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Semen Donor Selection by *In Vitro* Sperm Mobility Increases Fertility and Semen Storage in the Turkey Hen

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ABSTRACT: Commercial turkey production relies on the artificial insemination (AI) of pooled semen. However, semen quality ultimately depends on the quality of individual ejaculates. The purpose of this study was to evaluate semen from individual toms by means of an objective sperm-mobility assay. Semen was then pooled by mobility phenotype and inseminated into hens, and the percentages of fertile and hatched eggs were determined after egg incubation. To indirectly evaluate hens' sperm storage, we determined the number of sperm holes in the perivitelline layer (PL) of freshly laid eggs. Semen from individual ejaculates (two trials, total of 169 toms) was evaluated by use of the sperm-mobility test (SMT). Semen was diluted to 1×10^9 sperm/ml, in prewarmed N-tris-[hydroxymethyl] methyl-2-amino-ethanesulfonic acid (TES)-buffered saline, and was placed over 3 ml of a 2% (w/v) Accudenz solution at 41°C. After a 5-minute incubation period, the cuvette was placed in a densimeter, and percentage transmission was recorded after 1 minute. Semen samples from toms ranked, according to sperm mobility, in the highest 10% and the lowest 10%, after three evaluations, were pooled by group and were used to inseminate hens weekly (trial 1: $n = 20$ hens/group, for 10 weeks, AI dose = 150×10^6 spermatozoa inseminated fresh and after 24-hour *in vitro* storage at 5°C; trial 2: $n = 60$ hens/group, for 16 weeks, AI dose = 75×10^6 spermatozoa inseminated fresh). Each week, eggs from day 6 post-AI were evaluated for holes in the PL, vestiges of acrosomal induced hydrolysis. Spermatozoa from toms of different mobility phenotypes were also evaluated individually, for sperm chromatin structure and motility variables, by use of the Hobson Sperm Tracker. Toms characterized by high and low *in vitro* sperm-mobility phenotype were categorized as "high mobility" and "low mobility," respectively. The percentage of fertile eggs from hens inseminated with semen from the high-mobility toms was higher than the percentage of fertile eggs from the low-mobility group, in each trial (95.8 ± 1.3% vs. 90.4 ± 2.2%, and 88.7 ± 4.0% vs. 82.4 ± 0.4%, trials 1 and 2, respectively; $P < 0.05$). More sperm holes were observed in the PL of eggs fertilized by the high-mobility toms than in the PL of eggs fertilized by the low-mobility toms ($P < 0.05$). No differences in susceptibility of sperm nuclear DNA to denature *in situ*, as measured by the flow-cytometric sperm chromatin-structure assay, were detected between toms of differing mobility phenotypes. Sperm-motility variables, straight-line velocity, and average-path velocity were significantly greater for high-mobility toms compared to low-mobility toms ($P < 0.05$). Sperm-mobility differences between toms (detected by means of the SMT) influenced sperm storage, as indicated by the number of sperm in the PL and by the percentage of fertile eggs produced.

Key words: Motility, sperm storage, CASA.

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Sustained production of fertile eggs by turkey hens depends, in part, on spermatozoa entering the oviducts' sperm-storage tubules (SST). These specialized structures are located at the juncture of the vagina and the shell gland. A select population of motile spermatozoa reach this area, after insemination, and are stored in the SST (Bakst et al, 1994). Therefore, sperm motility is a variable that affects reproductive efficiency. Traditionally, the investigation of poultry-sperm motility has been constrained by either an inability or a limited ability to measure motility under physiologic conditions. An objective sperm-mobility assay, the sperm-mobility test (SMT), has recently been developed for the rooster (Froman and McLean, 1996). The mobility test is based on the ability of subpopulations of highly motile spermatozoa to penetrate a solution of Accudenz, from a sperm-suspension overlay, at body temperature. Therefore, the SMT measures sperm mobility more aptly than does the traditional estimate of sperm motility on a microscope slide. The present study was designed to determine whether *in vitro* sperm mobility was related to sperm storage, in the hen, and to fertility. We also wanted to determine whether selection of toms by mobility phenotype would influence spermatozoa fertilization potential after *in vitro* storage for 24 hours at 5°C. Sperm storage in the hen was estimated by means of a sperm–egg penetration assay that determines the number of holes produced by sperm hydrolysis of the egg membrane around the time of fertilization (Wishart and Wood, 1994; Bramwell et al, 1995; Donoghue, 1996). This assay is correlated with the ability of the hen to store spermatozoa in the SST (Brillard and Antoine, 1990; Bril-

