# ABSTRACT

Title of Thesis:   **THE EFFECT OF SPERM MOBILITY PHENOTYPE ON FERTILITY PERSISTENCE IN LAYER AND BROILER HENS**

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Domestic fowl (*Gallus gallus domesticus*) were studied to identify accurate predictors of potential fertility in two lines of broiler breeder males along with fertility persistency in layer and broiler hens. Sixty-four Hy-Line layer and thirty-seven broiler breeder hens were AI with identical amounts of high or low mobility sperm from FG males. Morphological measurements were taken to determine relationships of these with semen volume, concentration, and mobility.  We hypothesized that 1) semen quality would decline as males aged, 2) morphology would be positively correlated with semen quality, and 3) females AI with high mobility sperm would have a longer duration of fertility.  Results revealed a significant age*line interaction for semen volume (p=0.0307), sperm concentration (p=0.0003), and sperm mobility (p=0.0405). Morphological measurements were correlated with different semen parameters in both lines.  Fertility was positively correlated with semen quality.  Sperm mobility influenced fertility in layer hens but not in broiler breeders.

**THE EFFECT OF SPERM MOBILITY PHENOTYPE ON FERTILITY PERSISTENCE IN LAYER AND BROILER HENS**


By


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# Chapter 1: Literature Review

## *1.1 Genetic Selection for Production Traits and Fertility Implications in the Domestic Fowl*

The broiler breeder, *Gallus gallus domesticus,* has undergone intensive genetic selection over the years to improve growth rate, meat yield, and feed conversion (McDaniel, 1978; Rishell, 1997). The corporations that now dominate the broiler industry have been very successful in their attempts to improve these performance traits which resulted in an increase from 366 million to 8.4 billion broilers produced per year from 1945 to 2001 with average live weight increasing from 3.03 pounds to 5.06 pounds (National Agricultural Statistics Service, 2002). Over a similar time period (1923-1996) feed conversion ratio decreased from 4.7 to 2.0 lbs. feed/lbs. gain while market age declined from 16 to 6/7 weeks (Emmerson, 2000). Modern broilers go to market at a much younger age, weigh considerably more, and use feed more efficiently. While advances in nutrition, management, and health have contributed to this improvement, most advances are a result of a very successful genetic selection (Havenstein *et al.*, 1994a; Havenstein *et al.*, 1994b; Havenstein *et al.*, 2003). The incorporation of production traits such as growth rate, breast conformation, body weight and feed conversion, are highly heritable, therefore phenotypic characteristics are easily passed to the following generation (Hunton, 1990; Rishell, 1997). This is accomplished by direct selection of males possessing the desired phenotypic traits to produce the next generation

of birds.  Genetic selection is so intense that initially some lines only keep 1% of males and 10% of females (Hunton, 1990).

The increase in performance appears to have a negative impact on fertility as evidenced by the decline in reproductive fitness.  In opposition to performance, reproductive traits have a low heritability (Lake, 1989; Hunton, 1990; Barbato, 1999) requiring between three and four generations for these genes to appear at the producer level (Amann, 1999).  Nevertheless, the broiler industry has long ignored the relevance of selecting for reproductive traits as economic gains in production from genetic selection more than offset the resulting negative affects on fertility (Sexton, 1983). This approach has resulted in a significant decline of fertility over the years, a problem that is becoming more severe.  This decline in fertility (Siegel & Dunnington, 1985; Lake, 1989; Reddy & Sadjadi, 1990; Barbato, 1999; Pollock, 1999) is also accompanied by other problems such as decreased hatchability, decreased immune response, decreased cardiovascular health, an increased occurrence of skeletal abnormalities, mortality, and a general loss of vigor compared to their predecessors (Emmerson, 2000).

As the fertility decline becomes more severe, industry may be faced in the future with the possibility of having to switch over from natural mating to artificial insemination (AI) practices (Reddy & Sadjadi, 1990). However, given the volume of the broiler industry, the economic cost of this practice will be prohibitive.  Besides potentially compromising the future viability of the broiler industry, current costs of reduced fertility

has been estimated at \$18.75 million annually for each 1.3 % decline in fertility (Pollock, 1999).

The concept of switching over to AI is not new to the poultry industry.  Currently, all commercial turkey breeding operations rely solely on AI (Fontana *et al.*, 1990; Donoghue & Bakst, 1999). Multiple experiments on turkeys dating back to the 1950s indicated a high incidence of incomplete matings as intense selection for an increased body weight (Kondra & Shoffner, 1955; Ogasawara *et al*., 1962), increased breast size (Berg & Shoffner, 1954; Carte & Leighton, 1969), and inefficient transfer of semen during copulation (Smyth & Leighton, 1953; Hale, 1955b; Carte & Leighton, 1969) led to decreased fertility in toms and the resultant need to switch over to AI.  Different studies have shown, for example, that Jersey Buff turkeys exhibited 32.95% incomplete matings (Smyth & Leighton, 1953) and that four different lines of Broad Breasted Bronze turkeys had exhibited between a 6% to 34% decline in fertility when switched from AI to natural mating, with the largest decline observed in lines bred for increased body weight (Ogasawara *et al*., 1962).  Large White turkeys also showed a mean of only 29.6% completed matings (Carte & Leighton, 1969).   Researchers determined that a large discrepancy in body weights between male and female Broad-Breasted turkeys along with a decrease in female sex drive could account for the majority of incomplete matings (Smyth & Leighton, 1953; Hale, 1955a; Carte & Leighton, 1969).  Due to the large breast size of the tom, natural matings were no longer efficient enough in sperm transfer to ensure adequate fertilization of females. In recent years, scientific evidence suggests that

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a similar phenomenon is the reason for the reduced fertility observed in broiler breeder

lines (McGary *et al.*, 2002; McGary *et al*., 2003; Bilcik *et al*., 2005).

*1.2  Sperm Storage in the Female Reproductive Tract*

Reproduction in the domestic fowl is a complex mechanism with multiple

environmental and physiological factors interacting and contributing to successful

copulation and fertilization in a commercial setting.  Photoperiod, temperature, and

feeding program have to be strictly monitored by the breeder company (Emmerson,

2000).  For a male to be successful he must be physically able to copulate and transfer

semen along with providing quality semen that will ascend the hen's vagina.  Age, sperm

selection, timing between mating and oviposition, and sperm quality all affect the

chances of a successful insemination (Brillard, 2004).

Of critical importance is the storage of sperm within the hen.  Sperm storage is a

type of reproductive strategy employed by multiparous species that either do not copulate

during the mating season or have males and females with infrequent contact (Lake, 1975;

Bakst *et al*., 1994).  There are two locations within the female reproductive tract where

sperm are stored; the infundibulum and the utero-vaginal junction (UVJ) (Bakst *et al*.,

1994).  However the primary storage site is at the latter in sperm storage tubules (SSTs),

which consist in invaginations of the luminal epithelium (Bakst *et al.*, 1994).  Females

exert a two part selection process to determine which sperm will successfully reach the

UVJ (Bakst *et al*., 1994).  A mechanical selection process allows only motile and viable

sperm to successfully reach SSTs, dead or impaired sperm are unable to do so (Bakst *et al.*, 1994). An immunological selection prevents incompatible sperm from traversing the reproductive tract (Bakst *et al.*, 1994).

Studies have indicated that less than 2% of the initial sperm will reach the UVJ (Steele & Wishart, 1992; Brillard, 1993; Bakst *et al.*, 1994). Upon reaching the UVJ sperm are stored in SSTs until they are released to potentially fertilize an ovum (Bakst *et al.*, 1994). It has been proposed that the beating of cilia around the SSTs create a type of current that can carry sperm away from the SSTs and only sperm that are capable of traveling against this current can maintain their location in the SST to fertilize an ovum (Froman, 2003). In support of this theory, a study of the retention rate of sperm in SSTs, high mobile sperm were lost at a significantly slower rate (p=.049) than low mobility sperm (Froman *et al*., 2002). After a single insemination rooster sperm may reside in SSTs for up to thirty days and fertilize eggs (Romanoff, 1960).

## *1.3 Influence of Sperm Mobililty Phenotype on Reproductive Success*

Sperm mobility, the net movement of a sperm cell population against resistance at body temperature, is a quantitative trait of the domestic fowl (Froman & McClean, 1996; Froman & Feltmann, 1998). It is heritable (Froman *et al*., 2002; Froman & Kirby, 2005), independent of time (Froman *et al*., 1997; Froman & McClean, 1998; Bowling *et al*., 2003), and is the only trait that has been shown to be positively correlated with fertility in domestic fowl and turkeys (*Meleagris gallopavo*) (Froman & Feltmann, 1998; Froman *et*

*al*., 1999; King *et al*., 2000). A long-term study investigating high and average sperm

mobility phenotypes in the domestic fowl, found phenotypic repeatability to be 84%

(Froman & Feltmann, 1998). This same study yielded results indicating differences in

fertility between hens AI with high versus average sperm mobiliy samples (94% v. 64%).

In the turkey, hens AI with toms classified as having high sperm mobility had

significantly higher fertility compared to hens AI from males classified as having low

sperm mobility (King *et al*., 2000).

Research has shown that compared to average mobility sperm, high mobility

sperm consume more oxygen, have higher stearoylcarnitine content, and have higher

linear velocities (Froman *et al*., 1999). Basing a study on these results, subsequent

studies determined that straight line velocity (VSL) and motile sperm concentration are

both key to the expression of sperm mobility phenotype; sperm cells within an ejaculate

that have a VSL > 30 μm/s are not mobilie in vitro (Froman & Feltmann, 2000; Froman

et al., 2002; Froman *et al*., 2003). Males with low mobility sperm have half the motile

concentration, significantly higher levels of aberrant mitochondria (47% v. 4%), and a

threefold difference in oxygen consumption compared to males with high mobility sperm

(Froman & Kirby, 2005).

## *1.4 Impact of Sexual Selection and Social Dominance and its Impact on Reproductive Success*

Sexual selection is defined as the evolutionary process of selecting traits

(behavioral, morphological, or physiological) based on results of reproductive fitness of

the individual and results in differential reproductive success among members of one sex (Darwin, 1871). Reproductive success encompasses an individual's ability to reproduce along with its ability to produce viable offspring that can out-compete potential competitors (Darwin, 1871). The intensity of sexual selection is dependent upon an individual's number of copulation partners (mating success) and offspring production (reproductive success) (Bateman, 1948).

Differences between the sexes arise as a result of differences between male and female gametes. Males produce vast numbers of sperm, extremely small DNA carriers whose main function is to reach a female egg quickly and fertilize it first (Parker *et al.,* 1972). On the other hand, a female egg is extremely large and the numbers produced are limited over a lifetime. Eggs have evolved to be energy rich environments suitable for the development of an embryo (Parker *et al*., 1972). A study on the fruit fly (*Drosophila melanogaster*) indicated that it was more advantageous for males to mate with multiple females than vice versa due to the differential cost of gamete production between males and females (Bateman, 1948). As the number of copulation partners increased, the probability of achieving a higher reproductive success in males also increased. Since males generally have a higher reproductive potential and invest less in an individual offspring, sexual selection is more intense in males (Bateman, 1948); (Parker, 1979).

There are two main components to sexual selection: intrasexual selection and intersexual selection (Darwin, 1871; Andersson, 1994). As its name implies **intrasexual**

**selection** involves competition between members of the same gender for the privilege to mate with the other sex (Bateman, 1948). Intrasexual selection arises from the differences between the sexes in their reproductive potential (Bateman, 1948). In addition to increased competition for matings, a form of intrasexual competition that occurs post-copulation is sperm competition which can have a large impact on the reproductive success of males (Parker *et al.*, 1972). Sperm competition occurs when a female mates with multiple males in a single reproductive period and paternity of the clutch can be shared among males (Parker, 1970). Multiple matings can actually induce sperm competition within the female oviduct (Birkhead & Moller, 1998), conferring a differential reproductive advantage to the sperm that is better able to out-compete other sperm cells (Birkhead, 1998; Donoghue & Bakst, 1999). Multiple matings and the sperm competition generated also has reproductive advantages to females by ensuring that sufficient sperm are available to fertilize all of her eggs or by enhancing the genetic quality of her clutch (Birkhead & Moller, 1998).

The impact of sperm competition has been clearly demonstrated in experiments with domestic fowl and controlled inseminations in the number of sperm cells inseminated as well as the timing of the inseminations (Martin *et al.*, 1974). When equal amounts of sperm are inseminated within four hours of each other, the sperm mix and paternity results in an approximately 50% success rate for each male (Martin *et al.*, 1974). When the time period between inseminations is greater than four hours the percentage paternity of the second male (P2 value) increases substantially over 50%

(Warren & Gish, 1943; Compton *et al.*, 1978).  This is referred to as last male sperm precedence phenomena (Birkhead & Hunter, 1990).

