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Can the Fertility Potential of a Seminal Sample Be Predicted Accurately?*

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This paper highlights the most critical aspects of the problem of predicting fertility. To determine if a laboratory test(s) is highly correlated with fertility it is essential to have: a) specific, precise and accurate laboratory tests, and b) precise and accurate fertility data. Acquisition of precise and accurate data for laboratory tests and fertility of spermatozoa in the same sample is not easy. Data derived from *in vitro* fertilization are not tests of fertility, because only a subset of the attributes important for fertilization *in vivo* are tested. Because of deficiencies in fertility data, there probably is no valid report for human spermatozoa correlating results of laboratory tests and fertility, and very few valid studies for laboratory or domesticated animals. There is little doubt that objective measures of sperm motion, acrosomal status, or other characteristics are significantly correlated with fertility. However, establishment of the correlations between a group of attributes and fertility is not the question of interest. The goal is prediction of fertility. There has been no recent effort to develop a prediction of fertility or fecundity based on sperm characteristics, and achievement of this goal may be elusive.

Key words: fertility, laboratory tests, semen quality, computer analyses, binomial variation.

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Clinicians and research scientists continuously are asked if a given laboratory test or combination of laboratory tests can predict the fertility of spermatozoa in a given seminal sample, or of a given male. Unfortunately, this question has no simple answer. Since the turn of the century, there have been countless reports of correlations among the results of different laboratory tests of sperm quality, often correlating data from laboratory tests with fertility. The data in virtually all published papers, including some by this author, are of very limited value for establishing correlations between laboratory tests and fertility. The purpose of this paper is to highlight the most critical aspects of the problem of predicting fertility. Few solutions directly applicable to human spermatozoa will be presented. This paper is not a review of the literature on evaluation of semen quality, automated analysis of sperm motion, or fertility and fecundity.

Even if one has precise and accurate data that give a high correlation between one or several laboratory tests and fertility, the test or tests still might not be useful for predicting fertility. Attainment of that goal requires: a) development of a predictive equation derived from one set of samples, b) application of that

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equation prospectively to a different set of samples, and c) subsequent determination that the predicted results with the second series of seminal samples were reasonably correct.

To determine if one, or a series, of laboratory tests is highly correlated with fertility, it is essential to have: a) specific, precise, and accurate laboratory tests, and b) precise and accurate fertility data. Both laboratory and fertility data should be for the same samples from each of a number of males with a range of fertility typical for the population. Data for at least three samples per male are desirable so that variation associated with samples within males can be separated from that due to differences among males. Although variation among males of most species usually is greater than that for variation within males (Foote and Oltenacu, 1980; Saacke, 1982), the latter is not insignificant.

Acquisition of precise and accurate data for laboratory tests and fertility of spermatozoa in a series of semen samples is not easy. Accuracy refers to correctness of a value, relative to an absolute standard. Precision refers to exactness of a measured or observed value, and reflects consistency in evaluating a given sample. Measurements could be very precise, but inaccurate, or vice versa. In theory, every andrologist (clinician or scientist) should carefully validate all laboratory tests used to characterize semen quality, but this often is not done. There are systematic errors in recording semen volume (Amann, 1981). Variation among observers in the same or different laboratories is marked for subjective tests such as percentage of motile spermatozoa or percentage of morphologically normal spermatozoa. The precision of laboratory tests often is not monitored on a day-to-day basis once the test has been established and, hopefully, carefully validated. Because of deficiencies in fertility data, there probably is no valid report on human spermatozoa correlating results of laboratory tests and fertility, and very few valid studies for laboratory or domesticated animals.

All spermatozoa in an ejaculate are not functionally equivalent, but it is not known which attributes differentiate a fertile spermatozoon from an infertile one. Certainly, any one of a number of deficiencies or defects in a spermatozoon can render it infertile and, if a sufficiently large proportion of a population of spermatozoa is affected, that sample will be subfertile or might have a very low probability of fertilizing an oocyte. Although causes of subfertility differ from male to male, there are certain attributes of fertile spermatozoa regardless of species. These would include:

