Prospective Approaches to Avoid Flock Fertility Problems: Predictive Assessment of Sperm Function Traits in Poultry

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ABSTRACT This paper discusses why it is important to evaluate males as individuals and how advances made in understanding and measurement of sperm function can be used to improve reproductive efficiency in poultry. Commercial turkey breeding relies on pooling semen from multiple toms. It generally is assumed that sperm in good quality semen from all toms are equally fecund. (Fecund is defined, for males, as an individual whose semen contains a majority of sperm with the potential of producing fertilized eggs, which includes success at all steps in the fertilization process: sperm movement, storage in the hens’ sperm storage tubules, binding and penetrating the periviteline layer, and fertilization.) However, when DNA fingerprinting was used to determine paternity efficiency after pooling ejaculates from seven or more toms, it was found that 18 of 26 males produced very few, or no, offspring. In addition, the traditional measures of poultry semen quality: semen volume, sperm concentration, sperm viability, and subjective motility assessment, were poor predictors of paternity. In recent years, a concentrated effort has been made to develop and evaluate methods that quantify sperm function in poultry. Methods to measure some of these traits are reviewed: sperm motility, sperm storage in the hen, and sperm binding and penetration of the ovum. Data supporting use of these tools for managing flock fertility from the male perspective are explored.

(Key words: male reproduction, semen quality, fertility, sperm concentration)

INTRODUCTION

The purpose of this paper is to discuss prospective approaches to avoid flock fertility problems caused by the male. Utilization of stud farms for managing toms and improvements in lighting schedules and feed control for roosters have been optimized to maximize semen production (see reviews by Bagley, 1995; Sharp, 1995; Reddy, 1995). Yet, routine semen evaluation procedures that might predict the fertilizing potential of an individual are not performed before males are used for breeding. If fertility is to be managed and optimized from the male perspective, assessment of sperm quality and culling of poor males need to be incorporated into management procedures.

Individuals must be evaluated based on sperm traits rather than considered as part of a potential semen pool or “flock”. The argument has been made that, given the large number of individual birds, evaluation of individual fertility potential is too massive an undertaking to include in commercial poultry breeding programs. However, reproductive management in turkeys is already extremely labor-intensive because artificial insemination (AI) is used exclusively for propagation. In addition, if broiler males continue to increase in body weight and skeletal frame, AI may be the only economical means to produce hatching eggs (Reddy, 1995).

Semen evaluation tests are both under- and inappropriately utilized in management of roosters and toms. Adoption of appropriate tests, and culling males on the basis thereof, could improve the effectiveness of the males’ contribution in breeding programs.

In livestock production systems in which AI is practiced, semen analysis is fundamental to sire selection and reproductive management with the minimum goal of identifying males unlikely to produce progeny. However, for production of poultry, evaluation of semen from individuals is limited, at best, to visualization of semen color and crude measurement of ejaculate volume and, possibly, sperm concentration. None of these tests evaluate the quality of sperm! Methods to assess sperm quality and function for poultry are available, but they are not routinely practiced (Amann, 1999). What is the objective of any test of sperm quality?
It is to predict which individual will be of high or low fertility. Hence, they are diagnostic tests. Given this fact, resulting data should be used for predictions and the correctness of such predictions must be established. Success in a diagnostic test is not measured by correlations between values for the trait measured and actual eggs yielding a chick or poult. Correlations hide substantial error. As noted by Hammerstedt (1996), concepts familiar to those establishing endocrine or clinical assays, namely precision, specificity, and sensitivity, can be used to establish the correctness of predictions. Results from many sperm quality assays do not predict fertility (Wishart, 1995), and those that do usually are time consuming or technically difficult. Most methods appropriate for predicting fertility have not been adapted to large-scale application. On commercial farms, sperm quality tests must be compatible with the existing breeder management procedures. Further, many of these semen tests evaluate a single characteristic of sperm (Amann and Hammerstedt, 1993) and do not account for the complex process of fertility, which involves sperm transport and storage in the female tract and sperm binding and penetration of the ovum (Bakst et al., 1994; Robertson et al., 1998). This paper will illustrate why it is important to evaluate males as individuals. Recent advances in understanding and measurement of sperm function will be presented.

