

# SPERM MOBILITY IN BROILER BREEDERS

by

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(Under the Direction of Jeanna L. Wilson)

## ABSTRACT

The large-scale production of hatching eggs is the primary goal of the broiler breeder industry. Therefore, semen quality is of importance to the industry. The present work evaluated the effect of sperm mobility on semen quality in strains of commercial broiler breeders. Low and high sperm mobility phenotypes were identified within populations of broiler breeders. The phenotypes were not independent of age, yet remained distinct. Computer-assisted sperm motion analysis explained the mobility of sperm populations in terms of individual sperm cell motility. Fertility differed between phenotypes by 25%. Sperm from low mobility males contained more aberrant mitochondria when evaluated with transmission electron microscopy. Finally, males with heavier body weights had lower sperm mobility ( $P \leq 0.0001$ ). In summary, sperm mobility is indicative of semen quality in commercial broiler breeders. However, due to the negative relationship between body weight and sperm mobility, males should not be selected based upon sperm mobility alone.

INDEX WORDS: Sperm mobility, fertility, ultrastructure, broiler breeder male

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## INTRODUCTION

The profitability of the commercial broiler breeder industry is based, in part, upon the number of hatching eggs produced. Broiler breeders have been genetically selected for meat yield, which is inversely correlated to reproductive performance. Fertility in breeder flocks has decreased due to this selection for meat yield. The decrease in fertility often occurs when flocks are approximately 40 weeks of age, and roosters are primarily responsible due to the fact that fertility can be sustained by artificial insemination (Brillard and McDaniel, 1986).

Male fertility is based upon both the ability to perform a successful copulation and the quality of semen produced. The objective of measuring semen quality is ultimately to predict the fertilizing ability of the rooster. Many techniques are available to measure the semen quality of roosters, but most techniques do not correlate well with fertilizing potential. The sperm mobility test has been used extensively as a semen quality assessment in turkeys because it was found to be predictive of paternity success in a competitive fertilization trial (Donoghue et al., 1999). However, limited research has been done using sperm mobility in commercial broiler breeders or using sperm mobility as a predictor of flock fertility as a breeder flock ages.

The objectives of this research were (1) to determine sperm mobility in two different genetic strains of commercial broiler breeders, (2) to apply computer assisted sperm analysis (CASA) to a population of broiler breeders, and (3) to compare fertility, body weight, comb size, blood plasma testosterone, sperm metabolism, and sperm cell ultrastructure to sperm mobility within a population of broiler breeders.

## LITERATURE REVIEW

### Male Reproductive Tract

#### *Overview*

Avian males have testes that are located internally, anterior to the kidneys and attached to the dorsal body cavity. Because of the location of the testes, spermatogenesis occurs at body temperature in avian species. The left testis is often larger than the right (Lake, 1957; Lofts and Murton, 1973), and total testicular weight is approximately 1% of the total body weight, depending on the breed of bird (Sturkie and Opel, 1976). The testes contain interstitial tissue and seminiferous tubules. The interstitial tissue and surrounding connective tissue contain blood vessels, lymphatic vessels, nerves and Leydig cells while the seminiferous tubules contain the seminiferous epithelium, consisting of Sertoli cells (Osman et al., 1980; Bergmann and Schindelmeiser, 1987) and various stages of developing germ cells (Lin and Jones, 1990).

The epididymis is a series of ducts attached to the testis that empty into the deferent duct. Starting at the testis, the epididymis consists of the rete testis, efferent ducts, connecting ducts and epididymal duct. The deferent duct is a coiled continuation of the epididymal duct that is larger in diameter by 3-fold between the cranial epididymal duct and distal deferent duct (Tingari, 1971). The distal deferent duct straightens and widens at the cloacal juncture and terminates in the urodeum.

Spermatogenesis occurs in the seminiferous epithelium and is the process where stem cells produce diploid spermatogonia that undergo mitosis and then meiosis to divide into haploid spermatocytes. The spermatocytes then undergo meiosis to form spermatids. Spermatogenesis is ultimately controlled by neurons (Sharp and Gow, 1983), and depends on testosterone, follicle stimulating hormone (FSH), and the activity of Sertoli cells (Sharpe 1994). The transformation of spermatids into sperm cells is referred to as spermiogenesis and takes place during 8-10 morphological steps (Gunawardana, 1977; Tiba et al., 1993) in the seminiferous epithelium. Spermiogenesis includes the formation of an acrosome and axoneme, loss of cytoplasm and nuclear condensation of the cell (Nagano, 1962; Tingari, 1973; Gunawardana, 1977; Gunawardana and Scott, 1977; Oliva and Mezquita, 1986; Sprando and Russell, 1988).

The release of fully formed sperm cells from the seminiferous epithelium into the lumen of the seminiferous tubules is known as spermiation. Cells are suspended in fluid secreted by the Sertoli cells. Passage through the seminiferous tubules depends on hydrostatic pressure of the fluid and contraction of myoepithelial cells (Rothwell and Tingari, 1973). Sperm cell transport through excurrent ducts is estimated to take several days (Munro, 1938; de Reviere, 1975) and it is presumed that passage through the deferent duct is dependent upon peristalsis (de Reviere, 1975). The extragonadal sperm reserve is located in the deferent duct (de Reviere, 1975), where the concentration of sperm cells is highest (Clulow and Jones, 1988). Sperm cells are immotile at spermiation (Ashizawa and Sano, 1990). Sperm acquire the potential for motility as they pass through the excurrent ducts (Munro, 1938; Clulow and Jones, 1982; Howarth, 1983; Ashizawa and Sano, 1990).

### *Ultrastructure of Spermatozoa*

Using light microscopy, Romanoff (1960) described avian spermatozoa as elongated, flagellated cells that can be divided into the head, midpiece, and tail. Using the scanning electron microscope, Thurston and Hess (1987) described chicken spermatozoa as vermiform cells 0.5-0.7 $\mu$ m wide and approximately 90 $\mu$ m long. Fine structural changes during transit through excurrent ducts have also been observed in rooster sperm (Tingari, 1972). The fine structure of ejaculated sperm, including the observation of the midpiece surrounded by helically arranged, rectangular mitochondria was described by Lake et al. (1968). An in-depth structural assessment of the head, neck and midpiece of sperm from White Leghorns using a transmission electron microscope indicated that the midpiece contains approximately 30 mitochondria (Bakst and Howarth, 1975). Mitochondrial swelling in degenerative sperm (Lake et al., 1968) and significant positive correlations among mitochondrial status, midpiece integrity and fertilizing capacity of fresh semen (Xia et al., 1988) indicate that mitochondria within the sperm cell can be important indicators of cell integrity and quality.

### *Hormones*

Gonadotrophin releasing hormone (GnRH) is a secretory product from the hypothalamus that stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Sterling and Sharpe, 1984; Hattori et al., 1986) from the anterior pituitary gland. In avian males, FSH stimulates growth differentiation and activity of the seminiferous tubules while LH binds to cell-surface receptors in Leydig and Sertoli cells (Brown et al., 1975; Ishii and Furaya, 1975; Ishii and Yamamoto, 1976). The Leydig cells respond rapidly to LH (Maung and Follett, 1977) by synthesizing and

secreting both testosterone and a precursor to testosterone, androstenedione (Nakamura and Tanabe, 1972; Sharp et al., 1977) into the circulatory system. The increase of plasma androgens in the circulatory system has a negative feedback effect by acting upon both the anterior pituitary gland and the hypothalamus to suppress the release of both GnRH and LH (Braunstein, 1997). Both LH and FSH are essential for spermatogenesis (Brown and Follett, 1977). Testicular function is dependent upon the combined actions of FSH and testosterone (Sharp and Gow, 1983). Age related effects of the neuroendocrine system on male reproduction include depressed aromatase and vasotocin systems, which are important for sexual behavior (Panzica et al., 1996; Ottinger, 1997) and indicate that the aging rooster may demonstrate decreased libido.

Photostimulation results in Leydig cells in the testes producing testosterone and androstenedione, which are both released into the bloodstream. Testosterone stimulates spermatogenesis (Lofts and Murton, 1973) and is essential for maintenance of excurrent ducts, secondary sex characteristics, sexual behaviors, and the alteration in pattern of GnRH secretion. Testosterone can inhibit the GnRH-induced secretion of LH (King et al., 1988) and stimulate the production of FSH (Tsutsui and Ishii, 1978). Sexual accessory tissues (comb, wattles) in males of the avian species concentrate testosterone and then metabolize it to other hormones such as  $5\alpha$ -dihydrotestosterone (DHT) and  $5\beta$ -DHT (Martini, 1982). In broiler breeders, plasma testosterone levels peak around 4 ng/mL at 30 weeks and average 2.5 ng/mL between 40 and 60 weeks of age (Sexton et al., 1989; Hocking and Bernard, 2000).

## ***Measurements of Sperm Quality***

### *Sperm concentration*

Sperm concentration can be estimated by a packed cell volume method, or spermatocrit. Semen is drawn into a microcapillary tube and one end of the tube is sealed. The sperm cells are centrifuged and the percent packed cells are measured using a microcapillary reader (Taneja and Gowe, 1961; Maeza and Buss, 1976). Optical density is another indirect method to estimate sperm concentration. Bilgili and Renden (1984) found 550nm to be the optimum wavelength to estimate concentration in a spectrophotometer. Direct sperm cell counts can be made by use of a hemocytometer (Allen and Champion, 1955). There have been conflicting reports of the relationship of concentration to fertility. The majority of reports have found no relationship between the two (Sampson and Warren, 1939; Shaffner and Andrews, 1948; McCartney, 1956; Cooper and Rowell, 1957), while other researchers have seen positive correlations (McDaniel and Craig, 1959; Boone and Huston, 1963).

### *Sperm Motility*

Sperm motility has been subjectively measured with light microscopy. A drop of semen (raw or diluted) is placed onto a microscope slide and a score assigned (0-4) that estimates the percentage of motile sperm cells in the semen (Wheeler and Andrews, 1943; Cooper and Rowell, 1957; Boone and Huston, 1963). Wishart and Ross (1985) developed a complex spectrophotometric technique to estimate sperm motility that requires the use of a variable flow rate spectrophotometer. A semen sample is diluted in

a vial and drawn through a light path flow cell for 30 seconds. The optical density of the flowing sample is read at 550nm, and correlated with forward motility of the semen sample.

As with concentration, there are conflicting reports concerning the relationship between sperm motility and fertility. Most researchers have found a significant correlation between sperm motility and fertility (McDaniel and Craig, 1962; Boone and Huston, 1963; Kummerer et al., 1972; Wishart and Palmer, 1986), while Wall and Boone (1973) found no correlation.

#### *Sperm Viability*

The percentage of dead sperm in a semen sample has been measured using nucleic acid stains such as eosin and nigrosin (Cooper and Rowell, 1957; El Jack and Lake, 1966), congo red and nigrosin (Chatterjee et al., 1967), and trypan blue (Wilson et al., 1969). Bayyari et al. (1990) used a vital stain in conjunction with a nucleic acid stain to distinguish between dead and moribund cells within a sample, thus evaluating semen quality. Viability of sperm cells has also been estimated fluorometrically (Bilgili and Renden, 1984). For fluorometric determination, ethidium bromide is used to penetrate damaged sperm cells, and the viability of the sample is equal to the ratio of initial fluorescence to that measured after all cells are killed with digitonin.

#### *Sperm-Binding Assays*

A technique was developed to count the number of sperm cells that penetrate the perivitelline membrane of an egg in order to estimate the fertilizing capacity of an individual rooster (Bramwell et al., 1995). For the assay, the perivitelline membrane is removed around the germinal disc, rinsed in saline solution, fixed with 20% formalin and

stained with Schiff's reagent. Excess formalin and Schiff's reagent are washed off of slide with saline, a coverslip is applied to the slide and slide is blotted dry. Holes caused by sperm cells appear white on a dark purple background, and can be counted with a light microscope at a magnification of 100x. Age of bird has been found to negatively effect sperm penetration of the perivitelline layer (Bramwell, 1996).

Cramer et al. (1994) introduced a sperm-binding assay that was refined by Barbato et al. (1998). The perivitelline membrane is isolated from a freshly laid, unfertilized egg and heat solubilized. A sperm suspension is placed into a flat-bottomed microtiter plate containing the heat solubilized perivitelline membrane and incubated at 38°C for 180 minutes. The membrane is then decanted, rinsed, dried, and stained with 4', 6'-diamidino-2-phenylindole (DAPI) to enumerate bound sperm. Sperm binding is reported per square millimeter when viewed with microscope. Sperm-egg binding has exhibited a high correlation ( $r = 0.83$ ) to fertility (Barbato et al., 1998).

### *Sperm Mobility*

Sperm mobility is characterized as the net movement of a sperm cell population in a specific direction. Developed by Froman and McLean (1996), the sperm mobility test has been used in New Hampshire chickens (Froman et al., 1997; Froman and Feltmann, 1998; Birkhead et al., 1999; Froman et al., 2002) and commercial turkeys (Donoghue et al., 1998; Donoghue et al., 1999; King and Donoghue, 2000; King et al., 2000b) as a determinant of fertility. A limited study with broiler breeders also found sperm mobility to be a determinant of fertility (Froman et al., 1999). For the sperm mobility test, sperm concentration of a semen sample is determined by a spectrophotometer and the semen is then diluted. The suspension is overlaid upon pre-warmed 6% Accudenz<sup>®</sup> (a non-ionic,

biologically inert cell separation medium) in a semi-micro polystyrene cuvet and incubated for 5 minutes at 41°C. After incubation, the cuvet is placed in a spectrophotometer and optical density is determined at 550 nm. Absorbance is recorded as the sperm mobility score (Froman and Feltmann, 1998). Accudenz<sup>®</sup> is the trade name for N,N'-bis(2,3 dihydroxypropyl)-5-[N-(2,3 dihydropropyl) acetamido]-2,4,6-triiodo-isophthalamide. A field instrument, the ARS Densimeter<sup>™</sup> can also be used to accurately measure sperm mobility (King and Donoghue, 2000).

#### *Sperm Quality Analyzer<sup>®</sup>*

The Sperm Quality Analyzer<sup>®</sup> (SQA) was designed for use in determining sperm quality in humans (Bartoov et al., 1991). A 3 to 5-fold diluted semen sample is drawn into a capillary tube, which is placed into the SQA. Within 40 seconds, the device will give a sperm motility index (SMI) that is an estimate of sperm concentration, viability and motility. The SQA has been used in poultry and correlated with concentration, viability and motility (Wishart and Wilson, 1997; McDaniel et al., 1998). In addition, the SMI has been correlated to the fertilizing ability of males (McDaniel et al., 1997). Further work in the same laboratory concluded that selection of males based upon SMI at an early age improves flock fertility (Parker et al., 2000, 2002). More recently, Froman et al., (2003) found that the AviMate<sup>™</sup> SQA did not predict sperm mobility phenotype.

#### *Sperm Metabolism*

Sperm motion is partly a function of metabolic capacity (Ishijima and Mohri, 1990), and in humans, the motility of spermatozoa was found to be dependent on mitochondrial function (Auger et al., 1989; Folgero et al., 1993). Reduction assays have been used to measure the rate of metabolism by measuring the degree of integrity of the

oxido-reductase enzyme activities vital to normal metabolism of sperm cells (Giese, 1973). By measuring the reduction of resazurin, Cooper and Rowell (1957) found it may be possible to identify males with low fertilizing capacity, which could be due to the significant association found between reduction time of methylene blue and motility (McDaniel and Craig, 1962). More recently, reduction of the dye p-iodonitrotetrazolium violet (INT) to formazan was used to measure the metabolic activity of fowl sperm and predict fertilizing ability (Chaudhuri and Wishart, 1988; Kirby and Froman, 1991) while 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT)-reduction is significantly correlated to the aforementioned INT-reduction test (Hazary and Wishart, 2001).

Synthesis of ATP was found to be critical for motility in mammalian sperm (Ford and Rees, 1990). The ATP content of freshly ejaculated sperm was found to correlate with fertility (Wishart, 1982; Wishart and Palmer, 1986) and McLean et al. (1997) found subfertile rooster sperm to have an ATP concentration only 37% of the more fertile controls. More recently, ATP content was found to correlate with sperm mobility in chickens (Froman and Feltmann, 1998).