Intrasexual selection is the primary factor leading to the exaggeration of male secondary characters (Darwin, 1871; Bateman, 1948; Trivers, 1972; Smith, 1991). Secondary sexual characters may be used to fight with other males (spurs in the domestic fowl) for access to females or to signal male quality to females seeking to copulate (ornaments, tail length, plumage, etc…).  Monogamous female barn swallows (*Hirundo rustica*) for example will preferentially mate with males possessing longer tails (De Lope & Moller, 1993).   In the pheasant (*Phasianus colchicus*) females prefer to mate with males with longer spurs (von Schantz *et al*., 1989).  A female widowbird (*Euplectes progne*) prefers to mate with a male that possesses a long tail (Andersson, 1982). Interestingly some studies have shown that after copulating with an attractive male, peahens will lay more eggs as compared to after copulating with a less attractive male (Petrie & Williams, 1993).  The conspicuousness of these traits to predators and the resulting decrease in survival rates is the cost of carrying these traits.

**Intersexual competition** involves one sex choosing which members of the opposite sex to mate with and is generally more intense in females (Bateman, 1948). Males producing energetically less expensive sperm cells are not as choosy as females who invest a significant amount of energy and resources into producing an egg and caring for it after laid (Orians, 1969).  In species where extra-pair copulations are common, females will choose with whom to mate based on either the potential direct benefits or

indirect benefits that they perceive they may be receiving in the future (Kirkpatrick, 1987; Reynolds & Gross, 1990).  When females choose a male to mate with based on direct benefits, they look for a male that will in some way increase their chances of survival or fecundity at the present time (Reynolds & Gross, 1990) which  may include nuptial gifts as occurs in the blister beetle (*Neopyrochroa flabellate*) (Eisner *et al*., 1996), parental care, and territory amongst others.  Choosing based on indirect benefits results in genetic advantages gained by future offspring as opposed to immediate benefits gained by the female (Kirkpatrick & Ryan, 1991; Andersson, 1994).  Mate choice by females is frequently based on a male's secondary sexual characters (Zuk et al., 1990a; Zuk et al., 1990b; Zuk et al., 1995b).

Females can exert cryptic female choice (CFC), a post-copulatory mechanism of biasing one male's sperm over another's in the fertilization process (Thornhill, 1983); (Eberhard, 1996).  Three types of CFC have been identified.  One type allows females to bias the success of a male by ejecting sperm directly following copulation via cloacal contractions.  This was found in the female domestic fowl which often eject sperm when coerced to mate with a subdominant male (Pizzari & Birkhead, 2000).  A second type of CFC is a result of sperm-egg interactions within the oviduct and occurs in the comb jelly (*Beroe ovata*) (Carre & Sardet, 1984).  In this species sperm pronuclei become immobile after reaching the egg  Over several hours the egg pronucleus examines several pronuclei before deciding which one to fuse with (Carre & Sardet, 1984).  A third type of CFC is the differential use of sperm by a female (Sheldon, 2000) which occurs in the yellow

dung fly (*Scathophaga stercoraria*) (Ward, 1998).  Females of this species are able to store sperm from different males and release eggs fertilized from different males into different environments (Ward, 1998).

Domestic fowl are descendents of the red jungle fowl and have a nearly identical behavioral repertoire (Darwin, 1887); (Kruijt, 1964).  They are social animals that communicate both audibly (Wood-Gush, 1971) and visually with one another (Duncan, 1980).  The social structure of red jungle fowl consists of groups of multiple males and females along with one dominant male. There exists both a relatively stable male and female linear dominance hierarchy with males dominant over females (Guhl, 1962).  The peck order serves as a type of spacing system (McBride, 1970).  In confined enclosures the alpha male tends to locate himself in the center of the group.  Males will position themselves further away from the center as their dominance rank decreases (Craig & Guhl, 1969).  The lowest males in the peck order have a tendency to congregate at the sides of the enclosure.  Visual contact is more effective than vocalizations in maintaining social status among males (Mench & Ottinger, 1991).  When aggressive behaviors occur, they are more common among members of the same sex (Rushen, 1983).

Dominant males are generally larger (Collias, 1943), more aggressive (Marks *et al*., 1960; Siegel & Hurst, 1962; Graves *et al*., 1985; Leonard & Horn, 1995), have larger and more conspicuous secondary sexual characters (Domm, 1939; Collias, 1943; Graves et al., 1985; Ligon et al., 1990) , have a higher frequency of crowing (Salomon *et al*., 1966) and have a greater access to matings compared to subordinate males (Guhl &

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Warren, 1946; Pizzari & Birkhead, 2000; Pizzari, 2001).  In one study subdominant

males exhibited a tendency for reduced testes size (Siegel & Siegel, 1961) with the

consequent influence in the expression of male secondary traits (Ligon *et al*., 1990),

aggressiveness (Ligon *et al*., 1990), and sperm production (de Reviers & Williams,

1981). In the domestic fowl females often use a male's comb or wattle as a signal for

male quality and prefer to mate with males with larger and redder appendages (Zuk et al.,

1992; Zuk *et al*., 1995b; Ligon & Zwartjes, 1995b).  Comb size and testosterone level are

positively correlated (Allee et al., 1939; Collias, 1943; Witschi, 1961) as are testosterone

levels and aggression (Allee et al., 1939; Harding, 1983).  Comb size may therefore

signal to females a male's social status in the dominance hierarchy (Ligon *et al.*, 1990;

Zuk *et al*., 1995a). However high testosterone levels come at the cost of suppressed

immunity as evidenced in one study in which males of red jungle fowl with longer combs

had fewer circulating lymphocytes (Zuk *et al.*, 1995a).


It is clear that in the domestic fowl females choose dominant over subordinate

males to mate (Pizzari & Birkhead, 2000) and are also more likely to accept sexual

advances from dominant males and resist copulations from subordinates (Pizzari &

Birkhead, 2000).  When a subordinate attempts mating, the hen is more likely to resist

and utter a distress call (Pizzari, 2001).  Distress calling serves as a signal to dominant

males as to the attempted mating and results in a disruption of the copulation attempt

(Pizzari, 2001). However, if males are prevented from competing with each other for

access to females in red jungle fowl, a hen will choose to mate with the larger combed

male first but will subsequently mate with both males (Ligon & Zwartjes, 1995a).

Dominant males provide hens with higher levels of courtship, feeding, anti-predator vigilance, and protection from sexual harassment from subordinates (Pizzari, 2001). However a study by Froman *et al*. (2002) indicated that, contrary to the expectations, dominant males had lower sperm mobility and were often out competed by high mobility ejaculates from subdominant. They propose that sperm mobility may be under the control of an independent maternally inherited element.

The higher the sperm mobility, the longer the sperm resides in a female's SST and henceforth the longer the fertilizing efficiency of the ejaculate (Froman *et al*. 2002). Therefore, it appears that males may be following two alternative reproductive strategies. There will be males that invest more in secondary sexual traits which will attract females and secure more matings and the second type of males that invest more in sperm quality, therefore increasing their chances of fertilizing a female's ova despite limited mating access.

Another example of the degree of sophistication in reproductive strategies in the red jungle fowl is their ability to allocate sperm (Pizzari *et al*., 2003; Cornwallis & Birkhead, 2006). They can produce several small ejaculates or a few large ejaculates depending on the number of females in the area (Pizzari *et al*., 2003). If a male repeatedly copulates with one female, he decreases the amount of sperm he inseminates over time (Pizzari *et al*., 2003). However when presented with a new female, he responds by increasing the size of the ejaculate providing his sperm reserves are not depleted

(Pizzari *et al*., 2003). This phenomena is commonly referred to as the Coolidge effect

(Wilson *et al*., 1963; Ligon *et al*., 1998; Wedell *et al*., 2002; Pizzari *et al*., 2003).

Dominant males, after given the opportunity to mate with two females differing in

quality, in a second placing with the same two females will result in differential sperm

allocation in favor of the high quality female (Cornwallis & Birkhead, 2006). On the

contrary subordinates will invest the most sperm in the female he mates with first

(Cornwallis & Birkhead, 2006).  Dominant males have preferential access to females and

therefore can afford to invest less initially in a low quality female to see if a better one

comes along (Parker, 1983).  Subordinates, who are limited in their access to females are

more likely to invest heavily in the first female he mates with (Cornwallis & Birkhead,

2006).  Dominant males also preferentially allocate sperm to females with large combs

(Pizzari *et al*., 2003; Cornwallis & Birkhead, 2007b; Cornwallis & Birkhead, 2007a),

which they use as an indicator of maternal fitness (Pizzari *et al*., 2003).  In females comb

size is positively correlated with egg size and the amount of resources provided to an

embryo (Pizzari *et al*., 2003; Cornwallis, 2004).

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# Chapter 2: Semen quality and its correlation with morphological phenotypes

## *2.1 Abstract*

In the domestic fowl body weight and comb area have been shown to be reliable indicators of the fertility potential of a male but few studies have related these parameters with semen quality and with the changes occurring as a consequence of aging. The objective of this study was to evaluate semen quality defined according to semen volume, sperm concentration, and sperm mobility phenotype in two lines of primary broiler breeders known to have different levels of fertility. Sperm quality was evaluated to determine the effects of aging on semen parameters and was of interest to determine potential relationships between sperm quality and morphometrical phenotype. We hypothesized that semen quality would be poorer in the line known to exhibit lower fertility and would decline in both lines as the birds aged. We also hypothesized that morphological characters would be reliable indicators of semen quality and therefore of the males reproductive potential.

Sperm characteristics were analyzed in 30 fast-growth (FG) and 20 high-yield (HY) males. Semen parameters were measured and compared from 41 to 53 weeks of age. Morphological measures of birds were taken at 54 weeks of age. Results revealed a significant age by line interaction for semen volume (p=0.0307), sperm concentration (p=0.0003), and sperm mobility (p=0.0405). Regression analysis yielded a quadratic

relationship of semen volume (square root transformed) and age in HY males with volume peaking at 47 weeks of age. Concentration followed a linear increase with age in HY males (p=0.0226) and sperm mobility tended to increase (p=0.0596) as HY males aged. Age had no effect on volume or concentration in FG males, but mobility improved with age (p<0.0001) in these males. A potential explanation is that successive semen collections may help to maintain sperm quality with age.

HY males weighed significantly more than FG males (p=0.0028) and had smaller combs and wattles (p<0.0001) but body weight did not correlate to any of the measures of sperm quality. In HY males wattle length and area correlated with semen volume (r=.6364; p=.0261; r=.7203; p=.0082), but a negative correlation was established between wattle width and sperm mobility (r=-.6833; p=.0424). In FG birds there was a positive correlation between comb width and sperm concentration (r=.5466; p=0.0232) and a positive trend between comb perimeter and sperm concentration (r=.4559; p=0.0659). These results suggest that in HY birds males with larger wattle values are likely to produce higher volumes of semen, whereas comb width may serve as an indicator of higher sperm concentrations in FG males. However, the relationship between the size of secondary sexual characters and semen quality appear to be complex. Results indicate that body weight per se has no impact on sperm quality.

Key Words: sperm quality, domestic fowl, morphology, broiler breeder

## 2.2 Introduction

The broiler breeder, *Gallus gallus domesticus,* has successfully undergone intensive genetic selection over the years to improve growth rate, meat yield, and feed conversion (McDaniel, 1978; Rishell, 1997). Today's broilers go to market at a much younger age, weigh considerably more, and are much more efficient in their feed conversion rates (Emmerson, 2000). Unfortunately, economic production traits and reproductive traits appear to be negatively associated in broilers causing a decline in reproductive fitness. The broiler industry has long ignored the relevance of selecting for reproductive traits as economic gains in production from genetic selection more than offset the resulting negative affects on fertility (Sexton, 1983). This approach has resulted in a significant decline of fertility which is becoming more severe to the point of having a major economic impact in current production. Today's broilers not only exhibit decreased fertility levels (Siegel & Dunnington, 1985; Lake, 1989; Reddy & Sadjadi, 1990; Barbato, 1999; Pollock, 1999), but also decreased hatchability, decreased immune response, decreased cardiovascular health, an increased occurrence of skeletal abnormalities, mortality, and a general loss of vigor compared to their predecessors (Emmerson, 2000).

It has been shown that certain secondary sexual characters of a male fowl confer information on his health and reproductive status to females (Zuk *et al*., 1995a; Zuk *et al*., 1995b). The appendages a female uses to evaluate male quality are his comb and

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wattle (Zuk *et al*., 1995a; Zuk *et al*., 1995b).  The larger and more intense red coloration

of the secondary sexual characters, the stronger the female preference (Zuk *et al*., 1995b).

If these morphometrical characters prove to be reliable indicators of a male's sperm

quality, then it is possible that they could be used as phenotypic selection markers.  The

search for traits to identify a male's potential fertility has yielded various morphometric

traits including body weight (Siegel & Dunnington, 1985), wattle width (HY males)

(Bilcik & Estevez, 2005), and comb area (HY males) (McGary *et al.*, 2002) as possible

indicators of fertility.