EVALUATING PATERNITY EFFICIENCY

Why is it important to evaluate individual males? In turkeys, management practices dictate pooling of semen from ≥ 10 to 15 toms to provide sufficient semen to AI large numbers of hens on a weekly basis. Pooling semen is convenient and it generally is assumed that sperm from each tom are equally fecund. Although it is known that sperm competition exists, the probability for one male’s sperm to fertilize an ovum relative to the sperm of another male is ignored. To establish a relationship between male fecundity and semen characteristics, semen was pooled from multiple toms and DNA fingerprinting methods were used to determine paternity efficiency (Donoghue et al., 1998a). Ejaculates from individual toms also were evaluated using tests representative of those used by the most diligent breeders: semen volume, sperm concentration, viability (using dual fluorescent stains, Donoghue et al., 1995), membrane integrity (using ethidium bromide hypoosmotic stress test; Bakst et al., 1991), and a subjective sperm motility evaluation. Semen was pooled (n = 10 toms per trial) and used to inseminate hens. In all three trials, paternity efficiency was highly skewed (Table 1). In Trial 1, one tom produced 37 of the 70 poults tested (53% of progeny analyzed) and in Trial 2, two toms produced 83% of poults evaluated. In Trial 3, each tom produced progeny, yet 4 of 10 toms produced 76% of the offspring. Considering all three trials, 18 of 26 toms produced very few poults.

### Table 1. Paternity as determined by DNA fingerprinting after pooling semen from several toms

<table>
<thead>
<tr>
<th>Trial</th>
<th>Poults</th>
<th>Tom</th>
<th>Progeny</th>
<th>Percentage paternity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:</td>
<td>70</td>
<td>189</td>
<td>37</td>
<td>52.9 (%)</td>
</tr>
<tr>
<td>1:</td>
<td>186</td>
<td>11</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>1:</td>
<td>187</td>
<td>6</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>1:</td>
<td>184</td>
<td>5</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>1:</td>
<td>182</td>
<td>4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>1:</td>
<td>190</td>
<td>4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>1:</td>
<td>183</td>
<td>3</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>2:</td>
<td>30</td>
<td>191</td>
<td>14</td>
<td>46.7 (%)</td>
</tr>
<tr>
<td>2:</td>
<td>198</td>
<td>11</td>
<td>36.7</td>
<td></td>
</tr>
<tr>
<td>2:</td>
<td>199</td>
<td>2</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>2:</td>
<td>226</td>
<td>1</td>
<td>3.3</td>
<td></td>
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<tr>
<td>2:</td>
<td>196</td>
<td>1</td>
<td>3.3</td>
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<td>2:</td>
<td>197</td>
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<td>0</td>
<td></td>
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<tr>
<td>2:</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3:</td>
<td>45</td>
<td>230</td>
<td>11</td>
<td>24.4 (%)</td>
</tr>
<tr>
<td>3:</td>
<td>227</td>
<td>9</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
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<td>3:</td>
<td>232</td>
<td>6</td>
<td>13.3</td>
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<tr>
<td>3:</td>
<td>234</td>
<td>3</td>
<td>6.7</td>
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<tr>
<td>3:</td>
<td>229</td>
<td>3</td>
<td>6.7</td>
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<tr>
<td>3:</td>
<td>236</td>
<td>2</td>
<td>4.4</td>
<td></td>
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<tr>
<td>3:</td>
<td>235</td>
<td>1</td>
<td>2.2</td>
<td></td>
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<tr>
<td>3:</td>
<td>231</td>
<td>1</td>
<td>2.2</td>
<td></td>
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<tr>
<td>3:</td>
<td>233</td>
<td>1</td>
<td>2.2</td>
<td></td>
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</tbody>
</table>

*Semen collected and pooled by trial using standard industry methods and inseminated weekly for 3 wk into hens (n = 12 per trial).*
REVIEW OF SPERM FUNCTION

Sperm must be motile and survive the environment of the vagina to reach the sperm storage tubules (SST), crypts that store sperm in the hen for extended periods of time. This “reservoir” of sperm within the SST insures that sperm are available between inseminations and ideally secures the sustained probability of fertilization. Upon release from the SST and transport to the infundibulum, the site of fertilization, sperm must be capable of binding to and penetrating the perivitelline layer (a single acellular investment enveloping the ovum at ovulation) and then fertilize the ovum. Success at every step is imperative for fertilization (Figure 1).