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lard and Bakst, 1990; Wishart, 1995). Sperm chromatin stability and computer-assisted semen analysis were used to evaluate spermatozoa from toms of differing mobility phenotypes.

Materials and Methods

Animals and Semen Collection

Large White breeder toms and Large White and Medium White hen poults were purchased from a primary breeder (British United Turkeys of America, Lewisburg, West Virginia) and were maintained under standard management conditions during brooding and growing periods. At 28 weeks of age, toms were photo-stimulated, by increasing light exposure from a 12:12 to a 14:10 light-dark cycle, to stimulate semen production. Semen was collected from toms, by means of the abdominal massage method (Burrows and Quinn, 1935), beginning at 30 weeks of age.

Selection of Semen Donors

Donor selection was initiated at 31 weeks and was completed by 33 weeks of age. For each trial, semen donors were selected from a flock of approximately 100 toms. Toms included in the selection process were those evaluated three separate times by the sperm-mobility assay, (n = 75 toms, for trial 1, and n = 94 toms, for trial 2). Sperm mobility was measured by use of the SMT, developed by Froman and McLean (1996) and modified for the turkey. Semen from each tom was diluted, to 1×10^9 sperm/ml, with motility buffer (50 mM N-tris-[hydroxymethyl] methyl-2-amino-ethanesulfonic acid (TES) buffer, pH 7.4, 120 mM NaCl, 10 mM glucose, 2 mM $CaCl_2$). A 300-µl volume of diluted semen was layered on 3 ml 2% (w/v) Accudenz (Accurate Chemical and Scientific Corp., Westbury, New York) solution that had been prewarmed to 41°C in a disposable cuvette. The cuvette was incubated for 5 minutes in a 41°C water bath, and the percentage transmission was measured 1 minute after the cuvette was loaded into a densimeter (Animal Reproduction Systems, Chino, California). Results are expressed as "blocked transmission," which is equal to 100 minus the percentage transmission. Replicate scores were averaged and toms were ranked, from highest to lowest mobility, by blocked-transmission averages. Toms with the highest and lowest mobility rankings, based on mobility scores, were used in the studies. For trial 1, nine toms per mobility phenotype were used, and for trial 2, six toms per mobility phenotype were used.

Trial 1. Comparison of Mobility Phenotypes, on Percentage of Fertilized Eggs After Insemination with Fresh or 24-Hour Stored Semen, and Sperm Chromatin-Structure Assessment

Ejaculates from selected high- and low-mobility toms were pooled, by phenotype, and were extended 1:1 with Beltsville Poultry Semen Extender (BPSE). Diluted semen was evaluated for sperm concentration and mobility and was then placed in 10-ml Erlenmeyer flasks and covered loosely with foil. Flasks were placed in beakers that contained enough water to reach the upper level of semen in the flask, and beakers were placed on an orbital shaker (150 rpm.), in a refrigerator, at 5°C. Hens were inseminated either 3 hours after semen collection ("fresh") or after semen had been stored 24 hours *in vitro* ("stored").

Large White breeder turkey hens were photo-stimulated, at 32 weeks of age, by increasing the daily light-dark cycle from 6:18 to 14:10, to stimulate ovarian development and egg production. Hens were initially inseminated at 14 and 16 days after photostimulation and then once a week for the next 10 weeks. In each case, the insemination dose was 150×10^6 viable spermatozoa. The percent viable spermatozoa was determined by use of dual fluorescence, the SYBR/propidium iodide method for evaluation of turkey spermatozoa, validated elsewhere (Donoghue et al, 1995a). Four treatment groups, consisting of 20 hens per group, were established: high-mobile, fresh spermatozoa; low-mobile, fresh spermatozoa; high-mobile, stored spermatozoa, and low-mobile, stored spermatozoa. Eggs were candled, at 7–10 days of incubation, to determine whether they were fertile. Fertile eggs were incubated and were assessed 28 days later, to determine hatch of fertilized eggs.