(a) normal structure of vital functional components, although this cannot be completely determined by light microscopy;
(b) fully functional metabolic pathways to produce energy necessary for sperm motion and maintenance of membrane potentials, ionic microenvironments, pH, or other cellular functions;
(c) motility to enable penetration through the cervix and utero-tubal junction, for departure from storage sites in the mucus or isthmus of the oviduct, and to contact the oocyte and penetrate investments around the oocyte;
(d) peripheral or possibly integral proteins of the plasma membrane that might be termed "survival proteins" and are essential for, or facilitate, survival within the foreign environment of the female reproductive tract;
(e) appropriate responses to the microenvironment and stimuli provided by the female reproductive tract;
(f) proteins, probably integral to the plasma membrane, essential for "recognition" and binding of the spermatozoon to the zona pellucida and vitelline membrane;
(g) enzymes within the acrosome maintained as a proenzyme or in an inhibited form, but available at the appropriate time for aiding in penetration of oocyte investments;
(h) plasma membrane capable of being altered in a timely manner to enable fusion with the outer acrosomal membrane during the acrosome reaction, or to enable fusion of the spermatozoon and oocyte plasma membranes;
(i) precise timing of countless steps in the sequence of events between when a spermatid is first formed by division of a secondary spermatocyte until the same cell has entered the oocyte, and formed a male pronucleus whose chromosomes coalesce with those from the female pronucleus to produce an embryo with a maximum probability of survival; and
(j) DNA adequately stabilized by nucleoprotein, but capable of undergoing decondensation at the appropriate time in the process of fertilization.

To evaluate sperm quality with the goal of accurately predicting fertility, it is essential that tests of several independent parameters be made. Although it may not be feasible to assess each of the 10 attributes listed above, with the possible exceptions of metabolism, motility, response to female stimuli, and timing, probably it would not be cost effective to probe the same attribute using more than one good test. The timing of different events may be difficult to measure, but there probably are several crucial "time windows" through which a spermatozoon must pass in its transformation and development from a spermatid to a male pronucleus within the oocyte.

Laboratory Tests

For many laboratory tests applied to spermatozoa, there is no absolute "gold standard"; one can only establish the relative accuracy of a test. This is not a critical problem, although, when feasible, absolute accuracy should be established and periodically rechecked. Precision of each laboratory test, however, should be of concern to an andrologist using the results to advise a patient about his fertility or a client of the potential fertility of his animal. Precision of any laboratory test will be influenced by biologic variation, human error involved in running the test, performance of instruments, and by inherent mathematical variation associated with the distribution of "observed values" for any test about "the true value."

For tests from which the results are a continuous variable, precision can be estimated from the intra- and interassay coefficients of variation, which should be recorded and monitored over time. With appropriate standards and replication, it should be possible to ascertain the sensitivity (the smallest detectable value or change) of each test and the 95% confidence interval about a given result. Obviously, pregnancy is a binomial variable for which there are only two alternatives (pregnant versus nonpregnant). Some laboratory tests also are binomial variables. Therefore, an appreciation of binomial variance is essential.

Consider classifying 200 spermatozoa as either normal or abnormal. If the "true value" is 65% normal spermatozoa, based only on the mathematical variation associated with this binomial classification, the 95% confidence interval will include 6 to 7 percentage units above or below the mean (from 58 to 71% normal spermatozoa). Note that the midpoint of this interval is slightly closer to 50% than is the true value, since the confidence interval is not symmetrical about the mean. The exact range of the 95% confidence interval about the "observed value" will depend on the "true value" and the number of spermatozoa examined. Assuming that 100 to 300 spermatozoa per sample are evaluated, it will include between 10 and 20 percentage units. Even if one examined 1,000 spermatozoa, the 95% confidence interval still would include about 3 percentage units above or below the mean. In fact, experimental and biologic variance will increase the range of these intervals.

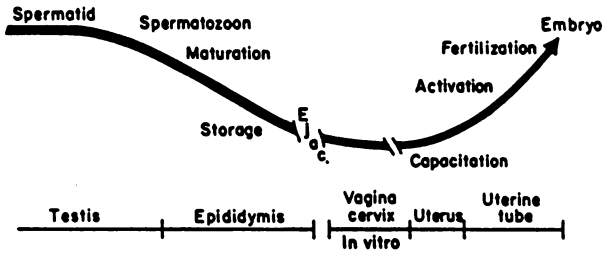

Fig. 1. The saga of the spermatid/spermatozoon. Many events influence ejaculated spermatozoa before and after examination. From Amann (1988c).

Regardless of whether the attribute is a binomial or a continuous variable, the "observed value" usually will differ considerably from the "true value." Also, distributions of values for a series of seminal samples usually are not normal. A logarithmic transformation often will normalize the data, but other transformations may be appropriate. Similarly, reporting data as the median with quartiles in a box and whisker plot, or some other innovative graphic presentation (Cleveland, 1985), may be more descriptive of the population than a mean and standard error.

For quantitative seminal characteristics, testicular size, age of the male, and abstinence interval are important factors influencing the observed value. Treatment of a semen sample prior to evaluation can profoundly effect qualitative tests. Although the most obvious factors are interval between ejaculation and evaluation, control of temperature during this interval, exposure to light, and composition of any diluting medium, there are numerous other factors. Since the goal is to relate results of laboratory tests to potential fertility, it is crucial to recognize the broad spectrum of events before and after an andrologist gains access to a sample (following ejaculation) and their potential effect on both the laboratory test and ultimate fertility (Fig. 1).

