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Laser scattering instrument for real time in-vivo measurement of ciliary activity in human Fallopian tubes*

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Based on a laser light scattering technique and fibre optic probe, we have developed and tested a simple and practical device for real time measurements of ciliary activity in human Fallopian tubes during laparoscopy and laparotomy. A further aim was to investigate the relationship between the ciliary beat frequency (CBF) and the morphology of the ciliary epithelium. The mean ± SE of CBF in the fimbria and in the ampulla were 5.4 ± 0.3 Hz and 5.0 ± 0.1 Hz respectively. Small pieces of fimbria and ampulla epithelium were taken from the same sites at which the CBF was measured, and the percentage of ciliary cells was determined by scanning electron microscopy. A high positive correlation was found between CBF and the percentage of ciliary cells in the fimbria (r = 0.84) and in the ampulla (r = 0.88). The instrument presented in this study provided, for the first time, a quantitative examination of the CBF in intact human Fallopian tubes and may be used for the investigation of ciliary activity in patients with infertility.

Key words: ciliary epithelium morphology/ciliary motility/laser light scattering

Introduction

Cilia are tiny hairlike appendages, about 0.25 μm in diameter, that contain a bundle of parallel microtubules at their core. They extend from many kinds of cells and are found in most animal species and some lower plants. Their primary function is to move fluid over the surface of cells or to propel single cells through fluid media (Albert *et al.*, 1994).

Tubal cilia have a critical role in ovum transport through the Fallopian tubes. In rabbits, the ability to transport the ovulated cumulus mass is quantitatively related to the proportion of ciliated cells. Odor and Blandau (1973) demonstrated a direct relationship between the transport of cumulus masses over the fimbria and the percentage of ciliated cells present in the epithelium. Fimbria with <44% ciliated cells usually did not affect movement of egg masses over their surfaces, while rapid transport occurred with 61% or more ciliated cells. Donnez and Casanas-Roux (1986) observed that the pregnancy rate in humans after tubal surgery is related to the percentage of ciliary cells.

Forty percent of women with infertility have tubal pathology, and pelvic inflammatory disease is the major cause of tubal infertility (Speroff *et al.*, 1994). Impairment of ciliary activity may produce infertility by interference with ovum pickup and transport in tubes that otherwise are patent and have a macroscopically normal appearance. Investigation of the Fallopian tubes in infertility is most often restricted to the evaluation of tubal patency by hysterosalpingography and laparoscopy. It seems likely that more information could be gained from evaluation of the ciliary motility in order to assess the efficiency of tubal function.

A number of techniques have been employed for accurate analysis of in-vitro ciliary motion: high speed cinematography or high speed video combined with optical microscopy allows analysis of both in-vivo and in-vitro ciliary motion (Sanderson and Sleigh, 1981). Dalhamn and Rylander (1962) were the first to describe a photometric method for measuring ciliary beat frequency (CBF), based on imaging at relatively high magnifications of light microscopy and electronic detection of transmitted light fluctuations produced by the beating cilia. Further development of this technique added signal processing to yield CBF, usually via Fourier transformation. The above methods demand short working distances and could be applied only to in-vitro CBF measurements. A laser light scattering technique was introduced by Lee and Verdugo (1976) for ciliary beat measurements. This technique has been shown to render highly accurate measurements of the CBF. Verdugo and Golborne (1988) and Svartengren *et al.* (1989) described the development and validation of the laser light scattering spectroscopy method to measure the movement of cilia *in situ*, inside the trachea and ampulla of animals.

The aim of the present study was to develop a simple and practical instrument for real time measurements of ciliary activity in the human Fallopian tubes during laparoscopy and laparotomy, and to investigate the relationship between the CBF and the morphology of the ciliary epithelium.

