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Review Article

How to Determine an Index of the Mechanical Energy of Sperm

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Abstract

Natural motion law of the free-swimming sperm has been reported by current author in 2007 as follows.

$$\left(\frac{1}{\mathrm{v}}\right)=a\left(\frac{1}{A}\right)+b$$

Here, V is VCL (curvilinear velocity); A is ALH (amplitude of lateral head displacement).

Mechanical Energy of the Sperms (E) and function of the Probability Density Distribution of Sperm (P) with respect to the ALH (A) was derived from new sperm energy theory by substituting the sperm motion law in 2012 as follows.

$$P=\frac{C}{A\left(n\lambda-A^2\right)^{\frac{1}{2}+nK\lambda}}\left\{\frac{n\lambda-A^2}{(n-1)A^2}\right\}^{KA^2}$$

Here, *λ* is the mean value of the square of the ALH for all sperm in a single visual field of CASA (Computer-Assisted Semen Analysis) and *n* is the number of motile sperm in a given field. *K* is a specific constant.

The SEI (Sperm Energy Index) is defined as an index of sperm mechanical energy as follows *SEI = nKλ/100.*

Current author have reported that SEI can serve as an index with which to predict natural conception. In the near future, the SEI can presumably be used to improve the rate of conception in artificial insemination and to improve the rate of fertilization in *in-vitro* fertilization.

The current review describes in detail the theory behind measurement of the SEI, calculation of the SEI, and assessment of the accuracy of that theory.

Keywords: CASA; Sperm energy; SMAS; SEI; Isobe plot

Introduction

Studies have reported the use of Computer-Assisted Sperm Analysis (CASA) to assess sperm motility [1-5]. The current author has devised an index of the mechanical energy of sperm, which has been designated the Sperm Energy Index (SEI), and the current author has reported that natural conception is more likely with a larger SEI in ejaculated semen samples. The probability of natural conception was approximately 60% with an SEI of >0.5, 70% with an SEI of >1, 80% with an SEI of >3, and 90% with an SEI of >6 [6]. Findings have revealed that SEI can serve as an index with which to predict natural conception. In the near future, the SEI can presumably be used to improve the rate of conception in artificial insemination and to improve the rate of fertilization in *in-vitro* fertilization.

The current review describes in detail the theory behind measurement of the SEI, calculation of the SEI, and assessment of the accuracy of that theory.

Theory (New sperm energy theory) [6]

According to the quantum theory, the energy that a material possesses is not a continuous value, but rather is given by a form in which a fundamental quantity, called an energy quantum is multiplied by a natural number. Conferring *r* energy quanta to a population of *n* sperm in a closed system may give us the energy possessed by a sperm population existing in a constant volume medium. First, if one energy quantum is placed in a closed system, the probability that any one sperm will receive that energy quantum is $1/n$. If a total of r energy quanta are placed in the closed system one by one, the probability that any specific sperm will have *x* energy quanta in the end follows the binomial distribution given below:

$$P(x)={}_rC_x\left(\frac{1}{n}\right)^x\left(1-\frac{1}{n}\right)^{r-x}\quad {}_rC_x=\frac{r!}{(r-x)!x!}$$

Where, x! is a factorial of x

Because there is an equal chance of this occurring for all sperms within the system, when a total of *r* energy quanta are placed into the system, *P(x)* in Equation (1.1) expresses the proportion in which sperm that have obtained *x* energy quanta exist. Equation (1.1) can be transformed as follows:

$$P(x)=\frac{r!}{(r-x)!x!}\left(1-\frac{1}{n}\right)^r\frac{1}{(n-1)^x}$$

If Stirling's formula is used, Equation (1.2) becomes the following:

$$P(x)=\sqrt{\frac{r}{2\pi x(r-x)}}\left\{\frac{(n-1)r}{n(r-x)}\right\}^r\left\{\frac{r-x}{(n-1)x}\right\}^x$$

According to the sperm spring theory [7], if we consider ALH (amplitude of lateral head displacement) to be *A* for a sperm population in a closed system, the amplitude of the waveform of the sperm tail to be *B*, and the forward velocity to be *V*, then the following relational expression is formed (Isobe's Law). Here, and are constants.

