AN ABSTRACT OF THE THESIS OF

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Title: Factors Affecting Semen Quality and its Effect on the Fertilizing Ability of Broiler Breeder Spermatozoa Using a Fluorometric Assay.

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Thomas F. Savage

Three distinct studies using an ethidium bromide-digitonin fluorometric assay were conducted to determine if the fertilizing ability of individually caged broiler breeder males (BBM) could be enchanced by nutrition, breeding and semen extender viscosity.

The first study evaluated the effects on the spermatozoal quality of BBM by feeding corn-soybean meal (CS) and corn-yellow pea (CYP) diets containing 16% crude protein (CP) fed on a daily restricted basis and the 7% CP fed ad libitum. The study was conducted from 30 to 43 weeks of age (WOA). Under the conditions of this study, feeding the diets containing CS and CYP did not have

detrimental effects on spermatozoal cell characteristics, fertilizing ability and hatchability of fertile eggs. This study demonstrated that BBM kept in cages could be fed yellow peas as well as 7% CP diets.

In the second study, BBM were divergently selected for a spermatozoal cell viability index score for three generations. Semen was collected and evaluated in each generation for semen viability, fertilizing ability and hatchability of fertile eggs. No differences ($P>.05$) were observed between the divergently selected lines in both generations (G1 and G2) for the semen characteristics evaluated. Attempts to explain the lack of response to divergent selection were discussed.

The third study attempted to decrease the conventional insemination dose of $100 \times 10^6$ spermatozoal cells to $33 \times 100^6$ per hen by increasing the viscosity of Beltsville Poultry Semen Extender (BPSE) by the addition of 4% (w/v) carboxymethylcellulose (CMC). The fertility at the lower insemination dose was not enchanced by increasing the viscosity. When the data was arranged according to the insemination dose and fertility was evaluated over the first seven days of the fertile egg collection period, no difference was observed ($P<.05$) according to the insemination dose. Therefore, the cost of maintaining the BBM could possibily be reduced by using $33 \times 10^6$ sperm cells for artificially insemination under the field conditions.

# FACTORS AFFECTING SEMEN QUALITY AND THEIR EFFECTS ON THE FERTILIZING ABILITY OF BROILER BREEDER SPERMATOZOA USING A FLUOROMETRIC ASSAY

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Salim M. Bootwalla

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Redacted for Privacy

Dean of Graduate School

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## CONTRIBUTIONS OF CO-AUTHORS

Dr. David P. Froman coadvised in these studies and under his direction the study on semen extender viscosity was performed. Dr. Vinai Rakphongphairoj was a co-worker in the experiment evaluating the effect of yellow peas on spermatozoal cell quality of broiler breeder males kept in cages.

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## CHAPTER I

## INTRODUCTION

Maintenance of breeder males for semen production is a substantial production cost in the breeder segment of the poultry industry. According to Sexton (1983), the estimated production cost per rooster was $30 in the year 1983. According to the USDA Agricultural Statistics (1986), there are approximately 3.4 million breeder males in the U.S. Assuming the present cost of breeder males to be the same as that of the year 1983, the overall cost of maintaining breeder males would be approximately 100 million dollars today. Thus, research directed at reducing the production cost of breeder males while maintaining semen quality is warranted.

The subject of male reproductive efficiency has been reviewed by Parker et al. (1942), Wilson et al. (1979), Sexton (1983), and most recently by Harris (1984) and Lake (1984). These authors have described how the reproductive efficiency of the male breeder can be manipulated by a variety of factors such as breeding, environment (daily photoperiod, temperature, and housing), nutrition,

frequency of semen collections, and the technique of artificial insemination (AI). In addition, these authors have also stressed the importance of evaluating the semen prior to insemination as a further activity to improve the reproductive efficiency.

This dissertation describes three distinct experimental approaches directed at improving the reproductive efficiency of broiler breeder males, by the use of a fluorometric technique to evaluate the fertilizing ability of spermatozoal cells:

1. The feeding of yellow peas (Pisum sativum L. var. Miranda) as an alternative protein source for maintaining the breeder males in production.

2. Genetic selection for enhanced spermatozoal cell viability.

3. Reduction in the insemination dose (spermatozoal cell concentration) in AI by increasing the viscosity of the extended semen.

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# CHAPTER II

# REVIEW OF LITERATURE

## ARTIFICIAL INSEMINATION

A factor that could enhance the reproductive efficiency of broiler breeders males (BBM) is the use of artificial insemination (AI). In chickens, AI is used mainly for research purposes, although Quinn and Burrows (1936) reported that high fertility (97%) could be obtained by inseminating chickens with .1 cc of undiluted semen. Wilson et al. (1979) reported that fertility from natural matings was poorly correlated with the physical characteristics of the male. Wilson et al. (1979) indicated that correlations between individual semen quality characteristics and fertility were not significant in natural mating, but with AI, spermatozoal cell concentration, percent dead spermatozoa, and motility were significantly correlated with fertility. Ansah et al. (1980) suggested that it was feasible to produce commercial broiler hatching eggs using AI. Renden and Pierson (1982a,b) observed that broiler breeder males housed in cages consistently produced larger volumes and more concentrated semen than males maintained on wood-shavings-litter-covered floors. They also noted that BBM

could be successfully used in an AI program through 105 weeks of age (WOA), with no significant reductions in volume, spermatozoal cell concentration, total numbers of spermatozoa per ejaculate, or its fertililizing ability.

## TECHNIQUES TO EVALUATE SPERMATOZOAL CELLS

According to Wilson et al. (1979), it would be beneficial to the commercial breeder if a rooster's semen could be evaluated before its use in the flock in order to select those males whose semen would improve fertility. Although the development of laboratory techniques for the determination of semen quality, such as spermatozoal cell concentration, motility, and number of intact spermatozoal cells, have been markedly improved during the last 20 years, there is still no single spermatozoal measurement that can be used for the accurate estimation of fertilizing ability. Various instruments are used to estimate the fertilizing ability of the spermatozoal cells: the microscope and, recently introduced, the fluorometer.

### Microscopic Evaluations

Microscopic evaluation of semen quality in poultry has been applied to study the spermatozoal cell morphology (Parker et al., 1942), subjective spermatozoal cell motility scoring (Parker et al., 1942; Allen and Champion,

1955; McCartney, 1956; Cooper and Rowell, 1957; Boone and Hudson, 1963; Soller et al., 1965; and Sexton, 1976), spermatozoal cell concentration (Parker et al. 1942; and Allen and Champion, 1955), and various staining procedures for the determination of live and dead spermatozoal cells (Cooper and Rowell,1958; El Jack and Lake, 1966; Kamar, 1960; Chatterjee et al., 1967; and Wilson et al., 1969). The major disadvantage of microscopic evaluation for semen quality is the subjectivity of error made by either different preparatory handling procedures or characteristics of different materials used for the evaluation of seminal quality (Cooper and Rowell, 1958; Wilson et al., 1969; and Cooper, 1969). The use of the microscope is still the major instrument for semen quality evaluation.

## Fluorometric Evaluation

A new method for semen quality evaluation is based on the principle of fluoresence and the use of the fluorometer. The function of the fluorometer in semen evaluation is the simultaneous determination of the spermatozoal cell concentration and the incidence of intact and damaged spermatozoal cells in an ejaculate. The two chemicals used in the fluorometric evaluation of semen are ethidium bromide (EB) and digitonin.

Le Pecq and Paoletti (1966) were the first to report

that the EB binding to nucleic acids (NA) increased its fluorescence quantum efficiency by a factor of 20 to 25. They further reported the increased fluorescence of an EB solution of a given concentration was proportional to the concentration of NA added. They concluded that EB markedly increased its fluorescence when it was bound to double stranded DNA or double stranded regions of RNA.

Halangk and Bohnensack (1982) reported that EB bound to the DNA of bull spermatozoa could be used for the estimation of spermatozoal cell intactness and concentration. These authors were the first to use digitonin, which allowed the spermatozoal cell membrane to become permeable to EB. They reported that digitonin removed the "permeability barrier" for EB in intact cells when used at a concentration of 50 ug/ml. Therefore, the measured fluorescence before and after digitonin treatment reflected the proportion of spermatozoal cells in a sample with damaged cellular membranes. The fluorescence after the addition of digitonin was highly correlated with spermatozoal concentration.

Based upon this same principle, Bilgili and Renden (1984) evaluated a flourometric method for its accuracy and validity with rooster semen for the simultaneous determination of spermatozoal cell concentration and the incidence of damaged cells. They reported a highly significant ($P<.001$) correlation coefficient ($r=.99$) for

the fluorometric reading and spermatocrit. Later, Bilgili et al. (1985) compared the fluorometric technique for estimating the percentages of dead chicken spermatozoal cells with the eosin-nigrosin staining procedure and the concentration of glutamic oxaloacetic transaminase in seminal plasma. Both procedures have been utilized as assays for spermatozoal membrane integrity. Bilgili et al. (1985) reported the correlation of percent dead spermatozoal cells determined by fluorometry and the eosin-nigrosin staining to be highly significant, $r = .99$ ($P < .001$). Thus, for determining spermatozoal cell concentration and the percentage of intact spermatozoal cells, the fluorometer offered potential. Also, this instrument could be used under field conditions.

## IMPROVEMENT OF MALE REPRODUCTIVE EFFICIENCY BY NUTRITIONAL MANIPULATION

### Dietary Protein and Energy Restriction

One of the first reports describing the effects of feeding low levels of dietary protein on the reproductive performances of male chickens was reported by Arscott and Parker (1963). They fed dietary crude protein (CP) levels of 16.9, 10.7, and 6.9% to White Leghorn roosters. They reported no adverse effects on the fertilizing capacity of spermatozoal cells as determined by AI, as well as on the subsequent hatchability of fertile eggs on the dietary

protein level as low as 6.9%. No additional information regarding feeding of low protein diets to the breeder males during semen production was available until the report of Buckner and Savage (1986). They reported that a diet containing 5% CP with 2314 kcal ME/kg, when fed ad libitum to individual caged BBM, did not adversely influence semen production. Later, Wilson et al. (1987a) reported that the low dietary CP had no significant effect on semen volume, spermatozoal cell concentration, number of spermatozoal cells per ejaculate, or testis weight of caged BBM. They concluded that the males could be fed 12 and 14% dietary CP on a restricted basis with no adverse effects on semen quality. In a sequential study, Wilson et al. (1987b) reported that higher percentages of caged BBM produced semen when fed 9% dietary CP level than either 12 or 15% CP on a restricted feeding program.

The first report of an effect of dietary energy restriction on the reproductive performance in male chickens was also reported by Parker and Arscott (1964). They indicated that a "harsh" dietary energy restriction reduced seminal production, testicular weights, and fertilizing capacity of White Leghorn males when fed ad libitum. There are no other reported investigations of the effects of low dietary energy feeds on semen production. Thus, there is a need for research on the effects of restricting protein, energy, and other nutrients on the

reproductive performance of male chickens.

## Alternative Poultry Feed

One of the main factors for the high cost of feed ingredients in animal feed deficient production areas of the United States is the cost of transportation. In the Pacific Northwest (PNW) corn and soybean are transported from the Midwest, which substantially increases the price for those ingredients. Therefore, it is important to develop as well as utilize new indigenous poultry feed sources. Thus, alternative feed proteins are those ingredients that can totally or partially replace corn and/or soybean meal as an energy and/or protein source. Yellow peas (*Pisum sativum L* variety Miranda) has been introduced in the PNW as a possible alternative protein source for poultry diets (Savage *et al.*, 1986a).

### Chemical Composition of Yellow Peas

Pea seeds, depending upon the variety and growing conditions, may contain (on a dry basis): 9 to 15% water, 18 to 35% protein, 24 to 60% carbohydrates, 0.6% to 1.5% lipids, 2 to 10% cellulose, and 2 to 4% minerals (Makashewa, 1983). A similar analysis by Cousin (1983) stated that dried peas contained 23 to 33% protein and 40% to 50% starch.

