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Sperm Mobility: A Primary Determinant of Fertility in the Domestic Fowl (*Gallus domesticus*)[1]

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ABSTRACT

Previous research demonstrated that sperm mobility is a quantitative trait of the domestic fowl. The trait is quantified by measuring the absorbance of an Accudenz solution after overlay with a sperm suspension and brief incubation at body temperature. In the present work, average and high sperm mobility phenotypes (n = 30 males per phenotype) were selected from a base population. Differences were found between sperm oxygen consumption ($p < 0.0001$), acylcarnitine content ($p < 0.05$), linear velocity ($p < 0.001$), and straightness ($p < 0.001$), a trajectory variable measured with the Hobson SpermTracker. Oxygen consumption and stearoylcarnitine content of sperm from the high-mobility phenotype were twice those observed with sperm from average males, implying a pivotal role for mitochondria. On the basis of these results, a graded relationship was predicted between fertility and sperm mobility. Males (n = 48) were chosen at random from another base population, sperm mobility was measured per male, and each ejaculate was used to inseminate 8–12 hens (8×10^7 viable sperm per hen). When fertility was plotted as a function of sperm mobility, data points approximated a skewed logistic function. The hypothesis that vaginal immunoglobulins constitute an immunological barrier to sperm transport was tested and rejected. Therefore, we concluded that sperm mobility is a primary determinant of fertility in the fowl.

INTRODUCTION

Sperm mobility, i.e., the net movement of a sperm population, is a quantitative trait of the domestic fowl [1]. This trait can be quantified by overlaying a sperm suspension on an Accudenz solution within a polystyrene cuvet, incubating the cuvet at body temperature for 5 min, and then measuring the absorbance of the Accudenz layer [2]. Whereas immotile sperm do not penetrate the Accudenz layer, motile sperm do so rapidly. However, the extent to which this happens varies among males. For example, 95% of the sperm within the overlay will enter the Accudenz solution in the case of some males, whereas less that 5% of the sperm will do so in the case of others. In contrast, the sperm mobility of most males within a population is between these two extremes. In any case, males categorized by sperm mobility have been shown to differ with respect to fertility [1–3]. Therefore, it was hypothesized [1, 2] that the sperm mobility assay had potential to identify highly fecund individuals within a population and to do so without previous information relative to the reproductive status of the individuals tested.

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Previous research [1] reported the following salient observations. First, a high correlation was observed between sperm ATP content and sperm mobility. Second, differences in sperm morphology could not account for differences in sperm mobility. Third, phenotype was independent of time. Fourth, phenotype was fully expressed in washed sperm when mitochondrial respiration was the only source of ATP. The pivotal role of phosphorylation reactions in the manifestation of the trait also was demonstrated by the effect of calyculin A, a protein phosphatase inhibitor. In a paired comparison, calyculin A enabled low mobility sperm to manifest average mobility, presumably by acting at the level of the axoneme. In summary, it was hypothesized that differential rates of mitochondrial ATP synthesis accounted for the extreme variation in sperm mobility observed within populations of roosters.

As reviewed by Bakst et al. [4], the behavior or fate of sperm within the hen's vagina constitutes a critical determinant of fertility in the domestic fowl. Motile sperm ascend the vagina and enter specialized sperm storage tubules (SST), which are located at the juncture of the vagina and shell gland. Sperm remain within the SST for a period of days to weeks. If the oviduct is patent upon their release, sperm pass rapidly up the oviduct, presumably by antiperistalsis, to the infundibulum, which is the site of fertilization in the hen. The predictive value of the sperm mobility assay was attributed [1] to its simulating a critical step for internal fertilization in the hen: the net movement of a sperm population against resistance. Consequently, this postulate constituted an alternative to the hypothesis that the net movement of motile sperm within the vagina is affected by selection exerted by the oviduct, in particular, an immunological barrier [5, 6].