Ideally, data for laboratory tests and for fertility should be from the same samples. Although this is possible for cattle, it is impossible for humans. For humans, and most mammalian species, it is necessary to base fertility data on results pooled from several seminal samples from a given male; fertility data based on inseminations from an individual ejaculate of human semen will be too imprecise to be meaningful. Therefore, it is crucial to establish that values for a given attribute of sperm quality are consistent over a short interval (possibly 2 to 3 weeks) so that the mean (median may be better) for each laboratory test can be correlated with the pooled value for fertility resulting from each of several semen samples collected and used during that short interval. By this approach, it may be possible to obtain meaningful correlations between laboratory tests and fertility for

a number of species. However, this does not circumvent the need for precise fertility data.

The ultimate question must be, are data from laboratory tests useful for predicting fertility? To obtain an answer to that question it will be necessary to evaluate critically correlative data between and among a number of laboratory tests and fertility. This should establish which tests have a high correlation with fertility and potential for predictive value, and also which tests are correlated one to another or independent of each other. The range of values for a given test should be considered. To have any success in predicting potential fertility, it would be prudent to select attributes for which individuals span a wide range of values that can be measured by laboratory tests with a high precision, and which appear to be independent one from another, but correlated with fertility.

Evaluation of Fertility

It is axiomatic that it is futile to study relationships between laboratory tests of sperm quality and fertility without good fertility data for the males. *In vitro* tests of sperm quality, such as those using zona-free hamster oocyte penetration, are not measures of fertility. Similarly, data derived from *in vitro* fertilization using homologous oocytes are not tests of fertility. In both cases, only a subset of the attributes presumed important for fertilization *in vivo* are tested. The *in vitro* situation circumvents the need for survival proteins, clearly alters natural time sequences, and probably alters the roles of "recognition proteins," enzymes for penetration of oocyte investments, or triggers for membrane fusion. Although such procedures have obvious relevance for evaluation of sperm quality, and an increasing role in establishing pregnancy, it is not precise to equate results from *in vitro* penetration of oocytes with fertility.

It is very difficult to obtain good fertility data either in a clinical setting or in a carefully designed experiment using animal models. Fertility data in most reports are of marginal value because of numerous problems including:

(a) males, females, or both are not representative of the population in general, and are far too few in number;
(b) confounding treatments may be superimposed, unreported or unknown;
(c) insufficient females are inseminated at the appropriate time relative to ovulation with spermatozoa from each semen sample;
(d) too few semen samples are evaluated in an appropriate manner from each male;
(e) inconsistent and/or excessive numbers of spermatozoa are used for each insemination;
(f) outcome of a given insemination is not reported as pregnancies per cycle of timely exposure.

If one is interested in predicting the fertility of males from a population at large, it does little good to obtain fertility data and compare it with laboratory tests, using semen only from subfertile males who have presented themselves to an andrology clinic. The alternative of basing conclusions on laboratory evaluations and fertility data from donor semen introduces bias, because donors are screened for the quality or freezability of their spermatozoa. Problems in obtaining representative samples of the male and female populations of a species, factors influencing fecundity (Sheps and Lapierre-Adamcyk, 1972), and the exact number of males needed for a valid test, are beyond the scope of this paper. However, these issues should be recognized and addressed in the design of any study.

The precision and accuracy of fertility data will depend on factors (b) to (f) listed above. The need to inseminate sufficient females with spermatozoa from a given sample cannot be achieved with human spermatozoa for ethical and biologic reasons. Nevertheless, it is essential that clinicians appreciate the problem. Too few inseminations are performed with semen from a given donor male to have valid data on fertility of an individual human. This is essentially the same problem a clinician faces in counseling an infertile couple; there are too few cycles of timely exposure of a cohabitating spouse to measure accurately the combined fertility of the pair even though a problem may be perceived and real.

Fertility is a binomial variable, and the ramifications of this for a clinician should be evident from Fig. 2. If the probability is 50% that a cohabitating couple will produce a pregnancy after 15 cycles of timely exposure, which might encompass 2 years of "trying," the 95% confidence interval for the probability of pregnancy is 24 to 76% (about ± 26 units; Fig. 2). This range is only for binomial variation and is an oversimplification of the factors involved (Sheps and Lapierre-Adamcyk, 1972). Nevertheless, binomial variation and not biology or medicine must have a role in "causing" and "curing" a considerable number of infertility problems.