According to Makashewa (1983), different pea

varieties contain varying amounts of amino acids (as a percentage of the decalcified proteins) as follows:

| | |
|---|---|
| Tyrosine | 2.3 to 3.3% |
| Cystine | 0.73 to 3.3% |
| Aspartic and glutamic acids | 26 to 59% |
| Methionine | 1.4 to 1.9% |
| Lysine | 3.7 to 6% |
| Tryptophan | 0.99 to 1.3% |
| Histidine | 2 to 2.6% |
| Arginine | 9.3 to 12.6% |

## Toxic Effects

Protease inhibitor: Naturally occurring trypsin and chymotrypsin inhibitors are polypeptides with molecular weights mostly between 8,000 and 12,000 or about 20,000 (Liener and Kakade, 1980; Weder, 1981). Trypsin and chymotrypsin inhibitory activities (TIA and CTIA, respectively) detected in different varieties of peas (Pisum sativum) have been measured and range from 7 to 13 TIA and 8 to 15 CTIA units, respectively. The TIA and CTIA units were expressed as milligram of enzymes by one gram of plant material (Weder, 1981). During feed processing most protease inhibitors are destroyed by wet heat (Kienholz et al., 1962; Liener and Kakade, 1980).

Lectins: Most leguminous seeds contain the heat-labile toxicants called lectins, but very few reports are available on their relative activities (de Muelenaere, 1965; Jaffe, 1980). Lectins are receptor-specific proteins and consist of two or four polypeptide subunits. They also

contain bivalent metal ions which are able to agglutinate a variety of cell types (Jaffe, 1980; Toms, 1981). Liener (1974) concluded that the toxic effects of lectins present in peas could be generally eliminated by proper thermal treatment, but it should be recognized that conditions might prevail whereby complete destruction of the lectins might not be achieved.

It is not known whether any of the toxins listed above has any effect on the semen quality of male chickens.

## The Use of Yellow Peas in Poultry

The field pea is an alternative protein source that has been included in the diet of market turkeys (Savage et al., 1986a). Peas are deficient in methionine; however, diets containing peas supplemented with methionine had comparable growth rates in chickens (Petersen et al., 1944; Bolin et al., 1946; Moran et al., 1968; Goatcher and McGinnis, 1972; Reddy et al., 1979). Lindgren (1975) observed that varieties (Lotta, Timo, Bello) of the pea (Pisum sativum) did not affect egg production of laying hens, but there was a significant negative correlation between tannin content and protein digestibility.

There was no adverse effect on the growth rate or feed efficiency in laying hens in which 5 to 35% of the corn or soybean meal in the control diet was replaced by

pea meal on an isocaloric and isonitrogenous basis (Vogt, 1984). He indicated that growing birds could be fed diets containing up to 45% raw or thermal treated peas (with methionine supplementation) without adverse affect on growth rates. Vogt (1984) also found that when laying hens were fed diets containing up to 30% peas, a decline in egg production resulted. Diets containing peas that were subjected to thermal treatment and supplemented with methionine had improved egg production, feed efficiency, and egg weights. Askbrant and Hakansson (1984) demonstrated that the digestibility of crude protein in diets of laying hens containing yellow peas was lower than that for soybean meal diets, while the digestibility of total carbohydrates was higher.

There are very limited reports on the effects of feeding yellow peas and the male reproductive system in poultry. Savage et al. (1986b) reported no difference in mean spermatozoal viability when Medium White toms were fed a diet containing yellow peas, when compared with a corn-soy diet. Rakphongphairoj (1987) reported significantly lower semen and packed sperm volumes for caged broiler breeder males fed 7% and 16% crude protein diets containing yellow peas when compared to their respective corn soy diets. Fertilizing capacity and hatchability of the eggs fertilized were not affected by feeding the males either corn soy or corn yellow pea

diets.

## IMPROVEMENT OF SEMINAL QUALITY BY GENETIC MANIPULATION

Semen quality is any measurable semen characteristic that provides a quantitative value to estimate the fertilizing ability of the spermatozoa. Some of these characteristics include spermatozoal cell concentration, spermatozoal cell motility, and the numbers of intact and damaged spermatozoal cells. One of the methods to improve semen quality is by the implementation of changes through genetic selection. Jones and Lamoreux (1942) studied the effect of intense selection for high and low fecundity lines based on egg production among strains of White Leghorn hens. Males obtained from the high fecundity line exhibited sexual maturity as early as 12 weeks of age (WOA) and had a higher number of males producing semen than the low fecundity lines when both the lines were raised under the same environmental conditions. The fertilizing capacity of spermatozoa in the high fecundity line, however, was lower than in the low line (19 and 43%, respectively). The authors concluded that the production of eggs and semen were the results of comparable genotypic characteristics because semen and egg production were controlled by the same "genotypes." Later, Siegel (1963), reported low heritability estimates for semen volume,

spermatozoal cell concentration, and motility of 0.14, 0.01 and 0.29, respectively. These estimates were obtained from a growth selected line in which selection pressure was not placed on semen characteristics. Similarly, Marini and Goodman (1969) determined semen quality characteristics of males divergently selected for high and low growth rates. The semen quality from the low line males was of higher quality than the semen from the high line. Although they did not report any heritability estimates, the repeatability estimates obtained for the semen characteristics (concentration, volume, motility, methylene blue reduction test, dead and abnormal spermatozoal cells) for both the lines were high. This finding indicated that the reproductive characteristics might also be due to comparable genotypes controlled by the growth rates. Siegel (1963) and Marini and Goodman (1969) did not select directly for semen quality. Soller et al. (1965) selected for semen characteristics in White Rock roosters and reported heritability estimates for semen volume, spermatozoal cell concentration, and spermatozoal motility of 0.41, 0.46 and 0.87. These results were based on one generation of selection. Siegel (1965) in a subsequent study, selected lines of chickens for divergence in reproductive behavior rather than on the semen quality characteristics. For six generations he selected for the divergence in the cumulative numbers of

completed matings (CNCM), establishing a line with a high frequency of completed matings (HML), and a low mating line with a corresponding low frequency of matings (LML). His selection criteria included the number of courts, mounts, and treads. During the first three generations there were no significant differences ($P>.05$) between the number of CNCM between the HML and the LML. With the F4 and subsequent generations, however, significant differences between the lines for CNCM were obtained. Siegel (1965) suggested that the absence of a response to divergent selection through the F3 generation was a concealed genetic response due to pleiotropy. Dunnington and Siegel (1983), reported continued bidirectional selection in the HML and LML for twenty-three generations and calculated a realized heritability of 0.18. However, no differences in semen quality were observed based up on the fertility trials using AI. This suggests that the reproductive behavioral patterns, like that of semen characteristics, could be manipulated by genetic selection. In view of the above reports, the manipulation of semen quality through genetic selection warrants further investigation particularly with emphasis on divergent selection for semen quality traits.

## IMPROVEMENT OF MALE REPRODUCTIVE EFFICIENCY BY SEMINAL MANIPULATION

Due to the high concentration and low volume of poultry semen, it is recommended that semen be diluted by increasing the semen volume. The first purpose of a dilution is to uniformly distribute spermatozoal cells so that the proper dose of the cells for insemination may be used. The second purpose is to sustain and protect spermatozoa for short or long term storage. Dilution permits maximum utilization of semen from superior males and can be used to extend semen during periods of low semen production. Thus, the efficiency of spermatozoal cells can be improved which ultimately results in lowering the insemination dose and at the same time maintains optimum fertility. Lorenz (1964) reported that the fertilizing capacity of undiluted semen is rapidly lost at 1-2 C, but dilution allows for storage at these temperatures. As a consequence, semen may be stored for considerably longer periods of time than undiluted semen stored under the same conditions.

According to Mann (1964) and Graham (1976) an ideal semen extender should have an optimum osmotic pressure (about 325 mOsm/kg) and pH (about 7), contain chelators that could sequester heavy metals harmful to spermatozoal cells, bind calcium ions to prevent the acrosomal reaction, and contain appropriate antibiotics to inhibit

bacterial growth. The extender should also be able to provide protection from the deleterious effects of cooling, especially under storage conditions.

Since the metabolic rate of spermatozoal cells tends to be proportional to absolute temperatures, reduced temperatures have been a major means of decreasing chemical reactions and prolonging life of the spermatozoa. Semen may be stored at 5 C for a short period of time or frozen at -196 C indefinitely. In both situations, manipulation of the semen extender is required to maintain the seminal quality.

Development of Semen Extenders for Short Term Storage.

Short term storage usually refers to holding the diluted semen from a few hours to a few days usually between 0 and 5 C. The effect of short term storage on the fertilizing capacity of extended fowl semen was first reported by Schindler et al. (1955) who stored semen for periods of 2, 4, or 6 hours at temperatures of 4, 10, and 41 C, using Ringer's solution, Locke's solution, whole milk, and an egg yolk-phosphate buffer. They reported that semen, undiluted and diluted with either Ringer's or Locke's solutions or with whole milk, retained full fertilizing capacity of almost 90% after 4 hours of storage at 4 C, whereas with the yolk-phosphate buffer, fertility values as low as 40% were obtained. During the

same year, Polge in 1955, (as cited from Van Wambeke, 1967), stored fowl semen for 24 hours in a glucose citrate solution with either milk or thin albumen. Fertility as high as 50% was obtained after storage. Wilcox and Shaffner (1958) used a chemically defined media containing phosphate buffer and antibiotics with a 1:10 dilution resuspended in buffer plus fructose before insemination. After semen had been stored for 48 hours at 2 C, fertility as high as 70% was obtained. Lake and McIndoe (1959) analysed fowl seminal plasma for the presence of various chemical substances and reported that glutamic acid and creatinine accounted for about 80% of the non-protein nitrogen and that glutamic acid had a major role in maintaining the osmolarity. A year later, Lake (1960) became the first to describe a diluent which resembled the rooster's vas deferens fluid closely in respect to positive inorganic ions and glutamate. He also used fructose as a source of energy and maintained the pH of the diluent at 7. He reported the possibility of maintaining sufficient spermatozoa <u>in vitro</u> to produce satisfactory fertility and obtained up to 64 and 47% after 24 and 48 hours of storage, respectively. This was achieved by providing the spermatozoa a suspension media similar to that of the vas deferens fluid in osmolarity (with a freezing point depression of -0.6 C) and the presence of potassium, sodium, magnesium, and glutamate

salts. He also reported a lack of fertilization of diluted stored semen containing no fructose. At this stage, partial success for the short term semen holding was achieved. However, according to Sexton and Fewlass (1978), Van Wambake (1967) was the first to demonstrate that diluted chicken semen could be stored in vitro for up to 24 hours at 0 C without a critical loss of fertility. To this stage in the development of semen extenders, fertility trials were performed with AI and using insemination doses of at least 100 million spermatozoal cells.

No attention was directed towards the importance of insemination dose for optimum fertility during short term storage research, until Sexton (1977) developed a phosphate buffered semen extender, Beltsville Poultry Semen Extender (BPSE). He reported a fertility value of more than 88% when White Leghorn hens were inseminated weekly with 20 million spermatozaol cells suspended in the BPSE. Fertility levels of more than 90% were achieved with diluted semen and an insemination dose of 100 million cells. He further reported that fewer sperm were needed in the insemination dose when diluted semen (1:2) was held at 5 C than at 25 C during a 30-minute storage time. Sexton and Fewlass (1978) subsequently determined the capability of BPSE to function as a storage medium by assessing the relationship of various diluent components

and certain additions to the BPSE on fertility of chicken semen stored at 5 C. Omission of sodium glutamate or potassium phosphate resulted in a significant change in the osmolarity of extended semen. Adjusting the osmolarities of the solutions did not improve the fertilizing capacity of stored semen when compared to BPSE controls. The fertilizing capacity of semen diluted with the BPSE containing fructose was significantly higher than in the BPSE containing other sugars when stored for 24 hours at 5 C. Later, Sexton (1978) studied the effects of storage temperature, presence of seminal plasma, presence of visible light, and semen to air ratio in the storage vessel on the fertilizing capacity of spermatozoa after 0, 4 and 24 hours storage at 5 C. He reported that the fertilizing capacity of chicken semen stored at 5 C, when diluted and exposed to light during the storage period, was equal to that of the unstored controls (0 hours). The presence or the absence of seminal plasma, or the semen to air ratio in the storage vessel did not affect the semen viability stored at 5 C. In addition, Sexton et al. (1980) studied the effect of antibacterials in control of bacterial contamination in chicken semen. He reported that gentamicin, kanamycin, neomycin, and tobramycin were the only antibacterials which controlled microbial growth without affecting sperm viability for up to 24 hrs. storage at 5 C. These advances and techniques that allow

for short term semen preservation (up to 24 hrs.) between 2 and 5 C will have little commercial application with chicken semen until commercial adoption of AI is achieved (Sexton, 1983).