The objectives of the present research were based upon the following arguments. First, if differences in sperm oxygen consumption existed between sperm mobility phenotypes, then mitochondrial respiration would indeed be pivotal to the expression of phenotype. A corollary of this argument was that levels of endogenous substrate, presumably a fatty acid, might differ between phenotypes. Second, if an attribute of individual, motile sperm differed between phenotypes, then a variable would be identified that might account for the differential behavior of sperm populations. Third, if fertility were found to be a function of sperm mobility, then sperm mobility would be a key determinant of fertility. Finally, if vaginal immunoglobulins induced loss of sperm viability in vivo, then this effect should be demonstrable in vitro.

MATERIALS AND METHODS

Evaluation of Base Population

The sperm mobility of individually caged New Hampshire roosters (n = 509) was evaluated using a modification of the method described by Froman and Feltmann [1]. Se-

men was diluted with 50 mM *N*-Tris-[hydroxymethyl]methyl-2-amino-ethanesulfonic acid (TES; Sigma Chemical Co., St. Louis, MO), pH 7.4, containing 128 mM NaCl and 2 mM $CaCl_2$ (TES-buffered saline). The 6% (w:v) Accudenz (Accurate Chemical & Scientific Corporation, Westbury, NY) solution upon which sperm suspensions were overlaid also was prepared with TES-buffered saline. Approximately 57 males were evaluated on each of 9 consecutive work days using a single batch of reagents. Before evaluation, diluent and 6% (w:v) Accudenz had been filtered through 0.2-μm Acrodisc filters into sterilized 8-ml screw-cap amber bottles (Fisher Scientific, Santa Clara, CA). This process was repeated twice. Thus, each male was evaluated three times between 25 and 31 wk of age. On the basis of an average score, each male was assigned to one of 12 frequencies, and the Kolmogorov-Smirnov test for goodness of fit [7] was used to determine whether observed frequencies approximated a normal distribution.

Selection of Experimental Animals

Estimates of the mean and standard deviation were used to calculate the normal probability density function [8], which was used for selection as follows. Roosters (n = 509) were ranked by their average sperm mobility scores. Any male whose average score was > 1.5 standard deviations above the population mean was categorized as having high sperm mobility. Likewise, any male whose average score was between 1 standard deviation below the mean and the mean was categorized as having average sperm mobility. Thirty males were retained per phenotype.

Metabolic Analyses

Sperm concentration was determined with a spectrophotometer [1]. A 25-μl sample of semen from each of 26 roosters per phenotype was diluted with TES-buffered saline so that a 2-ml sperm suspension was procured containing 2.5×10^8 sperm/ml. Oxygen consumption was measured over a 3-min interval at 41°C with a Model 5300 YSI Biological Oxygen Monitor (Yellow Springs Instruments, Yellow Springs, OH). Data were analyzed by single-classification ANOVA [9].

In a subsequent experiment, an ejaculate from each of 10 males per phenotype was microcentrifuged for 20 sec, the seminal plasma was discarded, sperm concentration was determined, and sperm were resuspended to a concentration of 3.0×10^9 sperm/ml in TES-buffered saline. Immediately thereafter, sperm suspensions were frozen at −80°C. Carnitine and acylcarnitine analyses were performed by the Center for Inherited Disorders of Energy Metabolism (Case Western Reserve University, VA Medical Center, Cleveland, OH). In brief, each sperm suspension was thawed, the sperm suspension was vortexed, a 60-μl sample was removed, and measurements were made with pre-column chemical derivatization HPLC. Data were analyzed by single-classification ANOVA [9].