Materials and methods

The laser instrument

A small portable unit including all the components necessary for laser illumination and optimal detection of the signal, was designed

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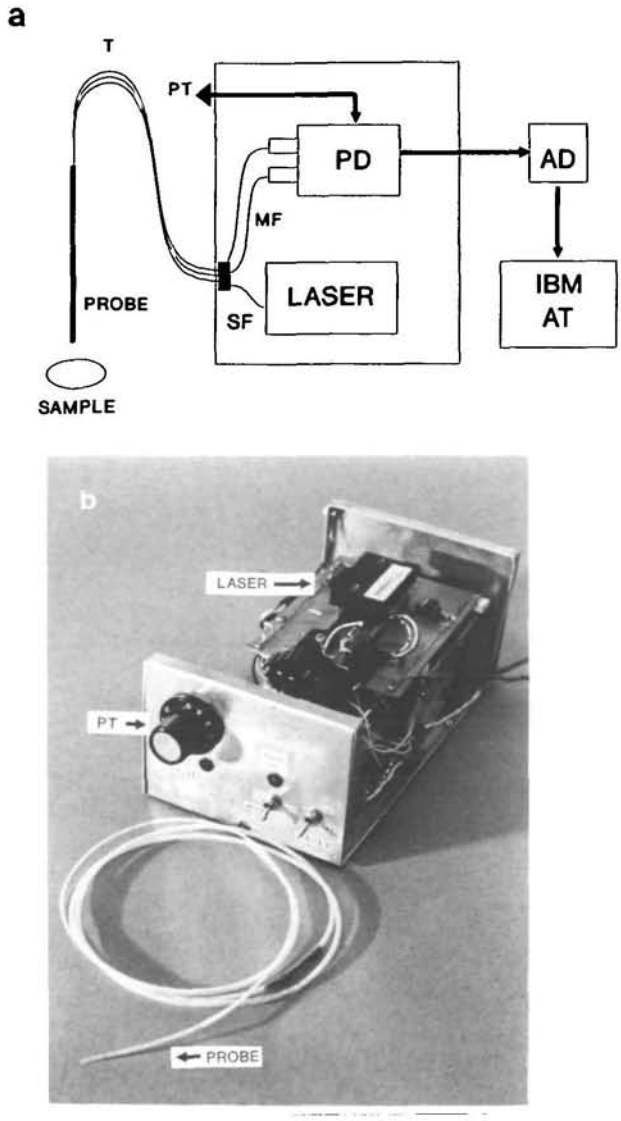

Figure 1. (**a**) Schematic diagram of the ciliary beat frequency (CBF) measuring instrument. (**b**) Photograph of the laser box (opened). AD, analogue to digital acquisition card; MF, multi-mode fibres; PD, photodiode detectors; PT, variable gain potentiometer; SF, single mode fibre; T, flexible tube.

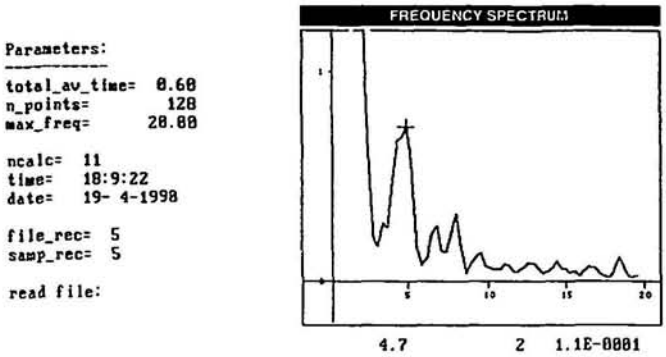

Figure 2. The computer monitor display during use of the instrument for ciliary beat frequency (CBF) measurement. The power spectrum is displayed on the right and the user-chosen parameters are displayed on the left. The CBF measured was 4.7 Hz. The presence of $1/f$ like spectrum below 3 Hz resulted from low frequency drifts in the detected signal due to motion.

and constructed (Figure 1). The light source is a low power helium–neon laser, wavelength 633 nm (Model 155, Spectra Physics, Oregon, USA), which was replaced later with a laser diode, wavelength 670 nm (FD 6700S, Gerhard Franck Optronik, Hamburg, Germany). The laser beam was transmitted into a single mode fibre which was selected in order to avoid speckle pattern fluctuations due to fibre movements. A flexible tube, ~1.5 m long, extending from a box, enclosed the single mode fibre and two multimode fibres guiding the back-scattered light from the probe tip to two photodiode detection units in the box. To collect as much of the scattered light as possible, thick multimode fibres were selected (numerical aperture of 0.316). The two collecting optical fibres enabled differential measurement between the two signals which reduced fluctuations due to relative motion between the sample and the probe. Two types of probes for laparoscopic use were designed: one was built from stainless steel tubing, 5 mm in outer diameter and 35 cm long, and the other probe was a flexible gamete intra-Fallopian transfer (GIFT) catheter, 1.6 mm in diameter and 50 cm long (Marrs Laparoscopic Catheter, Cook Ob/Gyn, Indiana, USA). Both were sealed to prevent air leakage during laparoscopy.