A recent study investigated the relationship between morphometric traits (comb

area, relative testicular weight, and testicular weight asymmetry) and fertility in two lines

of broiler breeders, a Cornish line bred for high breast yield (HY) and a line bred for fast

growth (FG) (McGary *et al*., 2002).  In FG birds relative testicular weight was correlated

with sample fertility and flock fertility.  In HY birds comb area was correlated with

sample fertility, flock fertility, and relative testicular weight indicating that in this strain

comb area may be a reliable indicator of male fertility (McGary *et al*., 2002).   McGary *et

al*. (2003) found the same negative association between dorsal pelvic width and fertility

in HY birds.  While the results did not show a direct correlation between body weight and

fertility, they did indicate that musculoskeletal changes to support increased breast yield

had negative consequences on male broiler fertility.  They are also indicative of possible

strain differences in reference to how these changes manifest themselves (McGary *et al*.,

2003).  A study by Bilcik and Estevez (2005) showed that competing males in a group

did show a large difference in individual mating frequency and results indicated that

those with the highest mating frequency did not necessarily father an increased

proportion of offspring, possibly a result of sperm competition (Bilcik & Estevez, 2005).

In this study they also found that heavier males exhibited a higher frequency of matings

without cloacal contact and a lower percentage of paternity.

Different analytical techniques have been utilized over the years to assess a

male's semen quality and to evaluate his fertility potential (Reddy & Sadjadi, 1990).

Volume is the total amount of semen produced; it is quick to measure and provides

information necessary to calculate dilution dose when performing an AI (Etches, 1996).

Concentration refers to the number of sperm cells per volume of ejaculate, (Etches, 1996)

whereas viability is a measure of the proportion of live, dead, and dying sperm cells from

a sample (Etches, 1996). It is often measured using live/dead stains via fluorescence

microscopy (Donoghue *et al*., 1995) or by the ethidium bromide exclusion procedure

(Bilgili & Renden, 1984). Sperm morphology is the microscopic assessment of individual

sperm cells and identifies problems with sperm such as missing acrosomes, bent necks, or

coiled tails that can render them unviable (Etches, 1996). Sperm mobility is the

progressive movement of a sperm cell population against a gradient and is analyzed most

accurately by the Accudenz (Accurate Chemical) procedure (Froman & McLean, 1996).

In the turkey industry workers typically look at a sample's color, consistency, and

presence of contaminants to quickly determine semen quality; males that produce

samples appearing pearly white and concentrated to the naked eye are retained while

males repeatedly producing watery, yellow, or contaminated samples (urates, blood, fecal

matter) are culled (Christensen, 1997). While the attributes used to cull males are

appropriate, those used to maintain males do not ensure a male is of high quality; a

sample that is viscous and white may contain sperm that are immotile, abnormal, or dead (Haye *et al*., 1981).

The objectives of this study were 1) to evaluate sperm quality in two lines of primary broiler breeder males with differing fertility levels and detect the effects of age on sperm quality and 2) to determine relationships between morphology and semen parameters. We hypothesized that semen quality would be reduced in HY males that have historically exhibited fertility problems, semen quality would decline as males aged, and morphological characters would be reliable indicators of semen quality.

## *2.3 Materials and Methods*

### *2.3.1 Facilities and Experimental Animals*

For this study we used two lines of primary broiler breeders housed at the primary breeder facility. Birds from the HY line are Cornish derived and have undergone an intense genetic selection for high breast yield whereas FG birds were selected for fast-growth. HY birds have shown increased fertility problems compared to FG birds (D.L. Pollock, personal communication). Birds were reared according to the breeder selection program. Until seven weeks of age all birds were raised as broilers (*ad libitum* feeding) with a photoperiod of 20L:4D. This is done in order to allow the full phenotypic expression of their growth traits. From 49 until 140 days of age access to feed was restricted to maintain a targeted body mass of 2500 grams with a photoperiod of 8L:16D. At day 140, 20 HY and 30 FG males were housed separately by line in single-sex stud

pens (4.88m x 6.10m) with a line of nipple drinkers.  Photoperiod was gradually

increased to 16L:8D.  Males were fed 150g/bird/day from 141 to 420 days of age to

increase their live weight to 4500-5000 grams at 35 weeks of age.  Birds were

individually tagged on one wing with a small metal tag to track male lines and one plastic

badge on the opposite wing to allow for visual male identification by the company.

## 2.3.2 Experimental Procedures

At 39 weeks of age, 30 FG and 20 HY broiler breeder males were trained for

manual semen collection (Burrows & Quinn, 1937) four times over a two week period.

Males were selected according to their response to semen collections.  Next, males were

screened to eliminate those with poor quality semen (small volume or sperm

concentration falling below the minimum threshold necessary to measure sperm

mobility).  From the initial group, 19 FG and 12 HY males were selected and collected

from twice a week through 54 weeks of age. Semen quality was evaluated by measuring

semen volume, sperm concentration, and sperm mobility. Volume was calculated by

drawing up preset volumes of sperm using a Gilson pipette and adding that value to the

total amount of the sample used in the concentration and mobility assays.  Concentration

was determined by measuring the optical density (OD) of 10μL of ejaculate mixed with

1.99 mL of 3% sodium citrate using an IMV Micro-Reader I[1] (Froman & Feltmann,

2000).  The OD reading was subsequently plugged into an equation from IMV

International specifically for broiler breeder sperm (Concentration = [11.34*OD] + 0.03).

Sperm mobility was assessed following the Accudenz procedure described by Froman

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[1] IMV International, Minneapolis, MN, Model 4025-001

and McLean (1996).  In short, each sample was diluted to $1 \times 10^9$ sperm cells/mL using warmed mobility buffer, layered over 1.5 mL of Accudenz and allowed to penetrate the medium for five minutes.  At this time the cuvette was placed into the IMV Micro-Reader I, the sample was given one minute to settle as a result of the move, and OD was measured.

At 54 weeks of age, all males were individually weighed outside the stud pen on a scale.  At that time a picture of the right profile of the head was taken with a digital camera.  The picture was taken against a grid composed of 1 cm x 1 cm squares that was attached to the wall of the pen.  Scion Image Analysis Software[2] was used to compute the following measurements: comb perimeter (CP), comb area (CA), comb width (CW), comb length (CL), left wattle perimeter (WP), left wattle area (WA), left wattle width (WW), and left wattle length (WL).  Perimeters and areas were measured by tracing the structure using a mouse.  Widths and lengths of combs and wattles, respectively, were calculated as the largest horizontal and vertical distances of the structures.

*2.3.3 Statistical Analysis*

Sperm volume, concentration, and mobility were analyzed using a mixed model repeated-measures analysis of variance (ANOVA) with age as the repeated measure (SAS Institute Inc. v 9.1 ed. Cary, NC). Bird line was considered a fixed effect and male ID was considered a random effect.  A compound symmetry covariance matrix was used. All data were checked for normality and homogeneity of the residual variance. All three

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[2] Scion Corporation Frederick, Maryland

measures were positively skewed and thus square root transformations were applied. Estimate statements were used to determine significant differences between lines for each parameter across age periods. Least square means and standard errors were back transformed for reporting purposes. In addition, analyses were performed to determine the regression of each parameter on age. A linear and a quadratic regression model on age were examined for volume, concentration, and mobility for each line to examine the effect of age on these parameters. A mixed model ANOVA was used to analyze differences between lines in CA, CP, CL, CW, WA, WP, WL, WW, and body weight (Wt). Least square means and their standard errors were reported. Spearman rank correlation analysis for each line was performed to determine relationships among morphometrical (CA, CP, CL, CW, WA, WP, WL, WW, and Wt), and semen quality parameters (volume (MV), concentration (MC) and mobility (MM)).

## 2.4 Results

### a) Semen quality

Mean volume, concentration, and mobility in HY and FG males were compared from 41 to 53 weeks of age. There were significant line*age interactions for mean volume, mean concentration, and mean mobility (Table 2.1). Compared to FG, HY males had a significantly lower volume (p=0.0152; Figure 2.1a) at 43 weeks of age, lower concentration (p=0.0003, Figure 2.1b) at 42, but a higher mobility at 43 and was close to significance at 50 weeks of age (p=0.0450 and p=0.0625; respectively, Figure 2.1c) and a significantly higher concentration at 52 weeks of age (p=0.0333; Figure 2-1b).

**Table 2.1:** Model ANOVA with degrees of freedom (df), F value, and p value, for volume, concentration, and mobility.

| Effect | df | F | p |
|---|---|---|---|
| **Volume** | | | |
| Line | 1,42.8 | 0.32 | 0.5755 |
| Age | 12,534 | 1.59 | 0.0914 |
| Line*Age | 12,534 | 1.91 | 0.0307 |
| **Concentration** | | | |
| Line | 1,41.1 | 0.03 | 0.8651 |
| Age | 12,469 | 1.58 | 0.0939 |
| Line*Age | 12,469 | 3.07 | 0.0003 |
| **Mobility** | | | |
| Line | 1,30.9 | 0.55 | 0.4620 |
| Age | 12,154 | 5.08 | <0.0001 |
| Line*Age | 12,154 | 1.88 | 0.0405 |

**Figure 2.1:** Comparisons between HY and FG males from 41 to 53 weeks of age for a) mean volume ± SE b) mean concentration ± SE and c) mean mobility ± SE. * indicates significant difference (p<0.05),

