

## ***In Vitro* Evaluation of Sperm Quality: An Opinion**

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Evaluation of sperm quality usually is linked with the desire to predict fertility in a clinical setting or to enable maximum number of offspring from a valuable sire. Such evaluations also are the basis of risk assessments relating to drugs, chemicals, or environmental factors, or management decisions affecting billion dollar industries (e.g., dairy, livestock, poultry). Such needs are not recent developments. For almost a century, and possibly longer, clinicians and researchers have striven to develop techniques to predict accurately the fertility of a seminal sample from an individual male. This goal has not been achieved and when phrased in its broadest context is unlikely to be achieved, because of the complex nature of fertilization and different definitions of fertility.\* However, there is an important role for *in vitro* evaluation of sperm quality in an andrology laboratory. Concepts presented herein are equally applicable to sperm from humans, laboratory animals, or livestock.

### ***What is the Goal?***

Evaluation of sperm quality probably can be considered in the context of one of three goals. The first goal can be achieved with present knowledge, the second probably could be achieved with appropriate use of existing biotechnology, and the third may never be achieved. These

goals are: (1) for a male of unknown fertility, predict correctly from one to three† samples of semen that he will have a fertility <40%† of the average value for males of the same type (breed or strain of animal, possibly race of human; age); (2) for a male of known fertility whose semen has been well characterized by appropriate laboratory tests, predict correctly that a given seminal sample from a male has a high probability of providing fertility ≥60%† of that typically achieved in the recent past by spermatozoa from that male; or (3) for a male on whom there are no fertility data, predict correctly from one to three† samples of semen, that his fertility will be greater than the mean fertility for the most fertile males (top 30%† of that species, strain, or breed). Satisfaction of a variant of the first or third goals is typically sought, but only the first two goals are achievable. It is essential that the semen evaluator clearly define his/her specific goal before deciding on which approach to use. Also, prospective prediction is not synonymous with retrospective correlation.

This opinion paper presents our rationale for these conclusions and should stimulate discussion about, and improvement of, approaches for evaluating fertility to achieve the first two of the three goals. In addition, we emphasize from concepts of integrated reproductive physiology why achievement of the third goal is unlikely. Issues raised are important for both clinical or research applications regardless of species of interest.

\* Ramifications of different definitions of fertility are beyond the goal of this paper, but measures used to quantify success or failure range from binding of sperm to investments of the oocyte, detection of embryonic tissues 1–15 days after fertilization *in vitro* or *in vivo*, to birth of a live offspring. The attribute measured, and especially its position in the temporal sequence, will influence success of any attempt to predict fertility. Similarly, the time-frame for success (e.g., one cycle or 1 year) affects predictions.

† Arbitrary values selected by the authors to reflect the data base frequently available and extent of discrimination desired. Other values might be equally or more appropriate.

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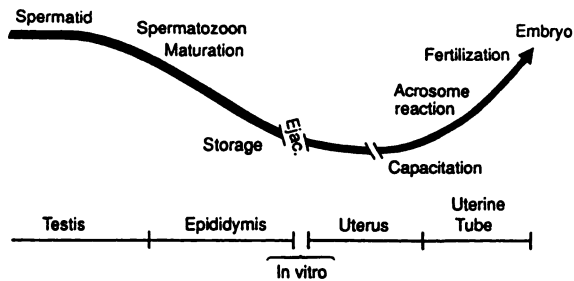
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### ***What Are the Problems?***

Success in predicting fertility is hampered by features of sperm, the process of fertilization, and the approach for *in vitro* evaluation of sperm quality. Understanding problem-causing elements of each feature is necessary for evolution of better procedures to evaluate sperm.

#### ***Spermatozoa***

To be successful a spermatozoon needs to adequately express, or suppress, a number of attributes in the correct temporal sequence to achieve fertilization. One or more attributes may limit fertility of a given cell, with the limiting



**FIG. 1.** The saga of the spermatid/spermatozoon. The function of each spermatozoon is dependent on processes initiated while the cell was a primary spermatocyte or spermatid or during changes induced by the extracellular milieu of the male and female reproductive tracts. Except for limited synthesis of protein within the mitochondria, no biosynthetic capability is retained after spermiation. Energy is produced by aerobic and/or anaerobic catabolism of carbohydrates and lipids and degradative actions of endogenous or exogenous enzymes can modify sperm. From Amann et al (1993).

factor depending on the individual spermatozoon, the male (or strain), how sperm are sampled and evaluated, and how sperm are used. A male germ cell undergoes a continuous process of modification, initiated in a primary spermatocyte (Fig. 1; Amann et al, 1993; Hammerstedt, 1993). This includes formation, development, and release of spermatids; maturation of spermatozoa within the proximal epididymis; maintenance and storage of fertile spermatozoa in the distal epididymis; admixture of sperm with seminal fluids at emission, initiating progressive motility; exposure to the environment of the female reproductive tract after ejaculation; exposure to the microenvironment of the oocyte, resulting in hyperactivation and the acrosome reaction; and exposure to the cytoplasm of the oocyte and formation of the male pronucleus (Amann et al, 1993).