Development of Semen Extenders for Long Term Storage

Long term preservation of diluted semen is achieved through cryopreservation which allows indefinite storage for years. This technique of semen preservation has a potential application especially for the broiler breeder industry regarding the transportation of semen. According to Lake et al., (1981) frozen semen provides a means for the relatively cheap transport of desirable genes on a world-wide basis, offering possibilities of servicing elite grand parent breeding programs at low cost, and the provision of semen banks (gene pools) that may be less expensive than maintaining experimental lines which could have future significance.

The discovery that glycerol was cryoprotectant during freezing by Polge et al. (1949) revolutionized the process of cryopreservation of spermatozoal cells. Polge et al. (1949) maintained motile chicken sperm up to 10 weeks by the addition of 10 to 15 percent glycerol and freezing at -79 C on dry ice. However, while motile spermatozoa were recovered in either case, the fertilizing ability of spermatozoa so preserved were not reported.

Later, Allen and Bobr (1955) reported a decrease in the harmful effects of high levels of glycerol on the fertilizing capacity of fowl sperm if surgical intrauterine inseminations were made. Intravaginal insemination were not successful. Later, the fertility trials (using intravaginal insemination) were performed by Clark and Shaffner (1960) and Watanabe (1967 and 1972) with some success, obtaining fertilities of 29, 30, and 59% fertility respectively. Clark and Shaffner (1960) were the first to establish that semen containing glycerol was harmful to the fertilizing ability of the spermatozoa if inseminated into hens.

Sexton (1976) used 4% dimethylsulfoxide (DMSO) as a cryoprotectant in a semen extender, similar in composition to BPSE, for improving the fertilizing capacity of frozen thawed chicken semen. He concluded that lower fertility levels with frozen semen might be associated with an insufficient number of viable spermatozoa surviving the freeze-thaw process rather than an inadequate number of insemination. Lake and Steward (1978) reported on an improved method for storing fowl semen using glycerol as a cryoprotectant and stored semen for 2.5 years in liquid nitrogen at -196 C. They obtained fertility levels as high as 80%. Later, Lake et al. (1981) modified the freezing equipment for use under field conditions. They reported fertility of over 90% from semen which had been deep

frozen at (-196 C) for 2 months. They concluded that the use of frozen semen was a feasible aid in the enhanced breeding of domestic fowl.

Even though both natural and synthetic components have been utilized in attempts to achieve an ideal diluent both for long and short term storage, no effort has been made regarding the alteration of any physical characteristic of the poultry semen extender. One of the physical characteristics that could be altered is extender viscosity.

## Viscosity

According to Kramer and Twigg (1979), viscosity is defined as the friction resulting from the resistance to flow between liquid layers or the resistance offered by a substance to deformation when subject to a shearing force. According to Kramer and Twigg (1979), the Ostwald viscometer is the best known instrument which measures the viscosity by flow in a capillary tube. Viscosity is determined by the time (seconds) required for a fluid to flow through a capillary of a given distance while the instrument is immersed in a constant temperature water bath. With the Ostwald viscometer, the determinations are made as relative viscosity in which water is commonly used as a reference solution. The viscosity of water at 20 C is 1.0050 centipoise and 0.8937 centipoise at 25 C (Kramer

and Twigg, 1979).

## Semen Viscosity

There are only two reports that described semen viscosity of bull semen. Roberts and McKenzie (1943), reported that semen color and spermatozoal cell concentration were significantly ($P < .05$) correlated ($r = .89$) with the viscosity. Later, Szumowski (1948) indicated that semen consistency is an indicator of quality and that viscosity allows the determination for dilution rate that will effect the viability of spermatozoa for storage. He reported a mean viscosity value of 3.74 in relation to that of water. He further reported that viscosity was a function of spermatozoal cell concentration which varied from 1.76 in a specimen containing 80,000 sperm/ul to 10.52 in a sample with 2,260,000 sperm/ul. Szumowski (1948) reported that bull semen with a high spermatozoal cell concentration survives longer because of the high viscosity than semen with a lower concentration and a lower viscosity. His results though were not statistically significant. He also reported that viscosity was negatively correlated with the methylene blue test ($r = -.85$) which reflected the metabolic activity of the sperm. There was a significant negative ($r = -.57$) correlation seminal viscosity and semen pH. Though unlike Rikmenspoel (1957), Szumowski (1948) found a positive correlation

(+.51) between viscosity and motility. There has been no information pertaining to the viscosity of chicken semen and its relation to the seminal quality characteristics.

## Viscosity of Seminal Diluent

The only research that has been reported on the viscosity of a diluent is that which was conducted on bull semen. Rikmenspoel (1957) indicated that viscosity of the fluid surrounding the spermatozoa is the only one that can be varied without influencing the physiologic conditions of the spermatozoal cells. He reported that mean motility of the bull spermatozoal cells was a function of the medium (i.e., inversely proportional to the viscosity of suspending media). He used diluents with increased percentages of egg yolk to obtain an increased viscosity. He also mentioned the use of two substances to increase viscosity of diluent which were known to have little physiological actions, dextrin and carboxymethylcellulose. Other substances were carboxymethylpyralidon, gum arabic pectin, polyacrylic acid, and polyacrylic alcohol, but all these substances precipitated in the presence of egg yolk.

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# CHAPTER III

## SEMEN QUALITY OF INDIVIDUALLY CAGED BROILER BREEDER MALES FED 16 AND 7% CRUDE PROTEIN DIETS CONTAINING YELLOW PEAS

S.M Bootwalla, V.J. Rakphongphairoj,

and T.F. Savage

# ABSTRACT

The semen quality of caged broiler breeder males (BBM) fed corn-soybean (CS) diets consisting of a daily restricted 16% crude protein (CP), a 7% CP fed ad libitum, and corn yellow pea (CYP) diets of a daily restricted 16% CP and a 7% CP fed ad libitum was evaluated using an ethidium bromide-digitonin assay. Spermatozoal cell motility (MS), concentration (SC), percentage of intact cells (ISC), and cell viability index (SVI) score of BBM were evaluated weekly from 30 to 43 weeks of age (WOA).

The SC was higher ($P<.05$) in the semen of males whose diets contained CS when compared with CYP diets. The CYP diet with 7% CP had a significantly higher SC than the corresponding 16% CP. The ISC percentages were higher ($P<.05$) for males fed CYP diets containing 16% CP. Neither the dietary protein source (soybean meal and peas) nor the dietary protein level (7 and 16%) influenced the MS, SVI, fertility, or hatchability. It was concluded that individually caged BBM could be fed diets containing yellow peas and the ad libitum feeding of 7% CP diets did not affect the semen quality.

# INTRODUCTION

In the Pacific Northwest (PNW) corn and soybean are transported from the Midwest which result in substantial increases in the prices for these feed ingredients. There are various approaches to reducing feed costs associated with broiler breeder male production. One approach is to develop and utilize new alternative feed sources. Alternative feeds can be regarded as those ingredients that can totally or partially replace corn and/or soybean meal (SBM) as sources of energy and/or protein, respectively. A variety of field peas (Pisum sativum L variety Miranda), locally referred to as yellow peas, has been introduced in the PNW as such an alternative protein source for poultry diets (Savage et al., 1986a). Feeding yellow peas to Medium White turkey breeder toms did not affect semen quality characteristics (Savage et al., 1986b). Rakphongphairoj (1987) also reported no difference ($P>.05$) in the fertilizing ability of broiler breeder males (BBM) fed corn-yellow pea diets containing 7 and 16% CP. There are no other literature reports describing the effects on the reproductive performance of breeder males fed diets containing yellow peas.

A second approach to reducing the cost of feed for BBM would be to reduce the level of dietary crude protein (CP) fed, provided semen quality was not adversely

affected. Arscott and Parker (1963) fed diets with decreasing CP levels of 16.9, 10.7, and 6.9% to White Leghorn males without affecting the fertilizing capacity of the spermatozoal cells. Buckner and Savage (1986) reported that a 5% CP diet fed ad libitum to individually caged BBM did not influence semen production. Wilson et al. (1987a) reported that feeding BBM daily restricted 12% and 14% CP also had no effect on semen volume, spermatozoal cell concentration, number of spermatozoal cells per ejaculate or testicular weights when compared with males fed 16 and 18% CP diets. In a sequential study, Wilson et al. (1987b) fed BBM males 9, 12 and 15% CP diets on a daily restricted basis. The percentage of males producing semen when fed the 9% CP diet was greater ($P<.05$) than those fed the higher CP diets. This demonstrated that the feeding of low protein diets to caged BBM sustained optimal semen production. Recently, Bilgili and Renden (1984) described a biochemical assay to fluorometrically evaluate the integrity, concentration, and fertilizing capacity of spermatozoal cells. Thus, the objective of this study was to evaluate, using a fluorometric technique, the effects on semen characteristics of BBM housed in individual cages and fed yellow pea (variety Miranda) diets with different levels of dietary CP and feeding programs.

# MATERIALS AND METHODS

Peterson BBM were raised on a wood-shaving-litter-covered-floor pen from day-old to 20 weeks of age (WOA), according to the breeder's recommendation, using an 8L:16D light-dark lighting regimen. At 20 WOA, 40 males were randomly assigned to individual cages (30.5 cm x 45.7 cm x 45.7 cm) within a windowless positive pressure-ventilated house and provided eight 15-minute watering periods at two-hour intervals during the light period (0400 to 1800 hours, daily). The males were allowed to adapt to the cages from 20 to 22 WOA.

At 22 WOA, ten BBMs were randomly assigned to each of four dietary treatments, a 16% CP corn-soybean (CS) diet with 2850 kcal/kg ME fed on a daily restricted basis, a 7% CP CS diet with 2414 kcal/kg ME fed ad libitum, a 16% CP corn-yellow pea (CYP) diet with 2859 kcal/kg ME fed on a daily restricted basis, and an ad libitum-fed 7% CP CYP diet with 2414 kcal/kg ME. The composition of the four diets are described in Table III.1. The dietary protein and energy levels of the four diets were obtained by the addition of a #70 grade sand. The amount of the 16% CP diets fed corresponded to 80% of the breeder's daily feeding recommendation for males in litter pens (126 grams per bird per day).

Between 22 and 29 WOA, semen was collected from the BBM weekly and the experiment was conducted between 30 and 43 WOA. The males were ejaculated using an abdominal massage technique. Semen was collected in plastic cups and the volume was not measured. The following spermatozoal cell characteristics were evaluated:

Motility Score (SM). Spermatozoal cell motility scores were subjectively determined according to the method of Cooper and Rowell (1958) using an unheated microscope slide stage. Motility was subjectively scored from 0 to 4. A score of 0 indicated all spermatozoal cells appeared immotile, and a score of 4, all spermatozoal cells exhibited vigorous and progressive motion.

Spermatozoal Cell Concentration (SC) and Percent Intact Spermatozoal Cells (ISC). These characteristics, cell concentration and the percentage of intact cells, were determined using the ethidium bromide-digitonin fluorometric procedure described by Bilgili and Renden (1984) with a Turner model 111 filter fluorometer.

Spermatozoal Cell Viability Index (SVI) Score. The SVI score was calculated as the arithmetic product of the spermatozoal cell motility score (MS) x cell concentration (SC) x percentage of intact cells (ISC).