Trial 2. Comparison of Mobility Phenotypes, With Fresh Semen, on Sperm Storage in the Hen, Percentage of Fertile Eggs, and Computer-Assisted Sperm-Motility Analysis

Semen was collected from toms that were selected for high- and low-mobility spermatozoa, pooled by phenotype, and processed as described for trial 1. Medium White turkey hens were photostimulated at 32 weeks of age, as described above. Hens were initially inseminated 14 and 16 days after increased light and then once a week, for the next 16 weeks, with 75×10^6 viable spermatozoa. Treatment groups consisted of 60 hens per group for high-mobile sperm and low-mobile sperm groups, and hens were inseminated within 3 hours of semen collection.

Determination of Sperm Holes in Freshly Laid Eggs

The perivitelline-hole assay was used as an indicator of hens' sperm storage and fertilization. Twenty eggs per treatment group were collected every week, on day 6 after AI, by use of a modified procedure (Donoghue, 1996) of Bramwell and coworkers (1995). The ovum was separated from the albumen. The chalaziferous layer over the vicinity of the germinal disc was removed by use of a Kimwipe. The ovum was placed in 2% (w/v) NaCl so that the germinal disc could come in contact with the solution for 30 seconds. A 40-mm × 20-mm piece (large enough to fit on a standard microscope slide) of perivitelline layer (PL) was cut such that the area over the germinal disc was in the center of the section. The PL was washed in phosphate-buffered saline (PBS), to remove any remaining yolk, was spread on a microscope slide, and was then fixed, with two drops of 3% formaldehyde in PBS. After 20 seconds, the slide was tipped, to allow fixative to drain off the slide. Two drops of undiluted Schiffs' Reagent (Sigma Chemical Co., St. Louis, Missouri) were then placed on the PL section. After the PL was stained a rich magenta, the slide was drained of the Schiffs' stain. By use of a 10× objective on a bright-field microscope, the germinal disc was centered in the field of view, and all holes in the field (circular unstained areas in the PL) were counted.

Table 1. *Results of AI with fresh or stored semen from high-mobility or low-mobility toms, trial 1*

Semen type and sperm mobility	Eggs (*n*)	Fertility* (%)	Hatchability* (%)	Hatch of fertile eggs (%)
Fresh semen				
High mobility	553	95.8 ± 1.3†	84.9 ± 2.9†	85†
Low mobility	665	90.4 ± 2.2‡	80.2 ± 1.8†	89†
Stored semen				
High mobility	698	75.5 ± 4.1§	60.7 ± 8.7‡	87†
Low mobility	610	74.7 ± 3.4§	66.4 ± 3.5‡	84†

See Materials and Methods for descriptions of high-mobility and low-mobility phenotypes.
* Mean ± SEM.
†, ‡, § Within columns, different symbols indicate significant differences ($P < 0.05$).

Table 2. *Mean density of sperm holes in the PL, and fertility and hatchability of eggs, after AI of turkey hens with semen from high-mobility or low-mobility toms, trial 2*

	High-mobility sperm	Low-mobility sperm
Sperm holes in the PL		
Eggs (*n*)	278	259
Sperm holes (*n*)*	10.6 ± 1.2†	6.4 ± 0.8‡
Fertility and hatchability		
Eggs (*n*)	3,856	3,985
Fertile eggs (%)*	88.7 ± 0.5†	82.4 ± 0.6‡
Hatchability (%)*	80.5 ± 0.6†	74.0 ± 0.7‡
Hatch of fertile eggs (%)*	90†	89†

See Materials and Methods for descriptions of high-mobility and low-mobility phenotypes.
* Mean ± SEM.
†, ‡ Within rows, different symbols indicate significant differences ($P < 0.05$).