Oxygen consumption of sperm cells can also be measured as a parameter related to metabolism because it can be used to determine metabolic rate. Research has shown that at body temperature, oxygen consumption of sperm from average sperm mobility males was only 54% of that compared to high sperm mobility males (Froman et al., 1999).

### *Computer Assisted Sperm Analysis (CASA)*

Computer technology has allowed for more objective sperm counts and sperm motility assessments in human cases with positive correlations to both *in vitro* (Holt et al., 1985) and *in vivo* fertilization (Barratt et al., 1993; MacLeod and Irvine, 1995). The Hobson Sperm Tracker® has successfully analyzed motile concentration and velocity parameters in semen from a boar (Holt et al., 1994), gazelle (Abaigar et al., 1999), rat (Moore and Aknondi, 1996), a turkey and a chicken (Donoghue et al., 1998, King et al., 2000a, McLean et al., 1997, Froman et al., 1999, Froman and Feltmann, 2000). Straight line velocity (the speed at which sperm cells travel) was found to differ between high and low sperm mobility males in both turkeys (Donoghue et al., 1998) and chickens (Froman et al., 1999).

### *Secondary Male Traits*

#### *Body Weight*

In broiler breeders, body weight is controlled through feed restriction to optimize reproduction, and weight may be associated with the social status of the male chicken (Allee et al., 1939). Other researchers have reported a negative correlation between sperm motility and rate of gain (Soller et al., 1965b; Seigel, 1963). Marini and Goodman (1969) observed that in a male line selected for increased body weight, sperm motility was decreased. A negative correlation was found between growth rate and mating activity (Rappaport and Soller, 1966). In a series of four natural mating trials and one artificial insemination trial, body weight was poorly correlated to fertility ( $r = -0.39$  to  $0.09$ ) (Wilson et al., 1979). This negative correlation between fertility and body weight

has also been seen in turkeys (Berg and Shoffner, 1954). A study by Edens et al. (1973) found decreased metabolic activity in sperm cells of a high weight line, which agrees with the decreased sperm motility and lower mitochondrial mass of sperm cells seen in a strain of chickens exhibiting high feed intake (Morrison et al., 1997).

#### *Comb Size*

Comb size has been used as a bioassay for plasma testosterone levels in males (Breneman, 1939; Emmans, 1939) because comb and wattle growth are androgen dependent (Dorfman, 1948; Zeller, 1971; Nakamura and Tanabe, 1973). Testosterone is a sex steroid produced by the testes, and is associated with sexual behavior of the male. McGary et al. (2002) found that male broiler breeders with large combs within specific strains were likely to have higher fertility. In addition, these researchers reported that wattle length correlated to sperm penetration of the perivitelline layer in a particular strain of broiler breeder (McGary et al., 2003). Contrary to those findings, it was observed that external characteristics such as size and color of wattles and combs are not reliable indicators of semen volume (Burrows and Titus, 1939). Fertility is poorly correlated to male physical appearance (Wilson et al., 1979). Schantz et al. (1995) found a negative correlation between comb size and testes mass.

#### *Testicular Size*

Traditionally, the only way to determine testicular size in birds was upon post mortem necropsy because of their internal location. However, with new ultrasound technology, it is possible to identify the size and, if present, the pathological state of internal organs. Ultrasound has been used to assess ovarian status and follicular development in hens (Melnychuk et al., 2002) as well as testicular size in roosters

(Richardson et al., 2002; Bowling et al., 2002). These researchers suggest that ultrasound measurements of testes width can accurately gauge size (width from epididymis) of the testes without causing harm to the bird. Testes weight has been positively correlated to body weight (Brown and McCartney, 1983; Wilson et al., 1988), semen volume, concentration, and total number of sperm (Lee et al., 1999). However, combined testes weight was not correlated to sperm mobility (Froman and Feltmann, 1998).

### *Body Conformation*

Skeletal conformation of the male broiler breeder has probably been altered because of intense growth selection (Soller et al., 1965b; Siegel and Dunnington, 1985; Hocking and Duff, 1989). These changes in body conformation may impede cloacal contact during natural mating and ultimately, semen transfer to the hen (Wilson et al., 1979; Hocking and Bernard, 1997). McGary et al., (2003) suggests that the dorsal pelvic width, or the distance between the outermost points of the pelvis, impacts semen transfer at copulation because fertility was negatively correlated ( $r = -0.30$ ) with dorsal pelvic width.

### ***Fertility Decline in Broiler Breeders***

#### *Females*

Egg production in hens usually declines after hitting a peak at approximately 35 weeks of age (Atwood, 1929; Etches, 1990; Robinson et al., 1990; Palmer and Bahr, 1992). Older hens are characterized by a continued decrease in fertility and production of hatching eggs laid post-peak production (Harper and Arscott, 1969; Kirk et al., 1980), with the most significant drop in fertility at 45-50 weeks of age (Kirk et al., 1980; Lake

1989; Hocking and Bernard, 1997; Walsh and Brake, 1997). Declining egg production and fertility can cause major economic losses in older flocks of broiler breeders.

Hens store sperm within specialized sperm storage tubules of the uterovaginal junction (Bobr et al., 1964) and infundibular region (Van Drimmelen, 1946). The decrease in fertility of older hens may be due to the decreased capacity of sperm storage tubules to store spermatozoa (Van Krey et al., 1967; Pierson et al., 1988). However, in a study performed with both young and old hens, Brillard (1993) showed that although the number of sperm residing in sperm storage tubules were the same in both groups of hens, the rate of sperm release from the sperm storage tubules in old hens was twice that of the young hens, indicating sperm must be released more often in older hens in order for fertilization to occur.

Maximal filling of the sperm storage tubules occurs 24-48 hours after insemination (Bakst et al., 1994), and insufficient filling of the tubules may be responsible for declining fertility in older hens (Etches et al., 1974). Changes in the uterine fluid associated with age may be related to the decline of spermatozoal viability in the oviduct (Dupuy and Blesbois, 1996). Bramwell et al. (1996) suggests that the hen-dependent decline in fertility may be due to the fact that sperm cells stored in old hens are viable for a shorter period of time than sperm cells stored in young hens. However, a study done with hens known to have short or long durations of fertility found that differences were not attributable to differing numbers of sperm storage tubules (Pierson

et al., 1988). If artificial insemination was an option in older flocks of broiler breeders, the age related decline of fertility in females could be reduced by inseminating a large number of sperm (de Reviere and Brillard, 1986) or by duplicate inseminations (Brillard and McDaniel, 1986; Brillard et al., 1989).

### *Males*

With aging broiler breeder flocks, the fertility decline may be due to the aging male, because fertility can be maintained through artificial insemination (Brillard and McDaniel, 1986) or introducing younger males into an older flock (spiking) (Wilson et al., 1979; Casonovas, 1999). In contrast, a study by Bramwell et al., (1996) found no difference in fertility when young (39 wk) and old (65 wk) roosters were used as semen donors, suggesting that spermatozoa from old males retain the physiological capability to fertilize, which agrees with research suggesting that males can be used for at least two production cycles (Renden and Pierson, 1982).

Older males have a reduction in both spermatozoa number and semen volume (de Reviere and Brillard, 1986; Lake, 1989; Sexton et al., 1989; Rosenstrauch et al., 1994). Reduction in sperm output may be caused by changes in the Sertoli cells that impair the regular release of spermatozoa (Rosenstrauch et al., 1994) or the decrease of activity seen in testosterone-dependent organs responsible for sperm production (Mann and Lutwak-Mann, 1975). In roosters, research shows that aging sperm have reduced fertilizing capabilities (Brillard and McDaniel, 1986), which may be explained by a possible decrease of motility due to the reduction of polyunsaturated fatty acids in spermatozoa

(Cerolini et al., 1997). Lack of control of male body weight gain is an important cause of declining fertility (Hocking and Duff, 1989) because natural mating may become physically difficult (Soller et al., 1965a,b; Wilson et al., 1979; Hocking and Bernard, 1997) for the overweight male broiler breeder.

### *Natural Mating*

Natural mating activity is high in young male and female broiler breeders and then declines with age (Duncan et al., 1990; Hocking and Bernard, 1997). Body weight may be a factor influencing lower fertility because overweight breeders were found to have reduced numbers of successful copulations (Hocking, 1990; Attia et al., 1993; Robinson et al., 1993; Hocking and Bernard 1997). High body weight males were found to have lower fertility (Burke and Mauldin, 1985; Hocking and Duff, 1989) and an increased incidence of musculoskeletal lesions (ruptured tendons and ligaments, dyscondroplasia, and destructive cartilage loss) making natural mating more difficult for the overweight roosters (Hocking and Duff, 1989). Natural mating problems may also be related to genetic strains of commercial birds. Parker (1961) found that the capacity of Cornish males to naturally fertilize pullets was below that of New Hampshire males.

### ***Fertility Trial***

#### *Insemination Dose*

High fertility has been achieved with an insemination dose of 40-70 million spermatozoa (Taneja and Gowe, 1962), but the industry standard for insemination dose in chickens and turkeys is 100 and 200 million spermatozoa per insemination, respectively (Etches, 1996). Older hens, however, require either duplicate inseminations, or more

than 250 million sperm per week to maintain fertility (Brillard and McDaniel, 1986). With artificial insemination, the quality of the spermatozoa is a more limiting factor for fertility than the number inseminated (Wishart and Palmer, 1986), and furthermore, sperm quality is more likely to determine fertility than oviduct selection (Froman et al., 1999). Therefore, a low (or minimal) dose of spermatozoa can be used in order to differentiate between sperm quality of males.

#### *Duration of Fertility*

Duration of fertility is a sensitive assessment of fertilizing potential following a single insemination (Shaffner and Andrews, 1948; Gowe and Hutt, 1949; Williams and McGibbon, 1956). A sigmoidal decline in fertility is expected over a period of 2 to 21 days post insemination (Kirby and Froman, 1990; Kirby et al., 1998) with a faster decline seen in older birds. The quicker decline with age suggests poorer semen quality of males (Bramwell et al., 1996). However, no difference in duration of fertility was found between flocks at 39 and 59 weeks of age (Fiser and Chambers, 1981), which would indicate that the duration of fertility is not related to age.

In the domestic hen, duration of fertility can range from 4 to 34 days (Curtis and Lambert, 1929; Romanoff, 1960). In a study by Taneja and Gowe (1961), meat strain birds were found to have a shorter duration of fertility than Leghorns, which was confirmed by Goerzen and others (1996) who found a negative correlation between hen weight and duration of fertility. An investigation of thirteen commercial breeder lines revealed a significant interaction between strain and duration of fertility (Fiser and Chambers, 1981). In contrast, no effects of duration of fertility were found in five commercial lines of broiler breeders (Kirby et al., 1998).

### *Fertility Assessment*

Fertility can be assessed at the hatchery before or after eggs hatch. By candling, one can assess flock fertility as well as other sources of hatch failure such as eggs set upside down, cracked eggs and embryonic mortality (Mauldin, 2002). Candling can be performed quickly using a table (mass) candler or more slowly (yet more accurately) using a spot candler. Egg breakout is the process where eggs that have been candled and deemed not viable are removed from the incubator and opened to assess fertility and embryonic development (Mauldin, 2002). Embryonic mortality is often classified as early dead, mid-dead or late dead (Wilson, 1995). Early dead embryos occur during the first week of incubation and are characterized by a blood ring or network of blood vessels. The embryo may also adhere to the side of the egg. Embryos that die during the second week of incubation are characterized by the presence of a hard beak and an egg tooth. Dead embryos that are fully covered in feathers occur during the third week of incubation (Wilson, 1995). Candling eggs and performing a breakout analysis is an estimate of flock fertility.

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CHAPTER 2  
VARIATION IN SPERM MOBILITY BETWEEN TWO STRAINS OF  
COMMERCIAL BROILER BREEDERS<sup>1</sup>

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<sup>1</sup>Bowling, E.R., D.P. Froman and J.L. Wilson. To be submitted to *Poultry Science*.

**ABSTRACT** Variation in sperm mobility phenotype was evaluated in two strains of commercial broiler breeders. Sample populations (n = 126 and 111 for Strains A and B) were evaluated with the sperm mobility assay. Sperm mobility was measured in absorbance units, and population data was  $0.260 \pm 0.159$  (mean  $\pm$  SEM) for Strain A and  $0.178 \pm 0.127$  absorbance units for Strain B. In each case, low and high sperm mobility males were evaluated weekly between 33-42 weeks of age. Sperm mobility phenotypes were distinct as measured by their weekly sperm mobility scores over the 10-wk period. High sperm mobility phenotype in both strains was independent of age ( $P \leq 0.05$ ) while the low sperm mobility phenotype in both strains tended to increase with age ( $P \geq 0.05$ ). Strain differences in sperm mobility were detected ( $P \leq 0.05$ ). Sperm motility was evaluated by a computer assisted sperm analysis method on a population-wide basis for the Strain A. Motile concentration, straight-line velocity, beat cross frequency and straightness were variables used for correlation analyses. In the present study, both beat cross frequency and straightness were highly correlated with straight line velocity ( $r = 0.94$  and  $0.88$ , respectively). Whereas motile concentration did not differ among low, average, and high sperm mobility phenotypes ( $P \geq 0.05$ ), average straight line velocity differed significantly ( $P \leq 0.05$ ) between average and high sperm mobility phenotypes. In conclusion, sperm mobility varied between commercial strains of broiler breeders, and phenotypic variation appeared to be largely due to differences in average straight line velocity.

*(Key Words: broiler breeder, sperm mobility, CASA)*

## INTRODUCTION

*Sperm mobility* is defined as the net movement of a sperm cell population against resistance at 41°C. This trait has been shown to affect male fitness in chickens and turkeys (Froman et al., 1999; Birkhead et al., 1999; Donoghue et al., 1999). However, sperm mobility has not been studied extensively in commercial broiler breeders even though broiler breeders were used to demonstrate that fertility is a function of sperm mobility following artificial insemination (Froman et al., 1999). Sperm mobility determination is useful in the commercial turkey industry due to the necessity of artificial insemination (King and Donoghue, 2000). Artificial insemination is most effective when semen donors have highly mobile semen, which can vary among strains of toms (King et al., 2000). Semen quality is also an important variable when insemination depends upon natural mating. To date, only preliminary work has been done comparing sperm mobility among different populations of broiler breeders (Rhoads et al., 1998). Therefore, one objective of the present work was to evaluate the range of sperm mobility in two commercial strains of broiler breeder males.

Previous research with computer assisted sperm analysis (CASA) in avian species has demonstrated that the number of sperm within an ejaculate having a straight line velocity (VSL)  $> 30 \mu\text{m/s}$  predicted sperm mobility phenotype (Froman et al., 2003). However, this experiment utilized a small number of males within a population. Previous experimentation with the New Hampshire breed (Froman and Feltmann, 2000) illustrated the value in applying CASA to a population of males. Because CASA methods have not been used with broiler breeders, the second experimental objective was to apply CASA to a population of commercial breeder males.

## MATERIALS AND METHODS

### *Bird Management*

Males from Strain A, (n = 126) were individually caged at 29 weeks of age (WOA) within a temperature-controlled poultry house equipped with evaporating cooling and forced air furnaces. The experiment was performed during the months of June through October, and set temperature inside the house was 70°F. The low and high ambient temperature averages were 62.1 and 83.1°F. Birds were provided 16 h light per d. Males were fed a standard breeder diet consisting of 15.9% crude protein and 2900 kcal ME/kg. Males were weighed weekly in order to determine feed amounts. Males were ejaculated by massage technique (Burrows and Quinn, 1937) twice a week (Tuesday and Thursday) from 29 to 32 weeks of age in order to condition the males for semen collection.

Males from Strain B (n = 111) were caged as above at 28 WOA. Birds were housed the following year in the same facility used for Strain A. This experiment was conducted during the months of June through October; set temperature inside the house was 70°F, low and high ambient temperature averages were 65.3 and 85.0°F. Strain B males were maintained on the same photoperiod and fed the same diet as Strain A. Males were ejaculated by massage technique (Burrows and Quinn, 1937) twice a week (Wednesday and Friday) from 28-32 WOA in order to condition the males for semen collection.