Computer-Assisted Sperm Motion Analysis

The attributes of individual, motile sperm were measured for each of 25 roosters per phenotype with a Hobson SpermTracker (Biogenics, Napa, CA). Immediately after a rooster was ejaculated, approximately 1–3 ml of blood was withdrawn from the right cutaneous ulnar vein into a heparinized Vacutainer tube (Fisher Scientific). One hundred microliters of blood was withdrawn from the Vacutainer tube and mixed with 900 μl TES-buffered saline. Sperm concentration was determined with a spectrophotometer [1]. Then, a 50-μl volume of semen was diluted to a concentration of 5×10^8 sperm/ml with TES-buffered saline. A 125-μl volume of the 1:10 dilution of blood was mixed with 1.121 ml of TES-buffered saline prewarmed to 41°C. Immediately thereafter, a 4-μl volume of the sperm suspension was added so that the ultimate sperm suspension contained approximately 1.6×10^6 sperm/ml in a 1:100 dilution of blood.

In each case, a sample chamber was filled in a prewarmed MicroCell (50-μm chamber depth; Conception Technologies, San Diego, CA). The erythrocytes instantly formed a monolayer over which motile sperm moved. Each of 4 fields within the sample chamber was viewed for 1 min at 41°C using a ×4 brightfield objective under a pseudo darkfield condition generated with a Ph3 annular phase ring. Inclusion of erythrocytes maintained sperm motility throughout the 4-min sampling period. Minimum track time was set at 1.6 sec. An average value for linear velocity and straightness of path length was estimated for each male on the basis of approximately 650 tracks per male. Before ANOVA, estimates of straightness were transformed to logits, $l = \ln [p/(1 - p)]$. Data were analyzed by single-classification ANOVA [9].

Fertility Trial

Broiler breeder males (n = 48) were selected randomly from each of two flocks containing birds at either 32 or 54 wk of age. Sperm concentration and viability were measured according to Bilgili and Rendon [10]. Sperm mobility was measured according to Froman and McLean [2]. Before insemination, semen was diluted to 1.6×10^9 viable sperm/ml with 50 mM TES, pH 7.4, containing 111 mM NaCl, 25 mM glucose, and 4 mM $CaCl_2$. Each of 8–12 hens per male was inseminated with 8×10^7 viable sperm. Eggs were collected over a 7-day interval, which commenced on the second day postinsemination. Fertility was determined by examining egg contents after incubation for 4 days. Fertility data were analyzed by ANOVA with a log-odds model [11]. Fertility was plotted as a function of sperm mobility.

Effect of Immunoglobulins on Sperm Viability

Anti-sperm immunoglobulins were produced with a modification of the method of Kirby et al. [12]. Nonimmune serum was procured from each of five New Hampshire roosters. Thereafter, each rooster was immunized against whole semen emulsified in Freund's complete adjuvant (Sigma Chemical Co.). Each rooster received 1 ml of the emulsion injected i.m. in 0.1-ml aliquots. This procedure was repeated a week later using Freund's incomplete adjuvant. Booster injections were given with Freund's incomplete adjuvant 5 wk after the initial injection. Blood was collected into nonheparinized Vacutainer tubes (Fisher) 12 days after the booster injections. Ejaculates from nonimmunized roosters (n = 3) were pooled, and semen was diluted to 5×10^8 with TES-buffered saline. A 100-μl volume of sperm suspension was added to each of 10 wells in a round-bottom microtiter plate. Then 100-μl volumes of nonimmune or immune serum were added to the wells. The presence of anti-sperm antibodies was determined by the immediate formation of a compact white button in the bottom of the well. Immune serum was frozen at −20°C in 0.5-ml aliquots.