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$$\left(\frac{1}{V}\right)=a\left(\frac{1}{A}\right)+b \text{ or } V=\frac{A}{a+bA}$$

The kinetic energy contained in a single sperm is then given by the following:

$$\frac{1}{2}mV^2+\frac{1}{2}k_1A^2+\frac{1}{2}k_2B^2$$

Where, m is the mass of a single sperm and K_1 and K_2 are constants determined by each sperm. The following relationship exists between A and B: $B = k_3A$.

Because the experimental value b is very small compared with a, Equation (2.1) can be approximated as V = A/a, and the kinetic energy possessed by a single sperm can be approximated with the following formula

$$\frac{1}{2}\left(\frac{m}{a^2}+k_1+k_2k_3^2\right)A^2$$

If the energy in 1 energy quantum is taken to be E_0, the following relational expression holds for sperm with x energy quanta that have head displacement of amplitude A. $K1+k_2k_3^2$ was rewritten as k.

$$xE_0=\frac{1}{2}\left(\frac{m}{a^2}+k\right)A^2 \text{ or } x=\frac{1}{2E_0}\left(\frac{m}{a^2}+k\right)A^2$$

Replacement with $\frac{1}{2E_0}\left(\frac{m}{a^2}+k\right)=K$ gives $x=KA^2$

Supposing that the sperm population in the closed system possesses a characteristic K value (Isobe coefficient), the mechanical energy of a sperm with displacement of amplitude A is expressed as $KA^2 \times E_0$

If the head displacement for each sperm is A_1, A_2, A_3 ….A_n the total energy $r \times E0$ for all sperm in the system is expressed by the following equation: $r \times E0 = (kA_1^2+KA_2^2+KA_3^2+\ldots+Ka_n^2) \times E_0$.

When the mean of is taken to be, the following relationship exists between the head displacement for each sperm and λ. $\lambda=A_1^2+A_2^2+A_3^2+\ldots+A_n^2/n$.

By substituting Equation (2.6) into Equation (2.5), the following equation is obtained. $r= nK\lambda$.

Accordingly, the total energy for all sperm in the system is expressed as $nK\lambda \times E_0$

Substituting Equations (2.4) and (2.7) into Equation (1.3) and replacing with, gives the following function

$$P(t)\propto\sqrt{\frac{n\lambda}{2\pi KtT}}\left\{\frac{(n-1)\lambda}{T}\right\}^{nK\lambda}\left\{\frac{T}{(n-1)t}\right\}^{Kt}$$

$$P(t)=\frac{C}{\sqrt{t}\sqrt{T}\times T^{nK\lambda}}\left\{\frac{T}{(n-1)t}\right\}^{Kt} \quad \text{(2.8) Isobe function}$$

$$P(A)=\frac{C}{A\left(n\lambda-A^2\right)^{\frac{1}{2}+nK\lambda}}\left\{\frac{n\lambda-A^2}{(n-1)A^2}\right\}^{KA^2}$$

C is a constant that fulfills $\int_0^\infty P(A)dA=1$

X takes a natural number, whereas the value of t or A is a rational number. Equation (2.8) is a continuous function for the variable t or A and $P(t)orP(A)$ expresses the existing probability density of sperm.

By expressing of Equation (2.8) with logarithm, the following equation is obtained.

$$\log P+\frac{1}{2}\log t+\frac{1}{2}\log T=-K\left\{T\log T+t\log t+t\log(n-1)\right\}+D$$

(D is a constant)

The value of K can be obtained as absolute value of the slope of the linear regression using the least square methods plotting *[TlogT+tlogt+tlog(n=1)]* on the x axis and $\left[\log P+\frac{1}{2}\log t+\frac{1}{2}\log T\right]$ on the y axis according to Equation (3.0) (Isobe plot).