Consider correlating fertility data with laboratory tests of sperm quality. If several semen samples with identical quality and fertility from one man were used to artificially inseminate a total of 10 women (one time each), the 95% confidence interval for the resulting "observed fertility" would exceed 25 per-

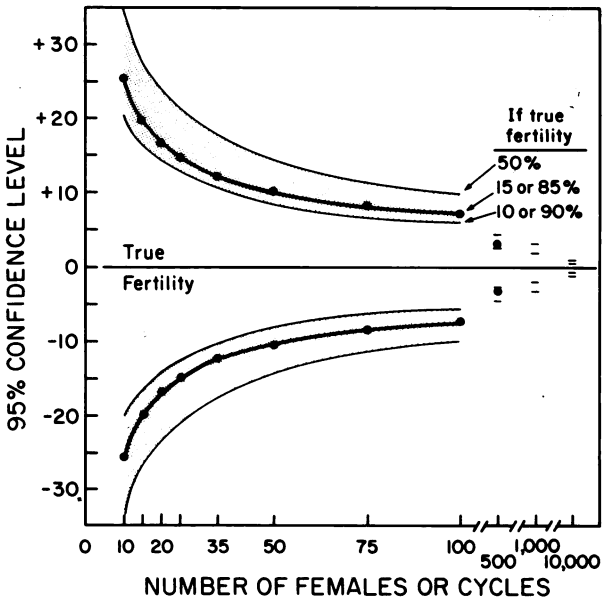

Fig. 2. The approximate 95% confidence interval for a binomial as influenced by sample size (number of females or cycles) and "true fertility." This figure depicts only binomial variance, and actual 95% confidence intervals for fertility would be larger because of biologic and experimental variation. For simplicity, the binomial variation is depicted as equally distributed above or below the mean, but actually it is asymmetric with a greater value on the side toward 50%; this error is negligible if $N > 20$.

centage units on either side of the mean (Fig. 2), assuming the man's "true fertility" was between 15 and 85%! Even if the "true fertility" for this donor was 50% and data for 20, rather than 10, cycles were available, the 95% confidence interval would be similar in magnitude. Given this information, should one have much confidence in published data correlating results of laboratory evaluations of human spermatozoa with fertility?

The problem of binomial variation in fertility data is exacerbated by a multiplicity of biologic and environmental factors that make precision of observed fertility data even less than depicted in Fig. 2. Based on extensive data for cattle (Foote and Oltenacu, 1980), imprecision resulting from biologic and random variation is at least 20% of that resulting from binomial variation. Thus, imprecision of fertility data might be 1.2 × that depicted in Fig. 2.

Probably cattle, and possibly rabbits, goats or sheep, are the only mammals for which precise fertility data can be obtained for ejaculated semen. It is possible to collect several ejaculates from a bull during an interval of 30 to 90 minutes and pool these into a single semen sample which then can be used for laboratory evaluations and artificial insemination of females. Using this strategy, a typical Holstein bull used as a semen donor for artificial insemination of cattle in North America would provide sufficient spermatozoa to prepare at least 300 insemination doses per semen sample. Processing of 600 or even 1000 doses from one pooled semen sample is not rare for bulls with a high daily sperm production and outstanding genetic value. Assuming a "true fertility" of 50%, the 95% confidence intervals for the "observed fertility" (binomial variation only) based on 1000 inseminations is 47 to 53%, as compared with 40 to 60% if based on only 100 inseminations. Obviously, fertility of a given sample of bovine semen can be established with much greater precision than for a given sample of human semen. Since a typical Holstein bull used for artificial insemination provides ≥ 500 doses on each of 2 or 3 days per week, using cattle it should be easy to establish what proportion of the sample-to-sample variation in fertility is attributable to sperm quality as compared with other factors.

The number of spermatozoa, or number of spermatozoa with a given or collective group of attributes, inseminated is an important factor contributing to the imprecision of fertility data. This is true for all species, whether the data result from copulation or artificial insemination. Normal males ejaculate $\geq$ 10-fold more spermatozoa than are needed to achieve maximum fertility and, when appropriate, litter size. Consequently, the number of fertile spermatozoa ejaculated could be reduced by $>90\%$ before a reduction in fertility might be detected. This fact serves as the physiologic basis for artificial insemination of millions of cattle, horses, sheep and turkeys annually. A normal human male might deposit 300 million spermatozoa in the vagina during copulation, but far fewer are necessary if artificial insemination is used. Similarly, a bull might ejaculate 15 billion spermatozoa into the vagina of a cow, but for most bulls normal fertility can be attained by artificial insemination of $<$ 10 million spermatozoa. However, with artificial insemination semen is deposited in the cervix or uterus rather than in the vagina. This reduces, but does not eliminate, loss of spermatozoa from the female reproductive system.