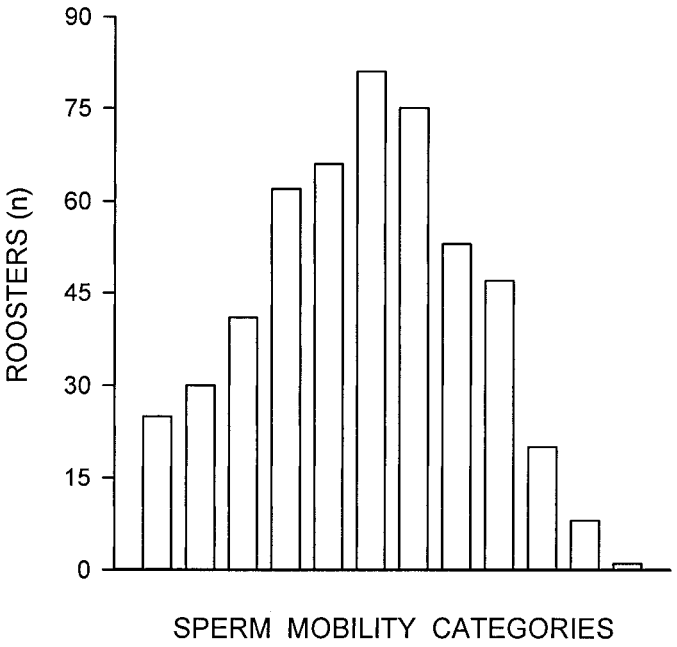

FIG. 1. Frequency distribution derived from categorization of sperm mobility data using an average score (n = 3 observations) from each of 509 New Hampshire roosters. Sperm mobility was quantified as described in *Materials and Methods.* Each category denotes an increment of 0.090 absorbance units from a baseline of zero. Thus, the category with the maximal number of roosters denotes a range of 0.451 to 0.540 absorbance units.

The effect of anti-sperm immunoglobulins on sperm viability was determined as follows. Serum samples were thawed and diluted 1:16 with TES-buffered saline. A portion of immune serum was held at 56°C for 20 min in an Eppendorf Thermostat Model 5320 (Brinkman Instruments Co., Westbury, NY). During this time, the nonimmune and the remaining immune sera were kept on ice. Ejaculates from

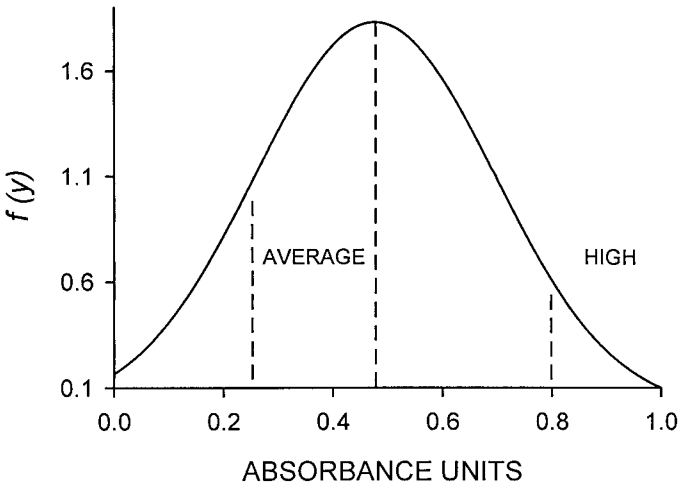

FIG. 2. Normal probability density function based upon estimates of 0.476 and 0.2180 for the mean and SD, respectively. The function depicts the distribution of fowl sperm mobility as measured by sperm penetration into 6% (w:v) Accudenz at body temperature using a base population of 509 roosters. Absorbance indicates the extent to which sperm penetrated the Accudenz layer after a 5-min incubation interval. Areas under the curve denoted by dashed lines represent two sperm mobility phenotypes. Thirty roosters representing each phenotype were reserved for a series of experiments in which sperm oxygen consumption, acylcarnitine content, linear velocity, and trajectory were measured.