Fertility and Hatchability. Hen fertility and the subsequent hatchability of fertile eggs were determined at 32 and 36 WOA, by artificially inseminating 4 individually caged Peterson broiler breeder hens with 100 million spermatozoal cells from each male. Eggs were marked and collected daily from each group of 4 hens for 9 consecutive days starting on the second day following a single insemination. The eggs were set in an incubator and candled after the tenth day of incubation. The contents of eggs lacking embryonic development were macroscopically examined to determine true fertility.

The MS, SC, ISC, SVI score, fertility, and hatchability were each analyzed separately using a 2 x 2 factorial repeated measures design (Snedecor and Cochran, 1980) with protein sources (CS and CYP) and crude protein levels (7% and 16%) as the main effects. Significant differences ($P<.05$) among the treatment means were determined by Tukey's Studentized range test (Steel and Torrie, 1980). Data determined as percentages were transformed to arc sin values prior to statistical analysis.

# RESULTS AND DISCUSSION

The means for the semen characteristics, measured from 30 to 43 WOA, and the fertility and hatchability at 32 and 36 WOA are summarized in Table III.2.

Motility Score. There were no differences ($P<.05$) observed in motility scores between the protein sources (CS and CYP) and the CP levels (7% and 16%). Also, there were no protein source by CP interactions.

Spermatozoal Cell Concentration. The concentration of the spermatozoal cells was higher ($P<.05$) in males fed diets containing CS at both dietary protein levels (7 and 16 %) when compared to their respective CYP diets. There was also no difference ($P>.05$) in cell concentrations between the two CS diets, 7% and 16%. When compared within the CYP diets, those males fed with 7% CP diet had higher spermatozoal cell concentrations than those fed the 16% CP CYP diet.

Intact Spermatozoal Cells. The intactness of cell membranes determined fluorometrically did not differ within dietary sources CS and CYP. When the percentages of intact cells were compared within protein levels, those of the males fed the 7% CP consisting of peas were

significantly (P<.05) lower than those of the males fed the 16% CYP diet.

Spermatozoal Cell Viability Index (SVI) Score. Dietary protein sources and protein levels did not influence the SVI scores.

Fertility and Hatchability. Both hen fertility and hatchability of fertile eggs resulting from the insemination of hens with semen collected from males fed the 4 dietary treatments were not different.

Based upon the results obtained using the ethidium bromide-digitonin, a fluorometric technique, BBMs can be fed diets containing yellow peas either as a daily restricted 16% CP or a 7% CP diet fed ad libitum. These results are in agreement with those of Savage et al. (1986b) who reported no difference in spermatozoal viability when Medium White turkey toms were fed a diet containing yellow peas. However, Rakphongphairoj (1987) reported lower semen volumes and packed sperm volumes of males fed 7 and 16% CP CYP diets, when compared with their respective CS diets. No differences (P<.05) were observed in the fertilizing ability and subsequent hatchability of eggs fertilized by semen from BBM fed either CS or CYP diets. The results obtained in this study also agree with those of Arscott and Parker (1963), Wilson et al. (1972),

Buckner and Savage (1986), and Wilson et al.(1987a,b) who reported that feeding of low protein diets do not reduce semen quality. None of their dietary treatments influenced fertility or hatchability.

According to Rakphongphairoj (1987) the CP intake of the caged BBM fed the ad libitum 7% CP diet containing CS or CYP were lower ($P<.05$) than that of the males fed restricted 16% CP diets; however, the daily ME intake of males fed 7% CP diets containing either CS or CYP were greater ($P<.05$) than the intake of males fed the 16% CP diets, because the dietary energy of the 7% CP diets was lower than the 16% CP diets. This difference in energy consumption was attributed to males fed the ad libitum 7% CP diets consuming almost 1.5 times the amount of feed when compared to the males fed restricted 16% CP diets. Under the conditions of this study, feeding BBM diets containing yellow peas (Pisum sativum L. variety Miranda) and the ad libitum 7% CP diets did not have any detrimental effects on semen quality, fertilizing capacity, and subsequent hatchability of fertile eggs. However, they consumed almost 1.5 times the amount of feed when compared to daily restricted 16% diets, which according to Wilson et al. (1987a) might not be feasible under field conditions.

Table III.1 Composition of broiler breeder male diets

| Ingredients | Corn-soy (CS) 16%CP 2855ME | Corn-soy (CS) 7%CP 2410ME | Corn-yellow pea (CYP) 16% CP 2855ME | Corn-yellow pea (CYP) 7%CP 2410ME |
|---|---|---|---|---|
| | % | | | |
| Corn, yellow | 67.88 | 69 | 33 | 45 |
| Peas, yellow (var. Miranda) | -- | -- | 61 | 14 |
| Soybean meal 47.5% CP | 19.2 | 1.5 | -- | -- |
| Alfalfa meal dehy., 17% CP | 5 | 1 | 1 | 1 |
| Silicate sand #70 grade | 5.05 | 25.06 | 1.78 | 30.13 |
| Animal fat | -- | -- | -- | 6.5 |
| Defluorinated phosphate 32% Ca:18% P | 1.65 | 1.95 | 1.7 | 2 |
| Limestone flour | .35 | .45 | .55 | .4 |
| Salt, iodized | .25 | .25 | .25 | .25 |
| Vitamin premix 1 | .4 | .4 | .40 | .40 |
| Trace mineral mix 2 | .07 | .07 | .07 | .07 |
| l-Lysine (50%) | .10 | .30 | -- | .20 |
| d,l-Methionine (98%) | .05 | .02 | .25 | .05 |
| Calculated analyses | | | | |
| Crude protein, % | 16 | 7 | 16 | 7 |
| Metabolizable energy, kcal/kg | 2850 | 2415 | 2859 | 2404 |
| Calcium, % | .82 | .84 | .82 | .84 |
| Available phosphorus, % | .41 | .42 | .42 | .42 |
| d,l-Methionine, % | .32 | .16 | .36 | .14 |
| Methionine & Cystine, % | .59 | .29 | .59 | .27 |
| Lysine, % | .82 | .34 | .74 | .35 |

1 Provided per kg of diet: Vitamin A, 6600 IU; vitamin D3, 2200 ICU; riboflavin, 6.6 mg; d-pantothenic acid, 11.0 mg; niacin, 44.0 mg; choline, 381.9 mg; vitamin B12, 11.0 mcg; vitamin E, 2.2 IU; vitamin K, 1.1 mg; folic acid, .44 mg; ethoxyquin, .12 g.

2 Provided per kg of diet: Ca, 136.5 mg; Mn, 84 mg; Fe, 28 mg; Cu, 2.8 mg; I, 1.68 mg; Zn, 38.5 mg; Co, .28 mg.

Table III.2. Influence of dietary treatments containing corn-soy (CS) and corn yellow pea (CYP) and 16 and 7% crude protein (CP) on the means of spermatozoal cell motility score (MS), cell concentration (SC), percent intact cells (ISC), semen viability index (SVI), measured weekly between 30 and 43 weeks of age (WOA) and fertility and hatchability measured at 32 and 36 WOA

| Protein Source | MS[a] | | SC (x100[4]/ml.) | | ISC[b] | | SVI[c] | | Fertility[b] | | Hatchability[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16% CP | 7% CP | 16% CP | 7% CP | 16% CP | 7% CP | 16% CP | 7% CP | 16% CP | 7% CP[d] | 16% CP | 7% CP[d] |
| CS | 2.6ax | 2.6ax | 4.4ax | 4.2ax | 70.6ax | 73.6ax | 8.2ax | 11.5ax | 73.1ax | 78.4ax | 66.6ax | 64.3ax |
| CYP | 2.7ax | 2.5ax | 3.0by | 3.7bx | 75.8ax | 64.1ay | 10.6ax | 8.5ax | 77.6ax | 75.8ax | 65.9ax | 63.7ax |
| SEM[e] | .2 | | .3 | | 10.6 | | 6.1 | | 3.7 | | 3.2 | |

a,b Means in the same column and x,y values in the same row having different lettered superscripts are different (P<.05).

[a]Based on subjective scoring from 0 to 4: 0, all the cells are immotile; 4, all cells exhibit a vigorous and swirling motion.

[b]Means expressed as arc sin.

[c]Product of motility score x spermatozoal cell concentration x % intact cells.

[e]Standard error of the mean.

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# CHAPTER IV

## DIVERGENT SELECTION FOR SPERMATOZOAL CELL VIABILITY IN BROILER BREEDER MALES

S. M. Bootwalla and T. F. Savage

# ABSTRACT

A divergent selection program for a spermatozal cell viability index (SVI) score was conducted for two generations using commercial broiler breeder males. The SVI score was obtained as the product of the spermatozoal cell motility score x cell concentration x percent intact cells. Three lines were developed based upon SVI scores, an unselected control (C) and those with high (H) and low values, respectively. Fertility and hatchability trials to evaluate the SVI scores were also conducted in all the generations. No differences ($P > .05$) were observed among the progeny of the C, H, and L in the two generations selected for semen viability based on the SVI score. Possible causes for the lack of response to divergent selection are discussed.

# INTRODUCTION

Semen quality in chickens is the combination of various quantitative characteristics such as spermatozoal cell concentration, spermatozoal cell motility, and semen volume, and these characteristics are inherited (Marini and Goodman, 1969; Lake, 1971). Siegel (1963) reported low heritability estimates for semen concentration, volume, and motility of .01, .14, and .29, respectively, in lines selected for body growth rates without emphasis on selection for semen characteristics. Soller et al. (1965), in contrast, reported high heritability estimates in lines when selection was applied to semen quality traits. Their heritability estimates for semen volume, spermatozoal concentration, and motility were .41, .46, and .87, respectively. Behavior associated with reproduction is also inherited. Siegel (1965) selected two lines of chickens for six generations based upon the cumulative numbers of completed matings (CNCM) and produced high mating (HM) and low mating lines (LM). Significant difference for CNCM between the two lines was not observed until after three successive generations of divergent selection. Dunnington and Siegel (1983) reported that following an additional 23 generations of continued divergent selection in the HM and LM lines, a higher number of CNCM's in the HM line was still observed.

A realized heritability value of 0.18 was calculated for the HM line. In contrast, selection for decreased numbers of CNCM in the LM line resulted in many males unable to mate. These results suggest that response to divergent selection for reproductive behavior is possible.

When genetic improvement of an economic trait is dependent upon more than one characteristic, a selection index may be used for the improvement of that economic trait. The contributing characters to the economic trait are usually merged together in a score or index which combines the total information in an "optimal manner." This combination of various traits simplifies selection since deliberations over the merits and faults of individual characteristics in an animal can be spared (Falconer, 1967, and Pirchner, 1983). Semen viability is an economic trait influenced by various individual and unrelated semen characteristics - spermatozoal cell motility, concentration, and the occurrence of intact or viable cells. There are no reported studies in the literature describing the use of a selection index for the improvement of semen viability. Therefore, the following study was undertaken to determine if broiler breeder males could be selected for high and low semen viability scores using a semen viability index.

# MATERIALS AND METHODS

Peterson broiler breeder males (BBM) were used for this study. The males were raised on a floor covered with wood-shavings-litter from day-old to 20 weeks of age (WOA), according to the breeder's recommended lighting regimen (8L:16D) and feeding program. At 20 WOA, 40 males were randomly assigned to individual cages (30.5 cm x 45 cm x 45.7 cm) in a windowless positive pressure ventilated house. Eight 15-minute watering periods at two hour intervals were provided daily during the lighting period (16L:8D). The males were allowed to adapt to the cages from 20 to 22 WOA and were fed in accordance with the breeder's recommendation. From 22 WOA, the males in the base population (P) were fed various levels of dietary protein as described by Rakphongphairoj (1987).