### *Sperm Mobility*

Sperm mobility was measured on two occasions for each male within each strain. The assay was performed according to Froman et al. (1999). Initial measurements were

made at 31 WOA. Briefly, a 50- $\mu$ L volume of semen was diluted to  $5 \times 10^8$  sperm/mL with pre-warmed mobility buffer. A 60- $\mu$ L volume of this sperm suspension was overlaid upon 600  $\mu$ L of pre-warmed Accudenz<sup>®1</sup> in a semi-micro cuvet. Then, the cuvet was incubated in a water bath at 41°C for 5 minutes. The absorbance of the Accudenz<sup>®</sup> layer was measured 1 minute after the cuvet was transferred to a Turner- 860 spectrophotometer<sup>2</sup>. Males within each strain were ranked based upon average sperm mobility scores. Any male with a sperm mobility score greater than one standard deviation above or below the respective strain mean was classified as either a high or low sperm mobility phenotype, respectively. These 40 select males (n = 10 per phenotype per strain) were assessed weekly over a 10-week period starting when birds were 33 weeks of age.

#### *Computer Assisted Sperm Analysis (CASA)*

Semen from each male classified as low, average or high sperm mobility phenotype within Strain A that was producing semen at 37 weeks (n=71) was analyzed with the Model 7V1B Hobson Sperm Tracker<sup>3</sup> as outlined by Froman and Feltmann (2000). In brief, neat semen was diluted to  $5 \times 10^8$  sperm/mL with mobility buffer. A 1:10 dilution of heparinized avian blood also was made with mobility buffer. A 125- $\mu$ L volume of diluted blood was mixed with 1.222 mL prewarmed mobility buffer within a culture tube. Immediately thereafter, 3 $\mu$ L of diluted sperm was added to the blood suspension. After mixing, a 7- $\mu$ L sample was withdrawn and placed in a pre-warmed (41°C) MicroCell<sup>4</sup> with a chamber depth of 50 $\mu$ m. The MicroCell was placed on a

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<sup>1</sup> Accurate Chemical & Scientific Corp., Westbury, NY 11590

<sup>2</sup> Barnstead/Thermolyne Corp., Dubuque, IA 52004-0797

<sup>3</sup> Hobson Vision Ltd., Baslow, Derbyshire, United Kingdom DE45 1RP

<sup>4</sup> Conception Technologies, SanDiego, CA 92121

microscope stage maintained at 41°C. CASA was performed with settings determined by Froman and Feltmann (2000). Data were collected for 45 s in each of 5 random locations within the MicroCell. The following variables were measured: straight line velocity (VSL), motile concentration (MOTC), beat cross frequency (BCF), and straightness (STR).

### *Statistical Analysis*

The Univariate procedure of SAS<sup>®</sup> was used to evaluate initial sperm mobility of each population. The General Linear Model of SAS<sup>®</sup> was used to compare sperm mobility scores between flocks, and for linear regression after scores of select males were plotted as a function of age. The correlation procedure of SAS<sup>®</sup> was used to correlate CASA variables.

## **RESULTS**

### *Sperm Mobility*

The population means  $\pm$  SD for Strain A and B were  $0.260 \pm 0.159$  and  $0.178 \pm 0.127$  absorbance units, respectively. Variation in sperm mobility within each strain was large, with coefficients of variation of 61 and 71% for Strains A and B, respectively. Sperm mobility differed between strains ( $P \leq 0.001$ ; Table 2.1). Selected males with low and high sperm mobility from each flock were evaluated from 33 to 42 weeks of age (Figures 2.1 and 2.2). In both strains, the high sperm mobility phenotype was independent of age ( $P \geq 0.05$ ). In contrast, the low sperm mobility phenotype in both Strain A and B improved slightly as a function of age ( $P \leq 0.01$ ) as evidenced by positive slopes associated with the regression lines. Nonetheless, low and high sperm mobility phenotypes were distinct ( $P \leq 0.05$ ) throughout the experiment. However, phenotypic

designations were strain-dependent. For example, the average score for the high phenotype in Strain A was 1.5 times greater than the average score for Strain B, with 0.582 versus 0.374 absorbance units. Likewise, means for the low sperm mobility phenotypes differed between strains ( $P \leq 0.0001$ ) with 0.178 versus 0.067 absorbance units for strains A and B, respectively.

#### *CASA*

The Hobson Sperm Tracker was used to evaluate VSL, MOTC, BCF and STR for Strain A. Correlation coefficients are shown in Table 2.2. Properties of individual motile sperm from low, average and high sperm mobility phenotypes are shown in Table 2.3. No difference was observed among phenotypes for MOTC ( $P \geq 0.05$ ). In contrast, VSL, BCF, and STR differed significantly between the high and average sperm mobility phenotypes ( $P \leq 0.05$ ).

### **DISCUSSION**

Few researchers have compared semen quality among broiler breeder strains. Agri Stats (2001) lists 15 male lines in the commercial market, with the percentage of fertilized eggs ranging from 75.7 to 87.5 %, indicative of variation among strains. In agreement, Fiser and Chambers (1981) investigated 13 commercial breeder lines and found a significant interaction between breeder strain and duration of fertility. In contrast, Kirby et al. (1998) reported comparable duration of fertility among commercial male broiler breeder lines.

The present research demonstrated a clear difference in sperm mobility between two commercial broiler breeder strains. Similar results have been observed in turkeys (King et al., 2000). Because of the number of male strains involved in the commercial

industry, additional studies are needed if sperm mobility guidelines are to be developed for broiler breeder males. Sperm mobility is a valuable measurement of semen quality because it is predictive of male fertility (Donoghue et al., 1999; Froman et al., 1999; Birkhead et al., 1999; Froman et al., 2002; Froman et al., 2003). Fertility and hatchability are critical components of the broiler breeder industry; hence a measurement predictive of fertilizing potential will have major economic impact. Additionally, sperm mobility could be used as a selection criterion to improve reproductive traits in commercial broiler breeders because the trait is heritable (Froman et al., 2002). It is interesting to note that over the 10-week period (33 to 42 weeks of age), the high sperm mobility males in both strains had sperm mobility scores that were different, but both were independent of bird age. With respect to low sperm mobility males, there was also a strain difference, but sperm mobility scores increased as the birds aged. This age effect has not been seen in previous research. No definitive explanation for this observation can be offered at this time, but perhaps further research comparing commercial broiler breeder males to New Hampshire males will elucidate these phenotypic differences.

High correlation coefficients for  $BCF \cdot VSL$  and  $STR \cdot VSL$  seen in this study agree with a previous study using the New Hampshire breed (Froman and Feltmann, 2000). The fundamental principle of CASA is to capture a series of pictures (60 per s with the Hobson Sperm Tracker) that document the progressive movement of the sperm cell head. It is known that fowl sperm move around their progression axis in a clockwise manner when viewed from head-on (Vernon and Woolley, 1999). The faster the velocity of the sperm head, the faster its head rotates around the progression axis. This relationship yields BCF. Consequently, BCF is a function of VSL. This relationship was

confirmed with the high correlation coefficient ( $r = 0.94$ ) for BCF\*VSL. Recently, Froman (2003) demonstrated that STR is actually a function of VSL because as VSL is increasing, STR increases towards an asymptotic value. In summary, the correlation seen between BCF\*STR can be explained by the correlations between BCF\*VSL and STR\*VSL; BCF and STR are not independent variables. In contrast, MOTC and VSL are independent variables.

In this experiment with broiler breeders, MOTC did not differ among sperm mobility phenotype (Table 2.3). In contrast, MOTC differed among sperm mobility phenotypes in New Hampshire chickens (Froman et al., 1999). Comparatively, the MOTC values were higher in broiler breeders even though the mobility scores of these males tended to be lower than those within a population of New Hampshire roosters. This difference can be explained by two phenomena. First, average VSL was higher in the case of the New Hampshire flock. Second, Froman et al. (2003) demonstrated that sperm mobility phenotype is actually determined by the number of sperm with  $VSL \geq 30 \mu\text{m/s}$ . This explains that a semen sample with high MOTC can have low sperm mobility if average VSL is low.

The use of CASA affords more consistent estimates of sperm motion than manual measurements and provides useful information on the quality of sperm motility (Holt et al., 1994). In humans, CASA variables were shown to have positive correlations to both *in vitro* (Holt et al., 1985) and *in vivo* (Barratt et al., 1993; MacLeod and Irvine, 1995) fertility. However, the coefficients of variation (Table 2.4) in the present study indicate that VSL, MOTC, nor STR alone can account for the variation in sperm mobility

seen in the population. Similar variation was observed by Froman and Feltmann (2000) for VSL and sperm mobility.

In summary, sperm mobility is a variable that can be measured in commercial broiler breeders. However, because classifications of both high and low mobility males differed significantly between the two strains studied, more experimentation is warranted to gather information from additional strains. The possibility of characterizing a male as having low or high sperm mobility in an industry setting will depend upon research that establishes a sperm mobility ranking system, and would probably be used to rid flocks of subfertile males. The use of CASA to measure properties including straight line velocity of individual sperm cells is a useful tool in research situations, but may not have practical implications for direct use in the broiler breeder industry.

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**TABLE 2.1. Single classification ANOVA of sperm mobility data from males representing two strains of broiler breeders<sup>1</sup>**

Source of variation	Degrees of freedom	Sum of squares	Mean square	<i>F</i> -value
Strain	1	0.267	0.267	12.45****
Error	165	3.538	0.021	

<sup>1</sup>Replicate size was n = 98 and n = 69 for Strains A and B, respectively.  
 \*\*\*\*P = 0.0005.

**TABLE 2.2. Correlation coefficients for CASA<sup>1</sup> variables**

	BCF <sup>3</sup> (cycles/s)	STR <sup>4</sup> (%)
VSL <sup>2</sup> (μm/s)	0.9431****	0.8766****
BCF (cycles/s)	-----	0.8110****

<sup>1</sup>Computer assisted sperm analysis.

<sup>2</sup>Straight line velocity.

<sup>3</sup>Beat cross frequency.

<sup>4</sup>Straightness.

\*\*\*\*  $P \leq 0.0001$ .

**TABLE 2.3. Sperm quality parameters sorted by sperm mobility in Strain A<sup>1</sup>**

	(n)	SMS <sup>2</sup> (absorbance)	MOTC <sup>3</sup> (x 10 <sup>6</sup> /mL)	VSL <sup>4</sup> (µm/sec)	BCF <sup>5</sup> (cycles/sec)	STR <sup>6</sup> (%)
Low	9	0.198 ± 0.01 <sup>b</sup>	0.703 ± 0.08	26.3 ± 1.71 <sup>b</sup>	1.5 ± 0.21 <sup>b</sup>	59.6 ± 2.9 <sup>b</sup>
Average	53	0.273 ± 0.02 <sup>b</sup>	0.758 ± 0.04	29.5 ± 1.02 <sup>b</sup>	2.0 ± 0.14 <sup>b</sup>	62.9 ± 1.4 <sup>b</sup>
High	9	0.600 ± 0.05 <sup>a</sup>	0.896 ± 0.05	36.1 ± 2.25 <sup>a</sup>	2.8 ± 0.32 <sup>a</sup>	72.2 ± 2.5 <sup>a</sup>

<sup>1</sup>Data expressed (mean ± SEM).

<sup>2</sup>SMS = Sperm mobility score.

<sup>3</sup>MOTC = Motile concentration.

<sup>4</sup>VSL = Straight line velocity.

<sup>5</sup>BCF = Beat cross frequency.

<sup>6</sup>STR = Straightness.

<sup>a,b</sup>Means within a column differed at  $P \leq 0.05$ .

**TABLE 2.4. Sperm motion parameters and coefficients of variation in Strain A<sup>1</sup>**

Variable	CV (%)
Sperm Mobility (absorbance)	56
MOTC <sup>2</sup> ( x 10 <sup>6</sup> /mL)	38
VSL <sup>3</sup> (µm/s)	25
STR <sup>4</sup> (%)	16
BCF <sup>5</sup> (cycles/s)	52

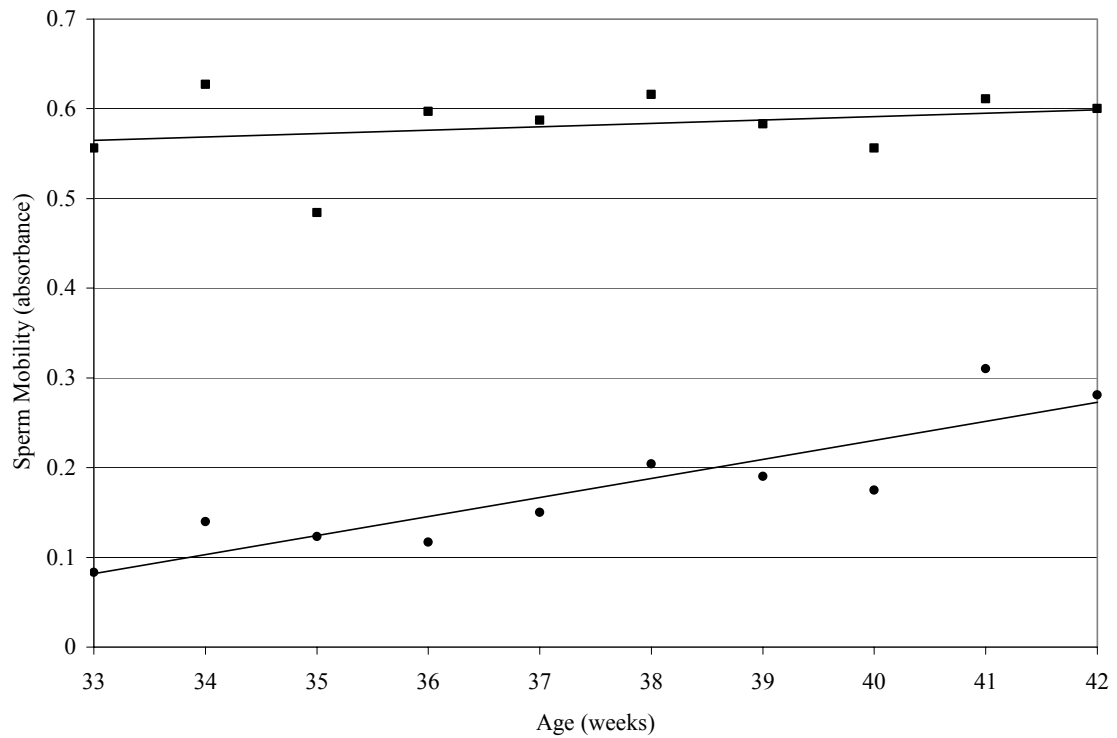
<sup>1</sup>Based on a population of 71 roosters.

<sup>2</sup> Motile concentration.

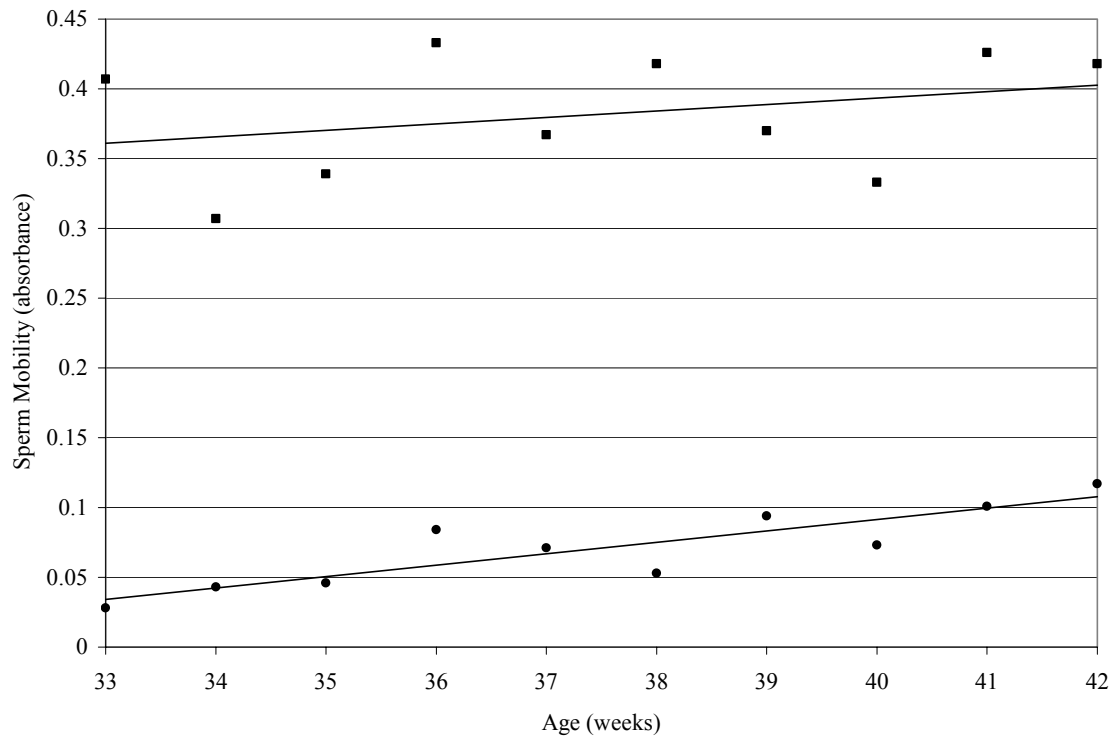
<sup>3</sup> Straight line velocity.

