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ANALYTIC MORPHOMETRY IN DIAGNOSTIC PATHOLOGY: UPPER DEGREE POLYNOMIALS AND FOURIER HARMONIC ANALYSIS IN INFERTILITY

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ABSTRACT

The shape of the seminiferous tubuli in maturation arrest is investigated by harmonic Fourier analysis and upper degree polynomials. The epithelial boundary line of the lumen is considered as a curve in a reference system after the points of the curve have been defined by coordinates: an automatic procedure (TV-camera interfaced with a computer) is utilized and the software expresses the profile as a Kth-order equation and submits real and calculated periodic function to Fourier analysis. Fourier analysis is very useful in shape trend investigations and its modifications are not dependent on perpendicular cross-sections. The method helps us to distinguish different types of maturation arrest.

INTRODUCTION

Maturation arrest, the most frequent type of testicular sterility, represents an abrupt cessation of spermatogenesis at one specific stage of the maturation process. It is possible to distinguish two levels of block: high or spermatocytic arrest, and low or spermatid arrest related to different etiologic factors. The prognosis is better in the latter type which often recovers spontaneously or after hormonal treatment.

In this study we have attempted to differentiate the two conditions employing morphometric methods which free us from the need of measuring only sections perpendicular to the length axis of the tubuli (Weissbach and Ibach 1976).

Seminiferous tubulus can be considered a cylinder and its section has a circular or an elliptic image. The contour of the lumen will be more or less irregularly shaped: more regular in high arrest, more complicated in low arrest, intermediate in normal tubuli.

We have aimed to study analytically such factors as the shape of the lumen, but our morphometric procedures may equally well be applicable to any biological structures with solid or hollow cylindrical shape.

In such cases the measurement of average diameter (or area or perimeter) is usually conducted by evaluating perpendicular cross-sections (Aherne and Dunnill 1982) or largest to smallest orthogonal diameter ratio is calculated. The results, however, do not describe the shape in detail and the diameters to be measured are not clearly defined.

So we suggest to study the luminal shape not by dimensional measurements but by analytic methods that essentially describe profiles (tubular lumen contour) utilizing polynomial equation of k-degree Fourier analysis.

The distribution curve of subsequent empirically known values may be described in two ways:

a) executing the exact matching by polynomial splines whose degree is usually not higher than third

b) giving the coefficient for an upper degree equation (Kth-order polynomial) reaching the best fit with the series of historical data. The typical format of a Kth-order polynomial is the following:

$$y = b_0 + b_1 x + b_2 x^2 + b_3 x^3 + \dots b_k x^k$$

where y is the dependent variable to be calculated, x the given independent variable; $b_0 \dots b_k$ are the coefficients.

Regression analysis takes the set of data and fits it in an equation while correlation analysis gives us evaluators of goodness of the fit.

For regression the least square method has been adopted in order to obtain the coefficient of Kth-order polynomial describing a curve with the same number of points as available in the original empirical data. The minimum deviation between the calculated value and the experimental value is looked for. Because deviation can be either positive or negative it is mathematically convenient to adopt square value of deviation.

The more irregular is the contour given by empirical data, the lower will the degree of fit be in the best-fit curve expressed by a Kth-order equation.

So, whatever contour, however oriented, becomes comparable, not with an ideal geometrical figure, but with the function curve corresponding to the equation whose degree assures the least value for the sum of square deviations.

The difference between the two curves may be expressed in numerous ways. We have selected two values: standard error between the two series of data (empiric and calculated) and the sum of the differences obtained by comparing both curves with Fourier harmonic analysis.

Fourier harmonic analysis considers the profile as a cyclic function obtained by summing sinusoidal (sine) waves of different amplitude, period and phase that represent the harmonic of a periodic function.

Fourier analysis arranges the harmonics in the order of importance in determining final function. Number of harmonics is directly proportional to the irregularity of the contour. The typical format of the harmonic Fourier analysis for a periodical function is:

$$y = a_0 + a_1 \sin x + b_1 \cos x + a_2 \sin 2x + b_2 \cos 2x + \dots$$

$$a_k \sin kx + b_k \cos kx$$

where k is the maximum order of the harmonic.

METHOD

We explored Bouin-fixed and paraffin embedded biopsies of infertile men and normal subjects (as controls) employing:

1 - Personal Computer (Apple II Europlus) with 48 kB of RAM connected with 2 disk-drives, CRT colour display, graphic printer, B/N TV camera with interfacing A-D converter and tablet digitizer;

2 - Dimmer controlled spot-lights

The software utilized includes 2 packages:

A) Original graphic tablet software (Apple Computer Inc.; modified after conversion to operating system 3.3) to obtain an array with x-y coordinate values for each point of the profile. Such array is then memorized in the text file and from it 2 separate sub-arrays for x and y values are created.

B) Original software written by the authors, and formerly published for other morphometric investigations (Pesce Delfino et al. a, b, c, 1983). This software includes 2 groups of routines. The first is dedicated to the procedure of dimension normalization and position standardization; the second to creation of textfiles.

x and y values are separately considered as dependent variables in the least square algorithm for Kth-order equations where independent variables are constantly positive integers from 1 to the total number of points.

The software automatically increases the degree of the polynomial, starting from a second degree equation and simultaneously performs variance analysis so that computation stops when the best-fit curve is reached.

The difference between the curve of empirical data and the calculated function curve is calculated in a different way. We have first an analytical indicator of the tubulus shape as a couple of coordinate series. Then graphic restitution of the 2 closed curves in performed. Finally the software performs Fourier harmonic analysis giving the sine-cosine coefficients of the series, the amplitude of the harmonics and the sum of differences between the sets of harmonics of the empirical curve and the function curve: this is another shape indicator obtained.