Between 22 and 28 WOA, the BBM were conditioned by ejaculating each male weekly. Semen evaluations were conducted and recorded on a weekly basis between 30 and 43 WOA. The semen samples were collected in individual plastic cups after applying the abdominal massage method of Burrows and Quinn (1935). The semen ejaculates were evaluated for subjective motility (SM) and scored from 0 to 4, in unit intervals (Cooper and Rowell, 1958). Both spermatozoal cell concentration (SC) and the percent intact spermatozoal cells (ISC) were determined using the

ethidium bromide-digitonin fluorometric assay procedures of Bilgili and Renden (1984). A spermatozoal cell viability index (SVI) score was calculated as the product of SM x SC x ISC. The fertilizing capacity of the spermatozoal cells and the hatchability of fertile eggs produced were determined at 32 and 36 WOA. Hens were inseminated intravaginally with $100x10^6$ spermatozoal cells, each. Eggs were identified by hen and sire and collected daily for 9 days starting on the second day following a single insemination. The eggs were set in a Jamesway 252 incubator and candled after the 10th day of incubation. The contents of eggs lacking embryonic development were macroscopically examined to determine true fertility. The above described activities were performed in each generation.

Selection from base population (P): Based upon the individual mean SVI scores from 30 to 43 WOA, 10 BBM with the highest and 10 males with the lowest SVI scores were selected as breeders for the high (H) and low (L) semen viable lines, respectively. An unselected control (C) line was established by the random selection and pooling of semen from 10 males selected from all the males available. To obtain the next generation of the three lines, Peterson broiler breeder hens were inseminated with semen from each male at 43 WOA.

Generation 1 (G-1): The chicks of the 3 lines produced from the parental founding generation were pedigree wing banded at hatching and reared to 20 WOA as detailed for the P generation. At 20 WOA, all birds were individually caged and fed a 16% CP breeder diet with 2850 ME kcal/kg. The males were provided with 126 grams of feed on a daily basis, which was equivalent to 80% of the breeder's recommendation. Twelve males with the highest SVI scores were selected among the 46 H line males, whereas all 13 males from the L lines and 16 males from the unselected C lines were evaluated for spermatozoal quality. Reproduction of the lines was accomplished at 43 WOA with the insemination of either 1 or 2 hens per male per line.

Generation 2 (G-2): The males of the 3 lines were reared and managed as described in generation G-1. Thirty eight males from the H line, 23 from from the L line and 10 from the unselected C lines were evaluated weekly from 30 to 43 WOA. Evaluations of fertility trials were determined using 4 Single Comb White Leghorn hens per male and line.

Statistical Analysis: The data for the spermatozoal cell characteristics (MS, SC, and ISC), SVI score, fertility, and hatchability were analyzed in each generation using a one-way analysis of variance, according to the Statistical Analysis System (SAS, 1982) using the following model:

$$Y(ijkl) = U + T(i) + A(ij) + B(ijk) + e(ijkl)$$

where

$Y(ijkl)$ = lth offspring of kth hen within jth male within ith line (C, H, and L lines)

$U$ = overall mean

$T(i)$ = effect of added response for ith line

$A(ij)$ = added effect of jth male within ith line

$B(ijk)$ = added effect of kth hen within jth male within ith line

$e(ijkl)$ = random error

# RESULTS AND DISCUSSION

The spermatozoal cell characteristics, SVI score, fertility, and hatchability for generations P, G-1, and G-2 are presented in Table IV.1. Within the P generation the spermatozoal cell MS, ISC, SVI score, fertility, and hatchability were higher ($P<.05$) in the H line than the L line irrespective of diet. The H line males also had a higher ($P<.05$) fertilizing capacity than that of line C. However, no difference ($P>.05$) was determined in SC among the C, H, and L lines.

Within the generations G-1 and G-2, respectively, there were no differences ($P > .05$) observed among the C, H, and L lines for any of the spermatozoal cell characteristics measured. Also, there were no differences observed in the fertility and hatchability among the 3 lines. There were variations among the lines for the SVI score, fertility, and hatchability, generation G-1 having more variability than generation G-2.

It is evident from these results that divergent selection for spermatozoal cell viability in broiler breeders males for two consecutive generations did not respond to selection based up on the SVI score. Since the index score was a composite of MS, SC, and ISC, a change in one of the spermatozoal characteristics would influence the overall index score. According to Pirchner (1983),

selection progress based on an aggregate economic value such as overall reproductive performance is decreased when selection is dependent upon more than one trait. This was the experimental approach of this study. The lack of selection response for spermatozoal viability could be due to either pleiotropy or unequal gene frequencies associated with semen characteristics or a combination of both factors, especially with the P population in which there was no history of selection for the traits in males of both the male and female lines. Falconer (1964) stated that if selection was applied to two characters simultaneously, the genetic correlation between them is expected to become negative due to pleiotropic effects. Another reason for the lack of response to the selection could be the small number of males in the base population P from which the selection for the SVI's were made. A further possibility is there could have been a lack of differential selection pressure for the two lines. The lack of selection response might also be due to the ethidium bromide-digitonin fluorometric assay in this study as a selection tool to estimate the semen viability. This biochemical assay might not have been an accurate indicator of semen viability unlike that of MS and SC. Therefore, the effect on the selection for the spermatozoal viability for more than two generations needs to be further investigated.

The lack of selection response for spermatozoal cell viability in this experiment differs from those of Siegel (1963), Marini and Goodman (1969) and Lake (1971) in which there were responses to selection for specific semen quality characteristics. The absence of response to selection in this study is similar to that observed by Siegel (1965), in that there may be a suppressed response for at least the first three generations when genetic selection is applied for a reproductive trait. Siegel (1965) suggested that the lack of response could be due to the intra and interallellic interactions, such as epistasis and linkage associated with the mating lines. He further suggested that the small difference in response to divergent selection to CNCMs in their study might be due to directional dominance and unequal gene frequencies of the loci concerned with high and low numbers of completed matings in the initial generation. Also, according to Siegel (1965), neither of these genetic factors would result in asymmetry of CNCMs in the first few generations of selection or until gene frequencies in the divergent lines were significantly different. It appears that several generations of selection may be necessary to change gene frequencies associated with reproductive behavior before response to selection, particularly in population where there is no history of artificial selection for the trait being selected. A similar type of

situation may have been present in the current study.

TABLE IV-1. Mean values of spermatozoal cell characteristics, semen viability index score, fertility, and hatchability of P, G-1, and G-2 generations of broiler breeder males for control, high, and low viability traits

| GENERATION | TREATMENT | MOTILITY SCORE[1] | SPERM CELL CONCENTRATION ($\times 10^4$/ml.) | INTACT CELLS (%) | SEMEN VIABILITY INDEX[2] | FERTILITY (%) | HATCHABILITY (%) |
|---|---|---|---|---|---|---|---|
| P | Control | 2.5 (.2)a | 4.2 (.6)a | 76.7 (4.1)a | 8.3 (1)b | 85.4 (7)b | 80.8 (7.5)a |
| | High | 3.2 (.3)a | 3.5 (.5)a | 91.3 (7.1)a | 10.4 (.5)a | 94.4 (.8)a | 89.5 (1.4)a |
| | Low | 1.6 (.5)b | 4.6 (.6)a | 71.3 (6.2)b | 5.3 (.1)c | 54.1 (1.6)c | 48.4 (1.3)b |
| G-1 | Control | 3.2 (.4)a | 2.9 (.4)a | 94.1 (.4)a | 11.0 (2.4)a | 76.9 (.5)a | 75.2 (7)a |
| | High | 2.6 (.2)a | 2.8 (.5)a | 90.8 (2.1)a | 7.2 (.8)a | 65.2 (6.3)a | 55.4 (6.1)a |
| | Low | 2.2 (.2)a | 2.8 (.9)a | 93.9 (.7)a | 6.0 (.6)a | 74.0 (10.3)a | 66.3 (10.6)a |
| G-2 | Control | 3.3 (.1)a | 4.0 (.2)a | 96.5 (.5)a | 12.6 (.8)a | 90.5 (2.1)a | 86.4 (2.4)a |
| | High | 3.1 (.2)a | 3.9 (.2)a | 95.1 (.7)a | 11.3 (.6)a | 80.9 (2.3)a | 79.0 (2.8)a |
| | Low | 3.0 (.1)a | 3.9 (.2)a | 95.1 (.7)a | 11.4 (.5)a | 89.2 (3.0)a | 81.4 (2.7)a |

a,b,c Different superscripts within column means of each generation are significantly different ($P < .05$).

( ) = Standard error of the mean.

[1]Score: 0 = all cells immotile, 4 = all cells exhibiting a vigorous and swirling motion.

[2]Product of motility score x spermatozoal cell concentration x % intact cells.

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# CHAPTER V

## EFFECT OF EXTENDER VISCOSITY ON INSEMINATION DOSE AND FERTILITY IN THE CHICKEN[1]

S.M. BOOTWALLA and D.P. FROMAN

[1]Oregon Agricultural Experimental Station Technical Paper No. 8309.

# ABSTRACT

An attempt was made to reduce the conventional insemination dose of 100 x $10^6$ spermatozoa per hen used for the artificial insemination (AI) of chickens by increasing the viscosity of extended semen. When semen was diluted 1:2 with Beltsville Poultry Semen Extender (BPSE) containing 4% (w/v) carboxymethylcellulose, the viscosity was 14 times greater than that of semen similarly extended with BPSE. Under these conditions, carboxymethylcellulose was not spermacidal ($P>0.05$). Fertility over 20 days following a single intravaginal insemination of 33 x $10^6$ spermatozoa per hen was not enhanced by carboxymethylcellulose ($P>0.05$). When data were compiled according to insemination dose, 33 vs. 100 x $10^6$ spermatozoa per hen, and fertility evaluated over the first seven days of egg collection, there was no difference ($P > 0.05$) due to insemination dose. Therefore, the cost of semen could be substantially reduced by using a minimal insemination dose for AI under field conditions.

# INTRODUCTION

Poultry semen must be extended before it can be used efficiently in an artificial insemination (AI) program. Use of an extender that would enable technicians to inseminate hens with a minimal insemination dose would decrease the cost of AI directly by reducing the number of males needed. For the AI of chickens, Sexton (1983) recommended an insemination dose of 100 x $10^6$ spermatozoa per hen per week. However, this dose may be excessive.

Allen and Grigg (1957) inseminated hens with $^{32}P$-labelled spermatozoa and reported that approximately 90% of recovered spermatozoa were lost in the feces in less than 1 hr. following intravaginal insemination. Since Allen and Grigg (1957) used insemination doses that were 1.6 to 3 times greater than that recommended by Sexton (1983), their effective insemination dose was < 30 x $10^6$ spermatozoa. This low dose in comparable to doses used by Sexton (1977) and Lake and Ravie (1987), who reported high fertility following weekly inseminations. Presumably, such inseminations were performed under carefully controlled conditions. For reasons already given, it would be desirable to use a minimal insemination dose under field conditions.

Sexton (1977) developed the Beltsville Poultry Semen Extender (BPSE), which has proven useful in a variety of

applications (Sexton, 1978; Sexton and Fewlass, 1978). While the effects of chemical composition, dilution rate, holding temperature, etc., have been studied, the effect of extender viscosity on fertility in the chicken is unknown. We reasoned that if high fertility could be obtained using a low insemination dose in conjunction with a viscous extender, then controlling the viscosity of extended semen would be warranted in AI programs. Therefore, our objectives were threefold: (1) to find an agent that would increase extender viscosity, (2) to use this agent to increase the viscosity of extended semen without damaging spermatozoa, and (3) to determine if the insemination dose could be reduced by inseminating hens with semen so extended.

# MATERIALS AND METHODS

Effect of Carboxymethylcellulose on Extender Viscosity. Beltsville Poultry Semen Extender was prepared according to Sexton (1977), and BPSE containing 4% (w/v) carboxymethylcellulose (C-8758; Sigma Chemical Co., St. Louis, MO) was prepared by mixing equal volumes of 2x BPSE with 8% (w/v) carboxymethylcellulose in distilled water. Fresh extenders were prepared on each of five days whereon semen was collected.