At the tip of the probe the stripped fibres were pooled together, embedded in a special epoxy resin and polished. The two multimode fibres were fixed at both sides of the single mode fibre and collected the laser light scattered from the cilia, guiding it to the detecting diodes (DF-633 or DF-670; EG&G Optoelectronics, Lake Forest, Canada). Outputs from the two photodiode pre-amplifiers were fed into a differential amplifier. A high pass sharp frequency cut-off at 0.5 Hz was introduced to prevent saturation of amplifiers due to slow movements. A low pass presampling filter eliminated high frequencies (such as electrical 50 Hz interference). Further amplification with gain ranges between 30× and 350× was user controlled. The spectral analysis of the signal and the graphic display were carried by an IBM-AT computer. An analogue to digital (A/D) data acquisition card sampled the output signal according to user-specified sampling parameters and stored the data in direct memory access mode. This enabled the computer to accumulate, process and display the analysed data simultaneously. On initiation of the program a parameter menu was displayed. User-chosen parameters were: averaging time (in min), N, the number of sampling points and F_{max}, the maximum frequency (cycles/s). For each array of N sampled data points, Fourier transformation and squaring yielded the power spectrum for all frequencies up to F_{max}. The averaging time determines the number of separate power spectra to be averaged in the final power spectrum. The resulting power spectra could be seen on screen in realtime (Figure 2), and were stored on disk for later analysis.

The instrument is user-friendly and no special training is needed. Optimal signals are obtained when the probe gently touches the fimbria mucosa and when it is inserted into the ampulla along its axis, without pressing the oviductal walls, which may mechanically impair ciliary beating.

Fixation and preparation for scanning electron microscopy (SEM)

Small pieces (1.5–2 mm in diameter) of fimbria and ampullary epithelium were taken from the same site where the CBF was measured. The samples were rinsed with phosphate-buffered saline (PBS) and put in fixative solution (2.5% gluteraldehyde in 375 mOsmol PBS, final pH 7.4). Fixation was carried out at room temperature for 3–5 h, followed by overnight fixation at 4°C. The specimens were rinsed thoroughly with PBS and post-fixed in 1% OsO_4 in PBS for 1 h at room temperature. They were dehydrated in ascending ethanol concentrations, passed into hexamethyldisalazane, air dried and visualized under the dissecting microscope. Specimens

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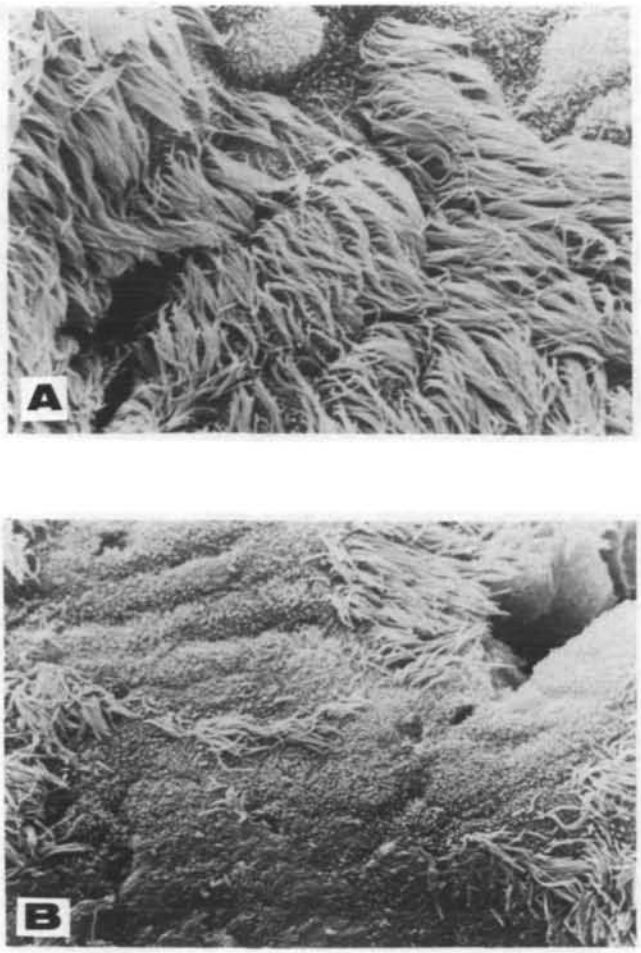