What sperm traits should be evaluated? Although simplifying the process, there are some key traits that can be identified. First, sperm must be motile to traverse the vagina and reach the SST. Sperm motility can be measured by many methods and will be reviewed herein. Second, sperm storage in the SST can be quantitatively evaluated. Third, the ability of sperm to bind to the perivitelline layer can be evaluated in vitro. This ability can be measured using a solubilized extract of perivitelline layer from chicken eggs to assess sperm binding in vitro (Barbato et al., 1998). Fourth, the number of sperm present at the site of fertilization at the time of ovulation can be estimated. Finally, retention of fertilization potential by a high proportion of sperm inseminated can be estimated. The number of sperm holes is correlated to number of sperm trapped in the outer perivitelline layer (Staines et al., 1998) and the number of sperm stored in the SST (Wishart, 1995), allowing an indirect measure of SST capacity.

There are many other methods to evaluate quality and function of poultry sperm. These methods include: determining adenoside triphosphate (ATP) or lipid peroxidation of sperm; evaluating sperm morphology, “cell viability” and plasma membrane integrity; and assessing the metabolic state of sperm from individual toms (see review, Wishart, 1995). Results from these assays have demonstrated, to varying degrees, a correlation with the fertility of individual males. However, most of these methods evaluate population averages rather than individual cells in a seminal sample, which is a serious flaw (Amann and Hammerstedt, 1993). Also, many of these methods require technical expertise, expensive equipment, or testing requirements that are not conducive to on-farm evaluation of flock fertility problems.

ASSESSMENT OF SPERM MOTILITY

Sperm are vehicles that carry DNA to the ovum and, therefore, their “motor” is important. Characteristics of sperm motion or percentage of motile sperm are reasonable indicators of sire potential. They have been used by several investigators to evaluate potential fertility (Wilson et al., 1979; Wishart, 1995; Wishart and Palmer, 1986; Froman and McLean, 1996; Donoghue et al., 1998b). Historically, the swirling movement of sperm placed on a microscope slide has been used as a subjective estimate of sperm motility. Data for the Swirl Method have shown good correlation with fertility in chickens and turkeys (Wilson et al., 1979).

An important distinction of some newer methods of assessing sperm motility is objectivity obtained via scoring with an instrument; bias is reduced. One of the first objective sperm motility tests available for poultry sperm was the spectrophotometer technique developed by Wishart and Ross (1985). Fertility of semen from individual roosters and differences in semen from individual toms were highly correlated with the change in light scattering patterns of sperm, measured by a spectrophotometer. This assay utilizes the physical properties of semen: 1) to align sperm in the direction of induced flow of a sample through the instrument “like sticks in a river”, and 2) the fact that sperm in parallel scatter more light than when randomly oriented. The assay measures the change in light scatter for sperm during parallel flow through a tube and then at intervals as they reorient after flow is stopped. The change in the light scattering relates to the velocity of sperm motion and the percentage of motile sperm. When assessing individual roosters, results from this motility assay were strongly correlated with fertility (Wishart and Palmer, 1986). Compared with measurement of sperm ATP concentration or sperm integrity (determined by nigrosin-eosin staining), this motility assay showed the strongest correlation with fertility (Wishart and Palmer, 1986).

The frequency at which sperm within a capillary tube alter a path of light can be used to determine a sperm motility index using a Sperm Quality Analyzer.2 Recently, it was used with avian sperm (Wishart and

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2Introtech, San Diego, CA 92122.
Wilson, 1997; McDaniel et al., 1998). According to McDaniel and coworkers (1998), the sperm motility index, which is influenced by sperm concentration, viability, and motility, is a good indicator of semen quality in breeder male. The Sperm Mobility Test and semen was collected and pooled within phenotype group. Hens were inseminated weekly.

A promising approach for the objective analysis of sperm motion was developed for rooster sperm (Froman and McLean, 1996) and modified for turkey sperm (Donoghue et al., 1998b). This Sperm Mobility Test is based on the ability of sperm to swim into a dense, inert, nontoxic diluent containing Accudenz®. Unlike motility assessment, in which the percentage of moving sperm is estimated, this assay is performed at body temperature (41°C), and measures the proportion of sperm with a powerful and relatively linear forward motion (motility). Such sperm have a greater probability of penetrating the dense solution. The assay might mimic conditions encountered and overcome by sperm in the hen’s reproductive tract. Male-to-male variation in sperm mobility phenotype was estimated using the Sperm Mobility Test and was repeatedly shown to be a normally distributed trait in roosters and toms (Froman and Feltmann, 1997a; Froman et al., 1997a; Froman and Feltmann, 1997b; Holsberger et al., 1998). Sperm mobility is a quantitative trait, and, most importantly, sperm mobility is one determinant of fecundity (Froman and Feltmann, 1998). When toms were selected out of a flock based on the extreme limits of sperm mobility, and used to provide pooled semen, fertility was higher or lower based on the extreme limits of sperm mobility, and used to provide pooled semen, fertility was higher or lower as predicted (Table 2).

