# Computer-Assisted Semen Analysis (CASA) of Epididymal Sperm from the Domestic Cat

JAMES J. STACHECKI, KENNETH A. GINSBURG, RICHARD E. LEACH, AND
D. RANDALL ARMANT

*From the C. S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan.*

**ABSTRACT:** Motion characteristics of epididymal sperm from domestic cats exhibiting a high (>60%; normozoospermic; $n = 21$) or low (<40%; teratozoospermic; $n = 6$) occurrence of structurally normal spermatozoa were correlated with morphology (MOR) using computer-assisted semen analysis (CASA). Mean values and standard errors for percent motility (MOT), curvilinear velocity (VCL), linearity (LIN), straight line velocity (VSL), and amplitude of lateral head displacement (ALH) were recorded for 3 hours. Average values for percent normal spermatozoa, MOT, VCL, VSL, and ALH were higher ($P < 0.01$) in samples from normozoospermic cats than from teratozoospermic cats at 0 hours, and there was no difference in motion parameters over the 3-hour incubation period in either group.

Strong correlations ($P < 0.01$) existed between MOR and VCL, VSL, ALH, or MOT, but not LIN, upon regression analysis. We conclude that (1) motion parameters of domestic cat sperm are significantly correlated with morphology and (2) abnormal motion parameters associated with low fertility potential in other species are prevalent in samples from teratozoospermic cats. The correlation between morphology and altered sperm movement found in this study suggests that motion analysis of spermatozoa by CASA may be useful in evaluating fertilization potential in felids.

Key words: Computer-assisted semen analysis, domestic cat, normozoospermia, teratozoospermia, sperm morphology.

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Recent investigations have revealed that several rare and endangered felids ejaculate high proportions of morphologically abnormal spermatozoa (Wildt et al, 1983, 1987a,b; Howard et al, 1984). Because morphologically abnormal spermatozoa are compromised in their ability to migrate through the reproductive tract and to penetrate the oocyte (Krzanowska, 1974; Nestor and Handel, 1984; Mahadevan et al, 1987), it is not surprising that some nondomestic felids reproduce poorly (Wildt, 1990). Although ejaculates from domestic cats contain less than 30% abnormal spermatozoa (Wildt et al, 1983), individual males sometimes exhibit teratospermia (Howard et al, 1990). The domestic cat has served as a valuable model for studying the impact of teratospermia on reproductive function in the Felidae (Howard et al, 1991b). Howard et al (1990) used this model to demonstrate that testosterone levels in teratospermic cats are 33% lower than in normospermic males, reflecting the decreased circulating testosterone levels found by Wildt et al (1983, 1987a,b, 1988) among teratospermic domestic cats, African lions, and

cheetahs. Decreases in genetic variability and heightened levels of aberrant sperm forms were documented in both the cheetah and lion populations. The findings of Howard et al (1990) and Wildt et al (1983, 1987a,b, 1988) suggest a relationship among androgen levels, genetic variability, and the occurrence of abnormal spermatozoa.

Decreased fertilization rates associated with structurally abnormal spermatozoa may be related to their altered motion characteristics; however, this has not been examined in felids. Computer-assisted semen analysis (CASA), a novel quantitative method for determining the motion characteristics of spermatozoa, has provided data relating sperm motion to fertilization potential in humans (Aitken et al, 1982b; Jeulin et al, 1986; Fetterolf and Rogers, 1990; Ginsburg et al, 1990). CASA has proven to be a useful diagnostic tool for infertility clinics, providing objective analysis of sperm motility and setting quality control standards for consistent semen analysis. Other investigators have used CASA to evaluate changes in motion parameters as spermatozoa undergo hyperactivation (Neill and Olds-Clarke, 1987; Ginsburg et al, 1990; Mbizvo et al, 1990; Suarez et al, 1991) and capacitation (Hoshi, 1988; Morales, 1988; Mortimer et al, 1988). Here, we have used CASA to analyze the motion characteristics of epididymal cat spermatozoa, especially with respect to possible differences between spermatozoa from normozoospermic and teratozoospermic domestic cats.