Determination of Sperm Chromatin Structure

Ejaculates were collected from selected high- and low-mobility–phenotype toms in trial 2, sperm concentration was determined, and 100 µl of neat semen was frozen in liquid nitrogen. Sperm DNA susceptibility to denaturation *in situ* was assessed by means of the sperm chromatin-structure assay (SCSA; Evenson and Jost, 1994), which was modified for turkeys. In brief, samples were diluted to 1–2 × 10^6 spermatozoa/ml and were stained by a two-step acridine orange (AO) staining procedure. AO (Polysciences Inc., Warrington, Pennsylvania) was dissolved, in double-distilled water, to a final concentration of 1 mg/ml stock solution. Acid-detergent treatment for the first step was composed of 0.15 M NaCl, 0.1% Triton X-100 (Sigma), 0.08 N HCl, pH 1.2, in double-distilled water. The buffer solution for the second step in the procedure contained 370 µl of 0.1 M citric acid buffer, with 630 ml 0.2M Na_2PO_4 buffer, 372 mg $Na_{(2)}$-EDTA, and 8.77 g NaCl, at pH 6.0. The staining solution consisted of 0.6 ml of AO stock added to 100 ml of buffer solution. Spermatozoa were stained by mixing 400 µl of Triton X acid solution at 4°C. After 30 seconds, 1.2 ml of the AO staining solution was added, and the samples were placed in a flow cytometer chamber. All flow-cytometric measurements were made as the cells passed through the quartz flow channel of a Cytofluorograf 30 that was interfaced to a 2151 Data Handler (Ortho Diagnostics, Westwood, Massachusetts). The flow cytometer was equipped with ultrasense optics and a Lexel 100-mW argon ion laser, operated at 35 mW, with an excitation wavelength of 488 nm. After staining, 5,000 cells were measured, by the flow cytometer, at a rate of approximately 200 cells/second. Recorded measurements were begun 3 minutes after staining, which allowed time for equilibration of the sample flow in the sheath flow. Red fluorescence (630 nm–long pass filter) and green fluorescence (515–530–band pass filter), emitted from individual cells, were separated optically, and digitized signals processed in peak mode were recorded as list mode on the computer disk.

Assessment of Sperm Motility by Computer-Assisted Sperm-Motility Analysis

Motile properties of individual spermatozoa, from ejaculates of 12 toms, were evaluated, in duplicate, with a Hobson Sperm Tracker (Biogenics, Napa, California). Sperm concentration was determined, and semen was diluted, to 25 × 10^6 sperm/ml, with motility buffer. Diluted samples were warmed to 41°C for 5 minutes, and then 3 µl was injected into a 20-µm microcell slide (Conception Technologies, San Diego, California). Sperm-motility variables were estimated, using a 20× objective, and 200 sperm tracks per sample were evaluated.

Statistical Analysis

Percent fertilized eggs and hatch of fertilized eggs were analyzed by use of the logs odd model described by Kirby and Froman (1991). Density of holes in the PL and motile attributes derived by computer assisted sperm analysis (CASA) of individual spermatozoa from differing mobility-phenotype groups were examined by use of analysis of variance and the general linear models procedure (SAS Institute, 1985).

Results

Mobility phenotype influenced the percentage of fertilized eggs, when hens were inseminated within 3 hours of semen collection, for both trials ($P < 0.05$; Tables 1 and 2). Donor phenotype did not influence the fertilizing ability of semen that had been stored 24 hours *in vitro* before insemination (Table 1). The overall percentage of fertile eggs was lower for stored semen ($P < 0.05$), over the 10 weeks of trial 1, than for fresh semen. When fertility was viewed by week in trial 2, there was no difference in the percentage of fertilized eggs during the first 4 weeks of the fertility trial; thereafter, the percentage of fertilized eggs was higher ($P < 0.05$) for the high-mobility phenotype than for the low-mobility phenotype, for 11 of the 12 remaining weeks of the study (Fig. 1). The percentage of hatched eggs was not different ($P > 0.05$) between phenotypes for trial 1 but was significantly higher in the high-mobility phenotype group in trial 2 ($P < 0.05$, Table