When sperm mobility was measured in semen from individual roosters over 20 wk (Froman et al., 1997) and from individual toms over 22 wk (Holsberger et al., 1998), the classifications remained consistent within phenotype. This is an important finding, as sire selection should take place early in production and the benefit of improving semen quality maintained over the entire breeding period. When evaluating individual toms over the course of semen production, Holsberger and coworkers (1998) found no relationship between sperm mobility phenotype and semen volume, sperm concentration, sperm viability, or sperm membrane integrity. Whereas the Sperm Mobility Test was predictive of fertility, data from other semen/sperm characteristics measured were correlated with this trait. When sperm velocity was measured using a computer-aided sperm analysis system, the Hobson Sperm Tracker,® toms ranked as high or average by the Sperm Mobility Test had sperm populations with significantly higher sperm velocity parameters than lower ranked males (King and Donoghue, 1998). Possibly one of the factors that make sperm competitive is the speed with which sperm maneuver through the hen’s reproductive tract.

The Sperm Mobility Test has the potential for predicting the fertilizing ability of potential sires in a commercial setting. Using the Sperm Mobility Test followed by fertility assessment of individual broiler breeder males (5 lines; 200 to 250 roosters), John Kirby’s group at the University of Arkansas found that sperm mobility was highly predictive of observed fertility ($r^2 = 0.88$ to 0.93; Rhoads et al., 1998). In addition, Froman and co-workers (1997) demonstrated that increasing the AI dose from roosters with average sperm mobility did not improve fertility when compared to males with high mobility (Figure 2). These findings clearly demonstrate that sperm mobility is predictive of fertility, and increasing the insemination dose using sperm with compromised function will not improve fertility. An advantage of the Sperm Mobility Test is that it is

### TABLE 2. Effect of sire selection on fertility of pooled semen from toms classified on the basis of sperm mobility phenotype.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Eggs</th>
<th>Fertility</th>
<th>AI Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>High mobile4</td>
<td>553</td>
<td>96 ± 1*</td>
<td>150 million</td>
</tr>
<tr>
<td>Low mobile4</td>
<td>665</td>
<td>90 ± 2</td>
<td>150 million</td>
</tr>
<tr>
<td>High mobile5</td>
<td>3,856</td>
<td>89 ± 4*</td>
<td>75 million</td>
</tr>
<tr>
<td>Low mobile5</td>
<td>3,985</td>
<td>82 ± 1</td>
<td>75 million</td>
</tr>
</tbody>
</table>

1From Donoghue et al. (1998b) with permission.
2Toms were classified on basis of the Sperm Mobility Test and semen was collected and pooled within phenotype group. Hens were inseminated weekly.
3AI = artificial insemination.
410-wk trial.
516-wk trial.
*Differs from Low mobile within trial ($P < 0.05$).

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![FIGURE 2. Comparative fertility of roosters classified by sperm mobility phenotype according to insemination dose. Black bars and clear bars represent fertility of hens inseminated with sperm from roosters classified as high or average mobility phenotype, respectively. Single Comb White Leghorn hens were inseminated weekly for 3 consecutive wk; eggs were collected daily and set over a 3-wk interval. Fertility obtained with the maximal insemination dose from the average phenotype was 9% less than ($P < 0.05$) that obtained with the minimal insemination from the high sperm mobility phenotype. Adapted from Froman et al. (1997) with permission. Mean dosages with no common letters differ significantly ($P < 0.05$).](https://academic.oup.com/ps/article-abstract/78/3/437/1548865)
SYMPOSIUM: MANAGING POULTRY REPRODUCTION TO SATISFY MARKET DEMANDS

OBJECTIVE, SIMPLE, REQUIRES LITTLE TECHNICAL EXPERTISE, AND IS CONSISTENT OVER THE REPRODUCTIVE LIFE OF A MALE.