Table 1. *Parameter settings for tracking felid spermatozoa using the Cell Track/s CASA system*

| Parameter | Setting |
|---|---|
| Frame rate (frames/second) | 60 |
| Duration of data capture (frames) | 40* |
| Minimum path length (frames) | 40* |
| Minimum motile speed (μm/second) | 10 |
| Maximum burst speed (μm/second) | 1,200 |
| Distance scale factor (μm/pixel) | 0.9348 |
| ALH path smoothing factor (frames) | 7 |
| Cent. X search neighborhood (pixels) | 4 |
| Cent. Y search neighborhood (pixels) | 2 |
| Cent. cell size minimum (pixels) | 2 |
| Cent. cell size maximum (pixels) | 9 |
| Path. max. interpolation (frames) | 2 |
| Path prediction percentage (percent) | 10 |

* Values changed to 5 when calculating percent motility (MOT).

## Materials and Methods

### Sperm Collection and Processing

Testes from castrated toms (8–36 months old; $n = 30$), provided by local veterinary hospitals, were collected in Eagle's medium (Sigma Chemical Co, St. Louis, Missouri) supplemented with 25 mM HEPES and 4 mg/ml bovine serum albumin (BSA) and kept at 23°C until processing. Epididymides were removed for sperm collection within 2 hours of castration. Blood vessels were dissected away to prevent blood cell contamination of spermatozoa, and epididymides were washed in Ham's F10 medium (Sigma) containing 4 mg/ml BSA. Spermatozoa were released into 2 ml of Ham's F10 through punctures made with a 30-gauge needle, concentrated by centrifugation (700 × $g$, 8 minutes) in a sterile 1.5-ml conical tube, resuspended in Ham's F10 to a working concentration of 40–70 million sperm/ml, and kept at 23°C until analysis. Normozoospermic ($n = 21$) and teratozoospermic ($n = 6$) samples were analyzed at 23°C immediately after preparation. Additionally, some samples were analyzed again ($n = 12$ and 4, respectively) following a 3-hour incubation at 23°C. The initial analysis was completed within 3 hours of castration.

### Morphological Assessment

A 10-μl smear preparation of each sample was heat-fixed, incubated with Papanicolaou stain for 2 minutes, and rinsed with water. At least 200 sperm/sample were examined using phase-contrast optics at ×1,000 magnification to assess the percent normal sperm morphology (MOR). Sperm were classified as normal or exhibiting one of the following structural deformities: macrocephaly, bicephaly, biflagellate, coiled flagellum, bent midpiece with or without a cytoplasmic droplet, bent flagellum with or without a cytoplasmic droplet, and cytoplasmic droplet. Sperm samples were classified as either normozoospermic, >60% normal sperm morphology, or teratozoospermic, <40% normal sperm morphology. Samples having intermediate MOR values were used only for regressional analysis.

### Motion Analysis

CASA requires the ability to identify and track spermatozoa over time and space. Accurate assessment of motion parameters for

Table 2. *Occurrence of morphologically normal and abnormal spermatozoa from normozoospermic and teratozoospermic domestic cats†*

| | Normozoo-spermic ($n = 21$) | Teratozoo-spermic ($n = 6$) |
|---|---|---|
| Normal spermatozoa (%) | 84.4 ± 1.5 | 27.8 ± 2.7* |
| Abnormal spermatozoa (%) | | |
| Microcephalic | 0 ± 0 | 0 ± 0 |
| Macrocephalic | 0.3 ± 0.1 | 0 ± 0 |
| Bicephalic | 0.3 ± 0.1 | 0 ± 0 |
| Biflagellate | 0.1 ± 0.1 | 0.7 ± 0.7 |
| Tightly coiled flagellum | 0.1 ± 0.1 | 1.2 ± 0.8* |
| Bent midpiece with droplet | 1.6 ± 0.6 | 10.6 ± 4.5* |
| Bent midpiece without droplet | 4.9 ± 0.7 | 8.8 ± 1.4* |
| Bent flagellum with droplet | 1.4 ± 0.5 | 10.5 ± 6.8* |
| Bent flagellum without droplet | 2.3 ± 0.6 | 42.3 ± 11.2* |
| Cytoplasmic droplet | 4.7 ± 1.2 | 1.7 ± 0.8 |

* Values are different ($P < 0.05$) from the normozoospermic group.
† Values shown are the mean ± SEM for each parameter determined.