The important point is that the number of spermatozoa entering the reproductive tract during copulation usually greatly exceeds that necessary for maximum fertility. This also is true for artificial insemination of females of any species. After all, the objective of donor artificial insemination is to get

females pregnant. This goal is exactly the problem with most fertility data.

Figure 3 depicts the dose-response curve for the fertility of Holstein cattle, although by changing the numbers on the X and Y axes, the figure could pertain to any species. The number of spermatozoa necessary for maximum fertility lies to the right of a "critical number" designated as C. The curve depicted in Fig. 3 also should be familiar as a typical dose-response curve from a biochemical test or radioimmunoassay. Obviously, with a laboratory test the sensitive area of the dose-response curve lies between points A and B. For reasons identical to those that necessitate measurement of every chemical constituent in semen on the sensitive portion of the dose-response curve, for maximum sensitivity it is essential that fertility be measured on the same portion of the curve.

To obtain valid fertility data for establishing a relationship with the results of a laboratory test, or to detect the deleterious effect of a suspected toxin, artificial insemination is essential to allow deposition of a "critical number of spermatozoa" in each of a series of females (Amann, 1986). Use of this strategy has ethical implications, and certainly requires informed consent of the individuals involved, or of the owners of the cattle or other species. The critical number of spermatozoa could be expressed as total number of spermatozoa or as total number of spermatozoa possessing a particular attribute, or combination of attributes. It is likely that the total number of spermatozoa with a given attribute is more important than the percentage of spermatozoa with that attribute (Pace et al., 1981). At least for progressively motile spermatozoa, the critical number is lower for high fertility bulls than for low fertility bulls (< 5 million versus ≥ 15 million, respectively; Sullivan and Elliott, 1968). The critical number of spermatozoa will differ from male to male within a species and also may depend on the fertility of the population of females. Nevertheless, establishment of the approximate location of point C for a given species or population of males is crucial to obtain valid fertility data. If the number of spermatozoa inseminated is near point B for the most fertile males, even if the number of spermatozoa per insemination for some males is between points B and C, or even slightly to the right of point C, the data will be better than if all inseminations are made with a number of spermatozoa approaching or exceeding point D. If most males of a species have normal fertility with insemination of a dose equivalent to C, but some males have low fertility using dose C, there probably is something unusual about their spermatozoa.

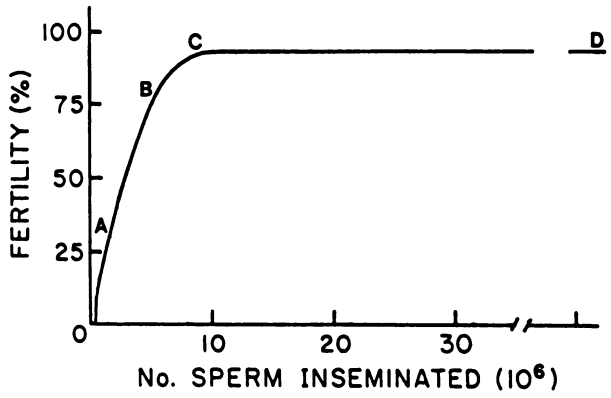

Fig. 3. The dose response curve for fertility as a function of total number of spermatozoa inseminated, or number of spermatozoa with a given attribute inseminated. Point C designates the critical number of spermatozoa needed for maximum fertility of a given male with a given population of females and point D approximates the number of spermatozoa ejaculated by the male during copulation. Point B designates the upper limit of the sensitive area of the dose-response curve which extends from about point A to point B. Insemination of a number of spermatozoa equivalent to point B will maximize the probability of detecting a difference in fertility among males, or a difference between two treatments imposed on spermatozoa from a given ejaculate or male, while minimizing the necessary reduction in fertility. If the number of spermatozoa inseminated is greater than point C, differences between males or treatments may be masked. Point C is not known for humans, but probably is about 7 million spermatozoa for dairy cattle using cryopreserved semen and intrauterine artificial insemination, and about 4 million spermatozoa for intracervical insemination of fresh spermatozoa from CF1 mice. Modified from Amann (1986) and based on concepts discussed by Salisbury and Van Demark (1961).