Ejaculates from 25 Single Comb White Leghorn (SCWL) roosters were pooled and gently mixed. Semen was then partitioned so that the viscosity of neat and extended semen, with and without carboxymethylcellulose, could be determined. When extended, semen was diluted 1:2. Viscosity was measured at 30 C with a sample volume of 5 ml using Ostwald viscometers (Salzberg et al., 1966).

Effect of Carboxymethylcellulose on Spermatozoal Viability. Two 100-ul semen samples were taken from each of 12 SCWL ejaculates. Each of these samples was diluted 1:2 in a 1.5-ml microcentrifuge tube with either BPSE or BPSE containing 4% (w/v) carboxymethylcellulose. After incubation at 30 C for 30 minutes in an Eppendorf Constant Temperature Heater (Brinkmann Instruments, Westbury, NY), 10 ul were removed from each tube with an M-50 positive

displacement pipet (Rainin Instrument Co., Inc., Emeryville, CA). Spermatozoal viability and concentration were determined fluorometrically according to Bilgili and Renden (1984). Preliminary work had shown that carboxymethylcellulose neither fluoresced nor quenched the fluorescence of spermatozoa under these conditions. Based upon concentrations of viable spermatozoa, a paired comparison was performed using a randomized complete block design (Sokal and Rohlf, 1969).

Effect of Carboxymethylcellulose on Spermatozoal Fertilizing Ability. Ejaculates from 10 SCWL roosters were pooled and gently mixed. Semen was then diluted 1:2 with BPSE or BPSE containing 4% (w/v) carboxymethyl-cellulose. Following admixture, spermatozoal viability and concentration were determined as above. Inseminations were performed with a positive displacement pipet. Two insemination doses were used for each type of extended semen: 33 and 100 x $10^6$ viable spermatozoa. Twenty SCWL hens were inseminated in each treatment.

Egg collection commenced on the second day following insemination and continued for a total of 20 d. Fertility was assessed by breaking eggs open on the fourth day of incubation and examining the contents for embryonic development. The percentage of fertilized eggs laid by each hen was transformed to arcsin and these data were

analyzed with a two-way ANOVA (Sokal and Rohlf, 1969).

# RESULTS AND DISCUSSION

Effect of Carboxymethylcellulose on Extender Viscosity. Mean viscosity for BPSE, neat semen, and semen diluted 1:2 with BPSE or BPSE containing 4% (w/v) carboxymethylcellulose are shown in Table V.1. Chicken semen was 27 times more viscous than BPSE, which had a viscosity equivalent to that of water. Diluting semen 1:2 with BPSE substantially reduced seminal viscosity. By diluting semen 1:2 with BPSE containing 4% (w/v) carboxymethylcellulose, extended semen was procured having a viscosity twice that of neat semen and 14 times greater than semen extended 1:2 with BPSE. We deemed these differences and the repeatability with which the viscous extended semen was prepared to warrant the use of carboxymethylcellulose in subsequent experiments.

Effects of Carboxymethylcellulose on Spermatozoal Viability. Spermatozoal uptake of ethidium bromide was not observed ($P > .05$) after ejaculates were diluted 1:2 with BPSE containing 4% (w/v) carboxymethylcellulose and then incubated at 30 C for 30 minutes. Therefore, we concluded that carboxymethylcellulose was not deleterious to chicken spermatozoa under our experimental conditions. This conclusion agreed with that of Rikmenspoel (1957), who reported that dextrans and carboxymethylcellulose

were suitable for increasing the viscosity of bull semen.

While Rikmenspoel (1957) observed that the velocity of progressively motile spermatozoa was inversely proportional to the viscosity of the surrounding medium, he concluded that there was no detrimental physiological effect due to increased extender viscosity. Furthermore, he suggested that the viscosity of the medium surrounding a spermatozoon is the only variable that can be substantially changed without altering the physiological status of the spermatozoon. Therefore, while seminal viscosity is generally reduced via extension in order to enhance the utilization of semen, we hypothesized that extended semen could be utilized more efficiently by increasing seminal viscosity.

Effect of Carboxymethylcellulose on Spermatozoal Fertilizing Ability. Fertility resulting from the combined effects of insemination dose and extended semen viscosity is shown in Table V.2. We had hypothesized that when an insemination dose of 33 x $10^6$ spermatozoa per hen was used, fertility would be enhanced by using extended semen containing 2% (w/v) carboxymethylcellulose. Thus, we expected to observe a significant interaction in the two-way ANOVA. However, such an interaction was not observed ($P > .05$). There were, though, significant differences ($P > .05$) in fertility due to insemination dose and

extender; regardless of the extender, insemination of 100 x $10^6$ spermatozoa per hen yielded higher fertility, and likewise, BPSE was superior to BPSE containing 4% (w/v) carboxymethylcellulose.

When data from the first seven days of egg collection were compiled according to insemination dose and analyzed with a one-way ANOVA (Sokal and Rohlf, 1969), there was no difference ($P > .05$) in fertility due to insemination dose. Fertility for doses of 33 and 100 x $10^6$ spermatozoa per hen was 98 $\pm$ 2.7 and 99 $\pm$ 2.2%, respectively. This observation corroborates Van Duijn (1964) and Sexton (1977), as well as Lake and Ravie (1987), in that insemination doses substantially less than 100 x $10^6$ spermatozoa per hen can yield high fertility.

In conclusion, while an inert agent suitable for increasing the viscosity of BPSE was found, increased extender viscosity did not enhance fertility in conjunction with a reduced insemination dose. Therefore, the variable of viscosity may not warrant further consideration in research designed to improve the efficiency of AI in poultry. However, reducing the cost of AI programs by minimizing the insemination dose, by whatever means, remains a reasonable objective.

TABLE V.1. Viscosity[1] at 30 C and coefficient of variation (CV), for distilled water, Beltsville Poultry Semen Extender (BPSE), neat semen, and semen diluted 1:2 with BPSE or BPSE containing 4% (w/v) carboxymethylcellulose (CMC)

| Sample | Viscosity (centipoise) | CV (%) |
|---|---|---|
| BPSE | 1.0 | 3.3 |
| Neat Semen | 27.0 | 9.7 |
| Extended Semen | 4.3 | 16.4 |
| Extended Semen Containing CMC | 60.1 | 12.2 |

[1]Each value represents the mean of five replicates.

TABLE V.2. Fertilized eggs (%) resulting from the combined effects of insemination dose and type of extender, Beltsville Poultry Semen Extender (BPSE) or 4% (w/v) carboxymethylcellulose (CMC) in BPSE, when semen was diluted 1:2 and inseminated intravaginally[1]

| | Spermatozoa Inseminated Per Hen | |
|---|---|---|
| Extender | 33 x $10^6$ | 100 x $10^6$ |
| BPSE & CMC | 44 (.2)ac | 52 (.1)ad |
| BPSE | 55 (.1)bc | 62 (.1)bd |

[1]Each value is reported as a mean (SEM) for 18 replicates over a 20-day egg collection interval.

a,b Denotes difference (P< .05) in fertility within a column.

c,d Denotes difference (P< .05) in fertility within a row.

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# CHAPTER VI

## SUMMARY AND CONCLUSIONS

Experiments were conducted using an ethidium-bromide digitonin fluorometric assay to assess the fertilizing ability of individually caged broiler breeder males (BBM). Within the parameters and conditions of these studies the following conclusions were summarized from the results of these studies.

### 1. Semen Quality of Individually Caged Broiler Breeder Males Fed 16 and 7% Crude Protein Diets Containing Yellow Peas.

The spermatozoal cell concentration was higher in broiler breeder males (BBM) fed the diets containing corn-soy (CS) when compared with corn-yellow pea (CYP) diets. The percentage of intact spermatozoal cell was higher ($P<.05$) for the males fed diets containing 16% crude protein (CP) than that for 7% CP diets containing CYP. Neither the dietary protein type (soybean meal or yellow peas) or the protein level (7% or 16%) influenced the spermatozoal cell motility, semen viability index score, fertility, and hatchability. It was concluded that individually caged BBM could be fed diets containing yellow peas as well as 7% CP without adversely affecting

semen quality.

2. Divergent Selection for Semen Viability in Broiler Breeder Males.

No differences (P>.05) were observed among the three lines C, H, and L in generations G-1 and G-2 selected for spermatozoal cell viability. The lack of response for divergent selection might be due to pleiotropy, unequal gene frequencies, or small numbers of males in the base population P from which selections were made. The lack of response might also be because the ethidium-bromide digitonin fluorometric assay which may not have been an accurate indicator of spermatozoal cell viability.

3. Effect of Extender Viscosity on Insemination Dose and Fertility in the Chicken.

Chicken semen was determined to be 27 times more viscous than Beltsville Poultry Semen Extender (BPSE), whose viscosity was equivalent to that of water. Diluting semen 1:2 with BPSE substantially reduced seminal viscosity. Semen diluted 1:2 with BPSE and containing 4% carboxymethylcellulose (CMC) resulted in a viscosity twice that of neat semen and 14 times that of semen extended 1:2 with BPSE. Hen fertility was not enhanced by using extended semen containing 2% (w/v) CMC. Insemination of hens with $100 \times 10^6$ spermatozoal cells yielded higher

fertility values than insemination with 33 x $10^6$ cells, and it was also concluded that BPSE was superior to BPSE containing 4% (w/v) CMC. When data from the first seven days of egg collection were compiled according to the insemination dose, no difference ($P>.05$) in fertility due to the insemination dose was observed. Increased viscosity of BPSE did not enhance fertility in conjunction with reduced insemination dose. However, a reduced insemination dose could be used for AI under field conditions.

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# APPENDICES

## DESCRIPTION OF APPENDIX ABBREVIATIONS

| | |
|---|---|
| BBM | Broiler breeder male(s) |
| BPSE | Beltsville Poultry Semen Extender |
| CP | Crude protein |
| CMC | Carboxymethylcellulose |
| CS | Corn-soybean |
| CV | Coefficient of variation |
| CYP | Corn-yellow pea |
| ME | Metabolizable energy |
| SEM | Standard error of the mean |
| WOA | Week(s) of age |

# APPENDIX 1

## SEMEN QUALITY OF INDIVIDUALLY CAGED BROILER BREEDER MALES FED 16 AND 7% CRUDE PROTEIN DIETS CONTAINING YELLOW PEAS

The following data listed in Appendix 1 provide more supplemental information about the study. Contained in Tables A1.1 through A1.6 are the mean weekly spermatozoal cell measurements, fertility, and hatchability by age in weeks.

Table A1.1. The effects of dietary treatments on BBM spermatozoal motility score[1] from 30 to 43 weeks of age

| TREATMENTS | WEEK OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| CS--16% CP | 2.6a | 2.4a | 2.7a | 2.9a | 2.9a | 2.7a | 3.0a | 2.8a | 2.8a | 1.8a | 2.9a | 2.9a |
| CS---7% CP | 3.5a | 2.9a | 2.9a | 2.5a | 2.5a | 2.3a | 2.1a | 2.6a | 2.9a | 2.4a | 2.5a | 2.7a |
| CYP-16% CP | 3.2a | 3.4a | 2.9a | 2.6a | 3.0a | 3.2a | 2.8a | 2.1a | 2.4a | 2.3a | 2.2a | 2.6a |
| CYP--7% CP | 1.7a | 2.4a | 2.8a | 1.9a | 2.9a | 3.1a | 2.6a | 2.0a | 2.6a | 2.2a | 2.7a | 2.8a |
| SEM[2] | .4 | .3 | .4 | .4 | .3 | .3 | .3 | .3 | .3 | .3 | .3 | .3 |

a Means in the same columns are not different (P > .05).

[1]Subjective scoring from 0 to 4; 0, all the cells are immotile; 4, all cells exhibit a vigorous and swirling motion.

[2]Standard error of the mean.