A pathophysiological or atypical event occurring during the developmental process depicted in Figure 1 *prior* to the point of evaluation, which typically is shortly after ejaculation, will suppress spermatozoal quality below that ideally achieved by a "perfect male." This might be detected. Any unanticipated event occurring *after* the time of evaluation could change subsequent spermatozoal modifications from those anticipated by the evaluator and increase or decrease the probability of fertilization from that typical for that individual or males of that breed (or strain). This might remain unknown, and in any case could not be factored into a prediction. Unanticipated events occurring after evaluation include an error in processing sperm for cryopreservation or *in vitro* fertilization, subtle pathophysiological changes in the uterus or oviduct, or an atypical oocyte.

### Fertilization

Fertilization is a probability event related to the presence of sufficient fertile sperm near an oocyte. There are two probabilities for fertilizing capability involved: (1) that

**Table 1.** A partial list of attributes of sperm essential for fertility\*

"Acceptable" morphology
Metabolism for production of energy
Progressive motility
Capacity for hyperactivated motility
Membrane lipids
Stabilize plasma and acrosomal membranes
Flippase enzyme activity
Facilitate timely fusion; but not premature fusion of membranes
Membrane proteins
Immunosuppressive factors
Attachment ligands available but masked to prevent premature binding
Acrosome reaction-inhibiting factor
Integral enzymes associated with fertilization
Enzymes modifying membrane glycoproteins
Acrosomal enzymes

\* In addition, the genome of the fertilizing spermatozoon may affect embryo development and apparent fertility. The genome package must contain genes needed for development and lack lethal mutations or extra genetic material preventing development.

for each spermatozoon, all essential attributes will be expressed at the right site and time; and (2) that for the total population of sperm, the population contains a given percentage of sperm with full fertilizing potential. An individual spermatozoon with unique attributes fertilizes the ovum, and its features probably do not reflect the population average attributes. Some attributes of a spermatozoon necessary for fertilization are recognized (Table 1), but other remain unknown. Attributes necessary for fertilization will depend on the methodology used for *in vitro* fertilization, the nature of storage and site of sperm deposition by artificial insemination, the mating strategy employed or allowed with natural mating, and female factors. Correct expression of fewer spermatozoal attributes is required with *in vitro* fertilization, and artificial insemination can reduce or eliminate certain restrictive interactions between sperm and the female environment. Use of cauda epididymal sperm rather than ejaculated sperm, cryopreserved sperm rather than fresh sperm, or intrauterine rather than intracervical artificial insemination usually will affect the outcome.

Because fertilization is a probability event, the biologically relevant question is: How many of the sperm exposed to oocytes completed *all* the steps of attribute expression, or suppression, necessary to achieve fertilization of oocytes under the conditions imposed, and retained "enough" molecules representing each attribute to provide a "combined effective amount"? (Amann et al, 1993). Recognition of this concept introduces two important problems: (1) some important attributes of a spermatozoon have not been identified, although many are known (Table 1), and (2) the amount of each attribute that is "enough" must be established.

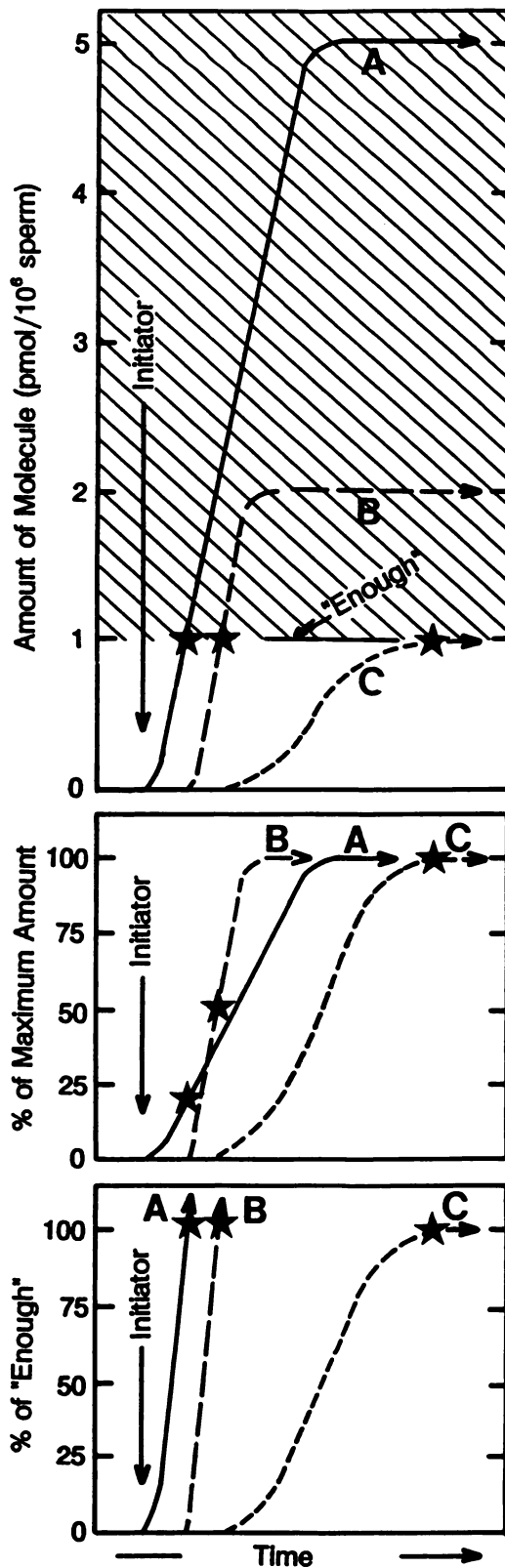


FIG. 2. Contrast between percentage of "enough" and maximum measured amount of each of three hypothetical molecules present in sperm. For each molecule (A, B, C), 1 pmol/10<sup>6</sup> sperm is "enough," and this value is designated by a star on the plots for each molecule in all