a)
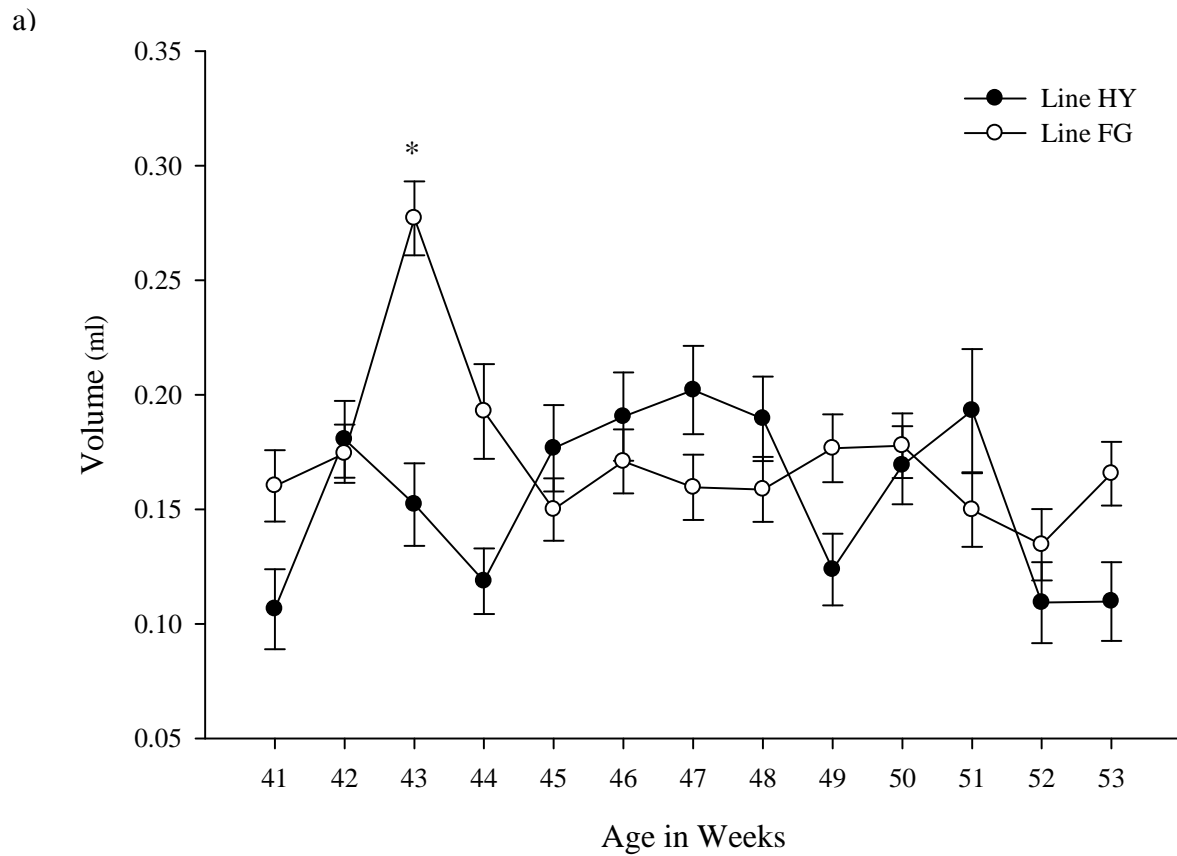
b)
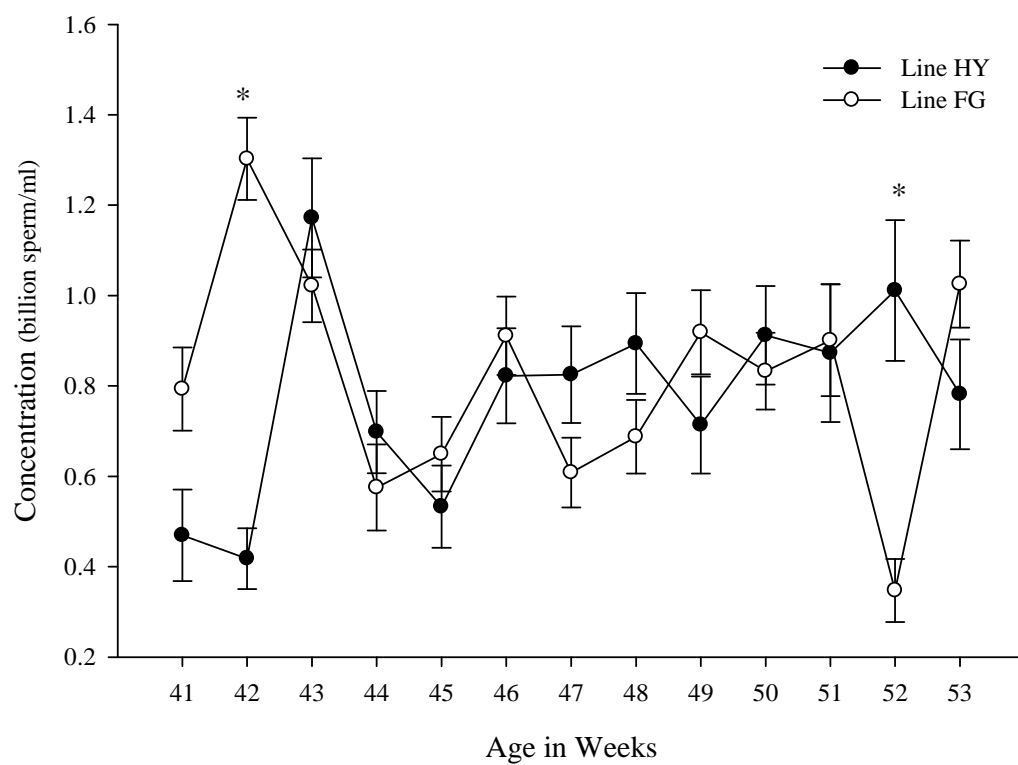
c)
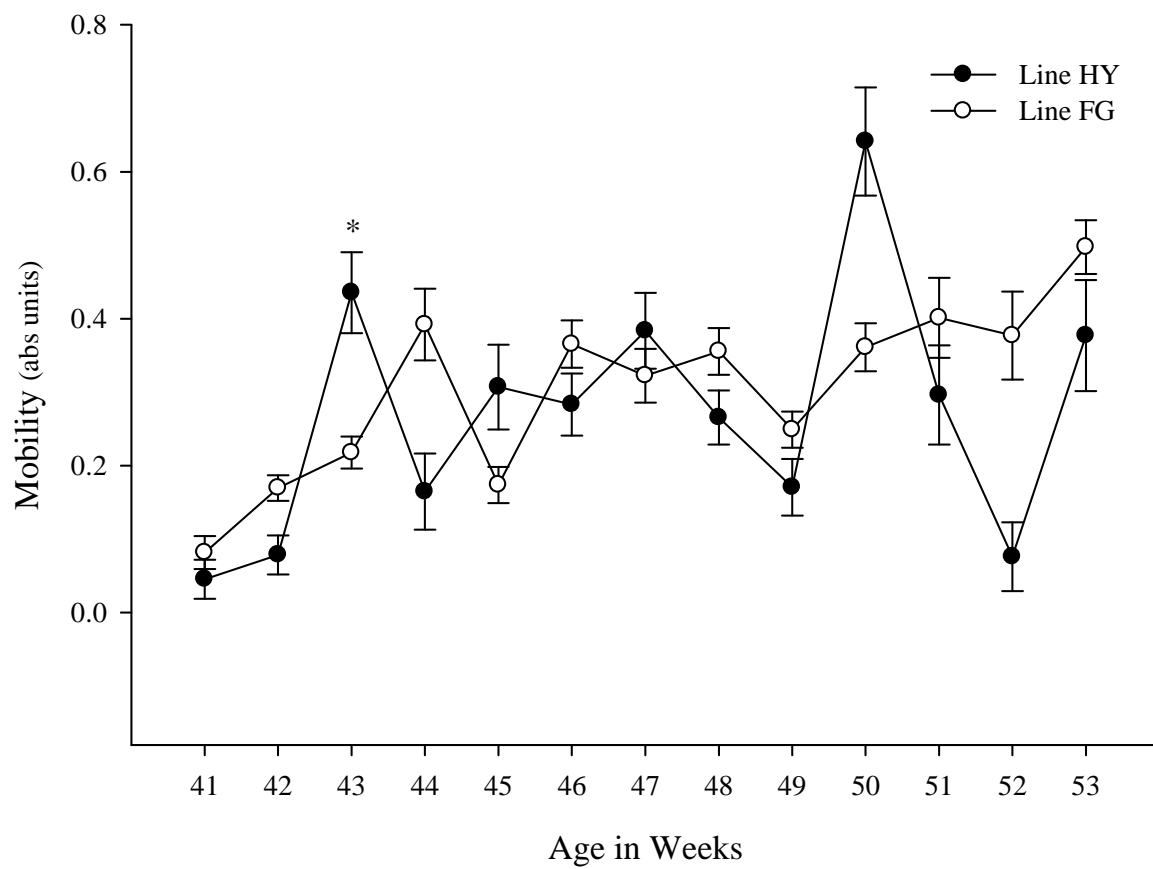
Regression analysis yielded a quadratic relationship between sperm volume (square root transformed) and male age in HY males. For every one week increase in age, the mean square root concentration increased by .0188 √(billion sperm/mL) (Figure 2.3). Age had no effect on volume or concentration (both square root transformed) at the significance level α=0.05 in FG males. Volume peaked at 47 weeks of age decreasing until the end of the experiment (Figure 2.2). Sperm concentration (square root transformed) slightly increased linearly with age (b=.01888; p=0.0226) in HY males. Regression of sperm mobility (square root transformed) with age was significant in FG males (p<0.0001) (Figure 2.4). Analysis indicated a trend for sperm mobility (square root transformed) to increase as HY males aged (p=0.0596). With each one week increase in age, FG males' mean square root of sperm mobility increased by √.02447 absorbance units whereas HY males' mean square root of sperm mobility increased by .01925 √absorbance units.

**b) Semen quality and its relationship with morphometrical parameters**

HY males weighed significantly more than FG males (5.200±.1233 v. 4.6788±.1007; p=0.0028). FG males had significantly larger values (p<0.0001; Table 2.2) for each of the following parameters: CA, WA, CP, WP, CL, CW, WL, and WW (Table 2.2). For HY males we found a significant positive correlation between semen volume and wattle area and length (r=.7203; p=.0082; r=.6364; p=.0261). There was a negative correlation of wattle width with sperm mobility (r=-.6833; p=.0424). Also in HY males there was a trend for males with a larger comb area to have lower sperm

**Figure 2.2:** Regression of square root transformed semen volume on age in HY males.  Y-axis on right represents back-transformed volume values for comparison purposes.



$$y=-4.29170+.20398x-.00220x^2$$
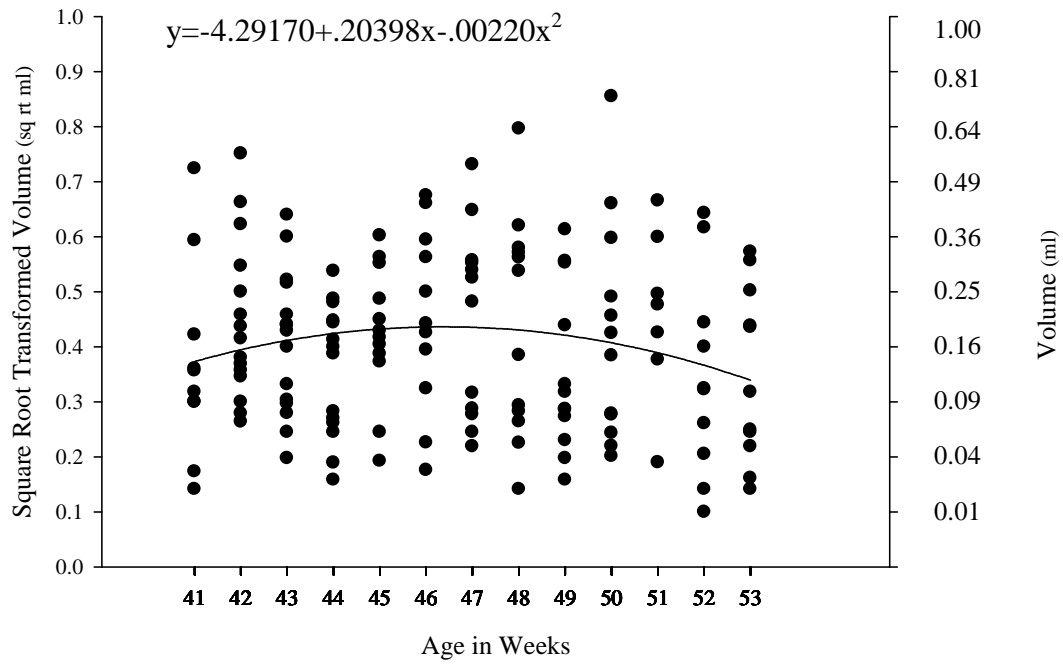
**Figure 2.3:** Regression of square root transformed sperm concentration on age in HY males. Y-axis on right hand side of graph provides corresponding concentration values back-transformed.
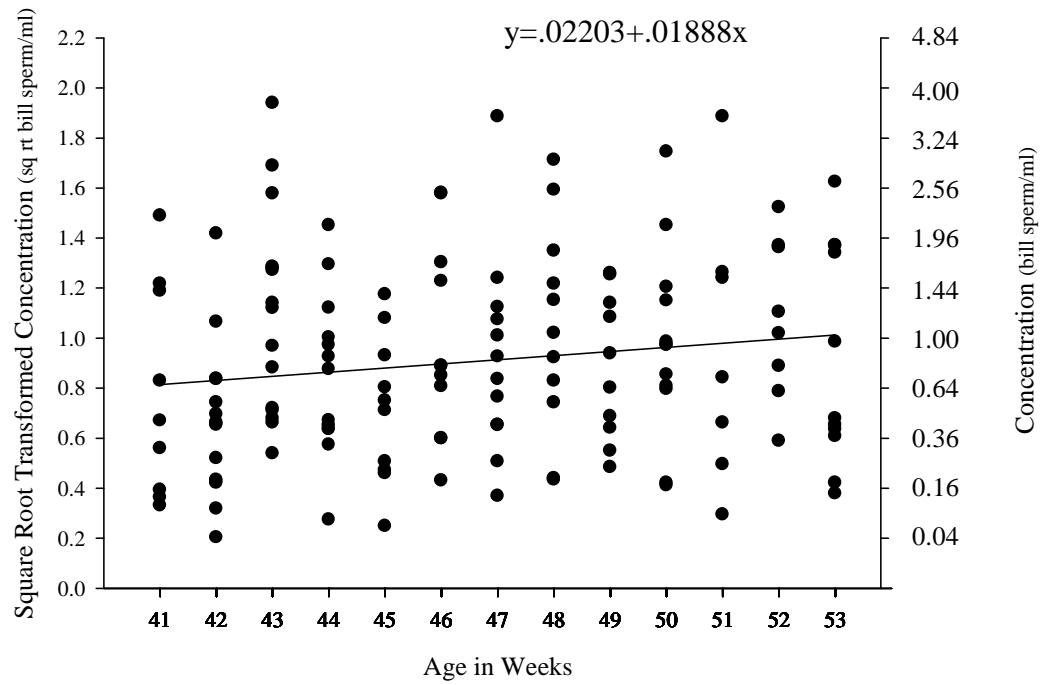
**Figure 2.4:** Regression of square root transformed mobility on age in HY and FG roosters.  Y-axis on right indicates mean mobility values back-transformed for comparison.