<sup>4</sup> Straightness of path.

<sup>5</sup> Beat cross frequency.



**FIGURE 2.1.** Weekly sperm mobility scores from low (●) and high (■) sperm mobility males in Strain A. The regression equation for low mobility males was  $y = 0.0219(x) + 0.64357$ . The regression equation for high mobility males was  $y = 0.00386(x) + 0.43686$ . Low sperm mobility scores tended to increase ( $P \leq 0.05$ ) with the increasing age of male broiler breeders while high sperm mobility scores did not change with increasing age.



**FIGURE 2.2.** Weekly sperm mobility scores from low (●) and high (■) sperm mobility males in Strain B. The regression equation for low mobility males was  $y = 0.0087(x) - 0.2559$ . The regression equation for high mobility males was  $y = 0.0073(x) + 0.1016$ . Low sperm mobility scores tended to increase ( $P \leq 0.05$ ) with the increasing age of male broiler breeders while high sperm mobility scores did not change with increasing age.

## CHAPTER 3

### COMPARISON OF REPRODUCTIVE TRAITS BETWEEN LOW AND HIGH SPERM MOBILITY BROILER BREEDER MALES<sup>1</sup>

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<sup>1</sup>Bowling, E.R., D.P. Froman, A.J. Davis and J.L. Wilson. To be submitted to *Poultry Science*.

**ABSTRACT** Individual sperm mobility scores were determined at 35 weeks of age (WOA) in a population of commercial broiler breeder males (n = 147). Males with scores below or above one standard deviation from the mean were characterized as low or high sperm mobility phenotype, respectively and were retained for the duration of the experiment. These males (n = 20 per phenotype) were evaluated for sperm mobility weekly through 45 weeks of age and every other week until 65 WOA. Body weight was measured weekly from 35 to 45 WOA and every other week from 46 to 65 WOA. Representative males (n = 10 per phenotype) were used as semen donors at 50, 54, 58, 62 and 65 WOA. Approximately 13 Single Comb White Leghorn hens were inseminated per male at each time point. Plasma testosterone concentration was determined at 53 WOA. Testes size was determined at 67 WOA. A negative correlation was found between sperm mobility score and body weight ( $r = -0.23$ ). Mean overall fertility ( $\pm$  SEM) was  $44 \pm 4.1$  and  $69 \pm 2.1$  % for low and high sperm mobility males, respectively ( $P \leq 0.0001$ ). As determined by transmission electron microscopy, the percentage of sperm with aberrant mitochondria was greater in the low sperm mobility phenotype ( $P \leq 0.0001$ ). No significant phenotypic difference was detected in plasma testosterone concentration, comb score, sperm metabolism or testis size. The results indicate that sperm mobility is a biologically significant predictor of broiler breeder semen quality. However, in view of the relationship with body weight, it may not be advisable to select commercial males based upon sperm mobility alone.

*(Key Words: broiler breeder, fertility, sperm mobility)*

## INTRODUCTION

Sperm mobility is a quantitative trait affecting male reproductive fitness in chickens (Froman et al., 1997; Froman and Feltmann, 1998; Froman et al., 1999; Froman et al., 2002). Sperm mobility can be quantified by the extent to which sperm penetrate an Accudenz<sup>®</sup> solution (Froman and McLean, 1996). Three important observations have been made in previous research regarding sperm mobility phenotype. First, sperm mobility phenotype was independent of a rooster's age (Froman et al., 1997; Froman and Feltmann, 1998). Second, sperm mobility phenotype can be explained in terms of the concentration of motile sperm and the straight line velocity of individual sperm cells (Froman and Feltmann, 2000; Froman et al., 2003). Third, in lines of New Hampshire chickens selected for average and high sperm mobility, oxygen consumption of sperm cells from average sperm mobility males was only 54% of that of sperm from high sperm mobility males, suggesting that sperm mobility is related to mitochondrial function (Froman et al., 1999).

Primary breeders of meat-type chickens select males based upon growth and feed conversion. Using conventional measurements of male reproductive potential such as semen volume, sperm concentration and sperm motility, negative genetic correlations have been observed between growth and reproduction (Siegel, 1963; Soller et al., 1965; Siegel and Dunnington, 1985). Recently, in a preliminary experiment with broiler breeders, male fertility was shown to be a function of sperm mobility phenotype (Froman et al., 1999). Thus, determining sperm mobility in broiler breeders could be a better measurement of male reproductive potential than previously used conventional measurements. Detailed experiments with broiler breeder males, however, need to be

conducted to confirm the relationship between male fertility and sperm mobility, and to determine if a relationship between sperm mobility and body weight exists. Therefore, the objectives of the present work were to: (1) identify low and high mobility phenotypes within a flock of broiler breeder males, (2) compare the fertility of these males, and (3) compare body weight, comb size, plasma testosterone concentration, sperm metabolism, testes weight, and sperm mitochondrial ultrastructure between the high and low sperm mobility groups.

## **MATERIALS AND METHODS**

### *Experimental Birds*

Broiler breeder males were obtained from a commercial broiler breeder at day of age, and reared in floor pens according to the breeder guideline. At 20 WOA, (n = 209) males were individually caged. Cage dimensions (L x W x H) were 60.96 cm x 45.72 cm x 59.69 cm. Each cage contained a perch and nipple drinker. Birds were maintained within a temperature-controlled poultry house equipped with evaporative cooling and forced air heating. Once caged, birds were maintained on 16 h light per d. The roosters were provided standard breeder diets (Table 3.1) throughout the experimental period. Feed allotments were based on weekly weight measurements from a sample of birds 37 to 45 WOA and biweekly weights from 46 to 65 WOA. Males were manually ejaculated (Burrows and Quinn, 1937) twice a week from 25 to 35 WOA in order to acclimate the birds to the semen collection technique and to assess semen production on an individual bird basis.

### *Sperm Mobility*

Males ejaculating  $\geq 0.1$  mL of semen by 35 WOA ( $n = 147$ ) were used as semen donors. Sperm mobility was evaluated using the method of Froman and Feltmann (1998). Each male was evaluated with the sperm mobility assay on two occasions, the scores were averaged, and sperm mobility phenotypes were assigned using the population mean and standard deviation. Specifically, males with scores below or above one standard deviation from the population mean were designated as exhibiting either low or high sperm mobility phenotype, respectively. From the population assessment, a total of 40 birds ( $n = 20$  per phenotype) were retained and evaluated weekly with the sperm mobility assay from 37 to 47 WOA and every-other-week from 48 to 65 WOA. Sperm mobility data were plotted as functions of time for each phenotype.

### *Male Fertility*

Representative males ( $n = 10$  per phenotype) were used as semen donors at 50, 54, 58, 62, and 65 WOA for artificial insemination. Sperm concentration was determined according to Froman and Feltmann (1998). Each ejaculate was diluted to  $1.5 \times 10^9$  sperm/mL with Poultry Buffer<sup>®1</sup>. Approximately 13 Single Comb White Leghorn hens were inseminated per male. Each hen was intravaginally inseminated with  $75 \times 10^6$  spermatozoa in a volume of 50  $\mu$ L. Eggs from the inseminated hens were collected on a daily basis from 2 to 14 days post insemination (DPI). Eggs were stored at 18°C and 75% relative humidity for up to 6 d prior to incubation. Eggs were incubated weekly and candled at day 12 of incubation. Each egg determined to be nonviable by candling was opened to determine whether the egg was infertile or contained an early dead embryo.

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<sup>1</sup> I.M.V. International Corp., Minneapolis, MN 55430

### *Other Reproductive Traits*

Comb size was scored subjectively at 40, 43 and 53 WOA as follows: 1 = small ( $\leq 2$ mm), 2 = medium (2 to 5mm), and 3 = large ( $\geq 5$ mm) (Wilson, personal communication). Relative comb size was determined at each time point by the same observer. A radioimmunoassay was performed to determine plasma testosterone concentration in males using procedures outlined in the TKTT2 Coat-A-Count<sup>®</sup> Total Testosterone Kit<sup>2</sup>, which has been used and validated in chickens (Sexton et al., 1989). For this assay, a 3-mL volume of blood was withdrawn from the brachial vein of each rooster (n = 39) at 53 WOA with a 22-gauge needle using heparin as the anticoagulant. Vacutainer<sup>5</sup> tubes containing the blood samples were kept on ice until all samples were collected, and then centrifuged at 2000 x g for 15 minutes. Plasma was recovered after centrifugation and stored in microcentrifuge tubes at -80°C until the testosterone radioimmunoassay was conducted. A Beckman 5500<sup>®</sup> gamma counter<sup>6</sup> was used to measure <sup>125</sup>I labeled testosterone as counts per minute. Plasma samples from males were measured in triplicate. Standard concentrations, measured in duplicate, were used to construct a standard curve (Figure 3.5).

Sperm ultrastructure was evaluated by transmission electron microscopy at 65 WOA in randomly selected males (n = 3 per phenotype). Ejaculates were collected and fixed in 2% (wt/vol) paraformaldehyde, 2% (vol/vol) glutaraldehyde, 0.2% (wt/vol) picric acid in 0.1 M cacodylate-HCl buffer<sup>7</sup>. On the following day, semen donors were killed. Spermatozoa were extracted using a glass pipet from a small opening made in the

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<sup>2</sup> Diagnostic Products Corp., Los Angeles, CA 90045-5597

<sup>5</sup> Becton Dickinson and Company, Franklin Lakes, NJ 07417

<sup>6</sup> Beckman Scientific Instruments Division, Irvine, CA 92713

<sup>7</sup> Electron Microscopy Laboratory, Veterinary Medicine, The University of Georgia, Athens, GA 30602

left deferent duct and then placed into a vial of fixative. Spermatozoa were fixed for at least 24 hours, and then concentrated by centrifugation at 6630 x g for 8 minutes three separate times. Samples were rinsed in 0.1 M cacodylate-HCl buffer three times before enrobing with 3% (wt/vol) molten agar<sup>8</sup> (Dawes, 1971) until hardened. Samples were post-fixed in 1% (wt/vol) osmium tetroxide in 0.1 M cacodylate-HCl buffer for 1 hour, rinsed, and then stained enbloc with 0.5% (wt/vol) aqueous uranyl acetate<sup>9</sup> before dehydrating with a series (50 to 100%) of aqueous ethanol and clearing in propylene oxide (Dawes, 1971). Samples were infiltrated and embedded in Epon-Araldite (Mollenhauer, 1963) and polymerized at 75°C for 24 hours. An ultramicrotome<sup>10</sup> equipped with a diamond knife was used to produce ultrathin sections (60-70nm) that were post-stained with 5% (wt/vol) methanolic uranyl acetate and Reynold's lead citrate (Reynold, 1963). Sections were viewed on a 200-mesh copper hex grid<sup>11</sup> using a JEM-1210<sup>12</sup> TEM at an accelerating voltage of 120 Kv. Approximately 400 transverse sections of spermatozoal midpieces were evaluated per sample. A midpiece was classified as aberrant if it contained swollen, disorganized mitochondria (Froman and Bernier, 1987).

Sperm metabolic activity was evaluated in randomly selected males (n = 15 per phenotype) when they were 66 WOA. Ejaculates were collected for use in the tetrazolium dye reduction test (TDRT) (Chadhuri and Wishart, 1988; Wishart, 1997).

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<sup>8</sup> Difco Laboratories, Detroit, MI 48212

<sup>9</sup> Ernest F. Fullam, Inc., Schenectady, NY 12008

<sup>10</sup> Leica, Inc., Deerfield, IL 60015

<sup>11</sup> Electron Microscopy Sciences, Fort Washington, PA 19034

<sup>12</sup> JEOL USA, Inc., Peabody, MA 01960

Semen concentrations were determined according to Froman and Feltmann (1998). The TDRT was performed as outlined by Wishart (1997) in triplicate for each bird, and formazan production was expressed as  $\mu\text{mol}/200$  million sperm/15 min.

At 67 WOA, the testicular width of remaining males ( $n = 19$  low- and 15 high-sperm mobility) was determined by use of an ALOKA-SSD900V<sup>13</sup> Ultrasound machine. A 1.0 cm linear surgical probe was inserted approximately 13 cm into the cloaca while the test subject was resting on its back. Images and measurements were recorded with a MaviCap<sup>14</sup>. Each bird was then killed and the testes were removed and weighed.

### *Statistical Analyses*

The General Linear Model (GLM) procedure of SAS<sup>®</sup> was used to analyze body weight, sperm mobility, duration of fertility, mitochondrial status, and comb size data. The least square means procedure was used to detect significant ( $P \leq 0.05$ ) differences between sperm mobility phenotypes. The correlation procedure of SAS<sup>®</sup> was also used to determine the relationship between body weight and sperm mobility score. Single classification ANOVAs were used to detect phenotypic difference in plasma testosterone, sperm metabolism, and both testis size and weight. Overall fertility data was analyzed with a log-odds model according to Kirby and Froman (1990).

## **RESULTS**

The population mean and standard deviation for sperm mobility were estimated to be 0.314 and 0.134 absorbance units, respectively. Sperm mobility scores of the initial population ranged from 0.054 to 0.700 absorbance units. Mean sperm mobility scores for the males selected for sperm mobility phenotype ( $n = 20$  per phenotype) from 35 to 65

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<sup>13</sup> Aloka CO., Ltd., Tokyo, Japan

<sup>14</sup> Sony Electronics Inc., Park Ridge, NJ 07656

WOA are shown in Figure 3.1. The regression lines (Fig. 3.1) for both phenotypes were distinct over the course of the experiment, even though an age effect was detected ( $P \leq 0.0001$ ). Duration of fertility after a single insemination declined as a function of age in both phenotypes (Figures 3.2 & 3.3). Since phenotypic differences in fertility were observed consistently throughout the experimental duration (Table 3.2), fertility data for all insemination periods were compiled (Table 3.3). Sperm mobility phenotype had a highly significant effect on fertility ( $P \leq 0.0001$ ). Breakout analysis of nonviable eggs indicated the occurrence of early dead embryos was significantly higher ( $P \leq 0.05$ ) in the low sperm mobility group at 65 WOA (data not shown).

The low mobility males were found to be significantly heavier ( $P \leq 0.0001$ ) than the high mobility males (Figure 3.4), and a negative correlation ( $r = -0.23$ ;  $P \leq 0.0001$ ) was found between sperm mobility score and body weight. Structural status of mitochondria was determined by the TEM in transverse sections of the spermatozoal midpiece. Figure 3.6 depicts two normal sperm midpiece sections, each containing 5 to 7 rectangular, compact mitochondria. Aberrant sperm midpiece sections were classified with swollen or disorganized mitochondria (Figures 3.7 & 3.8). No significant difference was found between the percentage of aberrant mitochondria before and after ejaculation in the high sperm mobility phenotype (Table 3.4). In contrast, the percentage of aberrant mitochondria was greater ( $P \leq 0.0005$ ) after ejaculation in the low sperm mobility males (Table 3.4). In contrast to body weight and mitochondrial ultrastructure, differences were not observed ( $P \geq 0.05$ ) for plasma testosterone concentration (Table 3.5), comb score (data not shown), sperm metabolism (Table 3.6), testis size (data not shown), and testis weight (Table 3.7) between the low and high sperm mobility phenotypes.

