respective quadratic regression equations are summarized in Table 2.

There existed significant correlative interrelationships between semen characteristics (Table 2). Live weight was positive and significantly correlated with semen volume $r = 0.42$, $p<0.01$), sperm motility $r = 0.55$, $p<0.01$), sperm concentration $r = 0.31$, $p<0.05$). Semen volume was significantly ($p<0.01$) correlated with sperm output $r = 0.84$, while sperm motility was negative but significantly correlated with percentage abnormal spermatozoa $r = -0.62$ ($p<0.01$), percent dead spermatozoa $r = -0.82$ ($p<0.01$), semen pH $r = -0.60$ ($p<0.01$).

Breed and season exerted some non significant ($p>0.05$) influence on the concentration of seminal plasma protein since the protein concentration was elevated in rainy seasons. Barred Plymouth Rock

Table 1: Predictive equations for egg hatchability and semen characteristics from seminal plasma total protein concentration in domestic fowl (*Gallus domesticus*)

Dependent variables (Y)	Seminal plasma protein concentration (X) (g/100 mL) regression equations	R^2	r
Sperm motility (%)	$Y = -0.3144X^2+2.1578X+74.324$	0.3506	-0.5920*
Semen pH	$Y = 0.0226X^2-2416X+7.6414$	0.2504	0.5004*
Sperm concentration ($x10^6$)	$Y = 0.0959X^2+0.7627X+2.1676$	0.2312	0.4808*
Sperm output ($x10^9$)	$Y = 0.0115X^2–0.0606X+0.7758$	0.0256	0.16
DSP ($x10^9$)	$Y = -0.041X^2+0.3757X+0.3292$	0.1757	-0.4192
DSP/g ($x10^6$)	$Y = -0.0013X^2+0.0129X+0.0427$	0.1628	-0.4035
Dead spermatozoa (%)	$Y = 0.0197X^2-0.5233X+4.1564$	0.9493**	0.9743**
Egg fertility (%)	$Y = 0.8977X^2-5.8289+81.628$	0.3971*	0.6302*
Egg hatchability (%)	$Y = -1.6376X^2+9.9293X+54.724$	0.5154*	-0.7179**
Sperm abnormality (%)	$Y = 0.357X^2+1.5364X+9.1886$	0.7098**	0.8425**

Df = 38; +: DSP = Daily Sperm Production, +: DSP/g = Daily Sperm Production efficiency; **:Values are significant (p<0.01); *:Values are significant (p<0.05)

Table 2: Correlation coefficient of semen characteristics in the domestic fowl (*Gallus domesticus*) raised in the humid tropics

		Dependent variables							
	Independent variables	8	7	6	5	4	3	2	1
1	Live weight	-0.04	-0.06	-0.30	0.39[a]	0.31[a]	0.55[b]	0.42[b]	-
2	Semen volume	-0.25	-0.23	-0.27	0.84[b]	0.28	0.27	-	
3	Sperm motility	-0.62[b]	-0.82[b]	-0.60[b]	0.23	0.11	-		
4	Sperm concentration	0.14	0.07	-0.25	0.62[b]				
5	Sperm output	-0.18	0.23	-0.27	-				
6	Semen pH	0.04	0.02	-					
7	% dead sperm	0.81[b]	-						
8	% Abnormal sperm	-							

Df = 38; All values are not significant except those superscripted a = (p<0.05) b = (p<0.01)

Table 3: Breed and seasonal influence on seminal plasma protein concentration (g/100mL) in the domestic fowl (*Gallus domesticus*)

Season	Barred plymouth rock	Nigerian indigenous breed
Late dry (Jan-Mar)	1.55±0.37	1.54±0.30
Early rain (April-Jun)	3.62±0.66	2.11±1.03
Late rain (Jul-Sept)	3.40±0.80	4.80±0.88
Early dry (Oct-Dec)	1.00±0.18	1.04±0.21

Values significant at p<0.05

Table 4: Concentration of seminal plasma protein (X) for maximum values of Y (Ymax)

