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STUDIES ON COCK SEMEN. II. EFFECTS OF FREQUENT EJACULATION AND BREED ON SOME BIOCHEMICAL CHARACTERISTICS

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Target Audience: Poultry farmers and breeders, animal scientists, veterinarians.

ABSTRACT

The effects of frequent ejaculation and breed on some biochemical characteristics of cock semen were studied using 18 cocks made up of 12 nine month-old exotic cocks (six Harco and six Anak strains) and six adult local cocks of unknown ages. The cocks were ejaculated once, twice or thrice daily for a seven-day period in a change over design. No significant breed influence was observed in seminal protein as well as spermatozoal phospholipid, protein and acetylcholinesterase activity. Frequent ejaculation resulted in significant ($P<0.05$) decrease in spermatozoal acetylcholinesterase activity. The study showed that biochemical composition of cock semen is stable among breeds and that frequent ejaculation could have a deleterious effect on the quality of ejaculates through a reduction in sperm motility.

Key words: Semen; cock; frequent ejaculation; biochemical characteristics.

DESCRIPTION OF PROBLEM

Reproductive inefficiency is recognised as the costliest and limiting constraint to efficient animal production. It is well known that both qualitative and quantitative characteristics of semen have a marked effect on egg fertility (1,2). In our earlier report, Gbadamosi and Egbunike (3) showed that frequency of ejaculation could have adverse effect on the quality of the ejaculates and hence the fertilizing capability, with the exotic cocks still having higher quality semen compared to the local cocks.

Spermatozoa are known to draw nutrients from two main sources - the seminal plasma and secretion of the female genital tract-during natural service (4). The main chemical constituents of spermatozoa are (a) deoxyribonucleic protein in the sperm nucleus and protein-bound mucopolysaccharide in the acrosome, (b) phospholipid (mainly plasminogen) in the mid piece and tail, (c) keratin-like proteins which compose the sperm membrane and fibrile and (d) a

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variety of enzymes and coenzymes which control the motility and metabolic activities of spermatozoa (5,6).

Evaluation of biochemical characteristics enhances the assessment of the functional state of the epididymis in which spermatozoa are stored awaiting ejaculation. Malfunction of both the epididymis and other accessory glands affects the fertilizing capacity of the spermatozoa adversely (7,8). Biochemical analysis of the seminal plasma can give information on the secretory function or capacity of the accessory sex glands, ejaculation process and the integrity of the sperm membranes. Studies on the biochemical characteristics of semen are thus essential for the understanding of sperm metabolism and the exchange of biochemical materials between the spermatozoa and the seminal fluid (8).

The objective of this study is therefore to evaluate the effects of frequent ejaculation and breed on spermatozoal protein, phospholipid and acetylcholinesterase activity.

MATERIALS AND METHODS

Experimental animals: A total of 18 cocks consisting of 12 nine-month old exotic cocks, made up of six Harco (2.57 ± 0.03 kg) and six Anak (2.33 ± 0.05 kg) strains, and six adult Nigerian indigenous local breeds of unknown ages (1.49 ± 0.07 kg), were randomly divided into three experimental groups with each strain being represented by two cocks.

Management of experimental animals: The cocks were kept singly in battery cage compartments measuring 0.15m x 0.12m x 0.15m in the Teaching and Research Farm, University of Ibadan with an equatorial and semi-hot climate (9). They were fed on commercial breeders' ration of 18% crude protein, about 130 g per cock per day, and given water *ad libitum* throughout the duration of the experiment.

Experimental design: Three experimental groups made up of two cocks of each breed were each subjected to three experimental ejaculation frequencies in a change over design. The ejaculation frequencies used were once a day, twice a day and thrice a day, each for seven days. This was done with thirty minutes between successive ejaculations and two-week rest period between frequencies.

Semen collection: Semen was collected in the dry rainy season by the double hand lumbar massage method as described by Burrows and Quinn (10). The cocks were subjected to a daily pre-experimental training period of three weeks to get them acquainted with the collection method.

Semen evaluation: The semen was centrifuged at 3,000g for three minutes and the plasma separated, diluted 1 in 10 with deionized water and stored frozen for analysis. Thereafter, the pellet was washed once with deionized water, again centrifuged, re-suspended in 1 ml deionized water and stored frozen for

analysis. Seminal fluid protein and spermatozoal protein, phospholipid and acetylcholinesterase activity were evaluated.

Protein concentration was determined in both seminal plasma and sperm cells by the biuret method of Weichselbaum (11). Acetylcholinesterase activity in sperm cells was measured by following the increasing yellow colour produced from thiocholine when it reacted with dithionitrobenzoate ion (DTMB). The absorbance of the 5-thio-2-nitrobenzoic acid was colorimetrically measured at 405 nm according to Ellman *et al* (12) and expressed as mmole/billion sperm cells/minute.