Figure 3. Scanning electron micrograph of fimbrial surface in woman with pelvic adhesions and right ovarian cyst (original magnification ×2000). (**A**) Right fimbria. The ciliary cell percentage was 86% and the ciliary beat frequency (CBF) was 7.2 Hz. (**B**) Left fimbria. The ciliary cell percentage was 24% and the CBF was 3.4 Hz.

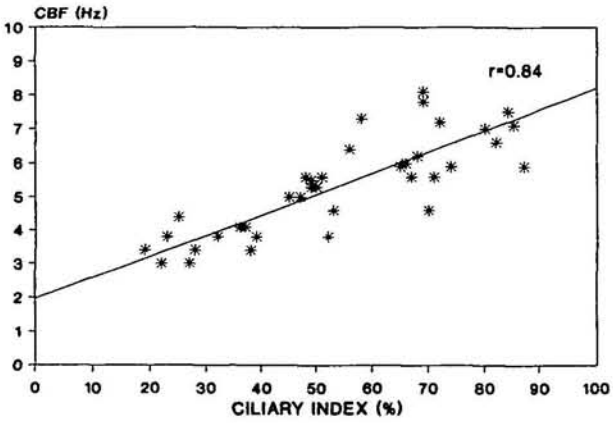

Figure 4. The relationship between the ciliary beat frequency (CBF) and the percentage of ciliary cells (ciliary index) in the fimbria.

were mounted on copper stubs with their epithelial side upwards, coated with 18 nm thick gold (Polaron coating unit E-5000), and viewed in a JEOL T-300 SEM operated at 25 kV.

The percentage of ciliary cells (ciliary index) was determined from three to four SEM micrographs which contained ~2000 cells per micrograph, as the fraction of the area covered by cilia from the total sample area, by using a digitizing system (Morphomat 30; Zeiss, Oberkochen, Germany).

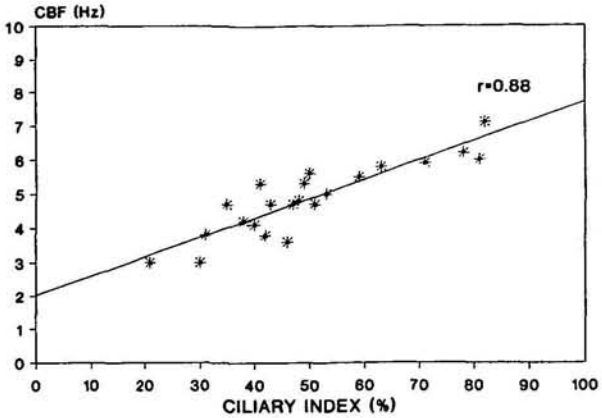

Figure 5. The relationship between the ciliary beat frequency (CBF) and the percentage of ciliary cells (ciliary index) in the ampulla.

Patient groups

Measurements were performed in 31 women volunteers undergoing laparotomy. In 24 cases total abdominal hysterectomy was performed because of uterine myoma. In four cases the indication for laparotomy was benign ovarian cyst. In three cases ovarian cystectomy and adhesiolysis was performed because of mechanical infertility and benign ovarian cyst. Microbiopsies from the ampulla were taken only in cases in which hysterectomy was performed. The clinical study was approved by the Bnai-Zion Medical Center Committee of Clinical Investigation.

Statistical analysis

The relationship between CBF and the percentage of ciliary cells was derived using linear regression analysis. The significance of differences was determined by Student's t-test and χ^2 test with Yates' correction when needed.