TABLE A1.2. The effects of dietary treatments on BBM spermatozoal cell concentration from 30 to 43 weeks of age

| | WEEK OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TREATMENTS | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| | x10⁶/ml | | | | | | | | | | | |
| CS--16% CP | 3.9a | 4.0a | 5.7a | 4.5a | 3.5a | 3.9a | 3.8ab | 5.7ab | 4.2ab | 6.0a | 3.5a | 4.4a |
| CS---7% CP | 3.4a | 3.6a | 4.3ab | 3.6a | 3.8a | 3.6a | 4.4a | 6.2a | 4.7a | 5.7ab | 3.4a | 4.3a |
| CYP-16% CP | 2.7a | 3.2a | 3.6c | 3.4a | 3.3a | 2.7a | 3.3ab | 3.7c | 3.1b | 2.6c | 2.7a | 3.1a |
| CYP--7% CP | 3.7a | 3.8a | 3.7bc | 3.4a | 3.6a | 3.6a | 2.8b | 4.4bc | 4.2ab | 4.3b | 5.0a | 3.7a |
| SEM[1] | .4 | .4 | .4 | .4 | .4 | .4 | .4 | .4 | .4 | .4 | .4 | .3 |

a,b Means in the same column having different superscripts are significantly different (P<.05).

[1]Standard error of the mean.

TABLE A1.3. The effects of dietary treatments on BBM intact spermatozoal cells (%) from 30 to 43 weeks of age

| TREATMENTS | WEEK OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| | % | | | | | | | | | | | |
| CS--16% CP | 79.8b | 81.7b | 45.7b | 73.9c | 95.2a | 87.3a | 93.9a | 94.2a | 91.9a | 92.9a | 92.9a | 96.5a |
| CS---7% CP | 93.2a | 81.5b | 92.7a | 89.2ab | 91.5a | 89.8a | 93.3a | 95.6a | 95.6a | 94.5a | 92.0a | 93.7ab |
| CYP-16% CP | 97.1a | 87.8a | 95.7a | 91.7a | 88.8a | 87.7a | 91.4ab | 94.8a | 96.7a | 94.9a | 94.2a | 97.3a |
| CYP--7% CP | 59.5c | 73.9b | 55.1b | 78.8bc | 90.3a | 78.0b | 78.0b | 96.4a | 87.4a | 91.0a | 89.0a | 86.4b |
| SEM[1] | 3.4 | 4.3 | 6.1 | 3.2 | 3.5 | 2.7 | 3.7 | 1.7 | 3.1 | 1.1 | 2.6 | 2.6 |

a,b,c Means in the same column having different superscripts are significantly different (P<.05).

[1]Standard error of the mean.

TABLE A1.4. The effects of dietary treatments on BBM spermatozoal cell viability index score from 30 to 43 weeks of age

| | WEEK OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TREATMENTS | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| CS--16% CP | 7.2bc | 7.5ab | 4.9c | 7.8a | 7.0a | 9.1a | 9.0a | 14.8a | 9.6b | 8.4a | 5.3a | 8.1a |
| CS---7% CP | 2.1a | 10.8a | 4.2ab | 7.7a | 9.7a | 7.6a | 5.2a | 14.6a | 17.4a | 10.1a | 7.2a | 11.5a |
| CYP-16% CP | 12.3a | 11.5a | 16.4a | 4.7a | 8.4a | 8.7a | 9.2a | 11.5a | 9.2b | 12.2a | 8.1a | 10.6a |
| CYP--7% CP | 3.3c | 5.7b | 6.1bc | 5.0a | 10.1a | 11.2a | 8.3a | 12.5a | 9.8b | 13.6a | 8.1a | 8.5a |
| SEM[1] | 1.9 | 1.4 | 2.2 | 1.3 | 2.8 | 2.0 | 1.5 | 2.3 | 2.0 | 2.4 | 1.7 | 2.3 |

a,b,c Means in the same column having different superscripts are significantly different (P<.05).

[1]Standard error of the mean.

TABLE A1.5. The effects of dietary treatments on BBM fertility determined at 32 and 36 weeks of age

| TREATMENT | WEEKS OF AGE | |
|---|---|---|
| | 32 | 36 |
| | -----%----- | |
| CS-16% CP | 88.0a | 95.6a |
| CS--7% CP | 96.3a | 97.9a |
| CS-16% CP | 88.6a | 92.4a |
| CS--7% CP | 91.6a | 98.8a |
| SEM[1] | 5.8 | 3.5 |

a Means in the same column are not different (P>.05).

[1]Standard error of the mean.

TABLE A1.6. The effects of male dietary treatments on the hatchability of fertile eggs at 32 and 36 weeks of age

| | WEEKS OF AGE | |
|---|---|---|
| TREATMENT | 32 | 36 |
| | -----%----- | |
| CS--16% CP | 84.5a | 84.0a |
| CS---7% CP | 78.6a | 83.7a |
| CYP-16% CP | 80.3a | 88.8a |
| CYP--7% CP | 85.3a | 87.3a |
| SEM[1] | 3.9 | 1.7 |

a Means in the same column are not different (P>.05).

[1]Standard error of the mean.

# APPENDIX 2

## DIVERGENT SELECTION FOR SPERMATOZOAL CELL VIABILITY IN BROILER BREEDER MALES

The following data listed in Appendix 2 provide further information about the study. Presented in Tables A2.1-A2.6 are the spermatozoal characteristics, viability index score, fertility, and hatchability of the foundation lines (P), and in generation-1 and generation-2 selected for controls, high, and low spermatozoal cell viability at the ages 30 to 43 weeks of age.

TABLE A2.1. The mean spermatozoal cell motility scores of BBM in generations P, G-1, and G-2 generation of the control, high, and low semen viable lines.

| Generation | Line | WEEKS OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| P | Control | 3.0a | 2.0a | 2.7a | 3.1a | 2.5a | 2.6a | 2.4a | 2.4a | 3.1a | 3.0a | 1.9b | 2.4a |
| | High | 3.5a | 3.5a | 3.4a | 2.9a | 3.6a | 3.5a | 3.1a | 2.9a | 2.8a | 2.3a | 3.2a | 2.6a |
| | Low | 2.4a | 1.4b | 1.8b | 2.4a | 1.6b | 1.3b | 1.4b | 1.8b | 2.1b | 1.1b | 1.0b | 1.7a |
| | SEM[1] | .5 | 1.0 | .8 | .4 | 1.0 | 1.1 | .8 | .5 | .5 | .9 | 1.1 | .5 |
| G-1 | Control | 3.4a | 2.4a | 3.2a | 1.6c | 3.0a | 4.0a | 3.2a | 3.6a | 2.8a | 4.0a | 3.0a | 2.8a |
| | High | 3.0a | 2.6a | 3.1a | 2.4a | 2.4a | 3.2a | 0.5b | 3.2a | 3.0a | 2.8a | 3.1a | 3.0a |
| | Low | 2.2b | 2.0a | 1.8b | 2.1a | 2.8a | 2.8a | 2.0a | 3.0a | 2.3b | 2.4b | 2.8a | 2.5a |
| | SEM[1] | .6 | .3 | .8 | .4 | .3 | .6 | 1.3 | .3 | .4 | .8 | .2 | .2 |
| G-2 | Control | 3.2a | 3.5a | 3.6a | 2.8a | 4.0a | 3.9a | 2.6a | 3.5a | 4.0a | 3.6a | 3.2a | 2.6a |
| | High | 3.5a | 3.0a | 4.0a | 3.2a | 3.6a | 3.1a | 3.4a | 3.6a | 3.1a | 3.8a | 2.8a | 3.2a |
| | Low | 3.0a | 3.4a | 3.6a | 3.0a | 3.8a | 3.1a | 4.8a | 4.0a | 2.6b | 3.5a | 3.1a | 2.8a |
| | SEM[1] | .2 | .3 | .2 | .2 | .2 | .5 | 1.1 | .2 | .7 | .2 | .2 | .3 |

a,b Means in the same column and generation having different superscripts are significantly different ($P < .05$).

[1]Standard error of the mean.

TABLE A2.2. The mean spermatozoal cell concentration of BBM in the P, G-1, and G-2 generations of control, high, and low semen viable lines.

| Generation | Line | WEEKS OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| | | x10⁹/ml | | | | | | | | | | | |
| P | Control | 3.1a | 3.6a | 4.0a | 3.1a | 3.8a | 3.7a | 2.6a | 4.1b | 4.6a | 4.6a | 6.1a | 3.1a |
| | High | 2.9a | 3.7a | 3.6a | 3.3a | 2.8b | 3.7a | 3.9a | 4.6b | 3.9a | 4.9a | 3.1b | 4.2a |
| | Low | 4.2a | 4.9a | 4.1a | 3.5a | 4.0a | 4.9a | 3.6a | 6.5a | 4.4a | 6.2a | 5.5a | 3.0a |
| | SEM[1] | .7 | .7 | .2 | .6 | .7 | .7 | .7 | 1.2 | .3 | .8 | 1.6 | .6 |
| G-1 | Control | 2.4a | 3.1a | 4.6a | 2.2a | 3.1a | 2.6b | 5.2a | 1.4a | 3.1a | 2.1a | 3.6a | 2.1a |
| | High | 3.2a | 3.6a | 3.2a | 4.6a | 4.2a | 4.1a | 4.4a | 2.1a | 3.2a | 1.6b | 2.8a | 2.6a |
| | Low | 3.2a | 4.0a | 2.2a | 2.1a | 3.2a | 4.2a | 4.6a | 2.4a | 3.1a | 3.1a | 2.6a | 2.4a |
| | SEM[1] | .5 | .4 | 1.2 | 1.4 | .6 | .9 | .4 | .5 | .1 | .8 | .5 | .2 |
| G-2 | Control | 4.9a | 4.2a | 5.6a | 3.1a | 5.8a | 5.3a | 3.8a | 2.4a | 4.1a | 3.3a | 3.7a | 5.2a |
| | High | 4.3a | 3.6a | 4.1a | 5.3a | 3.9a | 4.1a | 4.2a | 3.6a | 3.5a | 4.6a | 4.8a | 4.8a |
| | Low | 3.6a | 4.2a | 5.9a | 4.2a | 4.6a | 4.3a | 2.4a | 2.3a | 3.8a | 3.5a | 3.8a | 3.6a |
| | SEM[1] | .6 | .3 | .9 | 1.1 | 1.0 | .6 | .9 | .7 | .3 | .7 | .6 | .8 |

a,b Means in the same column and generation having different superscripts are significantly different (P < .05).

[1]Standard error of the mean.

TABLE A2.3. The mean of intact spermatozoal cells of BBM in the P, G-1, and G-2 generation of control, high, and low semen viable lines

| Generation | Line | WEEKS OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| | | % | | | | | | | | | | | |
| P | Control | 94.6a | 91.3a | 90.1a | 91.3a | 91.3a | 90.4a | 92.9a | 90.9a | 91.2a | 90.6a | 92.3a | 88.8a |
| | High | 97.0a | 83.8a | 97.9a | 80.8a | 95.7a | 89.1a | 93.8a | 97.5a | 96.4a | 92.6a | 93.7a | 96.0a |
| | Low | 69.9b | 53.0b | 60.9b | 81.9a | 79.8b | 84.1a | 84.0b | 82.9a | 87.3a | 80.5b | 78.5b | 86.7a |
| | SEM[1] | 15.0 | 11.7 | 11.2 | 5.7 | 8.2 | 3.3 | 5.4 | 7.3 | 4.5 | 6.5 | 8.4 | 4.8 |
| G-1 | Control | 88.0a | 100a | 80.0a | 100a | 100a | 84a | 100a | 86.6a | 100a | 100a | 95.5a | 90.6a |
| | High | 96.0a | 84.0b | 78.0b | 86.0b | 92.5a | 100a | 96.2a | 98.0a | 100a | 80.3a | 85.2a | 100a |
| | Low | 88.2a | 92.3a | 94.1a | 100a | 96.6a | 90.4a | 100a | 94.7a | 94.2a | 96.6a | 85.3a | 91.0a |
| | SEM[1] | 4.5 | 8.0 | 8.7 | 8.0 | 3.7 | 8.0 | 2.2 | 5.9 | 3.3 | 10.5 | 5.9 | 5.3 |
| G-2 | Control | 76.8b | 100a | 100a | 100a | 96.0a | 100a | 100a | 92.0a | 100a | 100a | 100a | 100a |
| | High | 92.8a | 100a | 100a | 100a | 89.9a | 91.6a | 88.2a | 100a | 97.0a | 100a | 100a | 88.2a |
| | Low | 90.2a | 92.3a | 96.8a | 100a | 98.1a | 92.0a | 100a | 88.6a | 90.1a | 100a | 100a | 100a |
| | SEM[1] | 8.6 | 4.4 | 1.8 | 0 | 2.4 | 4.7 | 6.8 | 5.8 | 5.0 | 0 | 0 | 6.8 |

a,b Means in the same column and generation having different superscripts are significantly different ($P < .05$).