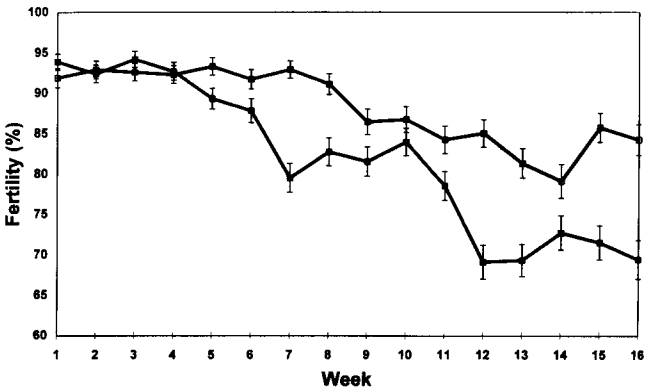

FIG. 1. Turkey hen fertility (%), by weeks, after AI with semen from high-mobility (□) or low-mobility (■) phenotype toms (trial 2). See Materials and Methods for descriptions of high-mobility and low-mobility phenotypes.

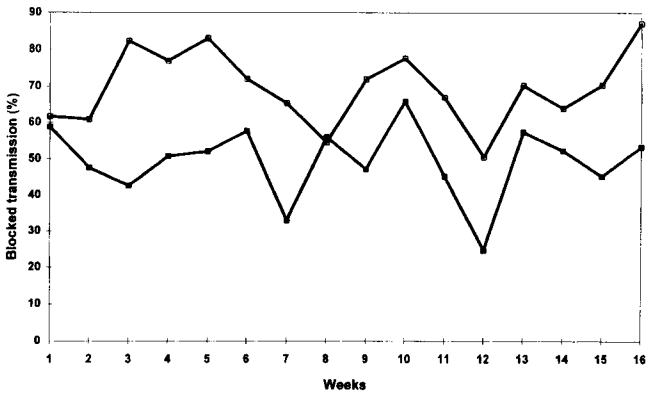

FIG. 2. Weekly sperm-mobility assessment of semen from high-mobility (□) or low-mobility (■) phenotype toms (trial 2). See Materials and Methods for descriptions of high-mobility and low-mobility phenotypes. Blocked transmission = 100 minus the percentage transmission.

2). Hatch of fertilized eggs was not different between groups, for either trial, or between fresh and stored semen (Tables 1 and 2).

Weekly mobility measurements for pooled semen were consistently higher for the high-mobility group than for the low-mobility group in trial 1 (data not shown), and this was also true in trial 2, except during week 7, when mobility was similar (Fig. 2). Density of PL sperm holes was consistently greater in eggs from the high-mobility group than in eggs from the low-mobility group, over the course of the study ($P < 0.05$, Table 2).

The SCSA measures the susceptibility of DNA, in sperm chromatin, to acid-induced denaturation. Acid-treated sperm were treated with AO and were exposed to 488 nm light. AO intercalated into the double-stranded (normal) DNA fluoresced green, whereas denatured, single-stranded DNA fluoresced red. The degree of normality/abnormality is expressed by the term "αt" which is the ratio of red to red + green fluorescence, for each sperm in the sample population (Evenson and Jost, 1994). Higher αt indicates increased levels of DNA abnormality. The αt for the high-mobility and low-mobility phenotypes appeared to be within the normal range for viable sperm from other species and were not different (170.7 ± .4 and 169.7 ± 1.7, respectively [$P > 0.05$]). Similarly, the SD of the distribution, a measure of the extent of chromatin abnormality, was not different ($P > 0.05$) between semen from toms of the high-mobility and low-mobility phenotypes.