TABLE 1. Oxygen consumption (mean ± SEM) of freshly ejaculated sperm at 41°C from roosters categorized a priori by sperm mobility phenotype

Phenotype	Male (n)	Oxygen consumption (μl O_2/min)
Average	26	0.62 ± 0.021[a]
High	26	1.14 ± 0.076[b]

[a,b] Means within a column differ ($p < 0.0001$).

nonimmunized roosters (n = 3) were pooled, and semen was diluted to 1×10^9 with TES-buffered saline. The sperm suspension was mixed 1:2 with nonimmune serum, immune serum, or complement inactivated immune serum. In each case, media were mixed within each of 25 wells within a flexible polyvinyl chloride microtiter plate floating in a water bath maintained at 41°C. Sperm viability was measured by ethidium bromide exclusion [10] at the onset of the admixture of media and at 30, 60, 90, and 120 min thereafter. Replicate observations (n = 5) were made at each time point for each treatment. Treatment effects were evaluated by plotting sperm viability as functions of time. Intercepts and slopes were estimated by the method of least squares [13].

The effect of vaginal immunoglobulins on in vitro sperm viability was determined as follows. Nonvirgin New Hampshire hens in lay (n = 125) were killed by cervical dislocation. In each case, the oviduct was excised, the vagina was straightened by severance of adherent connective tissue, and a ligature was tied at the junction between the shell gland and vagina. The vagina was lavaged with 3 ml of TES-buffered saline. Each lavage was microcentrifuged for 5 min. Supernatants were pooled. The resultant protein solution was placed in dialysis tubing (molecular size cut-off, 6–8 kDa; Fisher) and then concentrated 50-fold at 4°C by overlaying the membrane with polyethylene glycol flakes (15–20 kDa; VWR Scientific, Seattle, WA). Protein concentration was measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). The presence of both chicken IgG and IgA was determined by Western blotting using goat anti-chicken IgG and IgA (Bethyl Laboratories, Inc., Montgomery, TX) as the primary antibody. Sperm were incubated at body temperature with antibodies derived from the hen's vagina by mixing a sperm suspension 1:2 with the concentrated vaginal extract as described above. Control sperm were diluted with TES-buffered saline containing 3.3 mg BSA/ml. In each case, 5 replicate observations were made per time point as above. Sperm viability was measured and data were analyzed as described above.

RESULTS

Evaluation of Base Population

Frequencies of males categorized by sperm mobility scores are shown in Figure 1. Scores ranged from 0.010 to 1.051 absorbance units. The population mean and standard deviation were estimated to be 0.476 and 0.2180 absorbance units, respectively. The hypothesis that observed frequencies approximated a normal distribution was not rejected ($p > 0.05$).

Selection of Experimental Animals

The predicted normal probability density function is shown in Figure 2. Grand means for selected roosters were 0.300 and 0.854 absorbance units for average and high sperm mobility phenotypes, respectively. These phenotypes

TABLE 2. Acylcarnitine analysis of washed sperm from roosters categorized a priori by sperm mobility phenotype (mean ± SEM).

Phenotype	Males (n)	Ratio of acylcarnitine to carnitine	Stearoylcarnitine (nmol/ml)
Average	10	2 ± 0.3[a]	2.2 ± 0.72[a]
High	10	4 ± 0.4[b]	4.1 ± 0.55[b]

* Procedure explained in Metabolic Analyses (*Materials and Methods*).
[a,b] Means within a column differ ($p < 0.05$).

are illustrated in Figure 2 relative to the normal probability density function.

Metabolic Analyses

A pronounced phenotypic difference in oxygen consumption was observed ($p < 0.001$) at body temperature. As shown in Table 1, the oxygen consumption of sperm from males categorized a priori with average sperm mobility was only 54% of that of sperm from males characterized with high sperm mobility. Acylcarnitine analysis revealed similar differences (Table 2) in that the acylcarnitine:carnitine ratio for average males was 50% of that observed in high males. Likewise, sperm stearoylcarnitine content from average males was only 54% of that observed with high males.

Computer-Assisted Sperm Motion Analysis

Two properties of motile sperm differed ($p < 0.001$) between phenotypes (Table 3). The linear velocity of sperm from average males was only 77% of that of sperm from high males. Likewise, trajectories of sperm from average males were more meandering than those of high sperm mobility males. Linear velocity and straightness were highly correlated ($r = 0.9$) on the basis of an a posteriori observation in which proportional data were transformed to logits before correlation analysis.