each species is dependent upon the computer settings and the concentration of spermatozoa analyzed (Kunth et al, 1987; Mortimer et al, 1988; Boyers et al, 1989). Each calibration parameter of the Cell Track/s System (Version 3.2, Motion Analysis Corp, Santa Rosa, California) was optimized to track felid spermatozoa by evaluating prerecorded samples at various settings. Operating parameters were optimized to track all sperm and exclude debris (Table 1). Due to the similarities in cat and human sperm morphology, the chosen operating parameters were similar to those used in our laboratory for tracking human sperm. The principal difference between settings for tracking cat and human sperm was the maximum burst speed, reflecting the higher velocity of cat sperm. A video digitizing rate of 60 frames per second (fps) was used to gather 40 frames of data for calculating kinematics and 5 frames of data for determining MOT. All examinations were performed using an Olympus BH2 microscope (Olympus, New York, New York) with a ×10 positive phase-contrast objective.

Sperm concentration and analysis chamber depth were selected to assure accurate image analysis. We found that when using a 12-μm-deep MicroCell chamber (Fertility Technologies, Inc, Natick, Massachusetts), sperm concentrations in excess of 80 million sperm/ml failed to track accurately due to increased collision rates. Therefore, sperm concentrations of 40–70 million sperm/ml were used. The 12-μm depth was selected because it restricted sperm movement within the focal depth of our objective lens.

A 5-μl aliquot of each sample was loaded into a 12-μm-deep MicroCell chamber and the average curvilinear velocity (VCL; micrometers/second), linearity (LIN; 1–100%), straight line velocity (VSL; micrometers/second), amplitude of lateral head displacement (ALH; micrometers), and percent motility (MOT) were determined for at least 200 motile sperm.

### Statistical Analysis

Morphologies and motion parameters of normozoospermic and teratozoospermic samples were recorded as means ± SEM. Dif-

Table 3. *Morphological and kinematic characteristics of freshly collected epididymal spermatozoa from normozoospermic and teratozoospermic domestic cats†*

|  | Normozoospermic (n = 21) | Teratozoospermic (n = 6) |
|---|---|---|
| MOR | 84.40 ± 1.46 | 27.83 ± 2.66* |
| MOT (%) | 78.99 ± 1.58 | 56.93 ± 5.30* |
| VCL (μm/second) | 144.51 ± 3.26 | 95.92 ± 4.14* |
| LIN (0–100%) | 34.76 ± 1.01 | 30.83 ± 1.40 |
| VSL (μm/second) | 48.25 ± 2.19 | 26.68 ± 1.86* |
| ALH (μm) | 6.47 ± 0.14 | 4.35 ± 0.22* |

* Values are lower ($P < 0.01$) than the normozoospermic values.
† Values shown are the mean ± SEM for each parameter determined.

Table 5. *Examples of morphological and kinematic characteristics of epididymal spermatozoa from individual cats**

|  | Normozoospermic | | | Teratozoospermic | | |
|---|---|---|---|---|---|---|
|  | 1† | 2 | 3 | 4 | 5 | 6 |
| MOR | 78 | 82 | 95 | 17 | 23 | 33 |
| MOT (%) | 83.0 | 88.9 | 75.0 | 47.6 | 69.5 | 36.4 |
| VCL (μm/second) | 169.0 | 176.0 | 132.4 | 99.4 | 85.5 | 85.9 |
| LIN (0–100%) | 48.0 | 34.0 | 44.0 | 36.0 | 31.0 | 26.0 |
| VSL (μm/second) | 80.0 | 58.6 | 55.9 | 30.0 | 24.5 | 19.8 |
| ALH (μm) | 6.4 | 7.3 | 5.9 | 3.7 | 4.2 | 4.4 |

* Values shown are the mean of 200 or more sperm.
† Sample number.

ferences between the means were analyzed using the Student $t$-test. The effects of incubation time on MOT, VCL, LIN, VSL, and ALH were also determined using the Student $t$-test. Univariate linear regression was used to assess correlations between MOR and the various motion parameters.