Phospholipid phosphorus was determined by the colorimetic method outlined in the Boehringer (13) Diagnostic kit after Zilversmit *et al.* (14). Thereafter, the phospholipid phosphorus was multiplied by a factor of 25 to obtain the phospholipid concentration (15).

Data analysis: The raw data were analysed using a two-way analysis of variance (16) and the means were compared using Duncan's multiple range test (17).

RESULTS AND DISCUSSION

A total of 751 successful ejaculations was obtained. The total seminal plasma protein concentration appeared to be highest in the Harco strain compared to the Anak and local strains and remained stable even as the frequency of ejaculation increased. However, there was more protein in the sperm cells than in the seminal plasma (Table 1).

The results presented here are consistent with our earlier reports (18,19) with respect to breed differences in the biochemical composition of chicken spermatozoa. However, no significant difference was observed in seminal protein concentration among breeds possibly because the amount in cock semen is species specific as the values were highly stable between breeds. It is worthy also to note that protein concentration in sperm cells found to be higher than that of the seminal plasma is in agreement with the report of Mann (5) as the main chemical constituents of spermatozoa are deoxyribonucleic protein, protein-bound mucopolysaccharide, keratin-like proteins, phospholipid and a variety of enzymes and coenzymes.

No significant breed difference was observed in the sperm phospholipid with the values for the local birds being 1.15 ± 0.04 mmole/10^9 cells and the two exotic strains having 1.17 ±0.03. Increase in the frequency of ejaculation did not influence ($P>0.05$) sperm phospholipid content (Table 1).

The non-significant difference observed in phospholipid content of sperm cells within breeds and as ejaculation frequency increased may be attributed to the

Table 1: Variations of Some Cock Semen Biochemical Characteristics at Different Ejaculation Frequencies

	Breeds								
Seminal Protein (mg/10^9 cells) Spermatozoa Protein (mg x 10^9 cells)	Local			Anak			Harco		
Sperm phosphoeipid concentration (mmole/10^9 cells)	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3
Sperm Acetylcholinestera se (mmole/10^9 cells) activity	1.23 ± 0.04 2.11 ± 0.03 1.18 ± 0.10 5.05 ± 0.03[a]	1.24 ± 0.03 2.14 ± 0.03 1.21 ± 0.09 3.57 ± 0.03[b]	1.21 ± 0.06 2.15 ± 0.05 1.07 ± 0.04 2.52 ± 0.13[c]	1.24 ± 0.05 2.12 ± 0.04 1.23 ± 0.09 4.49 ± 0.09[a]	1.24 ± 0.04 2.14 ± 0.03 1.18 ± 0.04 3.44 ± 0.26[b]	1.26 ± 0.05 2.17 ± 0.03 1.09 ± 0.06 2.25 ± 0.16[c]	1.22 ± 0.05 2.13 ± 0.04 1.22 ± 0.09 4.41 ± 0.11[a]	1.25 ± 0.03 2.16 ± 0.02 1.19 ± 0.09 3.31 ± 0.24[b]	1.13 ± 0.03 2.08 ± 0.03 1.09 ± 0.05 2.34 ± 0.17[c]

F_1 = Once a day
F_2 = Twice a day
F_3 = Thrice a day

* Seminal pasma protein is expressed in mg/10^9 cells suspended therein.
abc: Values for each breed along the same row differently superscripted are significantly different ($P<0.05$)

highly stable phospholipid content within breeds as observed by Poulos et al. (20).

Sperm acetylcholinesterase activity (AChE) decreased significantly ($P<0.05$) as the frequency of ejaculation increased but was not influenced by breed (2.13 ± 0.01 mg x 10^9 cells for local birds compared to 2.14 ± 0.01 mg x 10^9 cells for Anak and 2.12 ± 0.02 x 10^9 cells for Harco). The decrease in AChE from one ejaculation a day to two ejaculations a day was 26.02% while that from the latter frequency to three ejaculations a day was 31.10%.

The observed significant ($P<0.05$) decrease in the value of this enzyme activity in cock sperm cells as the frequency of ejaculation increased is in agreement with earlier findings (18). A positive relationship was established between sperm AChE and motility (19). Thus, the decreasing motility which resulted from increased frequency of ejaculation (3) suggests that AChE plays a vital role in sperm motility. This is however in contrast with the findings of Sekine (21) and Egbunike (2) that a negative relationship exists between AChE and motility in mammalian spermatozoa. As inferred earlier (19), this may be an implication of some differences in the physiological responses of mammalian and avian spermatozoa to various elements in extension media during preservation as well as differences in the time required by these spermatozoa to undergo the maturation processes. All these are presently being investi-gated.

CONCLUSION AND APPLICATION

Based on these and our previous results, we may conclude that:

1. Breeds compare favourably in their spermatozoal biochemical constituents.
2. Increased frequency of ejaculation significantly decreased sperm acetylcholinesterase activity like sperm concentration and motility.
3. Inspite of the changes observed with the frequency of ejaculation, semen characteristics were still within the normal range adequate for natural or artificial insemination without any loss in fertility.

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