Measurement of the SEI

Sperm motility parameters were measured using a CASA system made in Japan known as a sperm motility analysis system (SMAS; DITECT Co., Tokyo, Japan). Raw data on the sperm of an individual patient can be processed by the SMAS in Excel (Microsoft, USA). Continuous measurements of 5 visual fields are possible in SMAS. Continuous measurements should be done in the sample with n (sperm count) of 100 or less because there is a possibility that Isobe plot Eq. (3.0) can't be made in small n. Four edges of the grid and the center of the grid should be chosen as measurements fields in the 5 visual fields measurements.

The Amplitude of Lateral sperm Head displacement (ALH) can be measured in a Makler chamber using CASA. A Makler chamber is used because experiments using three disposable chambers with different depths (12 μm, 20 μm, and 100 μm) and a Makler chamber (depth: 10 μm) indicated that CASA in a Makler chamber yielded the most accurate equation Eq. (2.1) for sperm motility. According to the new sperm energy theory, the total mechanical energy of sperm in a visual field assessed with CASA can be expressed as $nK\lambda \times E_0$ Eq. (2.7). Here, λ is the mean value of the square of the ALH for all sperm in a given field, and n is the number of motile sperm in a single visual field. E_0 is the mechanical energy of 1 energy quantum in each sperm. Candidate value of K (Isobe coefficient) can be obtained as the absolute value of the slope of the regression line Eq. (3.0) of an Isobe plot.

The SEI (sperm energy index) is defined as an index of sperm mechanical energy as follows. $SEI = nK\lambda/100$.

SEI indicates the total mechanical energy of sperm in a single visual field [7].

Calculation of the SEI

The frequency of ALH was determined every 0.2 μm along a distance of up to 4 μm. The determined frequency was divided by 0.2 to obtain the probability-density distribution of sperm. The probability density curve for the presence of sperm with respect to the ALH was plotted (e.g. frequency/0.2 with ALH>0.4 and <=0.6 was plotted on the Y axis as the probability-density distribution P of sperm when ALH=0.5 was plotted on the X axis). Pairs of P and ALH values were re-arranged in order starting with the highest probability density. The regression line in an Isobe plot of the 10 highest probability densities was determined using the least squares method, and correlation coefficients (R) were calculated from that line and the slope of that line. The absolute value of the slope of that line is Isobe coefficient. The 10 highest probability densities (frequencies) were used since plotting of low frequencies would yield a regression line with a large random error. The theoretical probability density curve for the presence of sperm with respect to the ALH can be plotted by substituting Isobe's