Fertility Trial

As shown in Figure 3, a logistic relationship was observed between sperm mobility and fertility. As evidenced by an ability to produce offspring, each male used in this experiment was fertile. However, fertility ranged from a minimum of 3% to a maximum of 100%.

Effect of Immunoglobulins on Sperm Viability

Anti-sperm immunoglobulins were produced in 1 of 5 roosters immunized against semen as evidenced by in vitro sperm agglutination. This effect was observed with serial dilutions of serum ranging from 1:2 to 1:16. Thus, a dilution of 1:16 was used to test the effect of fowl immunoglobulins on in vitro sperm viability. As shown in Figure 4, anti-sperm immunoglobulins were associated with a deleterious effect on sperm cells incubated at body temperature. However, this effect appeared to be mediated by complement, as immune serum heated to 56°C before admixture with sperm had little effect in comparison to nonheated immune serum. As evidenced by Western blots (data not

TABLE 3. Linear velocity and trajectory of freshly ejaculated motile sperm at 41°C from roosters categorized a priori by sperm mobility phenotype (mean ± SEM).

Phenotype	Males (n)	Linear velocity (μm/sec)	Straightness* (%)
Average	25	34 ± 1.8[a]	61 ± 2.0[a]
High	25	44 ± 1.2[b]	70 ± 1.2[b]

* Calculated by dividing the linear distance between the termini of the smoothed path by the length of the smooth path.
[a,b] Means within a column differ ($p < 0.001$).

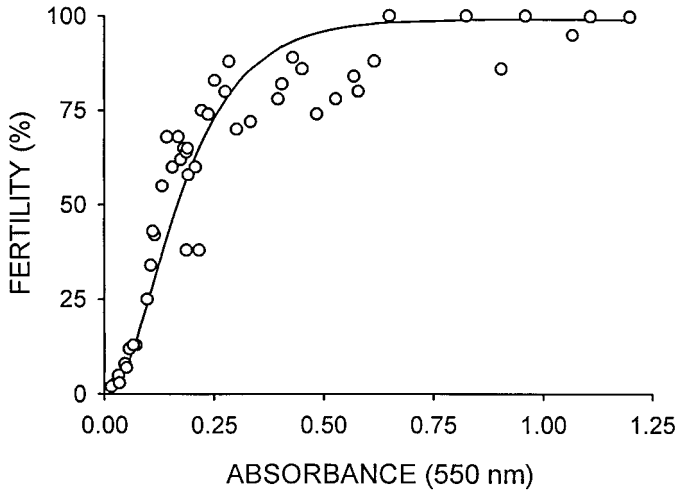

FIG. 3. Fertility plotted as a function of sperm mobility, in which sperm mobility was measured by a change in the absorbance of 6% (w:v) Accudenz after an overlay with a sperm suspension. Open circles denote data pairs for 48 broiler breeder males. Fertility was measured by inseminating 8–12 hens per male, collecting eggs for a period of 7 days following artificial insemination, and examining egg contents after 4 days of incubation. Raw data approximated a skewed logistic function rising from a minimum of 3 and reaching an asymptote of 100%. Therefore, Richard's function, $p(x) = 1/[1 + \theta e^{-\beta(x-\tau)}]^{1/\theta}$, was chosen as a reasonable model. Parameters were estimated by iterative least squares. The solid line is the fitted curve.