## DISCUSSION

Distinct sperm mobility phenotypes were identified within the commercially managed broiler breeder stock tested, which was consistent with previous observations of broiler breeders (Froman et al., 1999; Froman et al., 2003). However, both the low and high sperm mobility phenotypes decreased significantly ( $P \leq 0.05$ ) as the males aged. This change in sperm mobility with age had not been seen in previous research with New Hampshire roosters (Froman et al., 1997; Froman and Feltmann, 1998) or turkey toms (Holsberger et al., 1998), and the decrease in sperm mobility in commercial broiler breeders suggests that the environment or possibly male management may play a strong role in the phenotypic expression of sperm mobility in these males.

The present research indicated that duration of fertility, which is a good test for a male's future fertilizing capabilities (Gowe and Hutt, 1949; Williams and McGibbon, 1956), was longer in the high sperm mobility males. High sperm mobility males also had a higher percentage of overall fertility. Therefore, sperm mobility could be used as an indicator of fertility in commercial broiler breeders, which agrees with the finding of Froman et al. (1999). Although in the present research the fertility trial was not carried out to hatch, the higher percentage of early dead embryos in the low sperm mobility males at 65 WOA suggests that these males would have produced lower hatchability rates. Previous research with New Hampshire chickens indicated that hatchability was significantly higher in high sperm mobility males, explicable in terms of fertility rather than embryonic losses (Froman et al., 1997).

Genetic selection for rapid growth in commercial broiler breeders has negatively affected male reproductive characteristics and ultimately, flock fertility. In the current

research, there was a negative correlation ( $r = -0.23$ ) detected between body weight and sperm mobility, which is similar to the negative genetic correlation found between body weight and sperm motility (Soller et al., 1965; Siegel, 1963). Negative correlations between body weight and both mating activity (Rappaport and Soller, 1966) and fertility (Wilson et al., 1979) have been reported in chickens. Previous researchers reported that socially dominant males sired more offspring (Guhl and Warren, 1946), but more recent work indicates that dominant males have lower sperm mobility, but probably still sire more offspring (Froman et al., 2002). While Froman et al. (2002) did not measure the size of dominant males, Collias (1943) found that males are generally dominant to females and that genetic stocks differ in dominance ability. This author suggested that older, larger, and stronger individuals have greater success attaining dominance.

Transmission electron microscopy was used to assess sperm mitochondrial ultrastructure. Significantly more aberrant mitochondria were detected in motile spermatozoa of low sperm mobility males (Table 3.4), which supports previous research that indicated mitochondrial function is fundamental to the expression of sperm mobility phenotype (Froman and Feltmann, 1998). In addition, mitochondrial integrity of spermatozoa from the ductus deferens was assessed because sperm within the ductus deferens are essentially immotile (Ashizawa and Sano, 1990). Once again, low sperm mobility males were found to have a higher percentage of aberrant mitochondria, which indicates the defect in mitochondrial status may occur during spermatogenesis. Based upon ultrastructural evidence, the mitochondrion is a key organelle affecting sperm mobility phenotype in commercial broiler breeders as well as lines of New Hampshires selected for low and high sperm mobility.

Sperm mobility phenotypes did not differ with respect to comb score, sperm metabolism, plasma testosterone level, or testis size. Burrows and Titus (1939) also reported no significant correlation between comb size and semen production. However, more recently, McGary et al. (2002) reported that comb surface area was related to fertility in a particular male-selected primary breeder strain. In contrast to the present results, previous research has shown sperm metabolic activity to predict fertilizing ability (Chaudhuri and Wishart, 1988; Kirby and Froman, 1991). In a later report, Chaudhuri et al. (1988) suggested that the TDRT was not as highly correlated with fertility as other variables, which, along with present research, suggests that the TDRT as described by Wishart (1997) may not afford the most accurate estimate of sperm metabolism.

Plasma testosterone concentration did not differ between the sperm mobility phenotypes. In roosters, testosterone levels have been shown to peak at 30 weeks of age, and then decrease by 40 to 60 weeks of age (Sexton et al., 1989; Hocking and Bernard, 2000). Because the roosters in this experiment were older (53 WOA) and testosterone levels decrease with age, it is possible that a small distinction in testosterone concentration between low and high sperm mobility males could have been detected in younger males or if a greater sample size had been utilized. Thus, testosterone may correlate with the number of sperm cells produced (Penfold et al., 2000), but not sperm quality.

Left testis width was not found to be an indicator of sperm mobility in the present research. This finding agrees with previous research utilizing ultrasound in which testicular width had no bearing on sperm mobility phenotype (Bowling et al., 2002).

Additionally, the total testicular weight in the present research did not differ between phenotype, which confirms results seen in New Hampshire chickens (Froman and Feltmann, 1998). Neither testis width or total testes weight was a good measure of semen quality.

It will be difficult to improve fertility in broiler breeder flocks as long as genetic selection is based on breast meat yield (Siegel, 1962). However, it has been shown that sperm quality measurements (such as the sperm binding assay) could be used to cull the bottom 25% of roosters with very little impact on progeny performance (Barbato, 1999). Therefore, prediction of the fertilizing potential of commercial broiler breeder males could have a vast economic impact. Since fertility is a function of sperm mobility phenotype in broiler breeders, the possibility of using the sperm mobility assay as a selection tool in the commercial industry is valid, yet warrants future progeny experimentation given the negative correlation detected between sperm mobility and body weight.

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**TABLE 3.1. Ingredient composition and calculated nutrient composition of diets fed to broiler breeder roosters**

Ingredients	Breeder I <sup>1</sup>	Breeder II <sup>2</sup>
	g/100g diet	
	68.15	68.43
Soybean Meal (48% CP)	20.71	18.68
Poultry Fat	1.00	1.67
Limestone	7.41	8.37
Dicalcium Phosphate	1.46	1.50
Salt	0.54	0.54
Methionine	0.14	0.11
Trace Mineral Premix <sup>3</sup>	0.08	0.08
Vitamin Premix <sup>4</sup>	0.50	0.50
Copper Sulfate	0.01	0.01
Total	100.00	100.00
<u>Calculated Composition<sup>5</sup></u>		
Crude Protein (%)	15.90	15.00
Metabolizable energy (kcal/kg)	2900.00	2900.00
Calcium (%)	3.22	3.60
Available Phosphorus (%)	0.38	0.36
Lysine (%)	0.83	0.82
Methionine (%)	0.40	0.33
TSAA (%)	0.66	0.66
Sodium (%)	0.21	0.20

<sup>1</sup>Diet was provided from 23 to 47 weeks of age to all males.

<sup>2</sup>Diet was provided from 48 to 67 weeks of age to all males.

<sup>3</sup>Trace mineral premix provided the following (mg/kg) in diet: selenium, 0.3; manganese 121; iron, 75; iodine, 0.8.

<sup>4</sup>Vitamin premix provided the following per kg of diet: vitamin A, 11,000 IU; vitamin D<sub>3</sub>, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine, 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid, 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

<sup>5</sup>Values meet or exceed NRC recommendations for broiler breeder males.

**TABLE 3.2. Percent fertility in male broiler breeders separated by sperm mobility phenotype**

Phenotype	Weeks Post Insemination by Age (weeks)									
	50		54		58		62		65	
	1	2	1	2	1	2	1	2	1	2
Low	80.8	44.2	63.5	34.5	69.7	31.3	56.6	28.1	38.4	11.1
High	92.9	67.8	87.1	56.7	85.1	56.7	88.7	61.3	73.6	33.6
SEM <sup>2</sup>	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Significance	**	****	****	****	****	****	****	****	****	****

<sup>1</sup>Week Post-Insemination.

<sup>2</sup>Pooled standard error mean.

\*\*Mean fertility within a column differed significantly ( $P \leq 0.01$ ).

\*\*\*\*Mean fertility within a column differed significantly ( $P \leq 0.0001$ ).

**TABLE 3.3. Fertility<sup>1</sup> of low and high sperm mobility phenotypes**

Phenotype	Sires (n)	Hens per sire <sup>2</sup> (n)	Total eggs per phenotype (n)	Fertility <sup>3</sup> (%)
Low	10	14	5974	43.6 ± 4.076 <sup>B</sup>
High	10	11	5015	68.8 ± 2.144 <sup>A</sup>

<sup>1</sup>Eggs collected 2-14 DPI for 5 insemination periods ranging 50 to 65 weeks of age.

<sup>2</sup> Average number of hens inseminated per sire. Range was 4-15.

<sup>3</sup>Each value is expressed as mean ± SEM.

<sup>A,B</sup>Values differed significantly ( $P \leq 0.0001$ ).

**TABLE 3.4. Aberrant<sup>1</sup> spermatozoal midpiece sections found in the ejaculate and deferent duct samples of low and high sperm mobility roosters**

Phenotype	Sample	Cell sections <sup>2</sup> (n)	Aberrant <sup>3</sup> (%)
Low	Ejaculate	1176	21.8 ± 0.0043 <sup>a</sup>
	Deferent duct	1162	16.4 ± 0.0119 <sup>b</sup>
High	Ejaculate	1217	5.0 ± 0.0026 <sup>c</sup>
	Deferent duct	1219	4.3 ± 0.0028 <sup>c</sup>

<sup>1</sup>Midpiece classified as aberrant when at least one mitochondrion was swollen or disorganized.

<sup>2</sup>Total spermatozoal midpiece sections viewed with transmission electron microscope.

<sup>3</sup>Values within the column (mean ± SEM) differed significantly  $P \leq 0.0001$ .

**Table 3.5. Plasma testosterone concentrations for low and high sperm mobility broiler breeder males**

Sperm Mobility	n	Testosterone (ng/dL) <sup>1,2</sup>
Low	19	142.29 ± 12.605
High	20	132.29 ± 10.358

<sup>1</sup>Each value is expressed as mean ± SEM.

<sup>2</sup>Means within column did not differ significantly ( $P \geq 0.05$ ).

**TABLE 3.6. Metabolic activity<sup>1</sup> of sperm cells from broiler breeders categorized by sperm mobility**

Sperm Mobility	(n)	Formazan Production <sup>2,3</sup> ( $\mu\text{mol}/200$ million sperm/15 minutes)
Low	15	80.545 $\pm$ 4.205
High	15	64.755 $\pm$ 3.375

<sup>1</sup>Measured as the reduction of p-iodonitrotetrazolium violet to formazan.

<sup>2</sup>Each value is expressed mean  $\pm$  SEM.

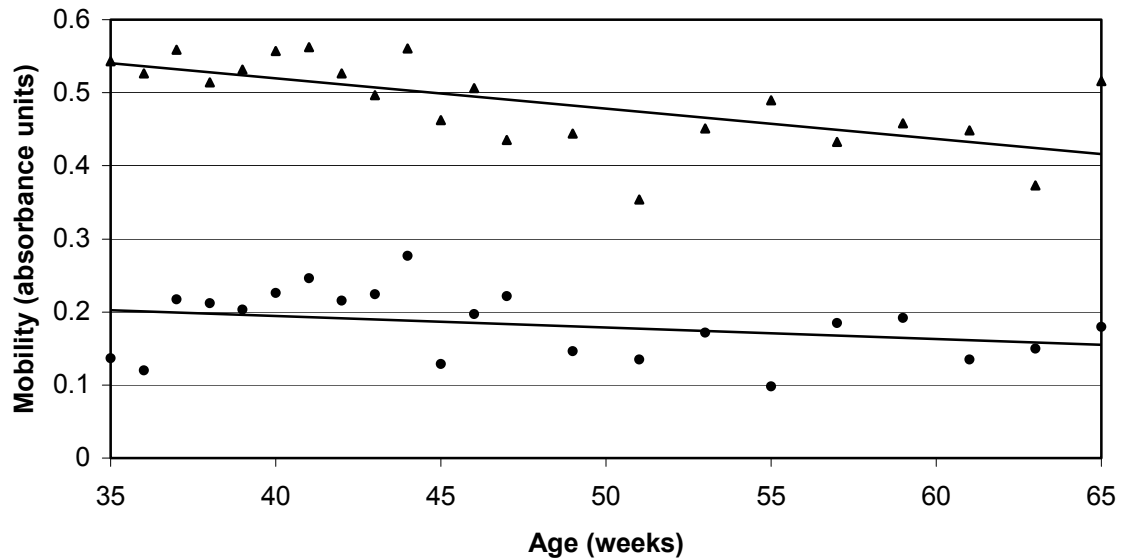
<sup>3</sup>Means within column did not differ significantly ( $P \geq 0.05$ ).

**TABLE 3.7. Combined testes weights of broiler breeder males categorized with low and high sperm mobility**

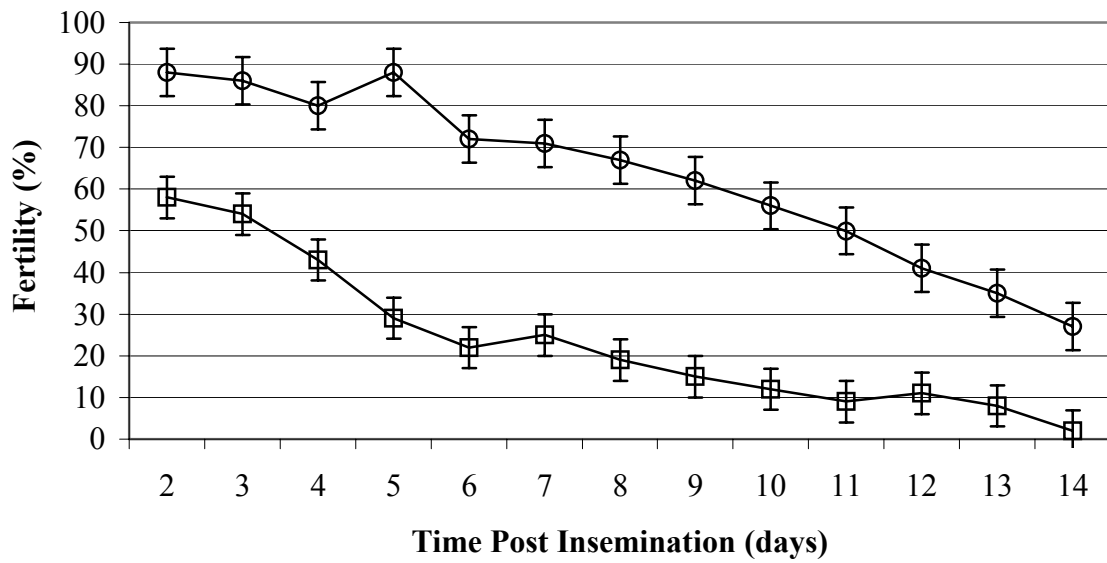
Sperm Mobility	(n)	Testes Weight <sup>1,2</sup> (g)
Low	19	18.25 ± 2.96
High	15	18.27 ± 1.70

<sup>1</sup>Values expressed as (mean ± SEM).

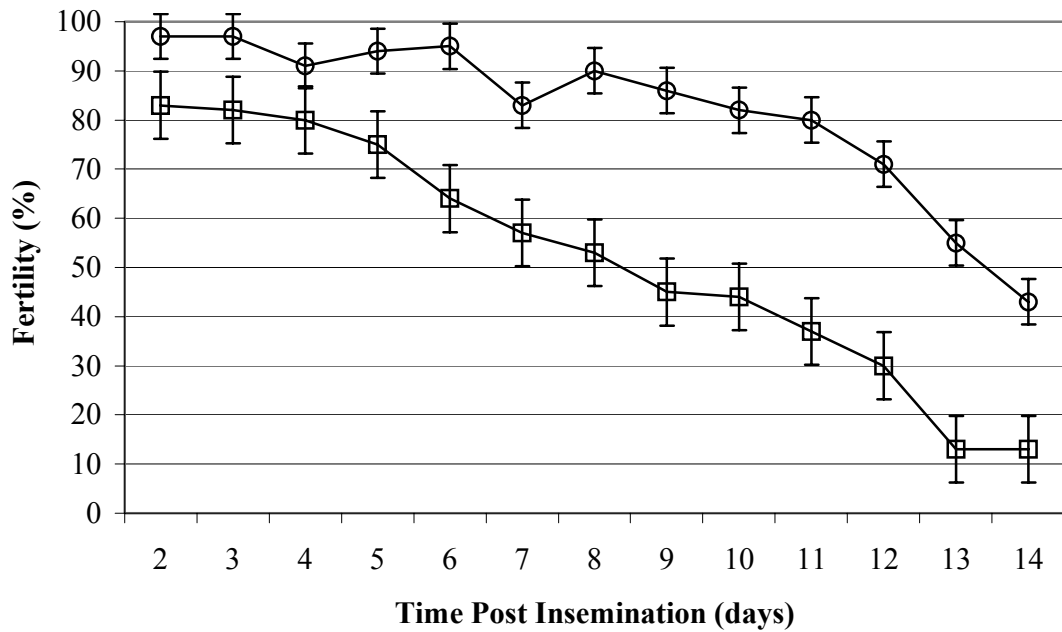
<sup>2</sup>Means within column did not differ significantly ( $P \geq 0.05$ ).