"Enough" represents the minimum amount, or range in amount, of a molecule or attribute a spermatozoon requires to induce a maximal response (Hammerstedt, 1989). "Enough" can have a high or low value, and for some molecules an amount greater than enough can preclude fertilization. Figure 2 facilitates understanding the term "enough" and shows comparisons in absolute and relative amounts for three hypothetical molecules representing attributes of a spermatozoon required for fertilization capability. Initial studies reveal the "need" for A, B, and C (plots in top and center panels; Fig. 2), and later analyses might reveal the critical molar amounts of each needed for the biological event under study—"enough" (stars on plots that for a population of sperm represent a range). Assume, in this example, that most sperm in a sample from a highly fertile male of a given species have a fivefold excess of molecule A, a twofold excess of molecule B, and no excess of molecule C; for simplicity, "enough" equals 1 pmol/10<sup>6</sup> sperm. Production or availability of 2×, 5×, or 50× of "enough" does not increase the response or probability of success in fertilization; *enough is enough*.

Typically, analytical data are reported in absolute or relative amounts (top and center panels, Fig. 2) and a reduction of X% is considered bad. This can be very misleading. For the example in Figure 2, a 40% reduction of each molecule would not affect functionality of attributes A and B; enough of these molecules remain. Conversely, only for attribute C is a value ~100% of the maximal amount needed (center and bottom panels, Fig. 2).

Until the value of "enough" is established, a potential problem in data interpretation can arise when simple dose-response relationships are dissected to reveal temporal response or predictions of success. In the example presented, attribute C is the only candidate (of A, B, and C) that never is expressed in excess. When each attribute is arrayed in terms of its maximum absolute value (top

←  
three panels. The vertical arrow designates an initiating event. As evident in the top panel, an event (in the epididymis, after mixture with seminal plasma, or in the female tract) initiates an increase in absolute amount of A; after 100% of "enough" A is present, A initiates a change in B; after 100% of "enough" B is present, B initiates formation of C. Amounts of A or B per sperm continue to increase, whereas the maximum amount of C never exceeds "enough." It is evident that the correct sequence is A → B → C. The middle panel shows the same data plotted as a percentage of the maximum amount. Data on sperm quality usually are expressed as in one of the top two panels, but from neither would it be evident that loss of 40% of the amount (top) or maximum value (center) of A or B would have no consequence on fertility. Clearly, loss of C would preclude fertility. The bottom panel depicts the same data expressed as a percentage of "enough." A change to less than ~100% of "enough" precludes subsequent events and will cause that spermatozoon to be infertile. Unfortunately, it is difficult to establish experimentally how much is "enough" until detailed studies have established the true sequence. Examples of precursor-product relationships were presented by Hammerstedt (1989).

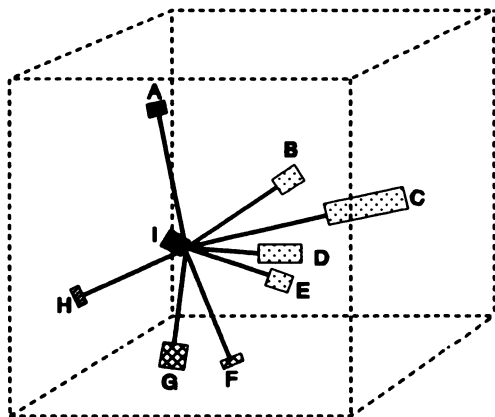


FIG. 3. Theoretical "combined effective amount" of 9 attributes in a highly fertile spermatozoon. Some compensation among attributes B, C, D, and E or F and G is possible, but there is no compensation across these two groups or with attributes A, H, and I. Amount is depicted by the length of the radiating vector line, and "enough" (range of amount consistent with fertility) is depicted by the box. For some attributes, this range encompasses much greater amounts than the absolute minimum amount needed, but this has no effect on fertility. Some attributes are quantal (A, B, D, F, H, I) and others dose-responsive (C, E, G). Direction of the vector line has no meaning. Modified from Amann et al (1993).

panel, Fig 2), the apparent order of action is  $A \rightarrow B \rightarrow C$ . But, when considered in terms of attainment of maximum value (center panel, Fig. 2) the apparent sequence is  $B \rightarrow A \rightarrow C$ . Only detailed analyses of the system will eventually reveal the value of "enough" (number of moles needed for maximum response; placement of stars in all panels) and enable normalization of data to percentage of "enough" (bottom panel, Fig 2). The "overshoot" in the production of A and B is obvious (center panel, Fig 2) and the correct sequence,  $A \rightarrow B \rightarrow C$ , is unequivocal (bottom panel, Fig. 2).

Essential attributes can interact in a synergistic, additive, or subtractive manner to achieve the maximum fertilizing potential for a given spermatozoon, termed "combined effective amount" (Fig. 3). To some extent, deficiencies in one attribute can be offset by expression of other attributes. The degree to which a deficiency or excess is overcome is reflected in the "combined effective amount."