HY: y=-.39925+.01925x
FG: y=-.60284+.02447x
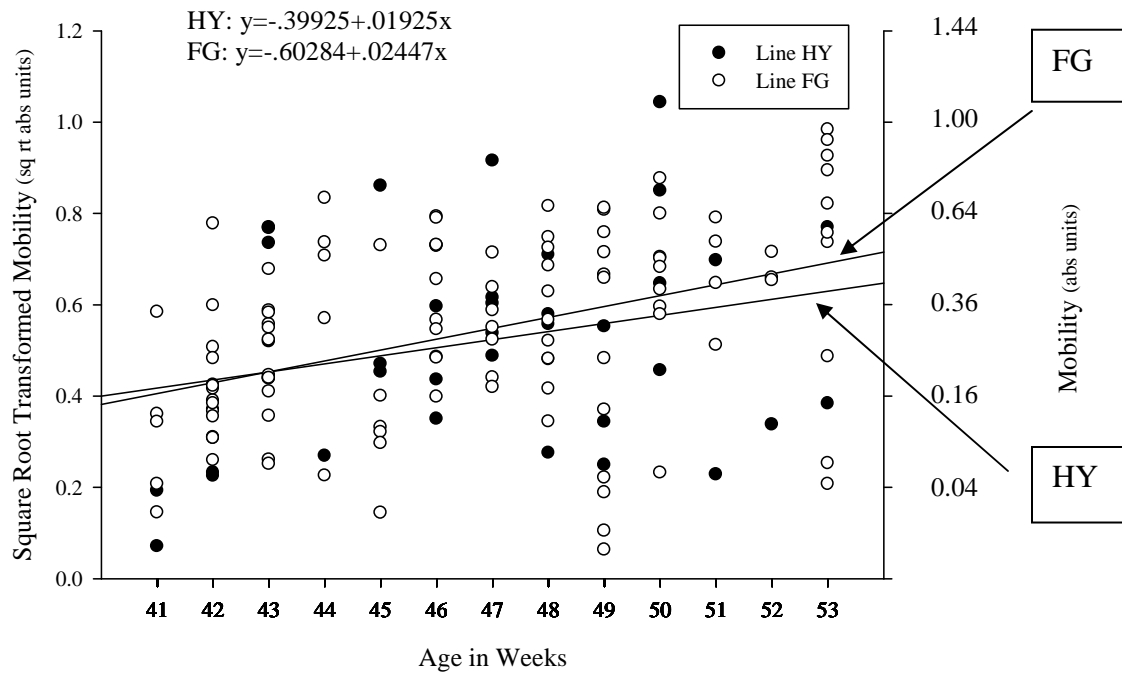
**Table 2.2**: Mean ± SE comparisons between lines for eight morphological parameters measured. Area measurements are in cm$^2$, all other measurements in cm.

| Measure | HY Line | FG Line | Line df | F | p Value |
|---|---|---|---|---|---|
| Comb Area (cm$^2$) | 5.241±2.704 | 50.330±1.824 | 1,27 | 188.80 | p<0.0001 |
| Wattle Area (cm$^2$) | 2.484±.991 | 15.787±.833 | 1,27 | 105.63 | p<0.0001 |
| Comb Perimeter (cm) | 11.051±1.240 | 46.777±1.042 | 1,27 | 486.76 | p<0.0001 |
| Wattle Perimeter (cm) | 7.360±.461 | 15.668±.387 | 1,27 | 190.74 | p<0.0001 |
| Comb Length (cm) | 3.445±.287 | 11.827±.241 | 1,27 | 499.99 | p<0.0001 |
| Comb Width (cm) | 1.673±.222 | 6.202±.187 | 1,27 | 243.81 | p<0.0001 |
| WattleLength (cm) | 1.635±.164 | 4.232±.138 | 1,27 | 147.01 | p<0.0001 |
| Wattle Width (cm) | 2.367±.153 | 5.201±.129 | 1,27 | 200.47 | p<0.0001 |

concentration (r=-.5359; p=.0725). All correlations between morphological parameters and parameters defining semen quality for HY and FG males are shown in Table 2.3. Neither mean body weight nor sperm concentration was significantly correlated with any other parameter in HY birds (P>0.05). For the FG individuals, results indicate a positive correlation between comb width and sperm concentration (r=.5466; p=0.0232) and a positive trend between semen volume and sperm concentration (r=.4180; p=0.0844). There was a positive trend between comb perimeter and sperm concentration (r=.4559; p=.0659). Correlations between morphological parameters can be found in Table 2.3. Neither mean body weight nor sperm mobility was significantly correlated with any other parameters at $\alpha$=0.05 level.

**Table 2.3**: Correlations between morphological parameters.  Speaeman correlation coefficients given when at least one of the two lines exhibited a correlation of the following significance values: * p<0.05, ** p<0.01, ***p≤0.001; T symbol indicates presence of a trend

| | | CP | WA | WP | CL | CW | WL | WW | MV | MC | MM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CA | HY | .8862*** | .1611 | .1261 | .7811** | .8932*** | | .1891 | | -.5359 T | |
| | FG | .9093*** | .5833* | .5784* | .7672** | .8137*** | | .6377** | | .4069 | |
| CP | HY | | .0979 | .1329 | .7133** | .8182** | | .2797 | | -.4336 | |
| | FG | | .6495** | .6103** | .7402** | .8750*** | | .6217** | | .4559 T | |
| WA | HY | | | .7483** | | .1399 | .7413** | .4266 | .7203** | | |
| | FG | | | .9118*** | | .5221* | .7914** | .8155*** | .2500 | | |
| WP | HY | | | | .0210 | .1678 | .5455 T | .5175 T | | | |
| | FG | | | | .4485 T | .5221* | .6761** | .8866*** | | | |
| CL | HY | | | | | .9441*** | | .3986 | | | |
| | FG | | | | | .8064*** | | .5359* | | | |
| CW | HY | | | | | | | .4685 | | -.3077 | |
| | FG | | | | | | | .5052* | | .5466* | |
| WL | HY | | | | | | | .3636 | .6364* | | |
| | FG | | | | | | | .5150* | .0822 | | |
| WW | HY | | | | | | | | | | -.6833* |
| | FG | | | | | | | | | | -.1412 |
| MV | HY | | | | | | | | | .0839 | |
| | FG | | | | | | | | | .4180 T | |

## *2.5 Discussion*

### 2.5.1 Semen quality

The purpose of this experiment was to investigate differences in semen quality and morphometric measures in two lines of primary broiler breeders, Line FG selected for a fast growth rate and Line HY selected for high breast yield. HY males possess a rose comb which has previously been shown to result in reduced fertility (Cochez, 1951; Crawford & Merritt, 1963; Crawford & Smyth, 1964; Buckland & Hawes, 1968). FG males have a single comb, are usually dominant over HY males, and have not exhibited fertility problems to the same extent as HY males. Based on previous information, in this study we hypothesized that HY birds would show reduced semen quality as compared with FG males, with differences between lines becoming larger as birds aged. We also predicted a general decline of semen quality as males aged in both lines.

We found a significant interaction between line and age for all semen parameters evaluated in this study, indicating that age affected the two lines differently. Early in the experiment, age played a more important part in establishing differences between the lines. Throughout the 13 weeks of the experiment age was able to explain the significant differences between lines a total of four times for semen volume, sperm concentration, and sperm mobility combined. One of these occurred at 42 weeks of age (FG significantly higher concentration) and two of these occurred at 43 weeks of age (FG significantly higher volume and HY significantly higher mobility). This early difference

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in male lines may be a result of not having been ejaculated regularly. Once males were

collected from on a regular schedule and were accustomed to handling and the collection

procedure, the two lines did not differ in semen quality. Contrary to our hypothesis, we

found that in general semen quality did not decline with age. Regression analysis

indicated a curvilinear relationship between semen volume and age in HY males which

peaked in volume production at 47 weeks. This is similar to the mean peak semen

production found at 44 weeks of age in a study of two meat-type strains of broiler

breeders (Harris *et al*., 1984). A relationship with age was not found in FG males.

Concentration increased linearly in HY males but in FG males there was no relationship

between sperm concentration and age. Sperm mobility increased in both lines of males

as they aged. This may be due to the fact that all males used were housed in same sex

stud pens with no access to females, allowing them to reach their full reproductive

potential during the time frame of our experiment. It is also possible, that being housed

in single-sex pens, males allocated more resources to sperm quality and not in sexual

characters used to acquire mates. It must be noted that we had a relatively small

population of males. When measuring sperm mobility in a flock, it is common to classify

high sperm mobility as one standard deviation greater than the sample mean and low

sperm mobility as one standard deviation below the sample mean. We excluded any

males not producing samples or producing low quality samples which may have biased

our results since we only worked with males of higher quality compared to flock mates.

**2.5.2 Semen quality and its relationship with morphometrical parameters**


In our study HY birds selected for high breast yield had a higher mean body weight than birds selected for a fast-growth rate. Throughout the course of the experiment HY males only differed significantly in sperm mobility once with FG males; at 43 weeks of age HY males had a significantly higher mean mobility (p=0.0450). These results, when combined with the fact that HY males exhibit more fertility problems than FG males, provides further evidence that increased body weight in broiler breeders has resulted in decreased fertility levels in hens as a result of inability to transfer sperm efficiently as opposed to having poor quality semen. Contrary to other studies, we did not find a negative correlation between sperm mobility and body weight (Soller & Rappaport, 1971; Bowling *et al*., 2003).


In our study three measurements were positively correlated with sperm concentration in FG males, comb width, comb perimeter, and semen volume. Two wattle measurements were positively correlated with semen volume in HY birds, area and length while wattle width was negatively correlated with sperm mobility. As comb area increased sperm concentration decreased. Contrary to our results, a previous study showed a positive correlation between wattle width and sperm mobility in HY birds (Bilcik & Estevez, 2005). Our results indicate that in FG males, comb width and perimeter can be used as indicators for selecting males with high volume production whereas in HY males a different comb parameter (area) may be used as an indicator to cull males with low reproductive potential (as a result of low sperm concentration). In

addition wattle area and length can be used as indicators for selecting HY males with high semen volume whereas wattle width can be used as an indicator to cull males with low reproductive potential (as a result of low sperm mobility). Both comb size and wattle size have previously been shown to be correlated with semen production (Burrows & Titus, 1939).

### 2.5.3 Conclusion

Sperm mobility increased in both lines of males as they aged. There is both a gonadal and gametic component to competing for mates; males either invest in developing larger and more attractive sexual characters to attract females or they may invest in sperm quality in an effort to out-compete other ejaculations post-copulation. In our experiment males were housed in same sex stud pens. As a result, males did not have to invest energy in resources such as mate guarding, courtship displays, or male-male competition for mates allowing them to invest more in sperm quality. The concept of investing in one strategy or the other, correlates with the results we found when investigating relationships between morphological characters and sperm quality. Hens have been shown to prefer males with longer combs (Zuk *et al*., 1990b) which in our study was negatively correlated with mobility in HY males. Being that HY males possess significantly smaller combs compared to FG males it may be in their best interest to invest more energy into producing quality sperm rather than further enhancing their secondary characters.

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# Chapter 3: Effect of sperm mobility on female fertility persistency

## 3.1 Abstract

Semen quality in poultry can be characterized by different traits including volume, concentration, mobility, viability, and sperm morphology.  To date, sperm mobility phenotype has been shown to be the most reliable indicator of male fertilizing potential in broiler breeder fowl (New Hampshire) under artificial insemination (AI) conditions. The objective of this study was to determine the effects of sperm mobility of broiler breeders on female fertility persistency in layer and broiler hens.  We hypothesized that hens AI with high mobility samples standardized for sperm concentration would retain fertility for a longer duration of time than hens AI with low mobility samples and that these effects would be similar across layer and broiler lines.  We also predicted that that percent and duration of fertility would be positively correlated with sperm quality.  In this study we used 64 Hy-Line layer hens, 37 broiler breeder hens, and 19 primary broiler breeder FG males selected for fast growth.  Samples were collected from 19 males, semen quality was analyzed, and samples were brought back to the university.  Over the course of the experiment a total of 12 high and 11 low semen pools were collected for AI.  All semen pools were used for layer AI, whereas five high and five low mobility pools were used for broiler AI.  Eggs were collected for 15 days post-AI and fertility was determined by egg candling during the second week of incubation.

Results indicated that overall percent fertility was significantly higher in layer than in broiler hens. As expected, there was a significant interaction between sperm mobility and day post-AI in percent fertility in layer hens. Layers AI with high mobility semen pools had higher overall fertility (17.23% ±2.19) than layers AI with low mobility semen pools (7.93% ±1.59). Layers AI with high mobility semen pools exhibited a 4.23% decrease in fertility each day post-AI starting at day two while layer hens AI with low mobility semen pools exhibited a 2.56% decrease in fertility each day post-AI starting at day two. Unlike layers, sperm mobility did not influence fertility in broiler breeder hens. Rather there was a significant effect of day and of semen pool on percent fertility in broilers. Broilers AI with high mobility semen pools exhibited a .8% decrease in fertility each day post-AI. There was a trend in layer hens for percent fertility to increase with increasing sperm mobility and a trend in broiler hens for percent fertility to increase with increasing sperm concentration.