Reporting pregnancy data as anything other than the number of pregnancies per cycle of timely exposure, or the percentage thereof, would appear to be ludicrous. Preferably, such data should include only the first cycle of exposure. Data for humans frequently are reported as pregnancies per year or per 5 years, and data for horses or sheep frequently are reported as pregnancies per breeding season. With repetitive exposure there is a reasonable probability that a female will get pregnant, even though the combined fertility of the male and female is relatively low. For example, if the fertility of a pair was 15%, after four cycles of exposure there is a 41% chance the female would be pregnant. This cumulative effect is a desirable feature from the perspective of a clinician, but a presentation that tends to misrepresent the actual situation. Also, if timely exposure of the oocyte to spermatozoa did not occur, it is illogical to include data for that ovulation in any compilation

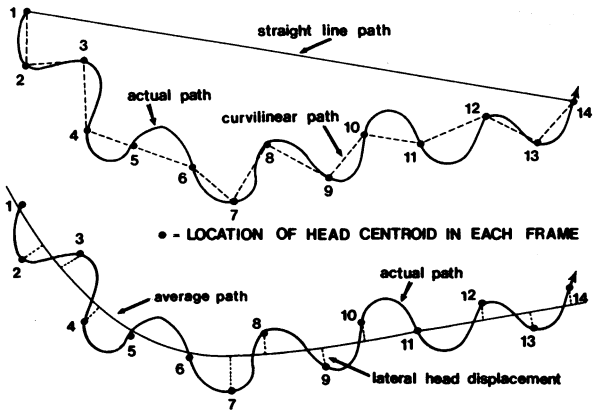

Fig. 4. Path of the centroid of a sperm head and some parameters measured or reported by CellSoft. Based on the location of a sperm centroid in each frame, the curvilinear velocity is calculated from the curvilinear path. Straight line velocity is calculated along the straight line path measured from the first to the last centroid. The function [100 (straight line path/curvilinear path)] is termed linearity and is a crude estimate of the straightness of the actual path. The average path is calculated using a smoothing algorithm. The mean amplitude of lateral head displacement is twice the mean deviation of the centroid locations from the average path (see Fig. 4B). Beat cross frequency is the number of intersections of the actual path and average path per second. Motile bovine spermatozoa were considered as those with a curvilinear velocity $\geqslant 20$ μm/sec. Progressively motile spermatozoa were those with a curvilinear velocity ≥ 20 μm/sec and a radius of curvature for the average path of $\geqslant 80$ μm. Further details are provided elsewhere (Amann, 1988a; Budworth et al., 1988). From Amann (1988a).

of fertility. Furthermore, it should not be assumed that all nonpregnant women will have 13 ovulations per year or that the fertility of a female is constant. Bias also can be introduced by exclusion of data for females leaving the study without becoming pregnant.

Motion Characteristics of Frozen-Thawed Bull Spermatozoa and Fertility

It now is possible to make rapid, objective evaluations of sperm motion. Within the next decade, advances in biotechnology should lead to more biologically sensible tests of sperm quality than have been used traditionally. Although motion is only one of many important attributes of a fertile spermatozoon, computerized evaluation of sperm motion currently is of great interest to clinicians and basic scientists. Thus, computerized evaluations of sperm motion will be used to illustrate the potential and limitations of laboratory tests for predicting fertility.

For a clinician or artificial insemination organization considering purchase of an instrument for analyzing sperm motion, the important question is, "will it enable better prediction of fertility?" For reasons outlined above, that question probably will never be addressed critically using human spermatozoa. Cattle probably are the ideal species for such evaluations. Availability of cryopreserved samples of bull spermatozoa for which fertility was known facilitated our research on whether computerized evaluation of sperm motion would be worthwhile for an andrology laboratory. Recent publications (Budworth et al., 1987, 1988) addressing this issue are summarized below, but the original publications or other sources (Katz et al, 1985; Katz and Davis, 1987; Amann, 1988a; Mack et al., 1988; Stephens et al., 1988; Davis and Katz, 1989) should be consulted for a detailed explanation of how computerized evaluations of sperm motion are conducted.

The general concept underlying systems for analysis of sperm motion is that the image produced by a negative-high, phase-contrast microscope is detected by a video camera or solid-state array and digitized. Typically, data for 20 to 30 video frames are acquired at 30 frames/second. Various algorithms are applied to distinguish spermatozoa from debris and to analyze sperm motion.

Until recently, there was no uniform nomenclature for similar data derived by different systems.[1] Comparisons of commonly used terms are available (Amann, 1988a, 1988b). Parameters discussed below are illustrated in Fig. 4. Both the sampling rate and duration of a scene will affect resulting data. In general, the higher the sampling rate, the closer the curvilinear path will approach the actual path of the middle of each sperm head or centroid (Fig. 4). Correctness of the curvilinear path will affect curvilinear velocity and correctness of the average path will affect the estimate of amplitude of lateral head displacement as a spermatozoon swims and rotates.