Three other features of fertilization (Amann et al, 1993) complicate evaluations. These are that: (1) some attributes need to be present at a maximal value while others must be reduced to a minimal value (i.e., "enough" can be high or low); (2) some attributes needed for fertility probably are quantal (like a conventional on-off light switch), whereas others probably allow a gradually reduced probability of success before going to the off position (analogous to a dimmer-type light switch); and (3) time. For a dose-responsive attribute, there is a threshold amount necessary ("enough") and an excess amount that provides the dose-response (Fig. 4); the excess or dose-

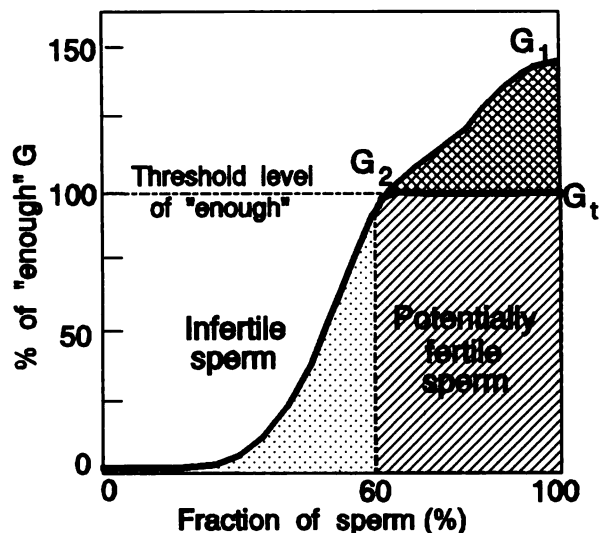
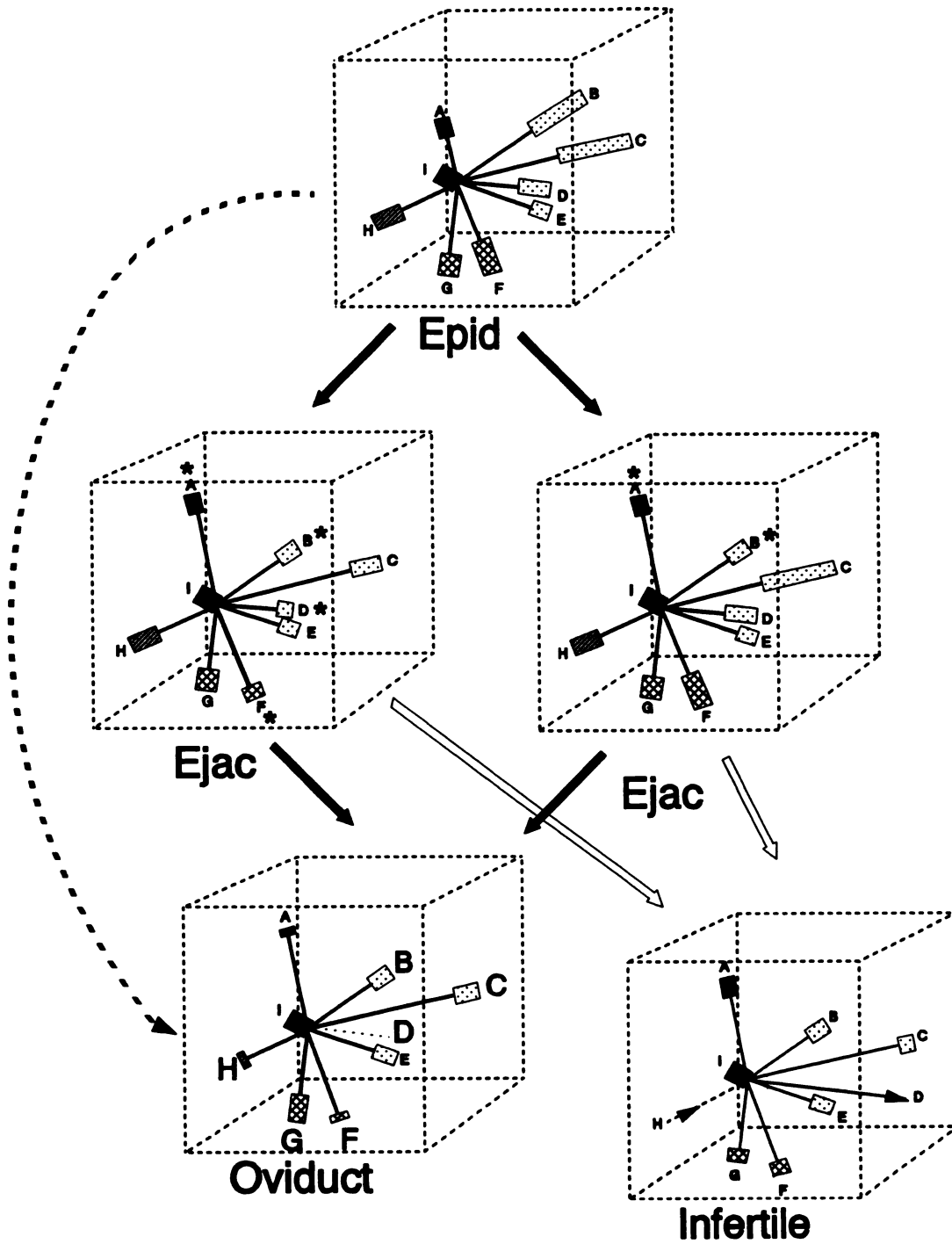


FIG. 4. Hypothetical distribution of attribute G, a dose-responsive attribute, in the subpopulation of sperm, which has "enough" of all other attributes necessary to fertilize an oocyte. Of all sperm in this subpopulation, 60% have insufficient G to be fertile (stippled) and 40% have "enough" G to be fertile ( $G > G_2$ ; striped area). However, because they contain more G, sperm such as  $G_1$  have a greater probability of fertilizing an oocyte than sperm such as  $G_2$ . The weighted average excess of attribute G (cross-hatched area) above the threshold of "enough" ( $G_t$ ) provides an estimate of the dose-response effect ( $G_{DR}$ ) for this attribute.

responsive amount integrated for all sperm in the sample provides a population estimate for that attribute ( $G_{DR}$  in Fig. 4). Similarly, for all dose-responsive attributes the integrated excess amount ( $\Sigma_{DR}$ ) provides an important measure of the quality of a sample.