[1]Standard error of the mean.

TABLE A2.4. The mean of spermatozoal cell viability index score of BBM P, G-1, and G-2 generation selected for the control, high, and low semen viable lines

| | | WEEKS OF AGE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Generation | Line | 30 | 31 | 33 | 34 | 35 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
| P | Control | 8.8a | 6.6b | 9.7b | 8.7a | 6.4b | 8.4ab | 5.8b | 8.9b | 13.0a | 12.5a | 10.7a | 6.5b |
| | High | 9.8a | 12.4a | 12.5a | 7.8a | 9.5a | 11.4a | 11.2a | 12.8a | 9.5bc | 10.3a | 9.1a | 10.4a |
| | Low | 7.1a | 3.6c | 4.5c | 6.9a | 5.1b | 5.6c | 4.1c | 9.9b | 7.6c | 5.5b | 4.3b | 4.4b |
| | SEM[1] | .8 | 2.5 | 2.5 | .5 | 1.3 | 1.7 | 1.3 | 1.1 | 1.6 | 2.0 | 1.9 | 1.7 |
| G-1 | Control | 7.2a | 7.4a | 11.7a | 3.5b | 9.3a | 8.7a | 9.8a | 4.3a | 8.7a | 8.4a | 10.3a | 5.3a |
| | High | 9.2a | 7.9a | 7.7a | 9.5a | 9.3a | 13.1a | 2.1b | 6.6a | 9.6a | 3.6b | 7.6a | 6.6a |
| | Low | 6.2a | 7.4a | 3.7b | 4.4b | 8.6a | 10.6a | 9.2a | 6.8a | 6.7a | 7.1a | 6.2a | 5.5a |
| | SEM[1] | .9 | .1 | 2.3 | 1.8 | .2 | 2.3 | 2.4 | .8 | .9 | 2.4 | 2.1 | .7 |
| G-2 | Control | 11.9a | 14.7a | 20.1a | 8.7a | 22.3a | 20.7a | 9.9a | 7.2b | 6.4a | 11.9a | 11.5a | 13.5a |
| | High | 13.8a | 10.8a | 6.4b | 17.0a | 12.3a | 11.6a | 12.6a | 13.2a | 10.5a | 17.4a | 13.4a | 13.5a |
| | Low | 9.7a | 13.1a | 20.4a | 12.6a | 17.1a | 12.3a | 6.7a | 8.1b | 8.9a | 12.2a | 11.8a | 10.1a |
| | SEM[1] | 2.0 | 1.9 | 8.0 | 4.1 | 5.0 | 5.0 | 3.6 | 3.2 | 2.0 | 2.3 | 1.0 | 1.9 |

a,b,c Means in the same column and generation having different superscripts are significantly different ($P < .05$).

[1]Standard error of the mean.

TABLE A2.5. The mean fertility of broiler breeder hens in P, G-1, and G-2 generation inseminated with BBM selected as a control, high, and low semen viable lines

| Generation | Line | WEEKS OF AGE 32 | 36 |
|---|---|---|---|
| | | -------%-------- | |
| P (A) | Control | 90.4a | 81.2a |
| | High | 94.8a | 96.4a |
| | Low | 55.6b | 52.6b |
| | SEM[1] | 12.4 | 12.8 |
| G-1 (A) | Control | 80.2a | 74.3a |
| | High | 70.6a | 63.2a |
| | Low | 76.1a | 72.4a |
| | SEM[1] | 7.8 | 5.4 |
| G-2 (B) | Control | 75.1a | 86.2a |
| | High | 82.0a | 78.6a |
| | Low | 91.6a | 87.8a |
| | SEM[1] | 8.6 | 4.9 |

a,b Means in the same column and generation having different superscripts are significantly different ($P < .05$).

[1]Standard error of the mean.

(A) Broiler breeder hens.

(B) Single Comb White Leghorn hens.

TABLE A2.6. The mean hatch of fertile eggs in generations P, G-1, and G-2

| Generation | Line | WEEKS OF AGE 32 | 36 |
|---|---|---|---|
| | | -------%-------- | |
| P (A) | Control | 82.2a | 80.1a |
| | High | 87.6a | 91.3a |
| | Low | 47.2b | 49.7b |
| | SEM[1] | 12.6 | 12.4 |
| G-1 (A) | Control | 78.2a | 71.6a |
| | High | 58.1a | 52.2a |
| | Low | 70.1a | 63.1a |
| | SEM[1] | 10.1 | 9.7 |
| G-2 (B) | Control | 89.7a | 83.4a |
| | High | 81.4a | 75.2a |
| | Low | 87.4a | 76.4a |
| | SEM[1] | 4.3 | 4.4 |

a,b Means in the same column and generation having different superscripts are significantly different ($P < .05$).

[1]Standard error of the mean.

(A) Broiler breeder hens.

(B) Single Comb White Leghorn hens.

# APPENDIX 3

## EFFECT OF EXTENDER VISCOSITY ON INSEMINATION DOSE AND FERTILITY IN THE CHICKEN

The data summarized in Appendix 3 provide information about the effect of carboxymethylcellulose on the intactness of spermatozoal cells (Table A3.1), the viscosity trials of semen and diluents (Table A3.2), and the effect of spermatozoal insemination dose containing BPSE or BPSE with 2% CMC on the duration of fertility (Table A3.3).

TABLE A3.1. Effect of BPSE and BPSE + 2% CMC on intact spermatozoal cell concentration after 30 minutes of incubation at 37 C

| EXTENDER | CONCENTRATION ($\times 10^6$/ml) |
|---|---|
| BPSE | 1.65a |
| BPSE + 2% CMC | 1.62a |

a No significant difference (P> .05).

TABLE A3.2. Viscosity of various samples measured at 30 C with their mean and coefficient of variation (CV)

| SAMPLE | TRIAL 1 | 2 | 3 | 4 | 5 | MEAN | CV |
|---|---|---|---|---|---|---|---|
| | Centipoise | | | | | | --%-- |
| Distilled water | 0.9 | 0.9 | 0.9 | 1.0 | 0.9 | 0.9 | 2.5 |
| BPSE | 1.0 | 1.1 | 1.0 | 1.1 | 1.0 | 1.0 | 3.3 |
| Extended semen (1:2) with BPSE | 4.8 | 3.5 | 4.7 | 3.6 | 4.9 | 4.3 | 16.4 |
| Neat semen | 27.1 | 27.9 | 26.0 | 24.0 | 30.1 | 27.0 | 9.7 |
| Extended semen (1:2) Containing 2% CMC | 54.5 | 59.4 | 70.4 | 52.2 | 64.1 | 60.1 | 12.2 |
| 4% CMC in distilled water | 93.7 | 68.5 | 68.2 | 69.7 | 73.3 | 74.7 | 14.5 |

TABLE A3.3. Duration of fertility for 20 days, hens inseminated (20 hens per treatment) with 8PSE or 8PSE containing 2% CMC with an insemination dose of either 100 or 33 x $10^6$ spermatozoal cells.

| EXTENDER | INSEMINATION DOSE | DAYS | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| | $\times 10^6$ | % | | | | | | | | | | | | | | | | | | | |
| 8PSE | 100 | 94.0 | 100 | 100 | 100 | 100 | 100 | 100 | 90.0 | 95.0 | 88.2 | 68.8 | 77.8 | 50.0 | 37.3 | 25.0 | 5.8 | 0.0 | 0 | 0 | 0 |
| 8PSE | 33 | 100 | 100 | 94.1 | 100 | 94.4 | 94.4 | 100 | 78.6 | 80.0 | 70.0 | 57.1 | 40.0 | 47.4 | 25.0 | 20.0 | 5.0 | 0.0 | 0 | 0 | 0 |
| 8PSE + 2% CMC | 100 | 85.7 | 100 | 87.5 | 86.7 | 77.8 | 94.1 | 86.7 | 84.7 | 82.4 | 61.7 | 61.1 | 27.8 | 33.3 | 25.0 | 12.5 | 6.7 | 0.0 | 5.3 | 5.6 | 0 |
| 8PSE + 2% CMC | 33 | 100 | 94.1 | 82.6 | 100 | 81.3 | 82.4 | 73.7 | 62.5 | 38.9 | 44.4 | 41.7 | 23.50 | 18.8 | 29.4 | 5.2 | 0.0 | 0.0 | 5.8 | 0 | 0 |

# APPENDIX 4

The information about the procedure for the fluorometric assay, viscosity measurement and composition of BPSE, ethidium bromide buffered solution, and digitonin solutions used in the different experiments are described.

Fluorometric assay for the determination of spermatozoal cell concentration and percent intact cells. In all the experiments a Turner filter fluorometer (Model 111) was used to measure the fluorescence and determine the concentration and percentage of intact spermatozoal cells based upon the methods and procedures described by Bilgili and Renden (1984). Excitatory light was maintained at 365 nm using a 7-60 narrow pass filter, and the emission wavelength at 560 nm was established by using a number 22 sharp cut-off filter. Cuvette tube filled with 4 ml of ethidium bromide (EtBr) phosphate-buffered-saline was placed in the fluorometer and the background fluorescence was adjusted to zero. Then 10 ul of semen using a positive displacement pipet was mixed with the EtBr buffer solution and the resulting fluorescence recorded. Next, 25 ul of a digitonin solution was added to each tube which allowed the spermatozoal cells to be permeable to EtBr. This reaction resulted in a second fluorescence value with an intensity proportional to the sperm concentration of the solution. The total sperm concentration of the sample was estimated from a standard curve* relating fluorescence units to sperm numbers. The percentage of the intact cells was estimated by the ratio of the first and second fluorescence reading minus 1 times 100.

*Y = 1.4786 + 4.0667(x)

where

y = spermatozoal cell concentration

x = flourometric reading

Preparation of the EtBr($C_{21}H_{20}BrN_3$) phosphate buffer:

| Ingredients | Concentration | |
|---|---|---|
| | mM | (g/l) |
| $NaH_2PO_4.H_2O$ | 1.9 | 0.2621 |
| $Na_2HPO_4$ | 8.1 | 1.4980 |
| NaCl | 128.2 | 7.4974 |
| EtBr | - | 0.0125 |

The above ingredients were mixed in one liter of distilled water using a mechanical stirrer until dissolved. The final solution was adjusted to a pH 7.4 if necessary. The osmotic pressure of the solution should be 230 mOsm/kg.

Preparation of the digitonin solution. .0125 g of digitonin was mixed in 25 ml of absolute ethyl alcohol and mixed with a mechanical stirrer until dissolved.

Measurement of viscosity. Viscosity by the capillary method is measured by the flow in capillaries and is expressed mathematically as follows:

$$n = \frac{pr^4}{8vl} t$$

where:

n = viscosity in poise
v = volume of fluid in cc, flowing through a capillary tube in time t
l = length of capillary tube in cm
r = radius of capillary tube in cm
p = pressure of the system in dyne/sq. cm
t = time of flow through capillary tube in second

(West, 1942)

Based on the above formula the viscosities of neat semen, semen diluted with BPSE, and semen diluted with BPSE containing 2% (w/v) carboxymethylcellulose were measured using an Ostwald viscometer* (Size 200, with constant of 0.1 centistrokes/sec.). The viscometers were immersed in a constant temperature (30 C) water bath and were filled with 5 ml samples. The time of sample flow (in seconds) through the capillary section of the viscometer was recorded.

* CANNON® Cannon-Fenske viscometer.