Evaluation of sperm-motility variables by use of the Hobson Sperm Tracker resulted in consistently higher values for sperm-velocity measurements for the high-mobility than for the low-mobility pooled samples. Straight-line velocity (VSL) and average-path velocity (VAP) measured 37% and 25% faster, respectively, over 400 sperm tracks for the high-mobility group than for the low-mobility group ($P < 0.05$; Table 3). Beat cross frequency was also higher ($P < 0.05$) for the high-mobility group, whereas amplitude of lateral head displacement was not different (Table 3).

Discussion

In vitro sperm-mobility differences between toms influenced the percentage of fertilized eggs hens produced when they were inseminated within 3 hours of semen collection. When toms were selected on the basis of the extremes of high or low sperm mobility, the percentage of fertilized eggs was higher or lower, respective of phenotype. Importantly, mobility of individual tom spermatozoa before the initiation of fertility trials remained consistent, within phenotype, when semen was pooled and used throughout the study. Sperm-mobility analysis could be

Table 3. *Sperm mobility variables, estimated by means of computer-assisted sperm analysis, for high-mobility and low-mobility toms, trial 2*

Phenotype*	Straight-line velocity (μm)†	Average-path velocity (μm)†	Beat cross frequency†	ALH (μm)‡
High mobility	45.5 ± 3.6‡	60.3 ± 4.1‡	16.6 ± 0.7‡	4.4 ± 0.3‡
Low mobility	28.8 ± 3.6§	45.1 ± 3.2§	11.3 ± 1.1§	4.9 ± 0.3‡

* See Materials and Methods for descriptions of high-mobility and low-mobility phenotypes.
† Mean ± SEM; 400 tracks per male, per phenotype.
‡, § Within columns, different symbols indicate significant differences ($P < 0.05$).

an important predictive test for selection of toms for fertilization potential, a characteristic observed when the SMT was used to differentiate between a population of roosters with higher-than-average subpopulations of mobile spermatozoa (Froman et al., 1997). When hens were inseminated with 50×10^6 spermatozoa per week over a 14-week study, fertility was 10% higher for hens inseminated with sperm from the high-mobility group than for hens inseminated with sperm from the average-mobility group (Froman et al., 1997), which demonstrates that males of high fertilization potential could be identified within a flock. Physiologically, the high-mobility spermatozoa may have a competitive advantage over the low-mobility spermatozoa, with respect to sperm selection, through the vagina, and storage in the SST. Conversely, low-mobility spermatozoa may not provide the numbers of spermatozoa necessary to maintain consistent production of fertile eggs between inseminations. Empirical observations suggest that embryo mortality increases as the hen approaches the end of her fertile period (Bakst, personal communication). Fewer spermatozoa in the SST, as a result of AI of low-mobility sperm, may mimic such an event. Although hatch of fertilized eggs was not different between mobility phenotypes, percentage of total hatchable eggs was different in trial 2 but not in trial 1. The larger number of eggs set (almost 4,000/treatment) in trial 2 compared to the smaller sample size in trial 1 (~250 eggs/treatment) may account for these differences. Reduced hatch of eggs from low-mobility–phenotype toms suggests a link between sperm mobility and early embryo mortality. Although not the focus of the current study, use of the SMT could provide a means to investigate male influence on early embryo development.

Interestingly, the percentage of fertilized eggs in trial 2 was similar between the two phenotypes during the first 4 weeks of insemination, and then fertility declined in the low-mobility group (Fig. 2), a pattern observed in *in vitro* stored-semen studies, in which fertility consistently drops after the first 4–7 weeks of egg production (Sexton, 1988; Bakst et al., 1991; Donoghue et al., 1995; Thurston 1995) with 24-hour *in vitro* stored semen. Since hens are most efficient at producing fertilized eggs at the beginning of egg production, the effect of using "suboptimal" (either low-mobility or *in vitro* stored) spermatozoa may not become apparent until several weeks into the production period.