Dilution of semen is desirable to minimize collisions among spermatozoa or to minimize "confusion" of the computer by particulate matter or lipid droplets. In our studies, the concentration of spermatozoa in thawed semen was reduced from that characteristic of cryopreserved bull semen (40 to 60 $\times$ 10^6/ml) to one appropriate for computerized evaluation of sperm motion (10 to 12 $\times$ 10^6/ml). Dilution was with similar extender that had been passed through a 0.2-μm membrane filter. Because the

[1]A meeting of experts familiar with computerized analysis of sperm motion was held in Houston, Texas, on March 28, 1988 and standard terminology was agreed to. These terms are used herein rather than those used in the original publications cited.

TABLE 1. Motion Characteristics of Frozen-Thawed Bull Spermatozoa and Correlations With 75-Day Nonreturn Rate*

Criterion	Mean (range)	*r*
Motile spermatozoa (%)	34 (26–44)	0.34
Curvilinear velocity (μm/sec)	68 (49–80)	−0.09
Straightline velocity (μm/sec)	42 (30–50)	−0.09
Linearity	60 (53–66)	−0.02
Amplitude of lateral head displacement (μm)	3.2 (1.9–4.3)	−0.01
Beat cross frequency (Hz)	15.9 (13.3–18.7)	0.05

*Data for 20 semen samples evaluated with CellSoft immediately after thawing; 75-day nonreturn rates based on 620 to 900 inseminations per semen sample. Modified from Budworth et al. (1988).

TABLE 2. Motion Characteristics of Frozen-Thawed Bull Spermatozoa and Correlations With Competitive Fertility Index*

Criterion	Mean (range)	r^2
Motile spermatozoa (%)	27 (9–42)	0.86
Curvilinear velocity (μm/sec)	71 (50–83)	0.68
Straightline velocity (μm/sec)	47 (25–56)	0.70
Linearity	62 (42–68)	0.60
Amplitude of lateral head displacement (μm)	3.4 (2.8–4.0)	−0.05
Beat cross frequency (Hz)	11.5 (8.2–14.3)	0.16

*Data for semen from nine bulls evaluated with CellSoft immediately after thawing; competitive fertility index was calculated from data for 229 calves born following heterospermic inseminations with mixtures of spermatozoa from different pairs of bulls (Saacke et al., 1980). Modified from Budworth et al. (1988).

samples evaluated had been frozen and thawed, the extender contained glycerol. Motion characteristics of these bull spermatozoa presumably were quite different from those that would have been measured for fresh bovine spermatozoa in a less viscous medium. Data reported herein were obtained with a CellSoft system (Cryo Resources, Ltd., New York) at a sampling rate of 30 frames/second with a scene duration of 1 second; 30 fields of view were examined for each sample (> 200 spermatozoa). We found that this system was reasonably precise and accurate for evaluating the motion of frozen-thawed bull spermatozoa, provided ⩾ 160 spermatozoa were evaluated per sample (Budworth et al., 1988).

To determine relationships among parameters of computerized analyses of sperm motion and their correlation with fertility, representative straws of cryopreserved semen previously used in two fertility trials were analyzed (Budworth et al., 1988). The first study was based on 20 seminal samples (Lorton et al., 1988a, 1988b). Fertility was based on the percentage of cows that apparently were pregnant 75 days after a single insemination during estrus (75-day nonreturn rate). Nonreturn rate data were based on 620 to 900 cows per semen sample and the range of nonreturn rates was 17.6 percentage units. Semen from representative straws of each sample was thawed, and within 2 to 10 minutes, spermatozoa in 10 preselected fields in each of three slides were recorded and subsequently evaluated. The samples were incubated at 37 C for 1.5 hours and again slides were prepared, videotaped and evaluated.

Data from motion characteristics of the frozen-thawed spermatozoa shortly after thawing and correlations of these data with 75-day nonreturn rate are summarized in Table 2. For characteristics of sperm motion evaluated by CellSoft, only the percentage of motile spermatozoa and curvilinear velocity differed among samples. This result might have been anticipated, since bulls providing this semen were of known high fertility and were in regular use by a commercial artificial insemination organization. No parameter of sperm movement was significantly correlated with the 75-day nonreturn rate (Table 2). The highest correlation of fertility was 0.34 for percentage of motile sperm cells. The multiple correlation based on six parameters analyzed by CellSoft shortly after thawing semen with 75-day nonreturn rate was 0.40. The similar multiple correlation based on six parameters analyzed after incubating thawed semen for 1.5 hours (data not shown) with 75-day nonreturn rate was 0.64.