Key Words: sperm mobility, broiler breeder, fertility

### *3.2 Introduction*

Fertility in domestic fowl hens is dependent on multiple factors. These include feed intake, photoperiod, temperature, environmental stress, and age (Brillard, 1993), but also on the mobility and viability of the inseminated sperm (Froman *et al*., 1999). To date, sperm mobility phenotype as measured by the Accudenz assay (Froman & McLean, 1996) has been shown to be the most reliable indicator of male fertilizing potential under

AI conditions in broiler breeder lines (New Hampshire) (Froman *et al*., 1999) in regards to semen characteristics.

The effect of selecting for a faster growth rate in broiler breeder hens has had a negative effect on female reproductive performance. High yielding broiler females usually lay between 160-175 settable eggs/year (Dr. F. Siewerdt, personal communication) whereas commercial laying hens lay in excess of 300 eggs/year (Hy-Line International). Broiler hens have lower fertility rates than laying hens. Part of this relates to the high incidence of abnormal eggs (extra-calcified shells, double yolks, and compress-sided eggs), defective egg syndrome, erratic oviposition, regression of developing follicles, internal laying, and chromosomal abnormalities (Chambers, 1990). In a previous study, researchers found that levels and duration of fertility were negatively correlated with body weight in broiler breeder hens (Bilgili & Renden, 1985). In a study examining the effects in broiler hens of selection over 27 generations for high and low body weight on hatch of fertile eggs and total eggs produced, high body weight hens exhibited an average decrease of .7% and .3% per generation while low body weight hens showed an average increase of .3% and .6% per generation (Dunnington & Siegel, 1985). The consistency of these results provides clear evidence that intensive selection for high growth rate in broiler breeders has come at the cost of an important decline in female fertility. In addition, the problem of reduced fertility is exacerbated if females are mated by males with poor sperm quality, as some of the reduced number of eggs laid by broiler hens may be lost because of lack of fertilization.

Multiple studies have found a positive correlation between sperm mobility and fertility (Froman & McLean, 1996; Froman *et al*., 1997; Froman *et al.*, 1999). Sperm mobility is a quantitative trait in the domestic fowl that represents the net directional movement of a sperm population (Froman & Feltmann, 1998). The Accudenz assay is a measurement tool used to determine sperm mobility (McLean & Froman, 1996). Accudenz is an inert medium that immobile sperm cannot penetrate. During the assay the Accudenz is kept at 41° C, the internal temperature of a hen. This is done to mimic the reproductive environment that sperm will be subject to after insemination (McLean & Froman, 1996). Upon insemination, dead or abnormal sperm are prevented from ascending the hen's vagina as are non-motile sperm. Less than two percent of the initial population inseminated actually makes it past this first barrier to be stored in the SSTs. Cilia at the end of SSTs create a current away from the SSTs. Only sperm that have mobility high enough to prevent being swept away by this current can maintain their position in the SSTs and wait to be released at an opportune time to fertilize an ovum (Froman *et al*., 2006).

While multiple studies have evaluated the impact of sperm mobility in laying hens, only a single study has been conducted in broiler breeder hens (Bowling et al., 2003). It is predicted that similar to layers, broiler breeder females inseminated with high mobility sperm will show higher fertility than those inseminated with low mobility sperm. It is speculated however that the impact of sperm quality, as determined by sperm mobility, may have a higher impact in lines with poor egg laying patterns. If only a few eggs have the potential to be fertilized, a loss of one due to poor sperm quality will have a

greater impact on percent fertility than in hens laying higher numbers of eggs. On the other hand, the more erratic egg laying patterns of broiler breeder hens may reduce the reproductive advantage of high mobility sperm as part of the sperm will be released when no egg may be available for insemination. Therefore the investigation of the impact of sperm mobility on the fertility patterns in layer and broiler breeder hens is of vital relevance to the poultry industry. We hypothesized that hens AI with high mobility semen pools would retain higher levels of fertility for a longer duration than hens AI with low mobility semen pools.

### *3.3 Materials and Methods*

### *3.3.1 Facilities and Experimental Animals*

On February 22nd, 2007 100 day-old female Line 1 Rock broilers and 100 day-old female leghorn layers were obtained from Perdue Farms and Hy-Line International, respectively and transported to the Animal Wing of the Animal & Avian Sciences Building at the University of Maryland, College Park campus. All females were randomly housed in 97.8cm x 68.6cm x 26.7cm (WxLxH) Petersime brooding cages (Petersime Incubator Co., Gettysburg, Ohio) in groups of ten individuals by line in four separate brooder units (rearing density of .067m$^2$/bird). Each brooding cage was partitioned with vinyl strips so that an area of 30.5cm x 68.6cm (WxL) would retain heat for the young chicks. After three weeks the birds were moved to grow-out cages (66.0cm x 71.1cm x 39.4cm) (WxLxH) and randomly divided into groups of five by line (rearing

density of .094m$^2$/bird). Grow-out cages contained a feed trough (63.5cm x 8.9cm x 5.1cm) (WxLxH) and a water trough (63.5cm x 8.9cm x 5.1cm) (WxLxH). On March 23$^{rd}$ two white strings were attached to each cage to discourage pecking of cage mates (Jones *et al*., 1997; Jones *et al*., 2002).

Finally broilers and layers were moved to individual layer cages (45.7cm x 45.7cm x 45.7cm) (Professional Pest Control Products) on June 20$^{th}$ and July 13$^{th}$, respectively. Each cage contained a feed hopper and a nipple drinker. After placement each bird was wing-tagged using Swiftack for Poultry Identification System (Heartland Animal Health, Inc. Fair Play, Missouri).

For the first 44 days of life, temperature was gradually decreased from 33.9° to 21.7° C. Chicks were kept under constant light for the first three days, at which point the light schedule followed was 8L: 16D. At 20 weeks of age photoperiod was adjusted by one-hour increments weekly, reaching the final photoperiod of 16L: 8D at 28 weeks of age. Four different diets were used throughout the course of the experiment: a starter diet (20% crude protein, 2915 kcal/kg metabolizable energy, 0-5 weeks of age), grower diet (18% crude protein, 3000 kcal/kg metabolizable energy, 6-9 weeks of age), finisher diet (16% crude protein, 3075 kcal/kg metabolizable energy, 10-16 weeks of age), and production diet (15.99% crude protein, 2975.7 kcal/kg metabolizable energy, 16 weeks of age through experiment completion). Broilers were feed-restricted according to the guidelines provided by the supplier. Briefly, each broiler was fed *ad libitum* for their first three weeks of life. Broilers were then provided approximately 23g/food/day and

amounts were increased by one to three grams each week.  Peak feed amount was

reached at 35 weeks of age when birds were receiving 145g/food/day.  Layers were fed

*ad libitum*.  Starting on March 26th when birds were four weeks of age, a random sample

of broilers was weighed weekly or bi-weekly to ensure birds were at the target weight

provided by the supplier.  If broiler weights were not on target, feed amounts were

adjusted accordingly.

*3.3.2 Experimental Procedures*

Nineteen FG males as described in Section 2.3.1 were used as semen donors.

Sperm was evaluated on site for volume, concentration and mobility. High and low sperm

mobility samples from FG males were individually extended 1:1 with Beltsville Poultry

Semen Extender (BPSE), placed in a cooler, and brought back to the university.  Based

on the average sperm mobility values obtained during the training period for the

collections, a value above .425 absorbance units was classified as high sperm mobility

whereas a value below .280 absorbance units was classified as low mobility.  Over the

course of the experiment 17 of the 19 FG males contributed to at least one of the semen

pools used for an AI; 12 males contributed to high sperm mobility pools, 7 males

contributed to low mobility pools, with 4 males changing sperm mobility phenotype

during the experiment.  Upon arrival at the university (a trip that ranged from a low of

two and a half hours to a maximum of six hours) samples from three males were pooled

based on mobility phenotype determined at the primary breeder facility earlier that day.

Sometimes we were unable to obtain three samples of either low or high sperm mobility

in which case we pooled the sperm of two males for AI. In general, each trip to the primary broiler facility resulted in one pooled phenotype available for AI at the university.

After pooling, the sample was gently mixed using a 200µL pipette (Gilson pipetman, France). Concentration of the pooled sample was calculated based on the concentration obtained at the primary breeder facility and the proportion of sperm cells each male contributed to the pooled sample. One person gently restrained the hen's legs with one hand while gently applying pressure in a posterior direction while she used her other hand to gently press the tail in an anterior direction. The pressure from each hand everted the hen's cloaca. The second person drew up 100 million sperm cells (as calculated by the pooled concentration, accounting for the 1:1 extension with BPSE) from the pooled sample and inserted the pipette into the everted cloaca to a depth of approximately 3cm from the cloaca's opening into the vagina. The person restraining the hen relieved the pressure applied to the oviduct at which point the inseminator dispensed the semen. We used 100 million sperm cells to AI an equal number of layer and broiler hens. Total volume AI ranged from 55µL to 124µL of an extended pool. All AI were done in the late afternoon or early evening (5:00 PM-9:00PM), a time frame conducive to successful insemination as during this time there is no egg in the reproductive tract that may prevent the successful uptake of sperm (Christensen & Johnston, 1977). A minimum of 21 days was allowed to pass before a hen was reused for an AI to ensure fertility could not be attributed to the previous round of AI.

Based on egg laying patterns at the beginning of the AI trials 37 broiler breeder hens and 64 Hy-Line layer hens were deemed suitable for the experiment. Females with erratic egg laying patterns were excluded from the experiment. Eggs from AI hens were collected starting one day post-AI and collection continued for a total of fifteen days. Eggs were collected daily and stored in a cooler at 7.8° C. The collection for the week was then transferred to the incubator. Settings for incubation were 37.5° C and 65-70% humidity incubated after day eight and after day fifteen post-AI. After seven/eight days of incubation eggs were candled with a small light to determine fertility. Eggs not appearing fertilized were broken open to determine if there was an early dead embryo. A total of 12 high and 11 low semen pools were obtained throughout the study for AI. Midway through the experiment broiler hens were euthanized. As a result, only five high and five low semen pools were available for their AI.

### 3.3.3 Statistical Analysis

Percent fertility was calculated by dividing the total number of fertile eggs by the total number of eggs candled for each female and was analyzed using a mixed model analysis of variance (ANOVA) (SAS Institute V 9.1). The Univariate procedure was used to check for normality and homogeneity of the residual variance. Percent fertility was arc sine transformed prior to analysis to satisfy requirements. Mobility phenotype of pooled sample (high and low), female line (layer and broiler), and their interaction were used as fixed effects, while semen pool nested to male mobility (12 high pools and 11 low pools) served as a random effect. Kenward-Roger was used as the degrees of

freedom method. Estimates statements were used to determine significant differences in percent fertility within and between lines for hens AI with the same or different mobility phenotype. Least square means and standard errors were back transformed for reporting.

A mixed model repeated-measures ANOVA was performed on each female line to examine the effect of day on fertility. Male mobility group, semen pool, day, and all interactions were used as fixed effects. Female ID was entered as a random effect with day of lay entered as a repeated measure. Least square means and standard errors were reported. Linear and quadratic regression models were analyzed for each female line to determine the regression of percent fertility on day of lay.

A Pearson parametric correlation analysis was performed for each female line to determine if a relationship existed between sperm quality of a semen pool (sperm concentration and sperm mobility) and the percent fertility obtained from hens AI with that particular semen pool.

### *3.4 Results*

Our results indicate that laying hens had a significantly higher overall percent fertility as compared to broiler hens ($12.20\% \pm 1.351$ and $0.36\% \pm .328$, respectively; Figure 3.1). There was a significant interaction between male mobility phenotype (High v. Low) and day of lay for percent fertility in laying hens ($F_{14,1857} = 2.70$; p=0.0006;

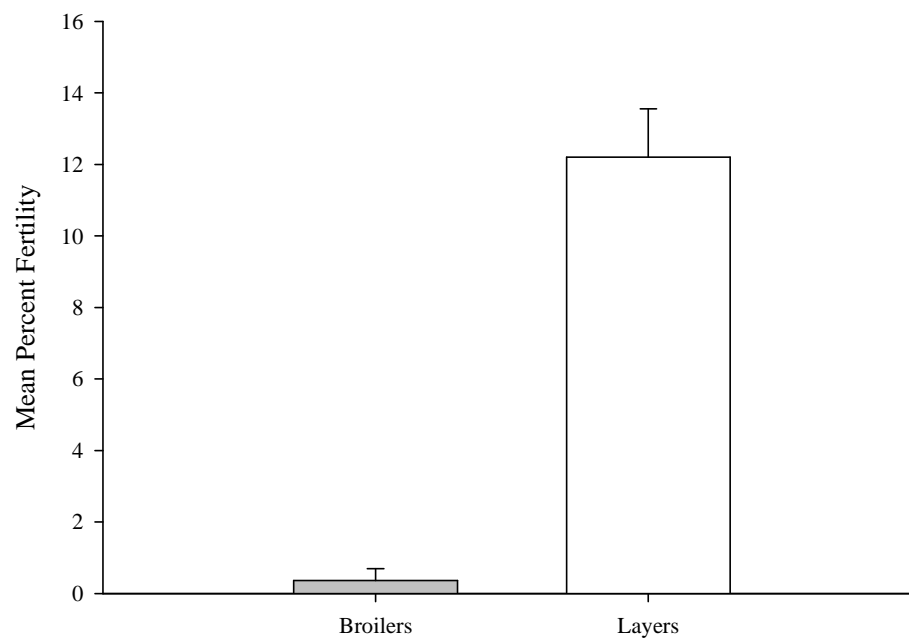**Fig 3.1:** Mean ± SE overall percent fertility for broiler (n=37) and laying hens (n=64)



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Figure 3.2).  In broiler hens there was a significant effect of day of lay ($F_{14,215} = 1.98$;

p=0.0205; Figure 3.3) and a significant effect of particular semen pool used for AI ($F_{9,215}$

= 2.49; p=0.0100; Figure 3.4) on percent fertility.  However, male mobility itself did not

have a significant effect on percent fertility.  Negative values in broiler hens are an

artifact of the type of analysis used.  Because broiler fertility was so low, estimates

obtained from fitting the data sometimes yielded a negative percent fertility in broiler

hens.