## Results

Morphological and kinematic analysis of domestic cat sperm revealed motility deficits among teratozoospermic males. The structural abnormalities most commonly found in both normozoospermic and teratozoospermic groups were in the midpiece and flagellum (Table 2). Among the teratozoospermic samples, coiled flagellum, bent flagellum, and bent midpiece defects were observed more frequently ($P > 0.05$) than in the normozoospermic group. In all samples examined there was a notable absence of head defects. When the two groups were compared using CASA, it was found that the average values for MOT, VCL, VSL, and ALH were higher ($P < 0.01$) in normozoospermic than in teratozoospermic cats (Table 3). Several sperm samples were incubated in medium at 23°C for an additional 3 hours (Table 4). The prolonged incubation period had no effect ($P > 0.05$) on any of the motion parameters within either group. Examples of ac-

tual CASA values for individual cats are presented in Table 5.

To further demonstrate a correlation between sperm morphology and CASA measurements, samples with MOR values at the extremes, as well as intermediate samples that had been excluded from the above analyses (Tables 3, 4), were subjected to linear regression. Regressional analysis demonstrated strong correlations ($P < 0.01$) between the occurrence of structural abnormalities and VCL, VSL, ALH, and MOT, but not LIN (Fig. 1).
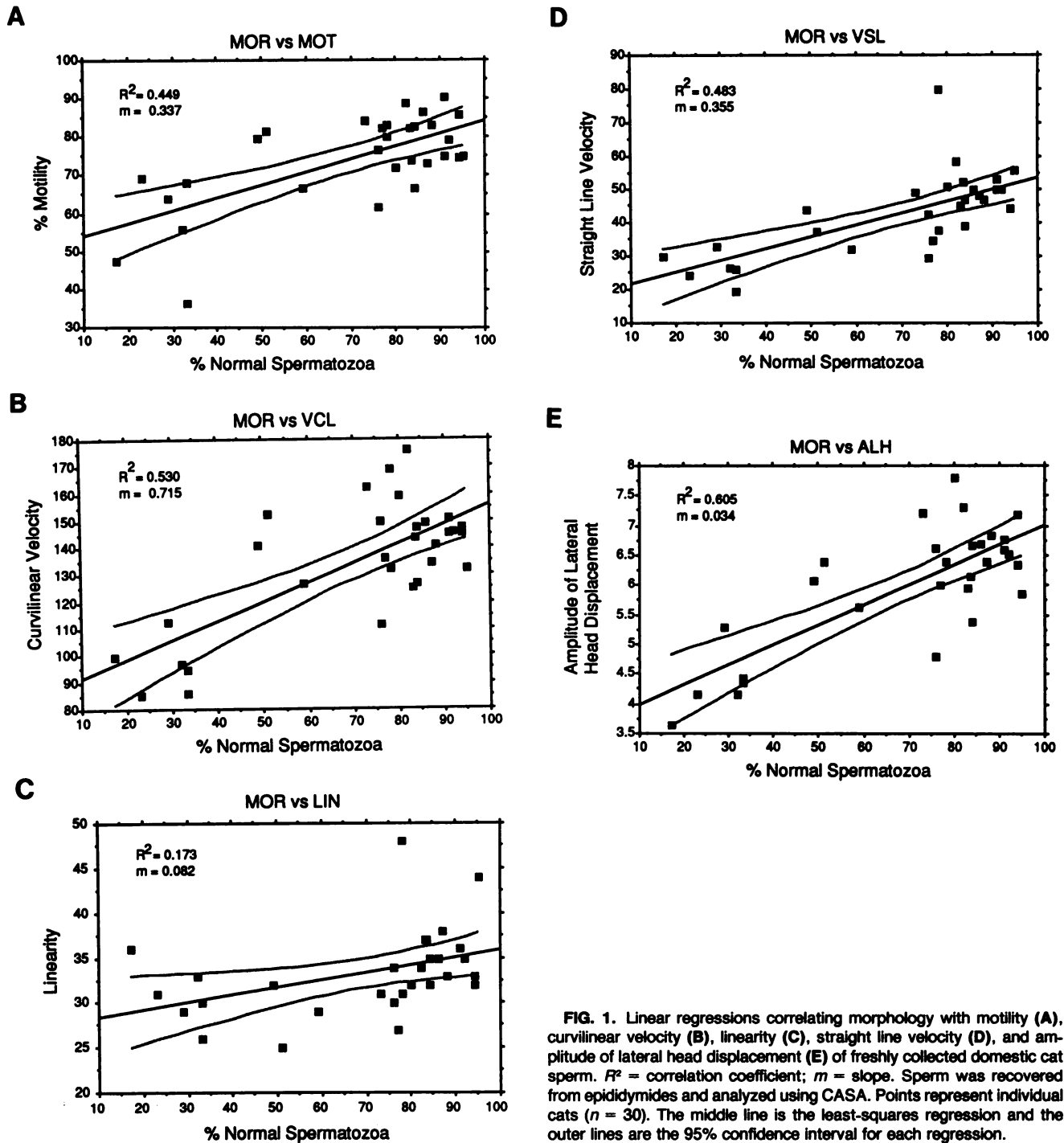
## Discussion