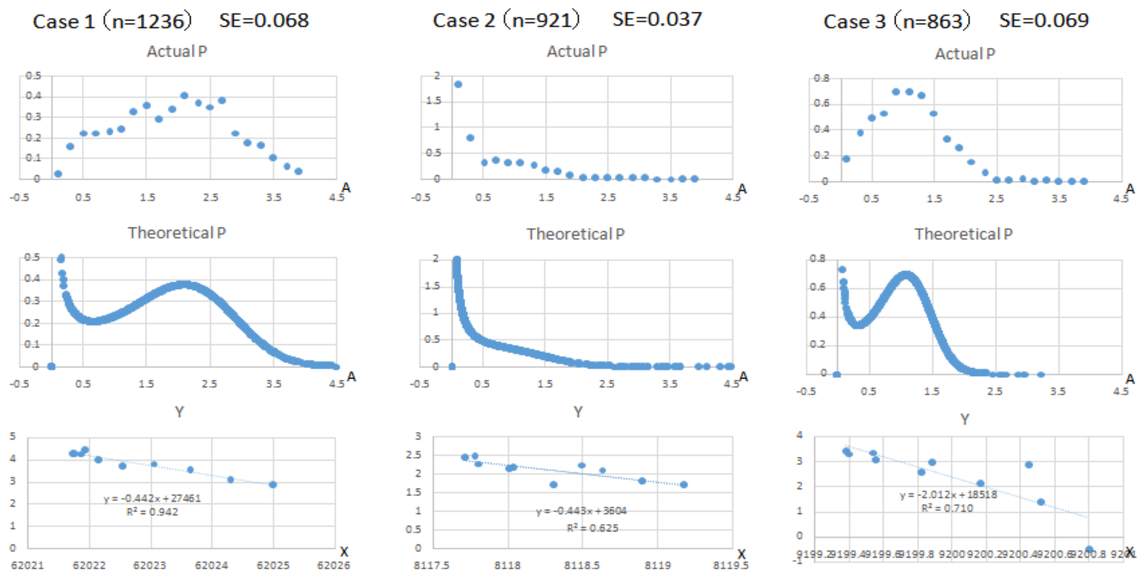

Figure 1: Actual distribution and theoretical distribution of sperm probability density with respect to ALH and Isobe plot for representative samples with various sperm counts Case 1.

coefficient into Isobe function Eq. (2.8). The constant C in Eq. (2.8) was determined so as to fulfill Eq. (2.9) by tallying the values of P × the interval between one ALH and an adjacent ALH after re-arranging those values starting with a smaller ALH. The Standard Error (SE) between the actual distribution and the theoretical distribution of the probability density was calculated with respect to the ALH (0.1, 0.3, 0.5 ····3.7, 3.9) used in an Isobe plot. When the correlation coefficient (R) obtained from an Isobe plot was less than 0.9, the Isobe coefficient was obtained by fine-tuning in order to yield the smallest SE based primarily on the candidate value of *K* obtained by Isobe plot. When the correlation coefficient (R) was more than 0.9, candidate value of *K* is notified as the Isobe coefficient. The aforementioned procedures to obtain the SEI have been designated the Bell-net method.

Assessment of the accuracy of the theory

Figure 1 shows the actual distribution of sperm and the theoretical probability-density distribution (P) of sperm with respect to the ALH (A) for representative samples with various sperm counts. According to new sperm energy theory, the theoretical probability-density distribution of sperm is expressed as Eq. (2.8). *X* and *y* are variables for an Isobe plot based on the new sperm energy theory.

When the ALH was extremely small, the probability density of the theoretical distribution was larger than its actual distribution. This presumably occurred because slow-swimming sperm were trapped by the chamber wall. Slow-swimming sperm have a small ALH according to Isobe's law Eq. (2.1), which governs the motility of free-swimming sperm. The new sperm energy theory was derived based on Isobe's law, so free-swimming sperm fit a theoretical distribution. As shown in (Figure 1), the SE between the actual distribution and the theoretical distribution was extremely small and the two distributions were in statistical agreement, indicating the accuracy of Isobe function. Since the SEI and Isobe function Eq. (2.8) are derived from the same theory, the SEI can be an index that accurately indicates the mechanical energy of sperm.

Conclusion

The SEI is an index of the mechanical energy of sperm. The SEI can be determined based on measurements using an SMAS and calculations done using the Bell-net method (Figure 1,2).

According to the new sperm energy theory, the theoretical Probability-Density Distribution of Sperm (P) with respect to ALH (A) and the total Mechanical Energy of Sperm (E) in a visual field assessed with CASA can be expressed as follows.

$$P = \frac{C}{A\left(n\lambda - A^2\right)^{\frac{1}{2}+nK\lambda}} \left\{\frac{n\lambda - A^2}{(n-1)A^2}\right\}^{KA^2}$$

$$E = nK\lambda \times E_0$$

Here, λ is the mean value of the square of the ALH for all sperm in a visual field, and n is the number of motile sperm in a visual field. E_0 is the mechanical energy of 1 energy quantum in each sperm (Figure 2). By expressing of Isobe function with logarithm, the following equation is obtained.