Regression analyses indicated a linear relationship of mean percent fertility on

days post-insemination in laying hens AI with either high or low mobility semen pools

and in broilers AI with high mobility pools.  The regression line had higher values for

high mobility pools in layers as compared with low mobility pools.  Laying hens AI with

high mobility pools exhibited a 4.23% decrease in fertility with each successive day post-

insemination (p<0.001; Figure 3.5).  Laying hens AI with low mobility semen pools

exhibited a 2.56% decrease in percent fertility with each successive day post-

insemination (p<0.0001; Figure 3.5).  Broiler hens AI with high mobility pools exhibited

a .8% decrease in fertility with each successive day post-insemination (p=0.0406; Figure

3.5).  Correlation analyses did not yield significant correlations at α=0.05 level in either

female line between percent fertility and either sperm mobility or sperm concentration of

a sample.  There was a positive trend in broiler hens between mean percent fertility and

sperm concentration (r=.58940; p=.0729) and a positive trend in layer hens between

mean percent fertility and sperm mobility (r=.36552; p=0.0863).

**Figure 3.2:** Mean ± SE percent fertility for laying hens AI with either high mobility or low mobility pooled semen samples beginning at two days post-insemination
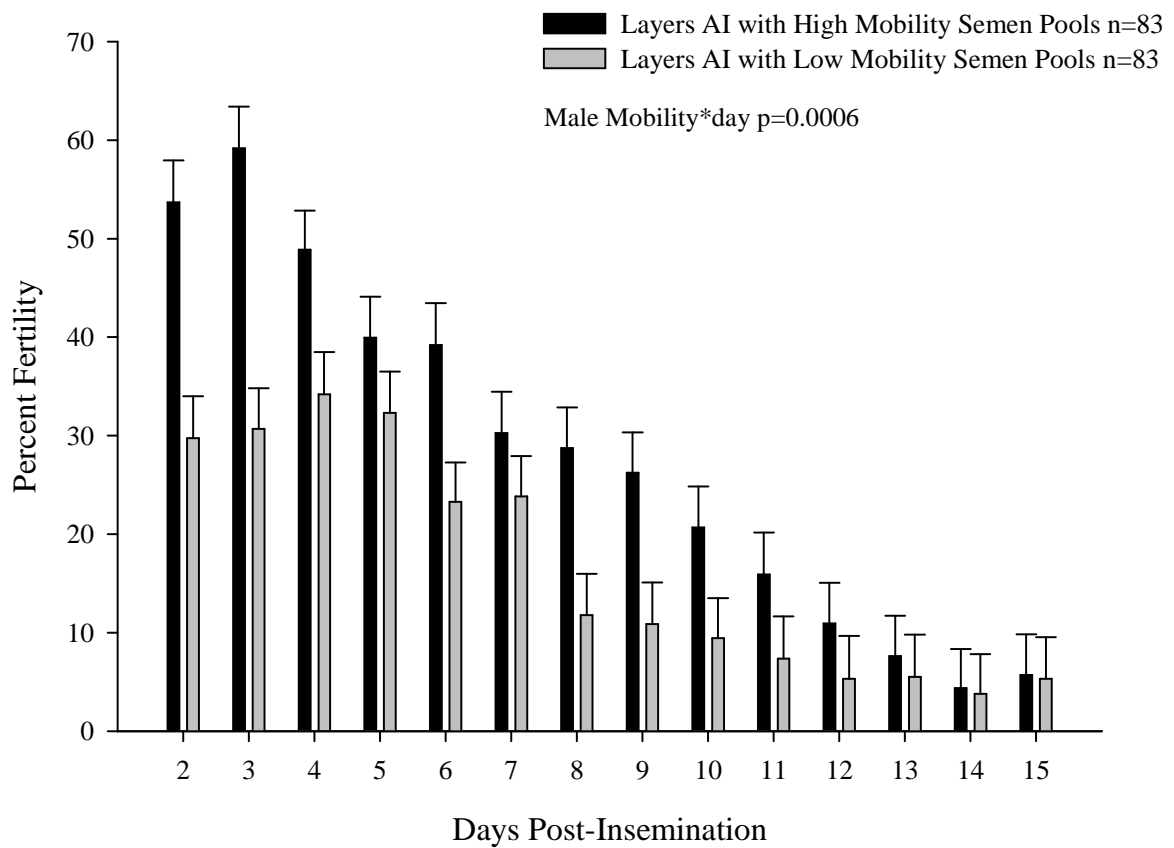
**Figure 3.3:** Mean ± SE percent fertility in broiler hens each day post-insemination starting at day two.

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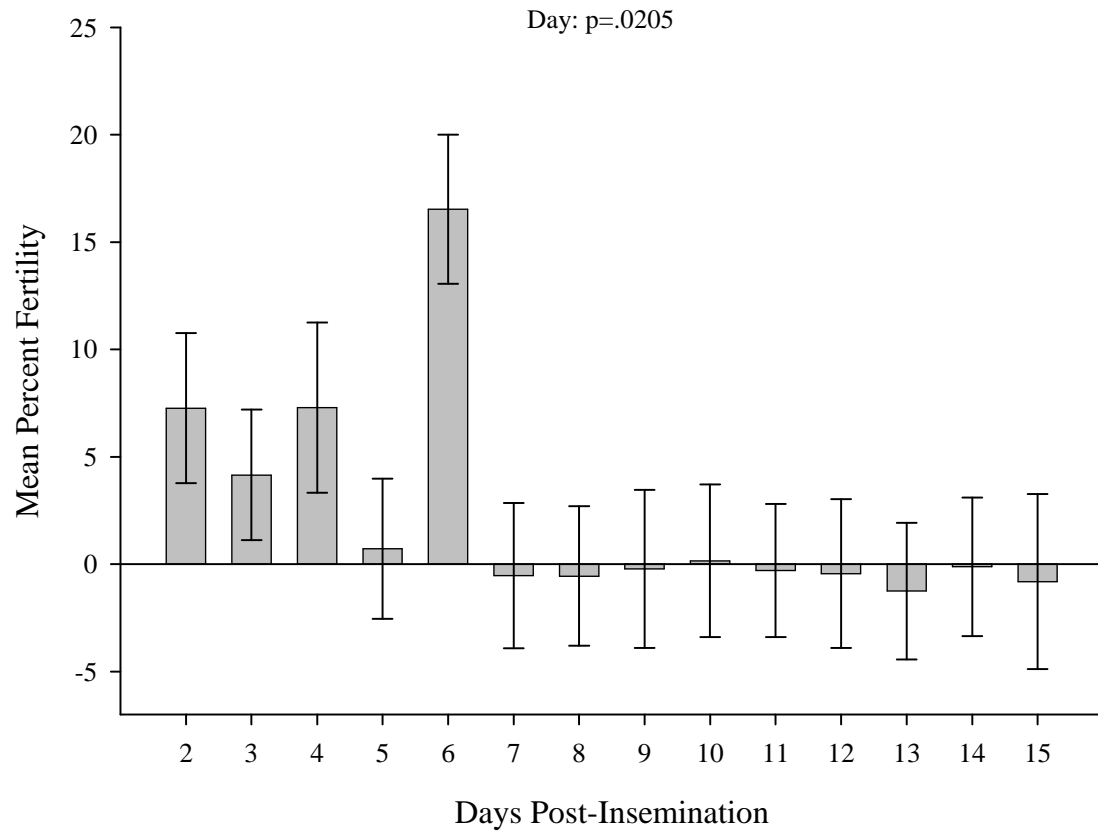
**Figure 3.4:** Mean ± SE percent fertility in broiler hens for each of ten different semen pools. H preceding a semen pool indicates it is a high mobility group and L indicates a low mobility group.

Male Mobility Group p=0.0100

**Figure 3.5:** Regression over days for laying hens AI with high and low pooled mobility samples and broiler hens AI with high mobility samples.



High broilers: y=.10243-.00818x
High layers: y=.61504-.04223x
Low layers: y=.34395-.02560x

## 3.5 Discussion

The purpose of this experiment was to determine the effects of broiler semen quality on fertility persistency in layer and broiler hens. We hypothesized that hens AI with high mobility sperm standardized for sperm concentration would ret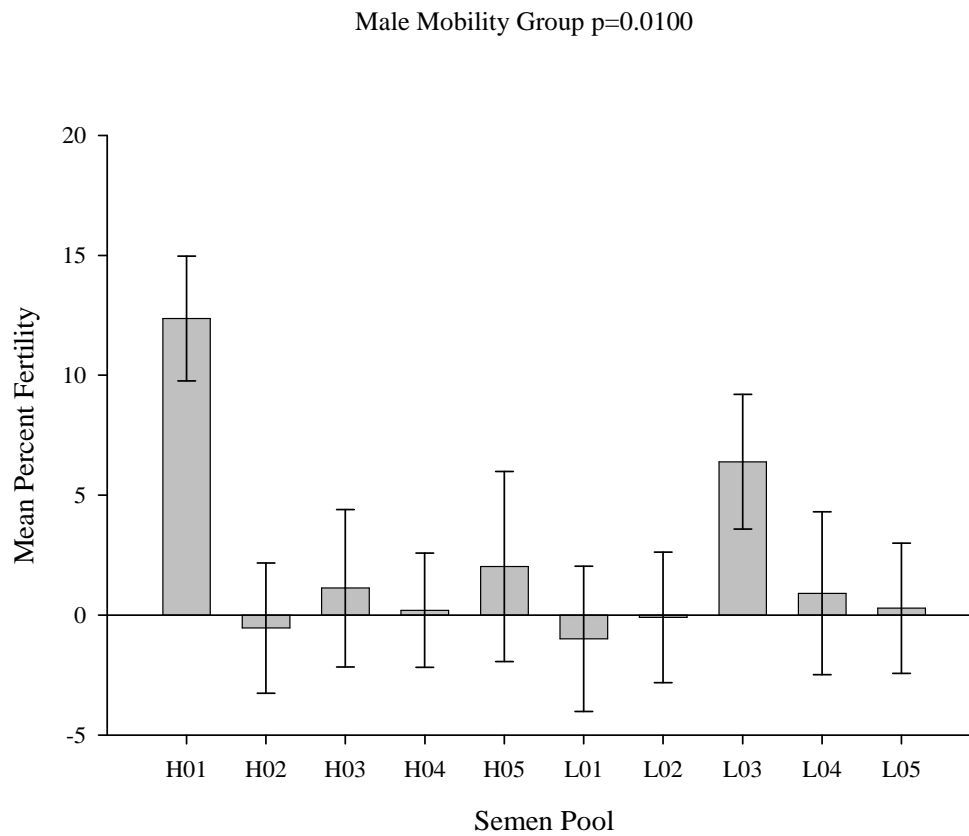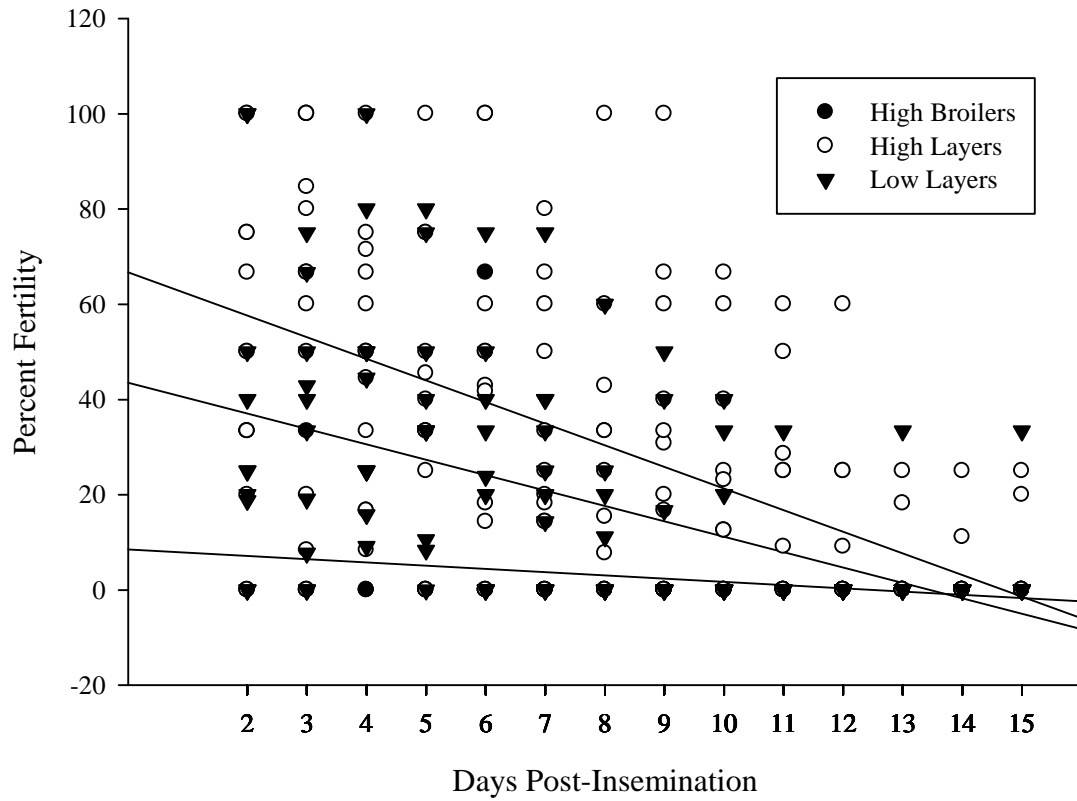ain higher fertility longer than hens AI with low mobility sperm and that these effects would be similar across layer and broiler lines. We also predicted that the impact of sperm mobility would be higher in layers due to the consistency of egg laying patterns as compared with broiler hens.