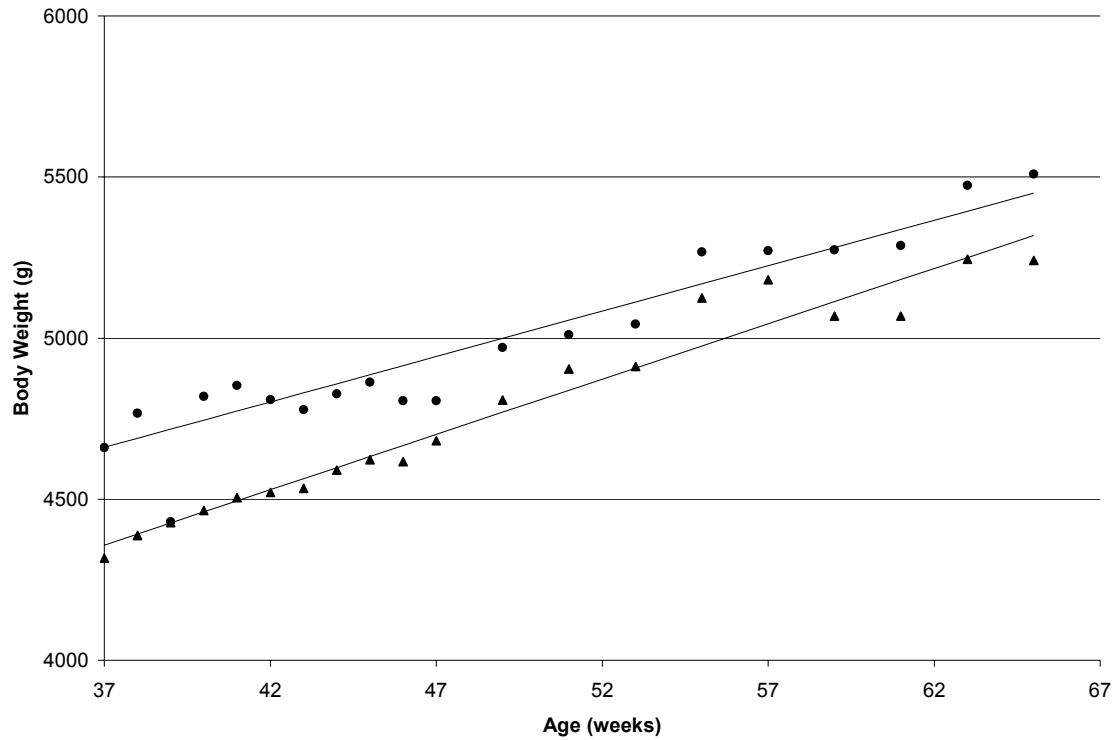
**FIGURE 3.1.** Mean sperm mobility scores and regression lines for low (●) and high (▲) sperm mobility males over the 35-65 week production period. The regression equation for low sperm mobility phenotype was  $y = -0.0015(x) + 0.25283$  and for high sperm mobility phenotype was  $y = -0.00429(x) + 0.69221$ . Neither phenotype was independent of bird age ( $P \leq 0.05$ ).



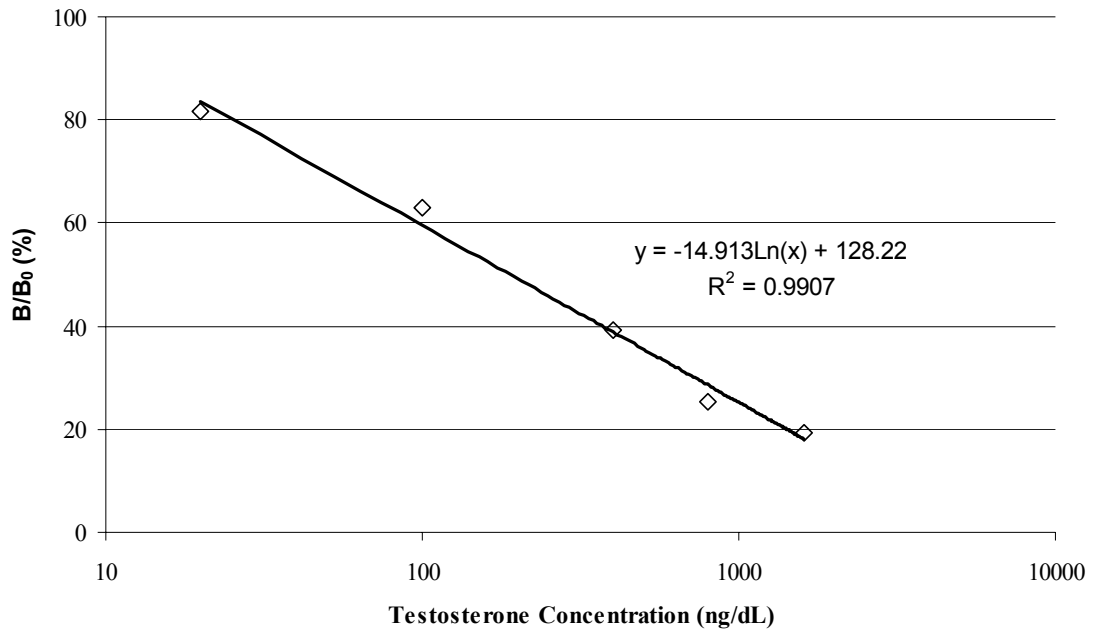
**FIGURE 3.2.** Duration of fertility for low sperm mobility males at 50(○) and 65 (□) weeks of age. SEM bars expressed at each day post insemination for both insemination periods. As evidenced, duration of fertility in the low sperm mobility males decreased with age ( $P \leq 0.05$ ). At 2 days post insemination, a 30% decrease in fertility is detected between 50 and 65 weeks of age.



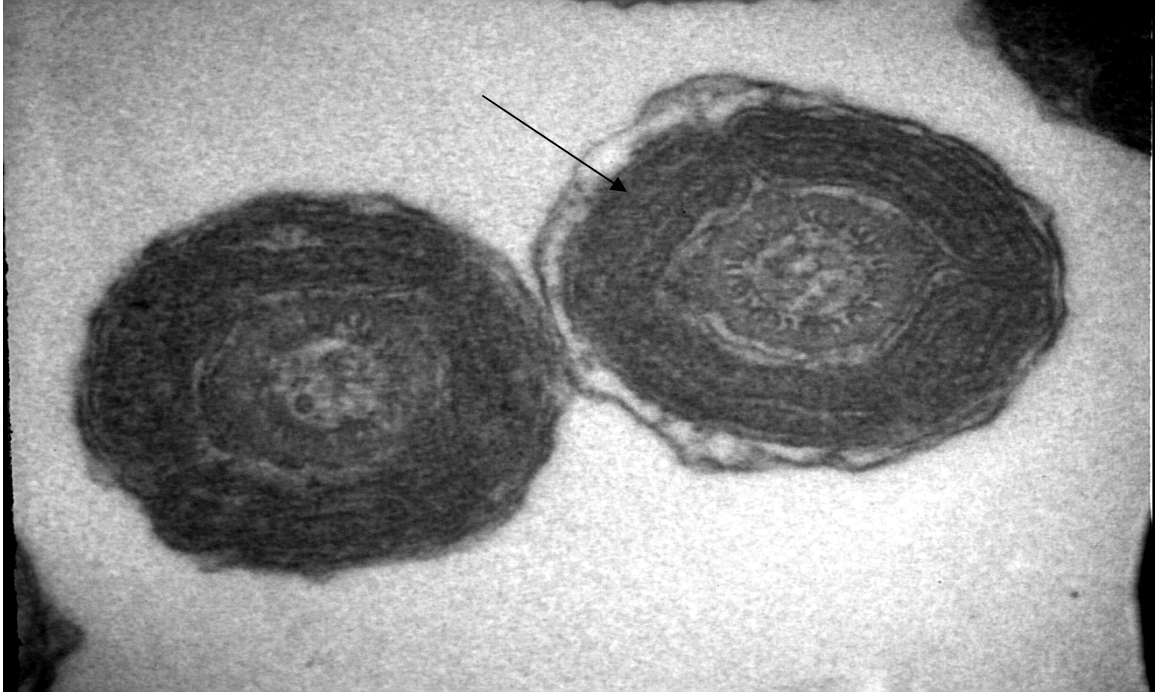
**Figure 3.3.** Duration of fertility for high sperm mobility males at 50(○) and 65 (□) weeks of age. SEM bars expressed at each day post insemination for both insemination periods. The fertility of high sperm mobility males decreased as the birds aged from 50 to 65 weeks ( $P \leq 0.05$ ).



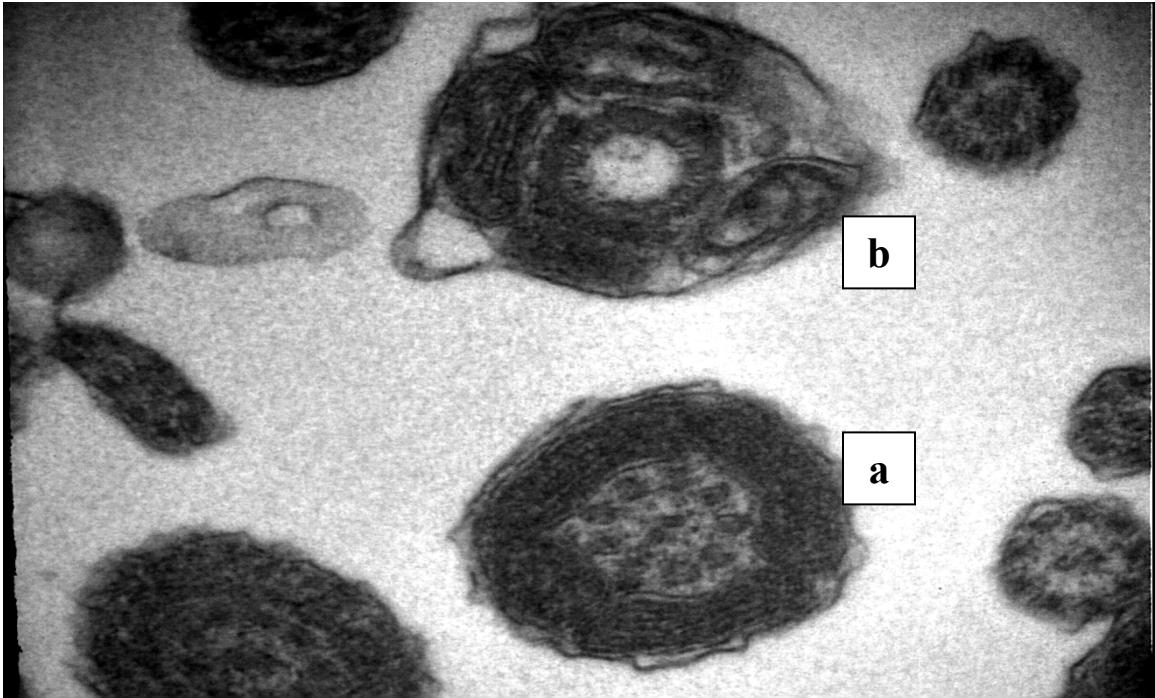
**FIGURE 3.4.** Mean body weights and regression lines for males in the low (●) and high (▲) sperm mobility phenotypes 37-65 weeks of age. Regression equation for low sperm mobility males is  $y = 28.18(x) + 3618.0617$  and for high sperm mobility males is  $y = 34.349(x) + 3086.468$ . Over the production period, low sperm mobility phenotype exhibited significantly ( $P \leq 0.0001$ ) higher body weights.



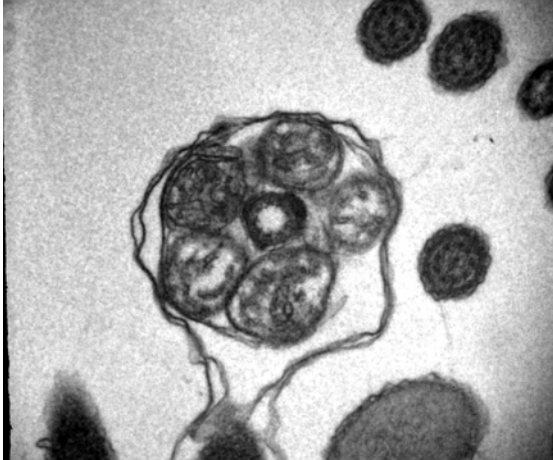
**FIGURE 3.5.** Standard curve for testosterone concentration. Radioimmunoassay was performed using <sup>125</sup>I-labeled testosterone.



**FIGURE 3.6.** Cross-sections of spermatozoal midpieces viewed by transmission electron microscopy. These sperm cells were ejaculated from a male with high sperm mobility. The ultrastructure is representative of 95% of sperm ejaculated from such males. The arrow indicates a mitochondrion containing well-organized cristae. Such mitochondria were tightly adherent to the axoneme. Magnification = 60K.



**FIGURE 3.7.** Cross-sections of normal (a) and aberrant (b) spermatozoal midpieces viewed with a transmission electron microscope. These sperm cells were ejaculated from a male with low sperm mobility. The ultrastructure represented in **a** contains normal mitochondria with well-organized cristae. The ultrastructure represented in **b** is classified as aberrant and characterized by swollen mitochondria. In low sperm mobility males, 22% of ejaculated cells are aberrant. Magnification = 50K.



**FIGURE 3.8.** Aberrant spermatozoal midpiece section collected from the deferent duct of a male characterized with low sperm mobility. Ultrastructurally, mitochondria within the midpiece are swollen, as evidenced by transmission electron microscopy. Low sperm mobility males were characterized with 16.5% aberrant cells in their deferent duct.

Magnification = 80K.

## CONCLUSIONS

These experiments evaluated sperm mobility in flocks of commercial broiler breeders. In both experiments, sperm mobility phenotypes were not independent of rooster age. However, the low and high sperm mobility males remained distinct. Evaluating two strains of males, it was determined that phenotypic distinctions in sperm mobility phenotype were strain dependent. Computer assisted sperm analysis (CASA) indicated that the phenotypic variation within a population of males was largely due to differences in average straight line velocity.

A separate flock of commercial males was then used to determine differences in fertility between low and high sperm mobility males. The fertility trial took place when males were 50-65 weeks of age. Overall fertility was higher in the high sperm mobility phenotype, and an age related fertility decline was observed for both phenotypes. Males in the low sperm mobility phenotype weighed significantly more and contained a higher percentage of aberrant mitochondria in their sperm cells when compared to high sperm mobility roosters.

Sperm mobility is a trait that can be measured in commercial broiler breeders, although strain differences must be taken into account when evaluating males. Because there is a natural decline in fertility as broiler breeder flocks age, the elimination of males

with low sperm mobility could increase the production of fertile eggs. However, the negative correlation seen between body weight and sperm mobility phenotype indicates that selection for sperm mobility alone could be detrimental to the meat production of the commercial broiler.

APPENDIX A

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ANALYSIS OF SPERM MOTION

**Sperm Mobility Phenotype Not Determined by Sperm Quality Index**<sup>1</sup>

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**Abbreviation Key:** SQI = sperm quality index; VSL = straight line velocity.