$$\log P + \frac{1}{2}\log t + \frac{1}{2}\log T = -K\left\{T\log T + t\log t + t\log(n-1)\right\} + D$$

The value of *K* (Isobe coefficient) can be obtained as absolute value of the slope of the linear regression using the least square methods plotting $[T\log T + t\log t + t\log(n-1)]$ on the x axis and $\left[\log P + \frac{1}{2}\log t + \frac{1}{2}\log T\right]$ on the *y* axis (Isobe plot).

When the correlation coefficient (R) obtained from an Isobe plot was less than 0.9, the Isobe coefficient was fine-tuned based primarily on the value of *K* obtained by Isobe plot in order to yield the smallest SE (Standard Error).

The SEI (Sperm Energy Index) is defined as an index of sperm mechanical energy as follows. $SEI = nK\lambda/100$.

SEI indicates the total mechanical energy of sperm in a visual field.

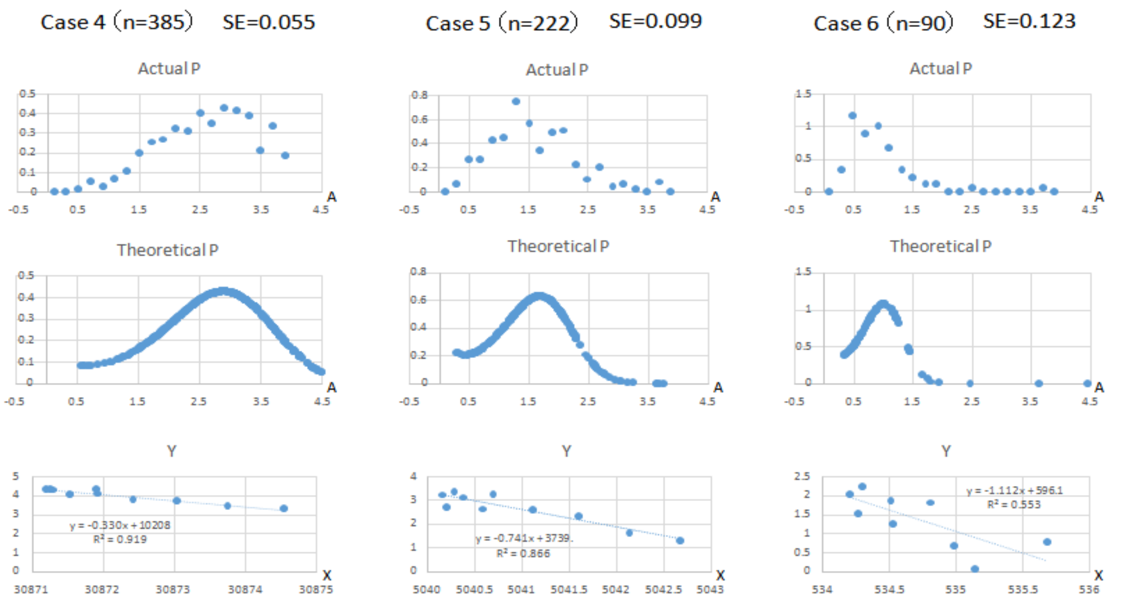

Figure 2: Actual distribution and theoretical distribution of sperm probability density with respect to ALH and Isobe plot for representative samples with various sperm counts Case 2.

When the ALH was extremely small, the probability density of the theoretical distribution was larger than its actual distribution. This presumably occurred because slow-swimming sperm were trapped by the chamber wall.

The SE between the actual distribution and the theoretical distribution was extremely small and the two distributions were in statistical agreement, indicating the accuracy of Isobe function. Since the SEI and Isobe functions are derived from the same theory, the SEI can be an index that accurately indicates the mechanical energy of sperm.

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