RESULTS

For the present study we use the procedure without previous normalization, so the results strictly refer to the luminal contour of each tubulus and classification of shapes is performed comparing the empirical scattered curves and the corresponding function curves derived from best-fit equations.

The degree of equation has been from 5 to 8 for value series from 60 to 250 points with determination coefficients never lower than 0.9. The number of harmonics for Fourier analysis has been 20.

In spermatocytic arrest variance between the two curves for the splitted x and y series varies from 0.15 to 1.12 (for x) and from 0.2 to 0.44 (for y) (Figs. 1 and 4); corresponding square roots of the mean square error are 0.44-1.08 and 0.56-70.83 and 24.7-36.35.

For normal tubules variance is from 0.25 to 6.63 (for x) and from 0.43 to 6.7 (for y); corresponding square roots of mean square error are 0.58-2.43 and 0.68-2.53, and Fourier sums of the differences are 110-220 and 104.5-202 (Figs. 2 and 5).

In spermatid arrest the variance is from 1.4 to 10 (for x) from 0.9 to 14.5 (for y); corresponding square roots of mean square error 1.2-3 and 1-3.75; Fourier sums of differences are 133.2-220.5 and 208.3-335.8 (Figs. 3 and 6).

If the examined classes are arranged, the normal class is situated in central position, the spermatocytic class to left (low value) and spermatid arrest to right (high value). High maturation arrest appears well separated from the normal class because indicators for x and y values are definitely inferior or seldom reach the corresponding lower values of normal class. On the other hand a wide overlap exists between normal and spermatid class even though the greatest values of the latter class are higher then the greatest values of the normal class.

At the end Fourier analysis appeared particularly useful for distinguishing spermatocytic arrest from normal. There is a correspondence between data obtained by the given purely analytic shape investigation and biological and anatomical background of the lesion: it is well known that high maturation arrest shows clear-cut morphological and prognostic differences from the normal condition; on the other hand the

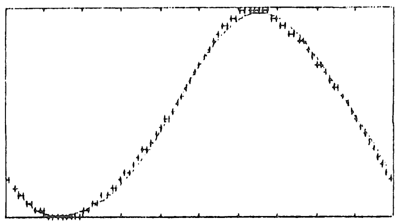

Fig. 1

Fig. 4

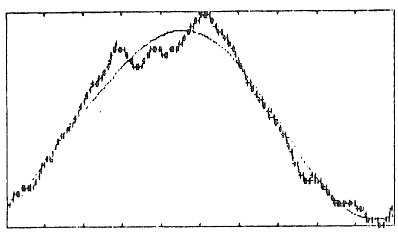

Fig. 2

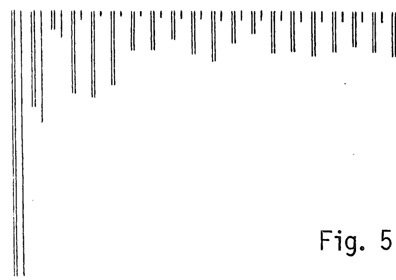

Fig. 5

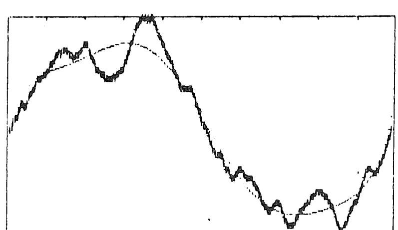

Fig. 3

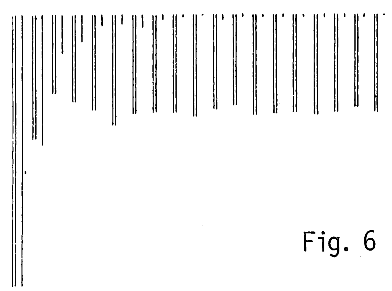

Fig. 6

Fig. 1. High arrest: slight irregular contour, almost perfect coincidence of empirical scattered (plus-signed) and function (point-signed) curves. 7th degree polynomial. No of points 87. Variance 0.2.

Fig. 2. Normal: intermediated situation of contour irregularity; increased distance between the curves. 6th degree polynomial. 200 points. Variance 3.8.

Fig. 3. Low arrest: strong irregular contour and distance between the curves. 7th degree polynomial. No of Points 271. Variance 8.43.

Fig. 4. High arrest: Fourier analysis of the empirical scattered (double barrelled bars) and function curves with almost identical trend. Sum of differences 23.6. First 7 harmonics describe the curve.

Fig. 5. Normal: only first 3 harmonics whose amplitude and number is greater than in high arrest (Fig. 4). Sum of differences 220.

Fig. 6. Low arrest similar to Fig. 5 with more complexity of the curves and maximal amplitude of the harmonics. Sum of differences 335.

border between normal and low arrest is very indistinctive.

It is necessary to investigate the biological meaning of the differences of the maximal values when normal class and low arrest are compared. A hypothesis is that tubuli with morphological pattern of spermatid arrest but characterized by values overlapping the normal class values, can be entirely normal tubuli scattered among tubuli with maturation arrest, or that they are pathological tubuli with so small lesions that full recovery is possible.

Application of Fourier analysis to seminiferous tubuli has been made not only for intrinsic interest but for a demonstration of the utility of this method in numerous fields. The results confirm the value of the procedure: in fact not only reconstruction of closed curves is topographically correct but the finding that normal tubuli are intermediately positioned between high and low arrest indicates a "very regular shape" for the former and a "very irregular shape" for the latter. This pattern is understandable because typical epithelial clumps are absent in high arrest, and because in low arrest spermatozoa are absent and do not fill the spaces among clumps of seminiferous epithelium.

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