Figure 5 shows hypothetical changes in sperm as a function of sampling site and also illustrates that even at one site, ejaculated semen, a different blend of attributes can constitute a "combined effective amount." Different amounts of an attribute may be appropriate (i.e., "enough") in epididymal sperm but excessive in ejaculated sperm (compare "B" in epididymal vs. ejaculated sperm in Fig. 5) or need to increase in amount (e.g., attribute "A"). It also is likely that the values for "enough" contributing to the "combined effective amount" differ slightly for individual sperm, as illustrated by the two ejaculated sperm in Figure 5. Given the concepts depicted in Figures 2-5, measurement of absolute amount of an attribute, in an attempt to predict fertility is of value only when: (1) the value of "enough" is known, and (2) the result obtained can be placed in the context of a "combined effect amount" and a given sampling site or treatment.

It is well established (Salisbury and VanDemark, 1961) that the apparent fertility of a given male (Pace et al, 1981; den Daas, 1992) or strain of animals (Robl and Dziuk, 1984) will follow a dose-response curve related to the total number of sperm (or the number of sperm competent



**FIG. 5.** Theoretical "combined effective amount" of attributes in sperm, illustrating changes in sperm as they evolve from the cauda epididymidis (epid) to ejaculum (ejac) and to the vicinity of an oocyte (oviduct). Representatives of two subpopulations of ejaculated sperm are shown, which differ in expression of attributes C, D, E, F, and G (compare location and length of boxes representing "enough"); both have a "combined effective amount" and are capable of fertilizing an oocyte. Subpopulations of epididymal sperm also exist (not shown). Differences between epididymal and ejaculated sperm are designated with a \*. Attributes for which "enough" differs between epididymal and oviductal sperm are designated with a large letter; oviductal sperm should not express attribute D (hence no box). Also shown is a spermatozoon (infertile) that has deteriorated because of senescent changes in expression or amounts of attributes D and H (arrow heads); excessive D and lack of H preclude this spermatozoon from having a "combined effective amount" of attributes.

for a given attribute) used for artificial insemination.‡ Typical data for bulls and mice illustrate that for many males the dose-responsive portion of the curve has a steep slope leading to maximum fertility of <100% (Fig. 6). These curves take the shape of an asymptotic curve, described by Equation 1,

$$y = ae^{-b/x} \quad (1)$$

as illustrated by Salisbury and VanDemark (1961) and Pace et al (1981) and confirmed by den Daas (1992). In this equation,  $y$  is fertility,  $a$  is the asymptotic limit,  $e$  is the natural logarithm base 2.7183,  $b$  is a function of the slope to the asymptote, and  $x$  is the number of "viable" sperm inseminated.

For bulls, insemination of  $10\text{--}40 \times 10^6$  sperm does not increase fertility above that achieved with  $\leq 10 \times 10^6$ . Insemination of additional sperm does not improve fertility of a subfertile bull (Sullivan and Elliott, 1968; Salisbury et al, 1978; Pace et al, 1981; den Daas, 1992). Hence, incomplete expression (or failure of expression) in most sperm of one or more of a series of attributes determines the slope of the left portion of the dose-response curve depicted in Figure 6 (component  $b$  in Equation 1), and for a given male or strain, there is an absolute maximum fertility (component  $a$  in Equation 1). The fact that the slopes in Figure 6 are not identical is evidence that some important attributes are of the dimmer-switch type (not quantal), and/or there are interactions among several quantal attributes resulting in graded responses. For purposes of modeling, we have assumed that the slope in Equation 1 is determined by the reciprocal of the integrated population value for the excess amount of all dose-responsive attributes,  $\Sigma_{DR}$  as defined above. Hence, Equation 1 might be rewritten as Equation 2, where  $\#e$  is the total number of sperm inseminated, each of which contains the "combined effective amount" of attributes.

$$y = ae^{-(1/\Sigma_{DR})/(\#e)} \quad (2)$$

We postulate that differences in the slopes of the lefthand portion of the curves for bulls A and B or mice strains R and S (Fig. 6, left and right panels, respectively) reflect subtle differences in attainment or loss of functional attributes essential for fertility (e.g.,  $\Sigma_{DR}$ ), only partially compensated for within the "combined effective amount." Further, it is likely that a slightly lower percentage of sperm from bull A retained a "combined effective amount" of attributes essential for fertility, so more sperm must be inseminated to reach maximum fertility than are required

‡ Recent data for cattle (Nadir et al, 1993) could be interpreted to be contrary to this concept, but the authors attribute higher fertility after insemination of  $100 \times 10^6$  bull sperm, rather than  $20 \times 10^6$ , to the lower dilution ratio and beneficial effects of a fivefold higher concentration of seminal plasma.