VARIABLES	CONCENTRATIONOFX(g/100 mL)	Y_{max}
Sperm motility (%)	3.43	78.03
Semen pH	5.35	6.990
SpermConcentration($x10^6$)	3.98	6.710
Sperm Output($x10^9$)	2.64	1.420
*DSP ($x10^9$)	4.58	1.190
**DSP/g ($x10^9$)	4.96	0.070
Dead Spermatozoa (%)	3.28	6.800
Egg Fertility (%)	3.25	72.17
Egg Hatchability (%)	3.03	69.82

*: Daily Sperm production **: Daily sperm production per gram testicular weight (Efficiency of sperm production)

1.55±0.37 g/100 mL, 3.62±0.66 g/100 mL, 3.40±0.80 g/100 mL and 1.65±0.18 g/100 mL for late dry, early rain, late rain and early dry seasons respectively. The Nigerian indigenous breed had 1.54±0.30 g/100 mL, 2.11±1.03 g/100 mL 4.80±0.88 g/100 mL and 1.04±0.21 g/100 mL for late dry, early rain, late rain and early dry seasons respectively (Table 3).

Based on the quadratic regression equations generated, the values of seminal plasma protein concentration (X) for which maximum values of the dependent variables (Y_{max}) could be obtained were predicted by differentiation in a quadratic equation $Y = AX^2+BX+C$ and setting the resultant equation to zero such that $dY/dX = 2AX+B = 0$, $X = -B/2A$. Applying this mathematical principle in the regression equation the values in Table 4 were obtained by substituting this entity in $Y = AX^2+BX +C$; $Y_{max} = A(-B)^2/2A+B(-B)/2A+C/1 = B^2/4A–B/2A+C/1 = B^2 -2B^2+4AC/4A$, $Y_{max} = -(B^2-4AC)/4A = B^2 -4AC/4A$. Thus, for maximum percentage hatchability, $Y = 1.6376X^2+9.9293X+54.724$; $dY/dX = 2(1.6376) X +9.9293 = 0$; Thus when $X = +9.9293/3.2752 = 3.0316$ g/100 mL, $Y_{max} = -1.6376 (3.0316)^2+9.9293(3.0316) +54.72 = -15.0505 +30.1453+54.724 = 69.82\%$

The highest percentage of sperm motility (78.03%) would be obtained at seminal protein concentration of 3.43 g/100 mL, Sperm concentration of 6.71×10^6 at 3.95 g/100 mL protein concentration. Maximum Sperm Output of 1.42×10^9 at 2.64 g/100 mL. DSP of 1.19×10^9 at 4.58 g/100 mL and the highest efficiency of sperm production (DSP/g) of 70×10^6 at 4.96 g/100 mL. 72% egg fertility and 70% egg hatchability would be obtained at seminal plasma protein concentrations of 3.25 g/100 mL and 3.03 g/100 mL, respectively.

DISCUSSION

From the results of this investigation neither breed of the domestic fowl nor season significantly affected seminal plasma protein concentration. This is in agreement with a previous report (Egbunike and Nkanga 1999). Seminal plasma protein concentration was observed to be negative and significantly correlated r = -0.5920 (p<0.05) with sperm motility, positively and highly correlated r = 0.9145 (p<0.01) with percent dead spermatozoa .The high coefficient of determination R^2 = 0.9493 is indicative that there is a 90% chance of semen with high seminal plasma protein containing

high percentage of dead spermatozoa. Dead and abnormal sperm cells viewed in Nigrosine-eosin vital stain exhibit damaged and compromised cell membrane (Orlu and Ogbalu, 2011) and proteins are integral components of cell membrane, indicative of the role of seminal plasma protein concentration in sperm maturation, capacitation and the presence of sperm abnormality. This is in agreement with the report of (Mogielnicka and Kordan, 2011) in mammals, (Novak *et al*., 2010a, b) in boars and stallions respectively. Seminal plasma protein concentration was not correlated with quantitative semen quality indicators; sperm output r = 0.16 ($p>0.05$), DSP r = - 0.4192, DSP/g r = -0.4035 ($p>0.05$) already reported (Orlu and Egbunike, 2009). All quantitative variables exhibited low correlation coefficients and low coefficients of determination (Table 1). This result agrees with earlier report by Egbunike and Nkanga (1999) who observed a lack of correlation between semen volume, sperm concentration and sperm output with seminal plasma protein concentration.