We have identified significant differences between the motion characteristics of sperm from normozoospermic and teratozoospermic cats. This represents the first study to report values for sperm motion parameters in domestic cats. Our data reveal a significant correlation between the morphology of epididymal sperm and their movement characteristics as quantified by CASA and show decreased values for MOT, VSL, VCL, and ALH in teratozoospermic cats as compared to normozoospermic cats (Tables 3, 5). These differences may contribute mechanistically to the overall poor reproductive capacity of teratospermic felids.

Table 4. *Morphological and kinematic characteristics of freshly collected and incubated epididymal spermatozoa from domestic cats**

|  | Normozoospermic | | Teratozoospermic | |
|---|---|---|---|---|
|  | 0 hours (n = 12) | 3 hours (n = 12) | 0 hours (n = 4) | 3 hours (n = 4) |
| MOR | 84.04 ± 2.20 | N.D.† | 26.25 ± 3.82 | N.D. |
| MOT (%) | 76.42 ± 2.16 | 71.93 ± 2.98 | 52.37 ± 6.98 | 56.13 ± 1.36 |
| VCL (μm/second) | 142.80 ± 4.17 | 144.44 ± 5.33 | 91.90 ± 3.62 | 97.05 ± 8.52 |
| LIN (0–100%) | 34.33 ± 1.09 | 31.54 ± 1.19 | 31.50 ± 2.10 | 33.50 ± 2.72 |
| VSL (μm/second) | 46.37 ± 2.23 | 44.19 ± 3.36 | 25.22 ± 2.13 | 29.00 ± 1.35 |
| ALH (μm) | 6.45 ± 0.24 | 6.24 ± 0.31 | 4.08 ± 0.15 | 4.02 ± 0.27 |

* Values shown are the mean ± SEM for each parameter determined.
† N.D. = not determined.

**A**

MOR vs MOT

$R^2 = 0.449$
$m = 0.337$

% Motility

% Normal Spermatozoa

**B**

MOR vs VCL

$R^2 = 0.530$
$m = 0.715$

Curvilinear Velocity

% Normal Spermatozoa

**C**

MOR vs LIN

$R^2 = 0.173$
$m = 0.082$

Linearity

% Normal Spermatozoa

**D**

MOR vs VSL

$R^2 = 0.483$
$m = 0.355$

Straight Line Velocity

% Normal Spermatozoa

**E**

MOR vs ALH

$R^2 = 0.605$
$m = 0.034$

Amplitude of Lateral Head Displacement

% Normal Spermatozoa

FIG. 1. Linear regressions correlating morphology with motility (A), curvilinear velocity (B), linearity (C), straight line velocity (D), and amplitude of lateral head displacement (E) of freshly collected domestic cat sperm. $R^2$ = correlation coefficient; $m$ = slope. Sperm was recovered from epididymides and analyzed using CASA. Points represent individual cats ($n$ = 30). The middle line is the least-squares regression and the outer lines are the 95% confidence interval for each regression.

Of the 30 sperm samples collected for this study, 23 were from cats between 8 and 12 months old. The male domestic cat enters puberty between the ages of 7 and 12 months, as determined by the presence of sperm in the ejaculate (Wildt, 1991). In some species, abnormal sperm production is high in young males and decreases with maturity. Because the occurrence of teratozoospermia and normozoospermia was distributed among cats of all age-

groups used in our study (data not shown), we could not conclude that teratozoospermia was age related. The morphological abnormalities that were most prevalent in epididymal spermatozoa were flagellar and midpiece defects, whereas head anomalies were rare. This distribution of pleiomorphisms is consistent with results obtained using ejaculates (Howard et al, 1990).