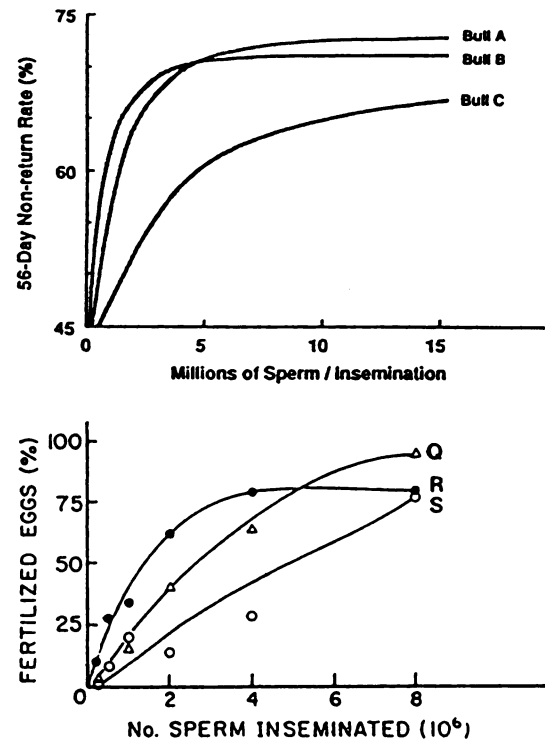


FIG. 6. The effect of number of fully competent sperm inseminated on apparent fertility of bulls (top) and mice (bottom). Depicted in the top panel are data for three bulls showing the effect of total number of sperm inseminated on pregnancy rate 56 days after artificial insemination. The percentage of fully competent sperm, those having a "combined effective amount" of essential attributes, is assumed to be similar for each insemination dose from a given bull, and the percentage of pregnant cows at 56 days is assumed to provide an estimate of fertilizing capacity of the sperm in which embryonic death between fertilization and day 56 is independent of number of sperm inseminated. The text details how differences in spermatozoal attributes are thought to result in curves such as these. Dose-response curves for bulls are based on data from den Daas (1992) and those for fertility of three strains of mice are based on data from Robl and Dziuk (1984).

for bull B. However, a high percentage of sperm from both bull A and bull B have a "combined effective amount" since fertility is excellent. If a quantal attribute was involved in reduced fertility of bull B, and especially bull C, insemination of sufficient sperm would raise fertility to the maximum value achieved by bull A. Clearly this is not the case.

Sperm from males with a reduced maximum fertility may not have all of the dimmer-type switches for dose-responsive attributes fully turned on. More likely, however, semen from such males contains a relatively high proportion of sperm with "enough" of each quantal attribute necessary for a spermatozoon to have a high probability of approaching an oocyte but do not have "enough" of a quantal attribute(s) required for fertilization. Hence, such sperm would diminish the probability of sperm with a "combined effective amount" of all attributes from fertilizing the oocyte. This could explain the relatively

low fertility of bull C or mouse strain R. Alternatively, the incorrect amount of one or more dose-dependent attributes could result in a timing error in embryonic development, and this, or a genomic defect, would cause increased early embryonic death. Absence of "enough" of a quantal attribute in most/all sperm would change slope rather than the asymptotic value.

#### Approach to Evaluation of Sperm Quality

Any seminal sample contains a heterogeneous population of sperm. At any instant, not all sperm in a population have the same fertilizing potential, some are "early" or "late bloomers," whereas others never acquire fertilizing potential or die before an oocyte is available. This probably facilitates success in fertilization in mammals where passage through the female reproductive tract and possibly a wait of several days are required before expression of terminal attributes of sperm function including egg binding, penetration, and provision of a male pronucleus (Amann et al, 1993).

Traditional approaches to evaluation of sperm quality ignore this heterogeneity and, hence, are seriously flawed. With conventional analyses (Salisbury et al, 1978; Saacke, 1982; Anonymous, 1992; Keel and Webster, 1992), the mean value for a population of sperm usually is considered for each attribute (e.g., percentage data or mass/10<sup>6</sup> sperm) or two or three attributes (Duncan et al, 1993), rather than values for multiple attributes of an individual spermatozoon (e.g., this spermatozoon has "enough" of each essential attribute and a "combined effective amount"). However, as noted above, the biologically relevant question is how many of the sperm exposed to oocytes have a "combined effective amount" of molecules in the matrix of attributes mandatory to allow a spermatozoon to fertilize an ovum in a temporally correct manner. Thus, a sample of semen contains a series of subpopulations of sperm, many of which lack "enough" of one or more attributes essential for fertility (Fig. 7). The population(s) with "enough" of each attribute must be enumerated.

Acceptance of concepts depicted in Figures 2–7 and the text associated therewith leads to the conclusion (Table 2) that conventional analyses can be used accurately only to identify males likely to be infertile or sterile; this would only achieve Goal 1, listed at the outset. § This conclusion is compatible with a previous detailed analysis (Foote and

§ Since the original submission of this manuscript, Jeyendran and Zaneveld (1993) editorialized their concerns about evaluation of sperm quality. We concur with their views and, especially, their points that (1) evaluation of quantal attributes is important provided each attribute represents a vital and different feature of sperm function, and (2) if all values for a limited number of population tests are normal, no definitive conclusion can be drawn concerning fertility of that sample of sperm. The latter point is evident in Table 2.