Only morphologically normal spermatozoa are capable of ascending through the hen's vagina to the region where the oviducts' SST are located (Bakst et al., 1994). To reach the SST, these spermatozoa must be motile. The ability of sperm to penetrate an Accudenz solution may reflect, to some extent, their ability *in vivo*. Logically, this indicates that toms with high-mobility spermatozoa have a larger subpopulation of spermatozoa that are capable of ascending the reproductive tract, entering the SST, and subsequently fertilizing eggs. We estimated the number of sperm in the SST by evaluating sperm-hole numbers in the PL of freshly laid eggs. A relationship between the number of holes in the PL and the number of spermatozoa in the SST exists, such that sperm holes can be used as an indicator of sperm numbers in the SST (Wishart, 1995). We observed 30% fewer holes in eggs laid by hens in the low-mobility treatment group. This pattern is also observed in eggs from hens inseminated with 24-hour *in vitro* stored semen: significantly fewer sperm holes were detected, and fertilization was lower (Donoghue, 1996), which suggests that the number of spermatozoa reaching the SST is lower.

What makes some spermatozoa highly mobile? At present, we do not know. No difference in either sperm viability or lipid-peroxidation susceptibility has been detected between phenotypes (Holsberger, 1998). The chromatin composition of male turkeys with varying mobility phenotypes, in the current study, did not differ, even though the SCSA has been used successfully in the assessment of mammalian sperm subfertility and semen quality (Evenson and Jost, 1994). These observations with turkey spermatozoa are consistent with recent research in roosters (Froman, unpublished data), in that sperm morphology was excluded as a possible underlying basis for differences in sperm mobility.

The sperm-velocity parameters, evaluated by the computer-assisted sperm-analysis system, were consistent with the mobility phenotypes. Velocity variables, VSL and VAP, were significantly higher for the high-mobility–phenotype toms. VSL has been strongly correlated, in many mammalian species, with fertility. Correlations both between VSL for boar spermatozoa and the number of piglets per litter (Holt et al, 1997), and between VSL for rat spermatozoa and *in vitro* fertilization results have been highly significant (Moore and Akhondi, 1996). The data reported here illustrate that sperm velocity may be meaningful in avian species as well.

Our original selections of toms were based on observation of freshly ejaculated spermatozoa. However, this selection criterion did not influence fertilizing ability after 24-hour *in vitro* storage, where there was neither a phenotypic difference in mobility nor a difference in fertilized eggs. Factors that enhanced the number of spermatozoa able to penetrate the Accudenz solution were lost during *in vitro* storage. Lower sperm mobility may be an indicator of why fertility is consistently reduced when stored spermatozoa are used for AI. Bagley (1995) suggested that semen from individual toms be evaluated for *in vitro* storage capacity after semen has been stored *in vitro,* a strategy we are currently pursuing with this assay. Graham et al (1984) reported that only a small proportion of toms produce semen capable of long-term *in vitro* stor-

age. The initial quality, based on sperm mobility, of semen was not maintained after 24-hour *in vitro* storage, which may explain, in part, the inadequacy of current methods for long-term turkey-semen storage *in vitro*.

Almost 80% of intravaginally inseminated spermatozoa are ejected from the vagina within 15 minutes of insemination (Howarth, 1971). The remaining spermatozoa are subjected to an intense selection process within the vagina (Wishart and Steele, 1992). Therefore, only a "fit" subpopulation of spermatozoa mobilize to the SST (Bakst and Brillard, 1995); high mobility may be a defining attribute of these spermatozoa. Investigators have shown that, in addition to mobility, the surface properties of spermatozoa are an important component of "fitness." The percentage of fertilized eggs is decreased when sialic acid residues from surface-associated glycoproteins are removed by neuraminidase (Froman and Thurston, 1984) or when surface-associated proteins or glycoproteins are removed with hypertonic NaCl (Wishart and Steele, 1992). A possible attribute of fit spermatozoa is their ability to move through the vagina quickly, so that they are less susceptible to detrimental effects in the vaginal environment. Although the mechanics of sperm motility has been studied in poultry spermatozoa (Wishart and Ashizawa, 1987; Ashizawa and Sano, 1990; Ashizawa et al, 1987, 1992, 1995; McLean and Froman, 1996), a comprehensive definition of cellular fitness has yet to be established. Such a definition would provide a new objective for research designed to improve semen preservation.

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