Morphology has been shown to be a good indicator of

fertilization ability. Percent normal morphology has been related to the success of the zona-free hamster ova penetration assay and to fertilization ability in humans and felids. Aitken et al (1982b, 1983) revealed that a significant decrease in the occurrence of morphologically normal sperm is associated with decreased penetration ability in the zona-free hamster ova test. Since that time, Rogers et al (1983), Shalgi et al (1985), and Kruger et al (1988) have all reported the predictive value of morphology in hamster oocyte penetration assays. In addition, high proportions of pleiomorphic sperm have been associated with decreased *in vivo* and *in vitro* fertilization of human oocytes (Aitken et al, 1982b; Mahadevan et al, 1983; Mahadevan and Trounson, 1984; Cohen et al, 1985). In domestic and leopard cats (Howard et al, 1990, 1991b), teratospermic individuals are compromised in the hamster penetration assay as compared to normospermic males. Although the percent motile sperm and concentration of normal sperm forms in the media were adjusted to be similar, penetration rates remained lower for teratospermic samples. In addition, only structurally normal cat sperm can penetrate completely through the zona of a homologous egg (Howard et al, 1991a). It is possible that physiological or genetic factors inherent to teratospermic samples may underlie the reduced oocyte penetration rates associated with teratospermic cats.

With the aid of high-speed video micrography, sperm movement can be divided into several categories, each measuring a different motion parameter. Using CASA, we observed significant decreases in all motion parameters of teratozoospermic cat sperm, with the exception of LIN. Because the forward progression of sperm is governed by flagellar beating, a high occurrence of tail and midpiece defects would affect both VCL and VSL. Normal sperm tend to move with symmetrical flagellar beats, with the beat frequency determining ALH. In a sample containing a high frequency of tail abnormalities, the ALH may be decreased. In the present study, the teratozoospermic samples, which exhibited a particularly high occurrence of tail defects, demonstrated significant decreases in VCL, VSL, and ALH. Sperm motility is essential for achieving fertilization and is correlated with hamster egg penetration rates and *in vitro* fertilization, possibly due to its influence on the number of collisions between spermatozoa and ova (Binor et al, 1980; Aitken et al, 1982a; Mahadevan and Trounson, 1984). Because of this correlation, CASA has been used to determine the motion characteristics that are most closely related to fertilization. In general, MOT, VCL, and ALH are of greatest importance. In this study we observed that MOR may impact less on MOT than it does on other motion parameters that can be determined using CASA, as indicated by the data in Table 3. This observation is further demonstrated in Table 5 where teratozoospermic sample #5 retains a

MOT of 69.5%, while VCL, VSL, and ALH are well below normozoospermic values. Aitken et al (1982a,b, 1983) have shown that ALH and the progressive velocity of spermatozoa are different between fertile and infertile men, and are related to overall fertilization ability. Jeulin et al (1986) have reported that ALH is an important factor in IVF success, and that ALH values are reduced in men with low fertilization rates. They suggest that the shearing forces required by sperm to penetrate cervical mucus and the oocyte may be reflected in the ALH value. Others have correlated ALH with bovine and human cervical mucus penetration *in vitro* and the ability to penetrate zona-free hamster ova (Aitken et al, 1985; Feneux et al, 1985).

A surprising finding in our study was that epididymal sperm maintained their motility longer than ejaculated sperm cultured under similar conditions. While ejaculated sperm exhibit a 60% loss of initial motility within 2 hours (Goodrowe et al, 1989), we observed no significant loss in the motility of epididymal sperm over a 3-hour incubation period. Longevity of ejaculated sperm can be extended up to 30 hours following swim-up processing (Wildt, 1991), indicating that factors acquired during ejaculation destabilize sperm motility.

The present study provides kinematic values for domestic cat sperm, revealing important differences between teratozoospermic and normozoospermic males. Our data demonstrate a significant correlation between morphology and motion characteristics. Because similar correlations have been found with human sperm, and were shown to be associated with fertilization potential, it is important to determine the relationship between motion characteristics and fertilization rates in domestic cats.

## Acknowledgments

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