**ABSTRACT** We tested the AviMate™ Sperm Quality Analyzer's capacity to predict sperm mobility phenotype. A preliminary experiment was performed to determine a sperm concentration that afforded maximal SQI values with sperm from high sperm mobility males. In order to facilitate comparison of sperm motility indexes, semen was diluted to a constant concentration of  $1.25 \times 10^6$  sperm/mL rather than a constant ratio of 1:75 (vol/vol) as recommended for the Sperm Quality Analyzer. Thereafter, sperm mobility, motile concentration, and SQI were measured from a single ejaculate collected from each of 70 broiler breeders. Coefficients of variation were 60, 37, and 30%, respectively. Neither sperm mobility ( $r = 0.01$ ) nor motile concentration ( $r = 0.14$ ) were highly correlated with SQI. Neither motile concentration nor straight line velocity differed ( $P \geq 0.05$ ) between roosters whose SQI values were below or above one standard deviation from the population mean ( $n = 10$  per group). In contrast, both motile concentration ( $P \leq 0.01$ ) and straight line velocity ( $P \leq 0.05$ ) differed between roosters whose sperm mobility values were below or above a standard deviation from the population mean ( $n = 10$  per group). Sperm mobility was a function of the size of a highly motile subpopulation of sperm. A replicate experiment was performed with a flock of New Hampshire roosters ( $n = 126$ ). Neither the properties of motile sperm nor fertility differed between males characterized by extreme SQI scores. We concluded that variation in SQI scores was enigmatic because sperm concentration was controlled in our experiments, sperm viability was invariant, and motile concentration did not differ between roosters characterized by extreme SQI scores. In summary, the AviMate™ Sperm Quality Analyzer did not predict sperm mobility phenotype.

*(Key Words: fertility, male fitness, sperm, sperm motility)*

## INTRODUCTION

Sperm mobility is a new quantitative trait in poultry (Froman and Feltmann, 1998; Holsberger *et al.*, 1998; Froman *et al.*, 2002). Phenotype is determined by the ability of motile sperm to auto-segregate from immotile sperm during a 5-min incubation at body temperature (Froman and McLean, 1996), hence the term *sperm mobility*. The size of the mobile subpopulation is quantified with a spectrophotometer. Extreme variation in sperm mobility has been observed among males within populations characterized by normal sperm morphology, concentration, and viability (Froman and Feltmann, 1998; Holsberger *et al.*, 1998; Froman *et al.*, 1999; Froman and Feltmann, 2000). Sperm mobility is a primary determinant of male fitness as evidenced by non-competitive and competitive fertilization (Froman and McLean, 1996; Froman *et al.*, 1997; Froman and Feltmann, 1998; Donoghue *et al.*, 1998; Froman *et al.*, 1999; Birkhead *et al.*, 1999; Donoghue *et al.*, 1999; King *et al.*, 2000). An alternative approach for evaluating the reproductive potential of male breeders is to measure sperm quality index (SQI; McDaniel *et al.*, 1998; Parker *et al.*, 2000; Parker *et al.*, 2002; Neuman *et al.*, 2002). Whereas both the measurement of sperm mobility and SQI depend upon the passage of light through a sperm suspension, SQI is measured immediately after dilution of a semen sample and requires only 20 s.

In previous work, computer-assisted motion analysis demonstrated that phenotypic differences in sperm mobility were attributable to variation in motile concentration and straight line velocity (VSL; Froman and Feltmann, 2000). However, the means by which these two variables interacted was not readily evident. Motile concentration appeared to be the more important variable as evidenced by a product-

moment correlation coefficient of  $r = 0.71$  when sperm mobility was correlated with motile concentration ( $n = 100$  roosters). Johnston et al (1995) used computer-assisted sperm motion analysis in conjunction with a Sperm Quality Analyzer to evaluate human sperm. These researchers reported that 69% of the variance in their data could be accounted for by motile concentration. Comparable data for fowl sperm, i.e. data based upon objective measurement of sperm motility, were not available. Therefore, we proposed the following hypothesis: if roosters categorized by SQI differed with respect to motile concentration, then measuring SQI might afford a rapid means of identifying sperm mobility phenotype.

## MATERIALS AND METHODS

A preliminary experiment was performed using semen donors ( $n = 10$ ) selected from a line of high sperm mobility males. Sperm concentration in each ejaculate was determined with a spectrophotometer according to Froman and Feltmann (1998). In each case, 10- $\mu$ L subsamples were diluted with 50 mM *N*-tris [hydroxymethyl] methyl-2-amino-ethanesulfonic acid (TES), pH 7.4, containing 128 mM NaCl and 2 mM CaCl<sub>2</sub> (TES-buffered saline)<sup>15</sup> to provide sperm suspensions containing 25, 50, 75, 100, 125, 250, and 500 x 10<sup>6</sup> sperm/mL. An AviMate™ Sperm Quality Analyzer<sup>16</sup> was used to procure an SQI score immediately after each concentration was prepared. Mean SQI scores were plotted as a function of sperm concentration. In order to facilitate comparison of sperm motility indexes, semen was diluted to a constant concentration of 1.25 x 10<sup>6</sup> sperm/mL rather than a constant ratio of 1:75 (vol/vol) as recommended for the Sperm Quality Analyzer.

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<sup>15</sup> Sigma Chemical Co., St. Louis, MO 63178

<sup>16</sup> Alpharma, Animal Health Division, Fort Lee, NJ 07024

A second preliminary experiment was performed with caged 35-wk-old broiler breeders characterized by high sperm mobility ( $n = 3$ ; average mobility score = 0.659, CV = 1.3%). A 50- $\mu$ L sample from each ejaculate was diluted to  $5 \times 10^8$  sperm/mL with TES-buffered saline at room temperature. Computer-assisted sperm motion analysis was performed with a Hobson SpermTracker<sup>17</sup> according to Froman and Feltmann (2000) as follows. Five replicate measurements of motile concentration were made at 1-min intervals at 25 and 41 C for each male. Motile concentration was analyzed with a randomized complete block design (Sokal and Rohlf, 1969a). Frequency distributions for straight line velocity (VSL) were generated for each temperature using pooled data, i.e. the VSL for each sperm track was assigned to one of 12 categories ranging from 0 to 120  $\mu$ m/s in increments of 10  $\mu$ m/s.

Thereafter, a base population of caged 43-wk-old broiler breeders ( $n = 70$ ) was used for a series of related analyses. Each male was ejaculated and sperm concentration determined as above. A 50- $\mu$ L volume of semen from each rooster was diluted to  $5 \times 10^8$  sperm/mL with TES-buffered saline. A 60- $\mu$ L subsample of this suspension was used to perform the sperm mobility assay as described by Froman and Feltmann (1998). A second 60- $\mu$ L subsample was diluted with 180  $\mu$ L of TES-buffered saline and used to determine SQI. A third 3- $\mu$ L subsample was diluted for computer-assisted sperm motion analysis (Froman and Feltmann, 2000). Each set of measurements per male was made within < 10 min of ejaculation. Sperm mobility and motile concentration were correlated against SQI scores (Sokal and Rohlf, 1969b). Sperm mobility, motile concentration, and average VSL were compared between groups of roosters whose SQI scores were below or above one standard deviation from the population mean. Data were analyzed by single

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<sup>17</sup> Hobson Vision Ltd., Baslow, Derbyshire, UK DE45 1RP

classification ANOVA (Sokal and Rohlf, 1969b) with 10 replicate males per group. A similar analysis was performed with roosters ( $n = 10$  per group) whose sperm mobility scores were below or above one standard deviation from the population mean.

Roosters representing the range of sperm mobility scores ( $n = 6$ ) were re-evaluated by the sperm mobility assay and computer-assisted sperm motion analysis. Three to four successive 5-min analyses were performed with the Hobson SpermTracker in order to accrue 500 to 1000 tracks per male. A VSL frequency distribution was generated for each male as above. Tracks with a straight line velocity  $> 30 \mu\text{m/s}$  were summed. This sum was divided by the total number of tracks within the distribution. The resulting proportion was multiplied by the motile concentration, and the rooster's sperm mobility score was plotted against this number. Data points conformed to a linear function. Therefore, the parameters of  $y(x) = \alpha + \beta(x)$  were estimated by linear regression (Sokal and Rohlf, 1969d).

A second series of experiments was performed with a base population of caged 30-wk-old New Hampshire roosters ( $n = 126$ ). Sperm mobility and SQI measurements were made with a single ejaculate from each rooster. The population mean and standard deviation were estimated for SQI scores. Roosters whose scores were below or above one standard deviation from the population mean were selected for further study ( $n = 15$  per group). A second ejaculate from each selected rooster was used to estimate sperm viability (Bilgili and Renden, 1984), motile concentration, and average VSL (Froman and Feltmann, 2000). Data were analyzed by single classification ANOVA (Sokal and Rohlf, 1969c).

Male fertility was compared in two fertility trials. In the first trial, roosters whose SQI scores were below or above one standard deviation from the population mean served as semen donors. Ejaculates were pooled by category and diluted to  $1.0 \times 10^9$  sperm/mL with TES-buffered saline. Each of 50 New Hampshire hens was inseminated with  $5 \times 10^7$  sperm. Egg collection began on the second day after artificial insemination. Eggs were collected for 7 d, incubated for 4 d, and then broken open for evaluation. Data were analyzed with a log odds model (Kirby and Froman, 1991).

In the second fertility trial, semen donors ( $n = 8$ ) were chosen to represent the range in sperm mobility phenotype. Two additional sperm mobility measurements were made per male on two consecutive days. Hens were inseminated on the following day. Each ejaculate was diluted as above and used to inseminate 15 to 20 hens with  $5 \times 10^7$  sperm per hen. Eggs were collected and treated as above. The fertility of each male was calculated as the quotient of eggs containing embryos and the total number of eggs laid per group of hens. Fertility was plotted as a function of average sperm mobility ( $n = 3$  observations per male). Data points conformed to an exponential function. Therefore, the parameters of  $y(x) = \alpha - \beta e^{-\lambda(x)}$  were estimated by iterative least squares (SAS Institute, 1996).

## RESULTS

A logistic relationship was observed when SQI was plotted as a function of sperm concentration in the first preliminary experiment (Fig. 1). In the second preliminary experiment with sperm from high sperm mobility roosters, neither a male nor temperature effect was observed with respect to motile concentration ( $P \geq 0.05$ ). Mean motile concentration was  $1.13 \pm 0.234$  and  $1.11 \pm 0.235 \times 10^6$  sperm/mL at 25 and 41 C,

respectively. The VSL frequency distributions generated with pooled data are shown in Figure A.2. Whereas 55% of tracks had velocities  $> 30 \mu\text{m/s}$  at body temperature, only 0.6% of the tracks had a velocity  $> 30 \mu\text{m/s}$  at 25 C.

Product-moment correlation coefficients were 0.01 and 0.14 when SQI was correlated against sperm mobility (Fig. 3) and motile concentration (Fig. 4), respectively. No differences were observed with respect to sperm mobility, motile concentration, or average straight line velocity when roosters were categorized by extreme SQI scores, i.e. those with a score one standard deviation below or above the mean (Table A.1). In contrast, when roosters were categorized by extreme sperm mobility scores, differences in motile concentration ( $P \geq 0.01$ ) and average straight line velocity ( $P \leq 0.05$ ) were observed (Table A.2). VSL distributions were skewed regardless of temperature (Fig. 2) or sperm mobility phenotype (Fig. 5). However, the shape of the skewed distribution varied with sperm mobility phenotype (Fig. 5). A linear relationship was observed when sperm mobility was plotted as a function of the number of highly motile sperm within an ejaculate (Fig. 6). No differences in sperm viability, motile concentration, or average VSL ( $P \geq 0.05$ ; Table A.3) were observed when New Hampshire roosters were categorized by extreme SQI scores in a replicate experiment. Likewise, fertility did not differ between groups ( $P \geq 0.05$ ; Table A.4). In contrast, fertility was a function of sperm mobility (Fig. 7).

## DISCUSSION

Our objective was to determine if sperm mobility phenotype could be rapidly assessed by SQI. There were three practical reasons for this objective. First, male reproductive potential is a commercial concern. Second, the recent discovery of sperm

mobility, a quantitative trait affecting male fitness (Froman *et al.*, 1997; Donoghue *et al.*, 1999; Birkhead *et al.*, 1999; Froman *et al.*, 1999; Froman *et al.*, 2002), affords genetic potential that warrants consideration in breeding programs. Third, while sperm mobility measurements are easy to make, the assay is moderately time-consuming and requires a modicum of equipment because it requires incubation at 41 C for 5 min. In contrast, SQI is measured in 20 s at room temperature.

Previous research with the Hobson SpermTracker demonstrated that motile concentration was a critical variable affecting sperm mobility phenotype (Froman and Feltmann, 2000). Motile concentration denotes the number of motile sperm within a volumetric analysis field. We hypothesized the AviMate™ Sperm Quality Analyzer would differentiate among roosters differing in sperm mobility phenotype because McDaniel *et al.* (1998) reported that such instrumentation detects sperm concentration and motion within a suspension of fowl sperm (McDaniel *et al.*, 1998).

According to the manufacturer's directions, semen should be diluted 1:75 before evaluation with the AviMate™ Sperm Quality Analyzer. However, SQI scores from high sperm mobility males were inexplicably low when this practice was followed in our initial attempts to use the instrument. Therefore, we conducted two preliminary experiments for the following reasons. First, the extent to which sperm motion contributes to an SQI score was not known. Second, if SQI scores were to be correlated with either sperm mobility or motile concentration, then an adequate range in SQI scores was needed. We suspected that such a range could be generated by using sperm

suspensions that were more concentrated. Third, we desired to conduct controlled experiments. Fourth, we did not know if motile concentration would be adversely affected at room temperature.

SQI scores were sensitive to changes in sperm cell concentration between 25 and  $150 \times 10^6$  sperm/mL when variation in motion was minimized by using high sperm mobility males as semen donors (Fig. 1). These semen donors were used due to the similarity of their phenotype, i.e. the CV of their sperm mobility scores was 11.3% (mean score = 0.602 absorbance units). We expected that diluting semen to a fixed concentration of  $125 \times 10^6$  sperm/mL would afford a range of several hundred SQI units when roosters representing a range in sperm mobility phenotype were studied. This goal was realized as evidenced by the range of SQI scores shown in Figure A.3. The comparison of data collected at room temperature to data collected at body temperature seemed legitimate in view of our demonstrating that a temperature of 25 C had no effect upon motile concentration ( $P \geq 0.05$ ) even though straight line velocity was affected (Fig. 2).

However, replicate experiments demonstrated that there was no relationship between SQI and motile concentration (Fig. 4; Tables A.1 and A.3). As expected, there was no relationship between SQI and average VSL (Tables A.1 and A.3). Our failure to observe a difference in fertility between roosters with extreme SQI scores (Table A.4) was unexpected in view of the report of Parker *et al.* (2002). A fertility trial was performed with males ranked by sperm mobility scores to confirm that this variable was biologically significant. Fertility was a function of sperm mobility (Fig. 7) as reported previously for broiler breeders (Froman *et al.*, 1999). To summarize, variation in SQI

scores was observed under our experimental conditions. We used a constant number of sperm in our experiments, and sperm viability was invariant. Consequently, the basis for variation in SQI scores observed among males was an enigma.

The differences in motile concentration and straight line velocity observed between sperm mobility phenotypes (Table A.2) corroborated the assertion that these variables were responsible for phenotypic expression (Froman and Feltmann, 2000). At first glance, our raw data contained contradictory information. For example, motile concentrations of  $1.17$  and  $1.02 \times 10^6$  sperm/mL were observed for roosters with mobility scores of  $0.115$  and  $0.700$  absorbance units, respectively. However, when corresponding average straight line velocities were considered, i.e.  $21$  and  $47 \mu\text{m/s}$ , it appeared that sperm mobility might be proportional to the size of a highly motile subpopulation of sperm within the motile population. The population mean was  $30 \mu\text{m/s}$  for average straight line velocity ( $n = 70$  broiler breeders). Therefore, sperm mobility was plotted as a function of the number of sperm with a straight line velocity  $> 30 \mu\text{m/s}$  (Fig. 6). We concluded that sperm mobility phenotype is attributable to the proportion of highly motile sperm within an ejaculate. Such an explanation implies that the *effective insemination dose can be highly variable among males* even if: (1) a constant number of sperm are inseminated per hen, and (2) the majority of these sperm are viable *and* motile. Our data and this explanation account for the relationship between sperm mobility and fertility shown in Figure A.7 as well as the outcome of competitive fertilization experiments in which semen donor selection was based upon sperm mobility phenotype (Birkhead *et al.*,

1999; Donoghue *et al.* 1999; King *et al.*, 2000). In conclusion, our data demonstrate that prediction of male fitness with an in vitro test warrants the use of physiological conditions and a context in which the *consequences* of variation in sperm velocity can become manifest in time.