In our study we found a significant effect of sperm mobility and days post-insemination for layer hens, whereas this relationship was not evident for broiler hens. Layers AI with high mobility sperm reached maximum fertility at three days post-insemination with 59% of eggs fertile, decreasing to 7% by day 15 post-insemination. Layers AI with low mobility sperm obtained maximum percent fertility on day four post-insemination with 32% of eggs fertile, and decreasing progressively by day 15 to 5%. These results are clear evidence of the impact of broiler sperm mobility on the rate of fertilization as hens AI with high sperm mobility maintained a higher rate of fertility as compared with those AI with low mobility sperm. This is in agreement with another study with broiler breeder (New Hampshire) males indicating that the higher fertility persistency of single white comb laying hens AI with high mobility sperm was due to the slower rate of sperm loss in AI with high sperm mobility as compared with low sperm mobility (Froman *et al.,* 2002). Previous research in turkeys also indicated that toms with

high mobility semen used for AI fertilized more eggs and allowed for a longer duration of fertility when compared to toms with low mobility semen (King *et al*., 2000).

In our study overall fertility in layers was and in broilers was substantially lower than results reported in other studies (12.20% ± 1.351 and 0.36% ± .328, respectively). In a study with leghorn hens, a fertility rate over 90% two days post-AI decreasing progressively to 35% by day 11 days post-insemination was reported (Froman *et al*., 1987). Although we observed a similar pattern of fertility decline over time, the lower overall fertility may be related to the timing between sample collection and the time of insemination. Collected samples had to be transported back to the University facilities for insemination of the hens which on average took between two and one-half hours and six hours. Due to biosecurity measures at the breeder facility all analyses were conducted in a separate building at the facility. As a result samples were neither analyzed nor extended immediately following collection.

While sperm mobility was an important determinant of fertility in laying hens it did not appear to affect the poor fertility levels observed in the hens from the broiler line suggesting that fertility problems may be attributable to the female as opposed to a male's semen quality. In a similar study where broiler breeder males of low and high sperm mobility phenotype were used to AI single comb white leghorn hens, researchers found results similar to ours: layers AI with sperm from high sperm mobility males had significantly higher levels of fertility compared to hens AI with sperm from low sperm mobility males (P ≤ 0.0001) (Bowling *et al*., 2003). In broiler hens we found an effect of

the day post-insemination and of semen pool used for AI, independently of whether it was high or low mobility sperm. However these results are quite difficult to interpret. Broilers exhibited their highest fertility levels on day six post-insemination. Since broilers were laying sporadically, it is possible that day six was when they laid their second egg, resulting in the higher fertility levels observed. Interestingly, of the 10 semen pools used to AI broiler hens, the highest percent fertilities were achieved with high mobility semen pool one (.615 absorbance units) and low mobility semen pool three (.219 absorbance units), whereas the lowest fertilities were achieved with low mobility semen pool one (.191 absorbance units) and high mobility semen pool two (.553 absorbance units). In one study looking at the effects of male line on average and duration of fertility, duration of fertility was shown to be primarily a male characteristic (Kirby *et al*., 1998). Five lines of broiler breeder males, one line of subfertile Delaware cross males, four lines of broiler breeder females, and one line of leghorn females were used in the study. In five replicate trials, five males from each line were randomly selected and used to AI 10-12 hens of one of the female lines with $80 \times 10^6$ spermatozoa, Eggs were collected for 21 days and fertility was determined via break outs between four to seven days post incubation. There were no significant male line effects on overall or duration of fertility but within a male line there were large differences among individual males in duration of fertility of hens they AI (durations of fertility between 3.4-14.5 days). Female line did not significantly effect duration of fertility but it did significantly effect 7-day fertility and 21-day fertility suggesting that overall fertility is dependent on females while duration of fertility is dependent on males which exhibit high levels of variation within lines (Kirby *et al*., 1998).

One possible explanation for a lack of sperm mobility effect on fertility in broiler hens may be that we had a smaller sample size (n=37) as compared with the layers with only four broiler hens laying at least one fertile egg. Another likely contributor to these findings was the health and condition of the broiler hens. Broilers reached sexual maturity a few weeks later than what is normal for them, due to an error in their feeding schedule. For a period of approximately 12 weeks (mid-July to mid-October, 20 to 32 weeks of age) birds received 50% less food that what is recommended. We immediately increased the amount of feed in small increments to speed up their development (D.L. Pollock, personal communication). Despite this inadvertent extreme three month feed-restriction, broilers were still overweight for the majority of the experiment. This was likely due to the lack of exercise as a result of being housed in individual cages. High body weight in broiler breeder hens has been shown to negatively correlated with both the number of fertile eggs produced and the duration of fertility (Bilgili & Renden, 1985) and broiler hens with full access to food were found to produce fewer fertile eggs than those restricted (Yu *et al.*, 1992). An increase in breast yield also has been reported to be negatively correlated with fertility in broiler females (Siegel, 1962).

Genetic selection for increased growth and yield traits has resulted in decreased reproduction efficiency in females due to occurrences such as defective egg syndrome and erratic ovulation (Chambers, 1990). The broiler hens in our study laid erratically. For example it was not uncommon to find two eggs laid on one day and then none for several days. We also found eggs with extra calcium depositions, and laid eggs lacking

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shells.  As a result the true effect of sperm mobility on fertility in broiler hens may have

been masked.  Regardless of the sperm mobility and quality of a semen sample, if a hen

is not producing normal eggs at regular intervals fertility levels will consistently be low.


We also found that in broilers there was a positive trend between mean percent

fertility and sperm concentration.  Since all hens were AI with the same number of sperm

cells, this may indicate that concentration is correlated with another parameter that may

positively influence fertility.  Samples that are more concentrated may potentially have

higher viability but this needs to be tested further.


**Conclusion**


In summary, similar to the results of other studies regarding the effect of sperm

mobility in layer lines, we found clearly higher fertility levels when using high mobility

(as compared to low mobility) sperm of broiler breeder males to inseminate laying hens

and these fertility levels remained higher throughout the post-insemination period. These

results are evidence that sperm mobility does impact fertility levels and that sperm

mobility can be used as an indicator of a male's fertilizing potential in broiler breeder

lines.  We also found that in broiler breeder hens used in our experiment, sperm mobility

was not a factor in decreased fertility levels.  This is indicative that fertility problems may

be attributable to females.  However, due to the low sample size, erratic laying, and

weight problems of our broiler hens, studies have to be repeated to determine the true

effect of sperm mobility on fertility for these hens.

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## Chapter 4: Summary and Conclusion

We found that for semen volume, sperm concentration, and sperm mobility, age affected the two lines differently. This was more prevalent early in our study. There were only four times throughout the experiment where males of the two lines differed significantly in their mean values, three of which occurred during the first three weeks of the study. This may be a result of males being housed in single sex stud pens with no access to females. After regular collections and becoming used to regular handling and collection techniques, males began to regularly produce samples. Being that it takes spermatids 14 days to fully develop into spermatozoa, it follows that it would take a period of time for males to produce quality semen samples (Lake, 1984; Kirby & Froman, 2000). These males had been kept in single sex pens for later use as replacement males. Prior to this experiment, males were never collected from. Our results indicate it would be beneficial for males housed in single sex pens to be collected from on a regular basis; this would allow males to be in full semen production upon placement with hens, likely resulting in higher fertility levels.

Our results indicate that contrary to our predictions, sperm quality (as determined by sperm mobility) improves with age (through 54 weeks of age). A possible explanation for increased sperm quality is that initial semen obtained may have been aged and as a result of inferior quality. Since our males were housed in single-sex stud pens with no access to females, there was no need to invest resources in copulation displays or to battle

others for access to females; it makes sense that they would invest more in sperm mobility, a sperm trait positively correlated with fertility.

We found that HY males possessing larger wattles (either in area or length) produced significantly larger ejaculates compared to conspecifics (r=.7203; p=.0082; r=.6364; p=.0261). HY males possess secondary sexual characters significantly smaller than FG males, indicating they invest significantly less in these traits. These males are adopting the strategy of investing more in gonadal components. This is further exemplified in our results indicating that wattle width was negatively correlated with mean sperm mobility (r=-.6833; p=.0424), a quantitative trait that is positively correlated with fertility. Males that did invest in wattle development suffered in regards to fertilizing potential. Further support is garnered in our finding that HY males with a larger comb area tended to produce ejaculates of lower concentration (r=-.5359; p=.0725). Our results indicate that in HY males the wattle can be used as an indicator to determine which males to cull.

Three positive correlations were found, all involving sperm concentration, in FG males and two of these were with comb parameters. Males with a wider comb produced ejaculates with significantly higher sperm concentrations (r=.5466; p=0.0232) as did those with a larger perimeter (r=.4559; p=.0659). Comb size appears to be an adequate predictor as to the concentration of a semen sample. This correlates with the fact that in the commercial turkey industry appendage size (such as the comb) and sperm thickness (concentration) are used to quickly decide which males to keep and which males to cull

(Christensen, 1997). However, sperm concentration is not correlated with sperm mobility, which is the best predictor of male fertility potential (Froman & Feltmann, 1998). It is highly possible that by using a 'quick' method to select highly fecund toms, the commercial industry may potentially be selecting against higher quality toms with higher sperm mobility.

Fertility results provided clear evidence of the impact of broiler sperm mobility on fertilization as layer hens AI with high mobility sperm maintained a higher rate of fertility throughout the post-insemination period as compared with those AI with low mobility sperm. In our study we found a significant effect of sperm mobility and days post-insemination for layer hens, but not for broiler hens. Layers AI with high mobility sperm reached a maximum fertility of 59% three days post-insemination steadily decreasing to 7% by day 15 post-insemination. Layers AI with low mobility sperm obtained maximum percent fertility of 32% on day four post-insemination and steadily decreasing to 5% at day 15 post-insemination. High mobility sperm are lost at a slower rate from a hen's SSTs as they are able to maintain their position against the current generated by cilia surrounding the SSTs. Low mobility sperm cannot maintain their position as long, causing them to be more quickly released from storage. As a result high mobility sperm retain their fertilizing capacity for a longer duration, explaining why layers AI with high mobility semen pools had higher levels of fertility that they maintained throughout the post-insemination period compared to layers AI with low mobility semen pools.

We found that while sperm mobility was an important determinant of fertility in laying hens it did not affect fertility in broiler hens. In broiler hens, we found an effect of the day post-insemination and of semen pool used for AI independently of whether it was high or low mobility sperm. However, due to the small sample size and erratic laying of the broiler hens, no definitive conclusions can be made. We also found that in broilers there was a positive trend between mean percent fertility and sperm concentration. Being that all hens were AI with the same number of sperm cells, this may indicate that sperm concentration is correlated with another parameter that may positively influence fertility in broiler hens.

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