Novak *et al*. (2010b) based on stepwise regression analysis reported that seminal plasma protein together with sperm citrate synthase were predictive of fertility ($r = 0.77$, $p<0.0001$) in stallions. Egg fertility and hatchability were correlated with seminal plasma protein, r = 0.6302 vs-0.7179 ($p<0.01$) until the concentration of 3.25 g/100 mL and 3.03 g/100 mL for fertility and hatchability, respectively. Higher concentrations of seminal plasma protein resulted in depression of both fertility and hatchability. This result is very similar to Thurston *et al*. (1992) who reported that when seminal plasma protein concentration was as high as or greater than 6 g/100 mL there was significant reduction in fertility and hatchability in Turkeys.

Season had no significant influence on egg quality, fertility and hatchability in the barred Plymouth Rock and Nigeria indigenous breeds of domestic fowl. Nkanga and Egbunike (1992) made similar observation in the Hubbard breed of the domestic fowl. Variations in fertility and hatchability are thus attributed to differences in semen quality. Quantitative semen characteristics were favored by dry season as already observed (Nkanga, 1996; Nkanga and Egbunike, 1988) so were daily sperm production (Orlu and Egbunike, 2009), testicular morphometry (Orlu and Egbunike, 2010a) and testicular histometric parameters (Orlu and Egbunike, 2010b).

Qualitative parameters such as sperm motility, percent dead and percent abnormal spermatozoa were favored by the cooler months of rain and were highly significantly ($p<0.01$) correlated with seminal plasma protein concentration. This is similar to Chacur *et al*. (2009b, 2010a) who reported that winter cooler months favored semen quality in stallions and bulls. The significant correlation of seminal plasma proteins with semen quality indicators is a reflection of its role in fertility and hatchability in the domestic fowl. Mogielnicka and Kordan (2011) shared this view and stated that among many components of seminal plasma, proteins and peptides play a specific role in regulation of the fertilization process, particularly through their ability to bind various types of ligands such as polysaccharides, lipids and ions while Therien and Manjunath (1997) reported that in humans, several seminal plasma proteins were found which serve as diagnostic markers of spermatogenesis, seminiferous epithelium state and azoospermia.

Muino-Blanco *et al*. (2008) on the other hand maintain that the role of seminal plasma in mammalian sperm function remains largely a matter of speculation as both inhibitory and stimulating effects have been reported. They reported that specific components of seminal plasma, particularly proteins, are adsorbed onto the surface of ejaculated sperm as they pass through the male and female reproductive tracts.

Maximal values of semen quality indicators (sperm motility, percent dead and percent abnormal sperm cells) as well as percent fertility and hatchability of the flock of domestic fowl can be predicted with known values of seminal plasma protein concentration. From Table 4 it is shown that the Y_{max} for each variable can be predicted by differentiation in a quadratic equation $Y = AX^2 + BX + C$ and by setting the resultant equation to zero such that the maximum values of each variable would be obtained by substituting in $Y = AX^2+BX+C$..

Thus at seminal plasma protein concentration of 3.03 g/100 mL a maximum percentage hatchability of about 70% can be predicted, 72% fertility and a minimum percent dead spermatozoa of 6.8% obtained. Values of seminal plasma protein concentration (X) greater than this become limiting and results in reduction in fertility and egg hatchability. Of particular interest was the observation that the maximum values (Y_{max}) of semen quality variables could also be predicted. Maximum sperm motility of 78.03% was obtained at X = 3.43 g/100 mL. This result showed that the best semen quality, highest percent fertility and hatchability would be obtained when the concentration of seminal plasma protein is >3.00 g/100 mL but less than 5.00 g/100 mL.

CONCLUSION

The concentration of seminal plasma protein can, therefore, be used as a biomarker for prediction of semen quality, fertility and hatchability and for formulation of diluents for artificial insemination in breeding programs of the domestic fowl.

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