Table 2. Prediction of fertility on the basis of spermatozoal quality, as reflected in amounts or localization of several attributes

Attributes and status*	Predicted outcome†
For individual sperm in a population, most have:	
A, B, C, D, E, F normal; G abnormal	Infertile
A, B, C, D, E, G normal; F abnormal	Infertile
A, B, C, D, F, G normal; E abnormal	Infertile
A, B, C, E, F, G normal; D abnormal	Infertile
A, B, D, E, F, G normal; C abnormal	Infertile
A, C, D, E, F, G normal; B abnormal	Infertile
B, C, D, E, F, G normal; A abnormal	Infertile
A, B, C, D, E, F, G normal; none abnormal	FERTILE
For the population of sperm, mean value:	
A normal	Unknown
A abnormal	Infertile
B normal	Unknown
B abnormal	Infertile
A and B normal	Unknown
A and B abnormal	Infertile

\* A, B, C, D, E, F, and G represent attributes evaluated in terms of amount per sperm or population of sperm or in terms of location on individual sperm. Normal designates "enough" of that attribute, and abnormal designates either an unacceptably high or low value.

† Outcome that can be predicted with reasonable certainty.

Oltenacu, 1980). It also should be evident that individual sperm could be infertile for different reasons, and it is well established that different males are infertile for different reasons (excluding oligospermia or azoospermia).

#### How to Evaluate Sperm Quality

The ideal *in vitro* evaluation of sperm quality would simultaneously quantify in several hundred, or preferably several thousand, sperm in a given sample the amount of each of a series (possibly 6–10; designated A through I in Fig. 3) of attributes representing different features required for fertility. Recall that attributes A, B, D, F, H, and I give a quantal response, and attributes C, E, and G give a dose-response above a threshold (see Fig. 3). Attributes present at "enough" in a spermatozoon might be designated as A<sub>e</sub>, B<sub>e</sub>, C<sub>e</sub>, etc. for quantal attributes, and spermatozoa with a "combined effective amount" of attributes as CEA. For dose-responsive attributes in sperm having a "combined effective amount," the value for the integrated excess amount of all dose-responsive attributes might be designated Σ<sub>DR</sub> (see cross-hatched area in Fig. 4 for attribute G).

Data for a given sample then could be expressed in terms of percentage of "enough" and categorized to provide the proportions of sperm having a "combined effective amount" (f<sub>CEA-s</sub>) or lacking "enough" of one or more attributes. With these data, knowledge of the value for the integrated excess amount of all dose-responsive

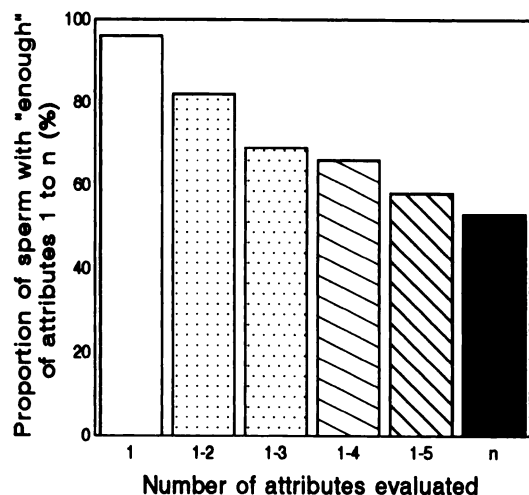


FIG. 7. Hypothetical distribution of subpopulations of sperm in an ejaculate. If only one attribute is evaluated, most sperm may be satisfactory, and accurate prediction of fertility would be impossible. As combinations of two or more attributes (1-2, 1-3, 1-4, 1-5, etc.) are evaluated, additional subpopulations of sperm become evident (each with a different character), and progressively fewer sperm will be satisfactory because individual sperm will lack "enough" of one or more attributes. Only sperm in population  $n$  (solid bar), for which all essential attributes were evaluated, have a "combined effective amount" of attributes necessary to fertilize an oocyte. The size of populations identified by evaluating  $<n$  attributes may not accurately predict fertilizing potential of the sample.

attributes ( $\Sigma_{DR,s}$ ), selection of insemination dose ( $Dose_s$ ), and prior knowledge of the fertility of that male (or strain of highly inbred animals), it is likely that one could achieve Goal 2—namely, accurately predict if a given seminal sample would be of high, average, or low fertility relative to others from that male.

What elements might an equation for prediction of fertility contain? Building on Equations 1 and 2, an equation similar to Equation 3 might have utility. Although Equation 3 includes features likely to be important, it cannot be tested because of limitations of available analytical procedures; other equations might be equally valid.

#### Predicted fertility of sample S

$$= \left( \frac{\text{Established maximum fertility of sire}}{\text{fertility of sire}} \right) (2.7183)^{-[1/(\Sigma_{DR,s})(Dose_s)(f_{CEA,s})]} \quad (3)$$

This equation assumes prior knowledge of fertility for the sire or strain of animal. Data for the sample under consideration, namely the fraction of sperm with the "combined effective amount" of attributes ( $f_{CEA,s}$ ), the value for the integrated excess amount of all dose-responsive attributes ( $\Sigma_{DR,s}$ ), and the total number of sperm to be inseminated ( $Dose_s$ ), can be used to predict fertility—achievement of Goal 2. Responses to changes in each element of Equation 3 are depicted in Figure 8. As compared in plots B and D, a decrease in  $f_{CEA,s}$  has little effect on fertility provided enough sperm are inseminated.