## **ACKNOWLEDGMENTS**

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TABLE A.1. Properties of sperm<sup>1</sup> from broiler breeders categorized by the sperm quality index (SQI)

SQI category <sup>2</sup>	Roosters (n)	SQI	Sperm mobility <sup>3</sup> (absorbance)	Motile concentration (x10 <sup>6</sup> /mL)	Average straight line velocity (μm/s)
Low	10	159 ± 8.6	0.275 ± 0.0676	0.78 ± 0.128	30 ± 2.5
High	10	425 ± 13.3	0.287 ± 0.0303	0.73 ± 0.079	26 ± 1.7

<sup>1</sup>Each value is a mean ± SEM.

<sup>2</sup>Roosters with scores less than or greater than a standard deviation from the population mean (n = 70).

<sup>3</sup>Measured by sperm penetration of 6% (wt/vol) Accudenz at 41 C from an overlay of extended semen; sperm penetration induced a change in absorbance at 550 nm.

TABLE A.2. Properties of sperm<sup>1</sup> from broiler breeders categorized by sperm mobility<sup>2</sup>

Sperm mobility phenotype <sup>3</sup>	Roosters (n)	Sperm mobility (absorbance)	Motile concentration (x10 <sup>6</sup> /mL)	Average straight line velocity (µm/s)
Low	10	0.080 ± 0.0097	0.55 ± 0.101 <sup>A</sup>	26 ± 2.3 <sup>a</sup>
High	10	0.629 ± 0.0355	0.96 ± 0.069 <sup>B</sup>	34 ± 1.7 <sup>b</sup>

<sup>1</sup>Each value is a mean ± SEM.

<sup>2</sup>Measured by sperm penetration of 6% (wt/vol) Accudenz at 41 C from an overlay of extended semen; sperm penetration induced a change in absorbance at 550 nm.

<sup>3</sup>Roosters with scores less than or greater than a standard deviation from the population mean (n = 70).

<sup>A,B</sup>Means within a column differed at  $P \leq 0.01$ .

<sup>a,b</sup>Means within a column differed at  $P \leq 0.05$ .

TABLE A.3. Properties of sperm<sup>1</sup> from New Hampshire roosters categorized by the sperm quality index (SQI)

SQI category <sup>2</sup>	Roosters (n)	SQI	Sperm viability (%)	Motile concentration (x10 <sup>6</sup> /mL)	Average straight line velocity (μm/s)
Low	15	280 ± 4.4	99 ± 0.4	0.96 ± 0.085	33 ± 1.4
High	15	502 ± 2.3	100 ± 0.5	0.86 ± 0.067	37 ± 2.8

<sup>1</sup>Each value is a mean ± SEM.

<sup>2</sup>Roosters with scores less than or greater than a standard deviation from the population mean (n = 126).

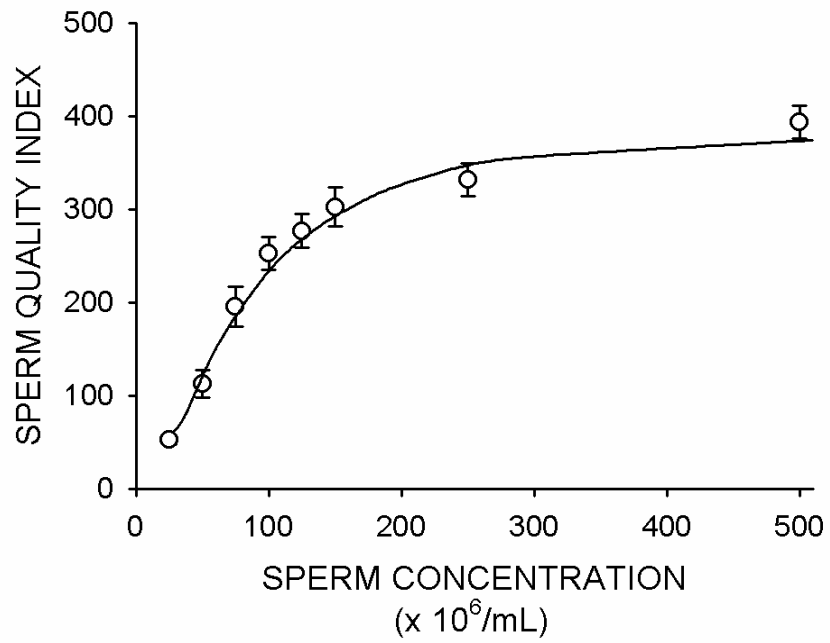
TABLE A.4. Fertility of roosters categorized by sperm quality index (SQI)

SQI category <sup>1</sup>	Hens (n)	Eggs <sup>2</sup> (n)	Fertility <sup>3</sup> (%)
Low	49	221	91 ± 2.6
High	47	208	93 ± 2.1

<sup>1</sup>Roosters with scores less than or greater than a standard deviation from the population mean (n = 126).

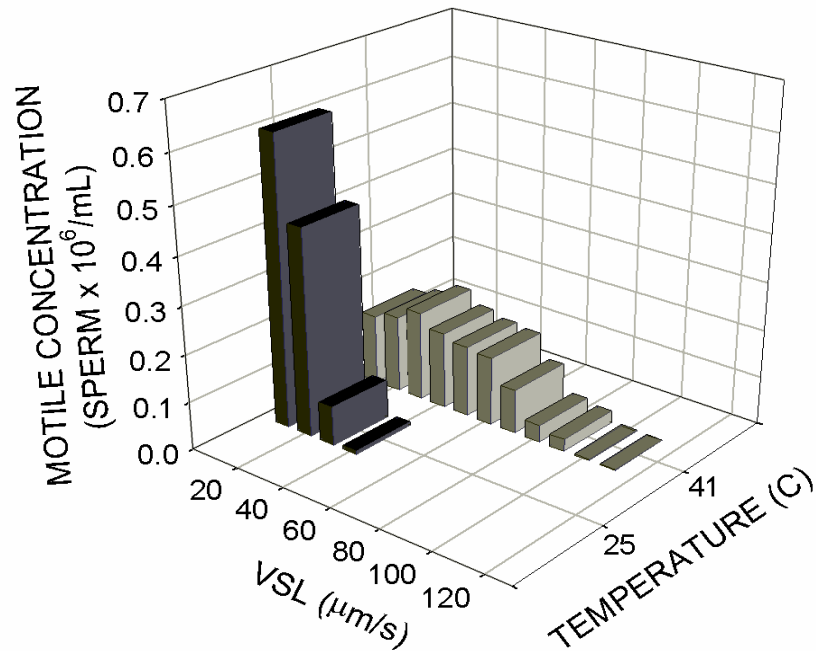
<sup>2</sup>Collected over a 7-d interval following a single insemination with pooled semen (n = 15 roosters per phenotype) and an insemination dose of  $5 \times 10^7$  sperm per hen.

<sup>3</sup>Each value is a mean ± SEM.



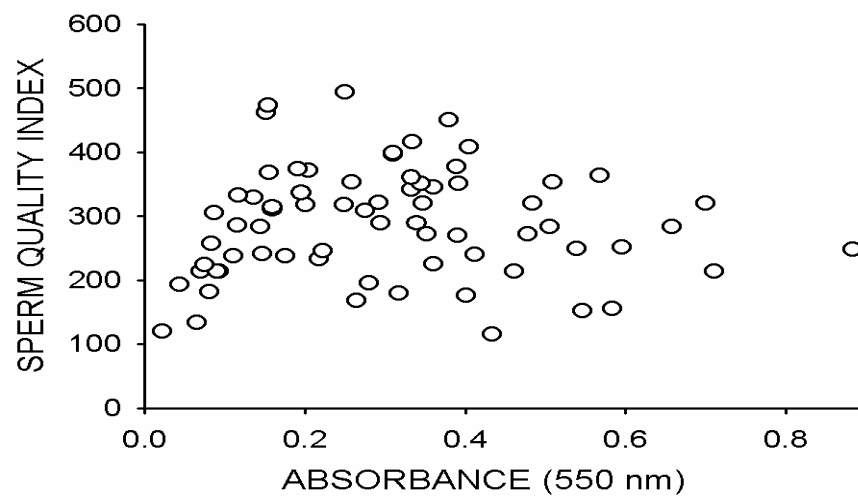
PS 2152 Figure 1

FIGURE A.1. Effect of sperm concentration on magnitude of sperm quality index scores using sperm from high sperm mobility males. Open circles represent means  $\pm$  SEMs (n = 10 roosters).



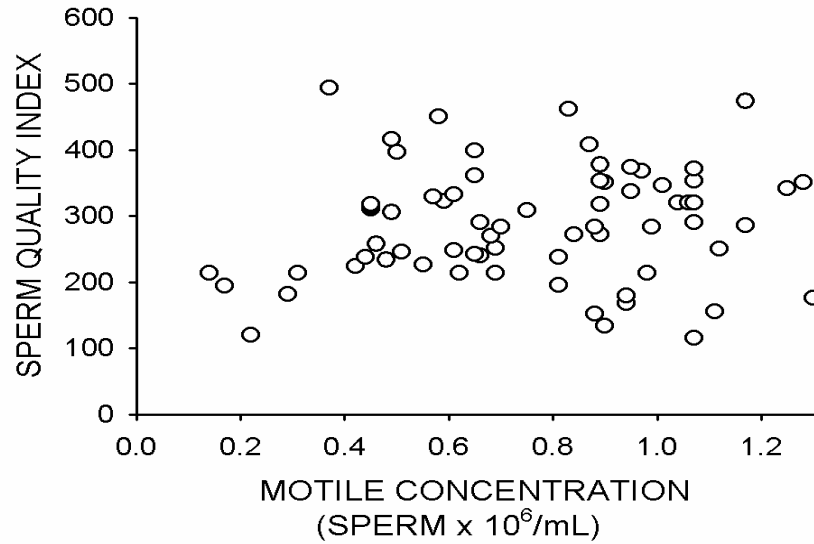
PS 2152 Figure 2

FIGURE A.2. Effect of temperature on straight line velocity (VSL) distributions. Motile concentration and straight line velocity were determined by computer-assisted sperm motion analysis. Total motile concentrations were equivalent at  $1.13$  and  $1.11 \times 10^6$  sperm/mL for  $25$  and  $41$  C, respectively.



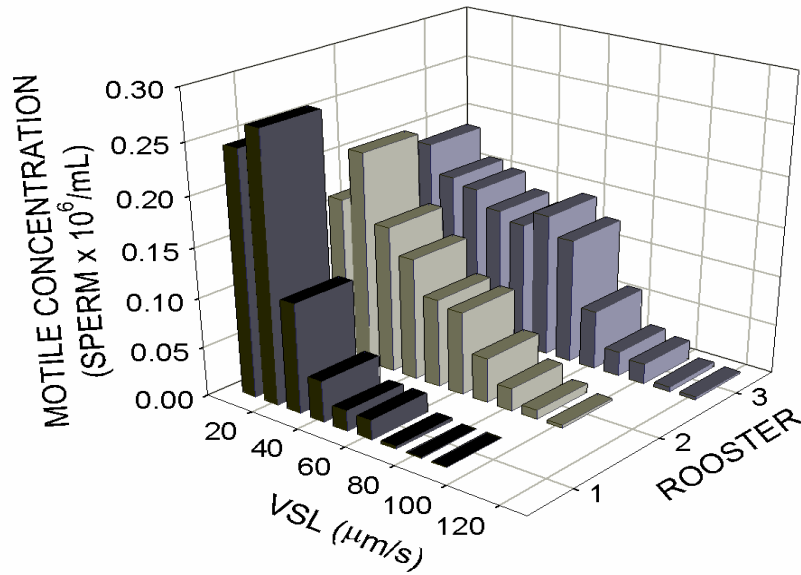
PS 2152 Figure 3

FIGURE A.3. Correlation between sperm mobility and sperm quality index (n = 70 broiler breeders). Sperm mobility was measured with a spectrophotometer. Consequently, the variable is expressed in absorbance units. Sperm quality index was measured with an AviMate™ Sperm Quality Analyzer. The product-moment correlation coefficient was 0.01.



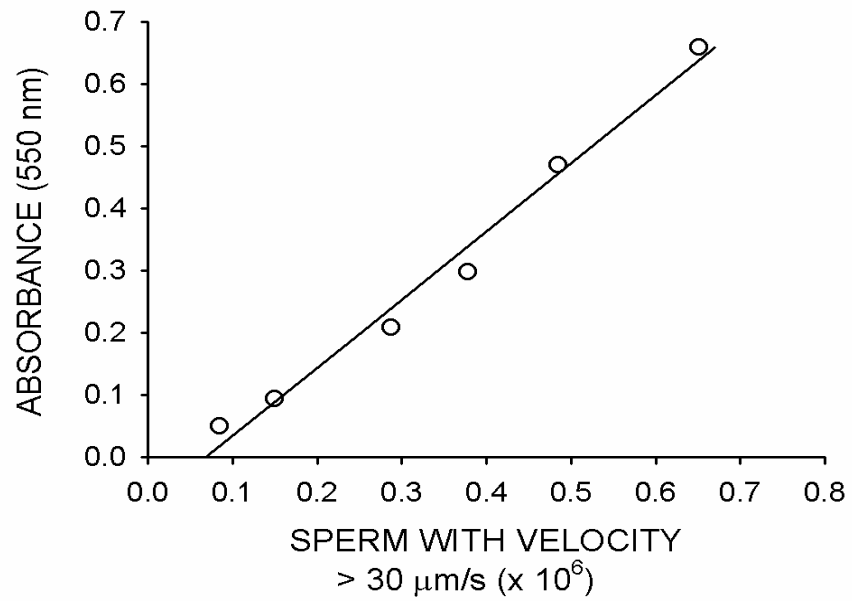
PS 2152 Figure 4

FIGURE A.4. Correlation between motile concentration and sperm quality index (n = 70 broiler breeders). Motile concentration was measured with a Hobson SpermTracker. Sperm quality index was measured with an AviMate™ Sperm Quality Analyzer. The product-moment correlation coefficient was 0.14.



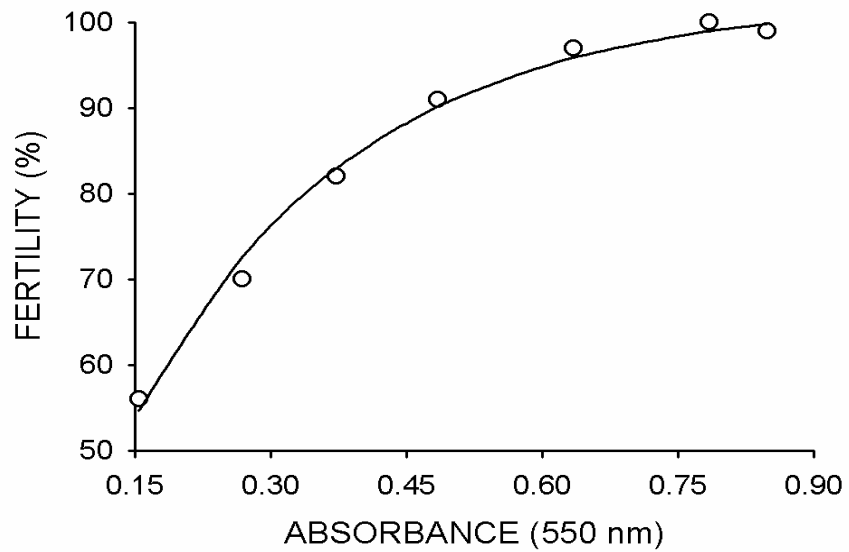
PS 2152 Figure 5

FIGURE A.5. Straight line velocity (VSL) distributions for broiler breeder roosters representing the range of sperm mobility phenotypes. Roosters 1, 2, and 3 were characterized *a priori* by low, average, and high sperm mobility, respectively. Motile concentration and VSL were measured at body temperature with a Hobson SpermTracker. VSL data were categorized in increments of 10  $\mu\text{m/s}$ .



PS 2152 Figure 6

FIGURE A.6. Sperm mobility, measured in absorbance units, as a function of the size of a subpopulation of highly motile sperm within an ejaculate. Each symbol denotes a data pair per broiler breeder rooster. The solid line represents the linear function  $y(x) = \alpha \beta(x)$ .



PS 2152 Figure 7

FIGURE A.7. Fertility as a function of sperm mobility, measured in absorbance units. Each symbol denotes a data pair per New Hampshire rooster. Fertility was determined with 70 to 80 eggs per rooster. The solid line represents the exponential function  $y(x) = \alpha - \beta e^{-\lambda(x)}$ .