QUANTIFICATION OF SPERM STORAGE IN THE HEN

Sperm storage in the hen is a critical component of sperm function because stored sperm maintain hen fertility between inseminations. An intensive effort has been made to quantify sperm in the hens’ reproductive tract to estimate flock and individual hen fertility (Brillard and Antoine, 1990; Brillard and Bakst, 1990; Brillard, 1993; Wishart, 1995). Methods have been developed that use freshly laid eggs to gain insight into sperm numbers in the SST (Brillard and Bakst, 1990; Bramwell et al., 1995, 1996; Wishart, 1995; Wishart and Staines, 1999). One such assay relies on determining the number of holes created by sperm hydrolyzing a passageway through the perivitelline layer of an ovum in the infundibulum during an approximately 15-min interval (approximately 24 to 26 h before oviposition; Bramwell et al., 1995). The number of sperm holes is correlated with the number of sperm stored in the SST, allowing an indirect estimate of SST capacity (Wishart, 1995; Wishart and Staines, 1995).

Although this assay has been used extensively to evaluate and predict flock fertility, it also has the potential to detect male-to-male differences. Semen from toms classified as high, average, or low mobility phenotype (based on the Sperm Mobility Test) was pooled by group and used to inseminate hens. The mean numbers of holes observed for eggs from hens inseminated with high or average sperm mobility samples were almost four times greater than that for low mobility samples (Figure 3). Hence, sperm storage in the hen or the number of sperm at the site of fertilization, is reduced in toms producing semen containing low mobility sperm (Donoghue, Holsberger and King, unpublished data).

Evaluating individual broiler breeders, Robertson et al. (1998) found striking differences among males in the number of sperm holes observed in freshly laid eggs using the traditional in vivo assay and also by a modified in vitro method. Fertility was highly correlated with the number of sperm holes when low insemination doses were used.

SPERM-BINDING ASSAY

The ability of sperm to bind to the perivitelline layer is a pivotal step in fertilization. A sperm-binding assay was developed to mimic this step in vitro and to quantify the number of sperm bound (Barbato et al., 1998). This assay utilizes microwell plates coated with an extract of chicken perivitelline layer. Depending on the sample, a percentage of sperm in the sample bind to the microwell, unbound sperm are washed away, and bound sperm are quantified. Differences in sperm quality for semen from individual roosters or toms can be detected. Differences in sperm binding are predictive of fertility in several different lines of chickens (Barbato et al., 1998; Barbato, 1999).

FIGURE 3. Frequency of the number of points of hydrolysis in the perivitelline layer of turkey hens inseminated (AI) with sperm from toms classified as High, Average, or Low Mobility Phenotype as determined by the Sperm Mobility Test. Hens (n = 8 per treatment) were inseminated once before the onset of lay and data were collected beginning Day 2 after insemination through Day 35.
When toms were selected based on percentage sperm bound using a commercial version of this assay, and their semen were pooled and used to inseminate hens on a weekly AI schedule (75 million sperm per dose), differences in fertility were apparent by the 8th wk of AI (Figure 4). Fertility from hens inseminated with sperm from toms classified as “bad” continued to decline through the end of the study, whereas the fertility for hens inseminated with sperm from males classified as “good” remained high. It is well-documented that fertility declines over the course of the production period. If the data in Figure 4 had been collected only during the first 3 to 6 wk, the differences in fertility might have gone undetected. This finding is an important point when analyzing any sperm assessment test; fertility should be monitored over an extended period of time.

Unlike the sperm hole assay, the Sperm-Binding Assay eliminates the hen effect while enabling identification of toms with low fertility. The binding assay requires some technical skill and time. Data can be gathered on-site by incubating a sperm suspension of known concentration on an assay plate, followed by washing away unbound sperm. Final analysis and ranking of males is done off-site and involves special equipment. It has been adapted and simplified for use in commercial flocks (Gill et al., 1998).

**CONCLUSIONS**

The impact of sire selection based on tests that predict sperm fertilizing potential would be extremely beneficial to the poultry industry. The ability to identify males with low fertility potential would provide a rational, objective approach to cull such toms from a flock. Determining what sperm traits are associated with the sperm-selection process in the hen could translate into methods of screening potential sires. The Sperm Mobilization Test, the Sperm-Hole Assay, and the Sperm-Binding Assay are predictive of fertility with sperm from roosters and toms and show promise as management tools for sire selection. Culling males with low fertility potential should increase number of chicks or poult produced and improve the overall efficiency of managing flocks.

**REFERENCES**