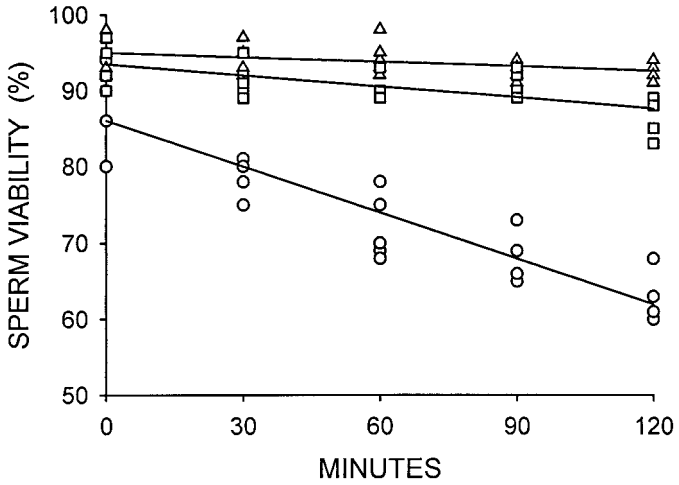

FIG. 4. In vitro sperm viability plotted as functions of time. A sperm suspension containing 1×10^9 sperm/ml was mixed 1:2 with a 1:16 dilution of nonimmune fowl serum (triangles), heated (56°C) immune fowl serum (squares), or immune fowl serum (circles). The resultant mixtures were incubated at 41°C. Sperm agglutination was observed within seconds of admixture of sperm with immune sera, and these sperm remained agglutinated for the duration of the experiment. Sperm viability was determined by ethidium bromide exclusion. Agglutinated sperm were dispersed before sampling. Solid lines represent the functions y(x) = 95–0.02 (x) for nonimmune serum, y(x) = 94–0.05 (x) for heated immune serum, and y(x) = 86–0.20 (x) for immune serum. In the case of nonimmune serum, sperm viability was independent of time ($p > 0.05$). In the case of immune sera, sperm viability decreased as a function of time ($p < 0.05$). However, the rates at which sperm viability decreased in response to immune sera differed between heated and nonheated immune sera ($p < 0.05$).

shown), both IgG and IgA were detected in a protein extract obtained from the luminal surface of the hen's vagina. The treatment effect observed when sperm were incubated with either vaginal immunoglobulins or BSA was equivalent to the effect of nonimmune serum shown in Figure 4; i.e., neither treatment had an effect on sperm viability ($p > 0.05$).

DISCUSSION

Previous research [1] had demonstrated that sperm mobility was highly correlated with sperm ATP content and that phenotype was fully expressed when ATP synthesis was limited to mitochondrial respiration. In the present work, we expected to detect a phenotypic difference in oxygen consumption. While such a difference was observed (Table 1), a phenotypic difference in stearoylcarnitine content was also detected (Table 2). In previous work with washed sperm in nutrient-free media [1], it was assumed that sperm motility, and hence mobility, was dependent upon an endogenous substrate, most likely a long-chain fatty acid. In order for long-chain fatty acids to be oxidized within the mitochondrial matrix, activated molecules first must be conjugated with carnitine on the exterior side of the inner mitochondrial membrane [14]. Presumably, the longest fatty acid moiety in a mixture of acylcarnitines would be the endogenous substrate. On a percentage basis, the contents of saturated long-chain acylcarnitine extracted from the sperm of high mobility males were 53%, 36%, and 10% for stearoyl-, palmitoyl-, and myristoylcarnitine, respectively. Therefore, we concluded that stearic or *n*-octadecanoic acid is an endogenous substrate within fowl sperm. Collectively, the data in Tables 1 and 2 provide additional evidence that mitochondrial function is pivotal to the expression of sperm mobility phenotype.

Our second objective was to determine whether one or more properties of motile sperm might provide an explanation for extreme differences in sperm mobility observed within a population (Fig. 1). Two variables measured with a Hobson SpermTracker, i.e., linear velocity and straightness, differed between phenotypes (Table 3). These data, along with the a posteriori realization that linear velocity and the logit transform of straightness were highly correlated, provided an explanation for phenotypic differences in sperm mobility. But more importantly, differences in the motion of motile sperm observed among phenotypes afforded a theoretical basis for the relationship between sperm mobility and male fertility. The search for such a relationship was our third objective. As shown by Figure 3, fertility was a function of sperm mobility. It appears that differences in fertilizing ability observed among males are explicable in terms of how effective their sperm are as self-propelled DNA delivery vehicles.