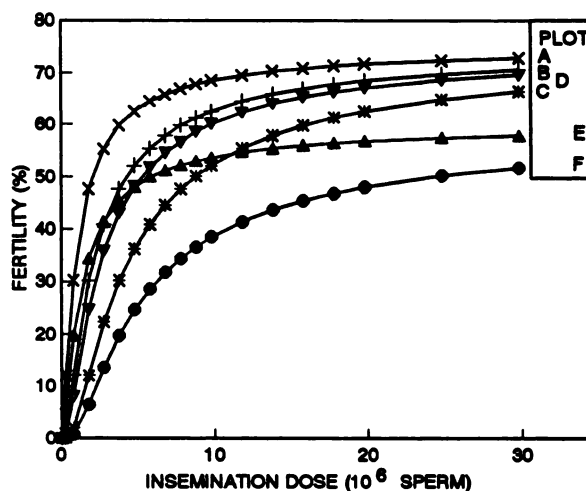


FIG. 8. Potential fertility as a function of number of sperm inseminated ( $Dose_s$ ), proportion of sperm with a "combined effective amount" of attributes necessary for fertilizing an oocyte ( $f_{CEA,s}$ ), and integrated population value for the excess amount of all dose-responsive attributes ( $\Sigma_{DR,s}$ ). Curves calculated using Equation 3. For plots A–D, the previously established maximum fertility was assumed to be 75%, but for plots E and F it was assumed to be 60%. For plots A–C and D–F, respectively,  $f_{CEA,s}$  was 0.55 and 0.45. Plots A–C illustrate effects of different values for  $\Sigma_{DR,s}$ , selected as 2.0, 1.0, and 0.5, respectively; the lower the value the shallower the slope. Plots B and D differ only in  $f_{CEA,s}$ , 0.55 and 0.45, respectively; both have values of 2.0 for  $\Sigma_{DR,s}$ . Plot F, when compared with plot E, illustrates the effect of a low value for  $f_{CEA,s}$ , 0.5 vs. 2.0, for samples of relatively low fertility; both plots assume 60% maximum fertility and a value of 0.45 for  $\Sigma_{DR,s}$ .

However, changes in  $\Sigma_{DR,s}$  affect the slope and, hence, predicted fertility at low insemination doses; not shown in Figure 8 is the fact that at a dose of  $>50 \times 10^6$  sperm, plots A, B, and C all approach the maximum fertility of 75%. This equation could be transformed to enable calculation of the insemination dose (total number of sperm) necessary to achieve maximum fertility. Information on number of sperm with a "combined effective amount" needed in the recent past to achieve this maximum fertility might make any prediction more accurate by modeling past responses. Further, effects of insemination dose on fertility could be modeled to maximize biological or economic benefit. Utility of such an equation or an earlier prediction equation (van Duijn, 1965) is uncertain.

Possibly the only accurate approach to prediction of fertility, to be obtained later under conditions of natural mating or artificial insemination, for a male about whom virtually nothing is known would be to add *in vitro* tests of oocyte penetration, chromatin decondensation, and pronucleus formation, and chromosomal anomalies associated with embryonic death to the list of attributes used in a prediction equation. Specific genetic limitations might be evaluated by probing recondensed sperm chromosomes or restriction fragment mapping. Such techniques might identify males similar to bull C in Figure

6, who is presumed to have a genetic defect(s) restricting his fertility.

As discussed elsewhere (Amann, 1989), any *in vitro* test(s) for evaluation of sperm quality can only be judged against robust fertility data. Because of biological and ethical limitations, validation of approaches for evaluation of sperm quality will be restricted to a few common species such as cattle, poultry, rabbits, and sheep. Extrapolations to other species of economic importance, humans, or species of interest in pharmacologic or toxicologic studies will be required because accurate fertility testing is logistically impossible. Prognostic value may decline as a consequence of lateral transfer from species A to species B.

### The Future

How is a future laboratory evaluation of sperm quality likely to be achieved? A two- or three-tiered approach seems logical, and each should emphasize the characteristics of individual sperm. The entry-level tier would be a microscopic assessment, either visually with a phase-contrast microscope or utilizing a simple computerized system (CASA system), which would address the questions if most sperm are progressively motile and if a high percentage of sperm have a normal morphology (low incidence of shape traits known to be associated with infertility). Failure to consistently pass this population test for several samples from a given male (see Table 2) probably would preclude further analysis (in most cases); the conclusion would be low fertility. This could achieve Goal 1, noted at the outset. Today, the secondary screen would test individual sperm by flow cytometric analysis. A better secondary screen might use new instruments and techniques to evaluate motion and morphology of sperm in a wet preparation concurrently with multiprobe assessment of biochemical attributes. Multiwavelength detection of probe quantity and localization by differential image analysis and motion-plotting strategies can evolve from current CASA systems. Such analyses could add data for three to five functional attributes to those for two to four selected attributes of sperm motion and morphology. Accurate prediction of either fertile or infertile status should be possible. Prediction of relative fertility, Goal 2, also should be possible. The tertiary test, presumably conducted on only selected samples, would include evaluations of oocyte penetration, pronuclear decondensation, and genetic limitations of the sperm or seminal donor. This level of analysis might provide an approach to Goal 3 but could be rendered invalid by unknown female factors.

The most important biologic and economic motivations for evaluation of sperm quality are to identify males with a high probability of reduced fertility or to ascertain if

fertility of a male is likely to increase or decrease (Goals 1 and 2). For these purposes, *in vitro* evaluations of sperm quality have been and will continue to be important for andrology.

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