A second study involved cryopreserved semen from nine bulls that previously had been used in an experiment in which a "competitive fertility index" was calculated (Saacke et al., 1980a, 1980b). Specially prepared cryopreserved semen was thawed and equal numbers of spermatozoa from two bulls were mixed to provide 25 combinations of mixed semen that were used to inseminate 785 beef cows. Based on the sire of each calf born, as established by phenotypic markers and blood typing, the competitive fertility index was calculated. This index ranked the nine bulls on the basis of their relative or competitive fertility with values ranging from −45.1 to 24.5. The competitive fertility index is a ranking based on relative fertility and is not an estimate of fertility, although the two are correlated. It is not known if this technique overcomes failure to inseminate with a number of spermatozoa that is less than a critical dose. However, the competitive fertility index does overcome many limitations of conventional fertility

data, including differences among females, by directly comparing the biologic quality of spermatozoa from pairs of bulls using several combinations of males. Males found to be of superior relative fertility by heterospermic insemination also tend to be of high fertility when used homospermically, but differences detected heterospermically frequently cannot be detected homospermically (Beatty et al., 1969; Saacke, 1982).

For parameters analyzed by CellSoft, percentage of motile spermatozoa, curvilinear velocity, straight-line velocity, linearity, amplitude of lateral head displacement, and beat cross frequency differed ($P < 0.05$) among bulls (Table 3). Many correlations between computer-determined characteristics of sperm motion and the competitive fertility index were significant. For percentage of motile cells at 0 hours, the coefficient of determination (r^2) for the competitive fertility index was 0.74. The multiple correlation between six swimming parameters analyzed by CellSoft at 0 hours and the competitive fertility index was 0.94. For similar evaluations performed after 1.5 hours of incubation of semen after thawing (data not shown), the multiple correlation coefficient was 0.99. Thus, these attributes accounted for > 88% variation in the competitive fertility index.

The variation of sperm quality among samples was less in Exp. 1 than in Exp. 2. This may be one reason why values for each parameter of sperm motion were not significantly correlated with 75-day nonreturn rates in Exp. 1. Also, the 75-day nonreturn rate typically has less variance than a competitive fertility index, and there was a difference in how the bulls were selected. For these and other reasons (Budworth et al., 1988), it is likely that data for Exp. 2 provided a more stringent and biologically accurate test of the potential usefulness of data obtained by computer for predicting fertility.

Inclusion of several attributes measured by CellSoft in a multiple correlation with the competitive fertility index gave a significantly better fit than use of any single attribute. However, using only the partly independent parameters of percentage of motile spermatozoa and straightline velocity, the multiple correlations with the competitive fertility index were 0.87 and 0.90 for data representing 0 and 1.5 hours, respectively, of incubation after thawing. These attributes also can be measured objectively by semiautomated evaluations of "track motility" (Budworth et al., 1987). Indeed, independent visual evaluations of "track motility" for different straws of cryopreserved semen from Exp. 2 made 8 years apart by Saacke et al. (1980a) and by Budworth et al. (1988), both gave high correlations with the competitive fertility index ($r = 0.93$ and 0.84, respectively). Values for percentage of spermatozoa with normal chromatin, a normal acrosome, or surface characteristics that prevented retention in a microcolumn of Sephadex G-10 ($r = 0.94$, 0.90 and 0.82, respectively) also were correlated with competitive fertility index, but correlations with amidase activity or extent of serum-induced agglutination were lower (Saacke et al., 1980a; Ballachey et al., 1988; Budworth et al., 1988).

The Path to Predicting Fertility

There is little doubt that objective measures of sperm motion, acrosomal status, or other characteristics (Saacke et al., 1980a, 1980b; Saacke, 1982; Budworth et al, 1987, 1988; Ballachey et al., 1988, and references contained therein) may be significantly correlated with fertility and, especially, with a competitive fertility index. Computerized evaluation of sperm motion appears to be useful. Improvements in image detection and analysis should result in measurement of attributes of sperm motion that probably would be highly correlated with fertility if accurate and precise fertility data could be obtained for the species of interest. Once important attributes of sperm function have been established, simplified tests or systems to evaluate objectively these parameters could be developed.

It is crucial to remember that establishment of the correlation between a group of attributes and fertility is not the question of interest. The goal is to develop laboratory tests that are predictive of fertility. This will require application of a predictive equation derived from one set of samples to a different set of samples. There is hope (Van Duijn, 1965) that this may be possible, although 25 years ago acquisition of the necessary data was extremely difficult and tedious. There appears to have been no recent effort to develop a prediction equation for fertility or fecundity based on sperm characteristics, and this may be an illusive goal for human spermatozoa. However, it should be possible to achieve this goal for spermatozoa from some other species. It is plausible that parameters that contribute most to an equation accurately predicting fertility of bull spermatozoa would be useful tests for human spermatozoa. Even if direct use of an equation derived from cattle is inappropriate for humans, it is likely that the same attributes will be important for spermatozoa from both species.

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