Results

A total of 99 CBF measurements was performed in 31 women, or about three measurements per woman. Each measurement took 36 s. If more than one measurement was performed at the same site, the mean difference between these measurements was 0.3 ± 0.03 Hz and the mean CBF was used for further calculations. The mean ± SE of CBF measurements in the fimbria of 38 Fallopian tubes was 5.4 ± 0.3 Hz, and the mean CBF in the ampulla of 22 out these 38 Fallopian tubes was 5.0 ± 0.1 Hz (t = 1.1, not significant).

The percentage of ciliary cells (ciliary index) in the fimbria and in the ampulla was determined from the SEM micrographs (Figure 3). Using linear regression analysis, a high positive correlation was found between CBF and the ciliary index in the fimbria (r = 0.84, $P < 0.0001$) (Figure 4) and in the ampulla (r = 0.88, $P < 0.0001$) (Figure 5). In the fimbria, in 21 of 22 measurements (95%) in which the ciliary index was >50% the CBF was 5.2 Hz or greater, and in 13 of 16 cases (81%) in which the ciliary index was ≤50%, the CBF was

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<5.0 Hz (χ^2 = 20.2, $P < 0.001$). In the ampulla, in 10 of 11 cases (91%) in which the ciliary index was >50% the CBF was ≥5.0 Hz, and in 10 out of 11 cases (91%) in which the ciliary index was ≤50%, the CBF was <5.0 Hz (χ^2 = 11.6, $P < 0.001$).

Discussion

Despite the critical function of the ciliary epithelium in the reproductive tract, there is not yet conclusive data about CBF range in human Fallopian tubes and the correlation between abnormal ciliary function and infertility. A reliable and simple method for investigating the ciliary epithelium could aid in the diagnosis of tubal infertility. Lee and Verdugo (1976) described laser light scattering spectroscopy as a reliable technique which presents several important advantages over methods currently used to measure the frequency of ciliary beat. It does not require high magnification microscopic imaging, and collects the average beat frequency of a large number of cilia. Using this technique, we developed a prototype of a compact and easy-to-operate medical instrument capable of real-time evaluation of CBF. The medical application had to overcome overwhelming low frequency signals originating from movements due to the breathing and heart beat of the patient, and due to movement of the surgeon's hands. The differential detection method was introduced to reduce these motion artefacts.

In this study, we describe for the first time, a quantitative examination of the ciliary beat activity in intact human Fallopian tubes during common gynaecological procedures. The mean ± SE of CBF measurements in the fimbria was 5.4 ± 0.3 Hz, and the mean of CBF measurements in the ampulla was 5.0 ± 0.1 Hz. Critoph and Dennis (1977), using a cinematographic technique in resected Fallopian tubes, also found slower ciliary motility in the ampulla compared to the fimbria, especially in the follicular phase. Donnez *et al.* (1984) observed a significant decrease in the percentage of ciliated cells in the fimbria during and after salpingitis. Patton *et al.* (1989), using a laser light scattering technique, measured the CBF in human mucosal tissue culture. They found that the CBF was significantly reduced in women with overt pelvic inflammatory disease or silent pelvic inflammatory disease as compared with controls. In the present study, a high correlation was found between the CBF and the ciliary index. These results suggest that the same physiological (i.e. hormonal) or pathological (i.e. salpingitis) factors may affect both the morphology and the function of the ciliary epithelium. Based on previous studies (Verhage *et al.*, 1979; Vasquez *et al.*, 1983; Donnez and Casanas-Roux, 1986), we defined a ciliary cell percentage >50% as normal. We also defined CBF ≥5.2 Hz in the fimbria and CBF ≥5.0 Hz in the ampulla as normal values of CBF. Using these criteria, the predictive positive and negative values of our test in detecting an abnormal percentage of ciliary cells were 93 and 88% respectively in the fimbria, and 91 and 91% respectively in the ampulla.

The instrument described in the present study might provide a new and convenient method of studying the ciliary activity in the Fallopian tubes. These studies may contribute substantially to the diagnosis of impaired ciliary function in cases of otherwise unexplained infertility.

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