Our fourth objective was to evaluate the effect of vaginal immunoglobulins on sperm viability. As shown in Figure 4, an experimental method was validated for studying the effect of homologous anti-sperm immunoglobulins on sperm viability in vitro. As reviewed by Bakst et al. [4], an immunoglobulin-dependent mechanism has been advanced for vaginal sperm selection. It has been known since the 1950s that only motile fowl sperm ascend the hen's vagina [15]. Steele and Wishart [16] proposed that sperm movement through the vagina was thwarted by luminal immunoglobulins. This proposal was based on the observation that IgG was bound to 82% of sperm recovered by lavage 2 h postinsemination and that 66% of recovered sperm were dead. Recently, Yoshimura et al. [17] demonstrated the presence of B lymphocytes and plasma cells positive for IgG within the mucosal epithelium and subepithelial stroma of the hen's vagina. However, Steele and Wishart did not determine whether sperm were dead because of IgG binding or whether IgG had bound to dead sperm. Consequently, we reasoned that if vaginal immunoglobulins killed sperm in vivo, then this effect would be demonstrable in vitro. However, vaginal immunoglobulins had no effect on sperm viability in vitro. Therefore, it is questionable whether vaginal immunoglobulins constitute a selection mechanism in vivo.

Our experimental outcomes have three implications. First, it appears that fertility in the domestic fowl is more likely determined by attributes inherent to sperm rather than by selection exerted by the oviduct. In this regard, while numerous attributes unquestionably play important roles in male fertilizing capacity following artificial insemination, e.g., sperm-egg binding [18], it appears that sperm mobility is the salient attribute (Fig. 3). The second implication pertains to the initiation of sperm motility. According to Ashizawa and Sano [19], fowl sperm are essentially immotile before ejaculation. Consequently, fowl sperm are uniformly immobile before ejaculation. In contrast, extreme variation in sperm mobility exists immediately after ejaculation (Figs. 1 and 2). This variation may stem from inherent differences in the capacity of mitochondria to synthesize ATP in spite of the phenotypic difference in sperm stearoylcarnitine content (Table 2), for phenotypic differences in sperm mobility are not ameliorated by millimolar amounts of exogenous glucose. However, an alternative mechanism that might account for variation in postejaculation sperm mobility might be a differential response to whatever induces motility at ejaculation.

Finally, we propose that sperm mobility is pivotal to sperm sequestration within the hen's SST. While sperm sequestration has been studied by numerous researchers since the 1960s [4], the mechanism responsible for sperm egress from the SST has remained a mystery. Ironically, sperm egress may be explicable in terms of sperm immobility. This hypothesis is tenable for a number of reasons. First, sperm within a sperm storage tubule are always oriented with their acrosomes toward the blind end of the tubule and their long axes parallel to the long axis of the tubule [4]. Second, sperm mobility under physiological conditions is highly correlated with sperm ATP content [1]. Third, sperm metabolize fatty acids, and the large lipid droplets located within the apical cytoplasm of SST epithelial cells [4] appear to be a likely source of exogenous fatty acids. The SST are blind-end tubules. If SST epithelial cells secrete a fluid into a tubule's lumen, then a current would be generated within the lumen. We hypothesize that sperm reside within the SST by actively maintaining their position against a current. In such a case, low intracellular ATP content would result in the egress of viable sperm from the SST. This hypothesis is parsimonious with respect to mechanisms of sperm movement into and out of the SST. The hypothesis is also consonant with protein half-life, for one outcome of lost enzyme function in a cell with condensed nuclear DNA would be decreased ATP synthesis.

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