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Multiple exposure photographic (MEP) technique: an objective assessment of sperm motility in infertility management

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Summary

Multiple exposure photography (MEP), an objective technique, was used in determining the percentage of motile sperms in the semen samples from 41 males being investigated for infertility. This technique was compared with the conventional subjective ordinary microscopy method of spermatozoal motility assessment. A satisfactory correlation was observed in percentage sperm motility assessment using the two methods but the MEP estimation was more consistent and reliable. The value of this technique of sperm motility study in the developing world is discussed.

Résumé

Exposé multiple à la photographie (MEP), une technique objective, fût utilisée pour déterminer le pourcentage de spermatozoïdes se déplaçant dans le liquide séminal de 41 échantillons mals dont on analysait la stérilité. Cette technique fût comparée à la méthode ordinaire et conventionnelle d'étude du déplacement spermatozoïdal. Grace à une étude comparative des deux méthodes analysant le taux de spermatozoïdes se déplaçant, on aboutit à la conclusion que la technique du MEP est plus consistante et plus rationnelle. La valeur de cette technique d'étude du déplacement du spermatozoïdes dans les pays sous développés est discuté aussi.

Introduction

There is a growing awareness that the spermatozoal motility is one of the most important parameters in evaluating the fertility potential of a semen specimen [1]. Several studies have shown that the accuracy of the conventional subjective method of assessing the spermatozoal motility is limited [2, 3]. Jeguier and Ukombe [4] demonstrated clearly that there were significant variations in the results obtained by different technicians performing an analysis of semen from the same patient. In order to overcome this problem, many objective methods of assessing sperm motility in semen (samples) have replaced the routine subjective technique in some laboratories [5–7]. One of these objective techniques (MEP) was used in this study. The purpose of this communication is to document our experience of these two techniques in Manchester, U.K., and to examine the place of multiple exposure photographic method as an objective way of assessing spermatozoal motility in the management of infertile males in the developing countries such as Nigeria.

Materials and methods

Method of semen collection

Semen specimens were collected from 41 male partners of infertile couples. All samples were obtained by masturbation after at least 3 days of abstinence. The men were requested to collect the whole ejaculate into a wide-bore sterile glass jar, with a metal cover. This allowed for a strictly standardized condition and reduced the risk of contamination. All specimens were analysed within 2 h of collection, according to Eliasson [8].

Microscopic method

After allowing for liquefaction, which occurred

within 15–30 min, the specimens were thoroughly mixed by gentle shaking of the jar, and a drop of the semen was placed on a clean glass slide. This was covered gently to prevent air bubbles under the cover-slip. One hundred sperm were counted under the microscope in five or more fields of vision to determine the percentage of motile sperm. The sperm count was also estimated using the Haemocytometer as described by Eliasson [8].

Multiple exposure photographic method or Makler technique

A drop of semen was placed in the centre of Makler's chamber (10 μm/cm chamber) and covered with a special cover-slip.

The cover-slip was engraved with 1 × 1 mm grid and subdivided into 100 squares (each measuring 0.1 × 0.1 mm). This can serve as a scale for true sperm velocity determination and as a frame of reference for rapid and easy sperm count. In this procedure, both motile and non-motile sperm were photographed with the aid of a phase-contrast microscope, with an attached still camera loaded with Ilford XPA-100 black and white film. With the aid of a Pl 10 × 10 objective and a 20 × 20 eye-piece, the frame within the viewer of the camera was able to cover an area approximately 0.5 × 0.35 mm. This was a satisfactory magnification as it allowed a substantial number of sperm to be included in a frame of that size, while the images of the moving spermatozoa appear big enough to be traced and measured with adequate accuracy.

After a drop of semen from a well-mixed specimen was placed on the lower disc of the

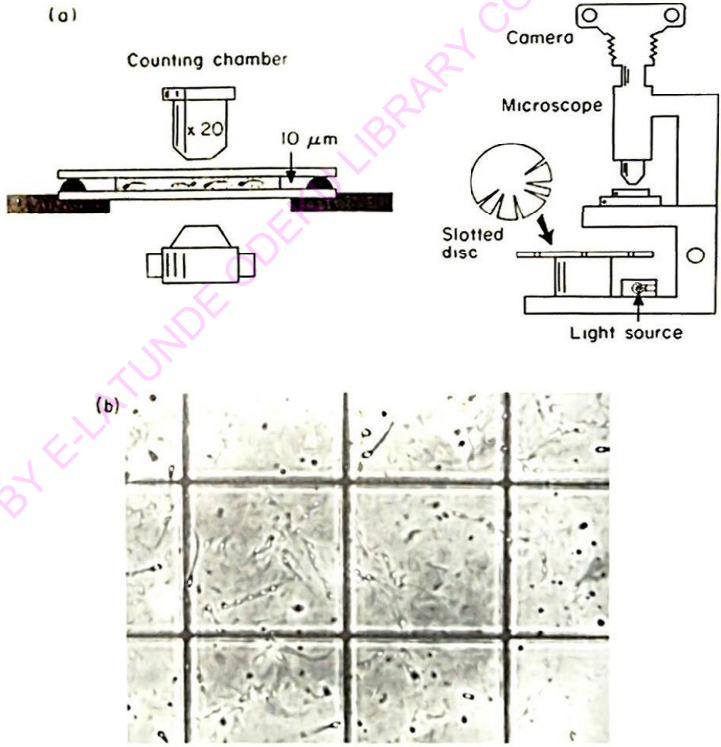

Fig. 1. (a) Diagrammatic representation of the counting chamber. This device allows the precise recording of sperm as the chamber is 10 μm deep and retains sperm as a single layer kept in focus. (b) The motile spermatozoa in chains. The resultant picture has bright immobile sperm and strings of sperm heads produced by motile sperm. The velocity may be calculated by measuring the path length with reference to the graticule on the counting chamber.

chamber and covered properly, the chamber was left for about 1 min to allow the current of fluid to subside completely, thus enabling non-motile sperm to be absolutely stationary. This eliminated the appearance of blurred images on the film (Fig. 1). The film was exposed for 1 sec by six stroboscopically induced light pulses each of 1/60 sec (Fig. 2). The multiple exposure photographic technique was achieved by a stroboscope made of a six-sector black disc placed between the light source and the condenser of the microscope (Fig. 2) and rotated at 60 r.p.m. by an electric motor.

The stroboscopic apparatus was constructed from an electric motor and a rotating 12-cm diameter black painted metallic disc from which six radially diverging 1-mm wide slots had been cut from only one hemi-circle [9]. The photographic procedure was performed when the shutter control of the camera was set on B, which meant that the shutter was kept open as long as the plunger was depressed. Whilst the electric motor was rotating, its plunger was depressed at a point when the light source was obscured by the solid point of the disc (Fig. 2). The disc thus completed one full turn while the slots were crossing the light. The film was, therefore, automatically exposed by all six pulses.

When the disc was at position A (Fig. 2), the plunger was released and the shutter closed. Blurred images induced by transmission of vibrations from the rotating motor to the microscope were eliminated by mounting the stroboscope on a special trolley, which kept the stroboscope apparatus steady (Fig. 2). In order to achieve correct exposure of the film the light intensity was set using a photometer. This elaborate photographic technique was based on the modification of Makler's method [7].

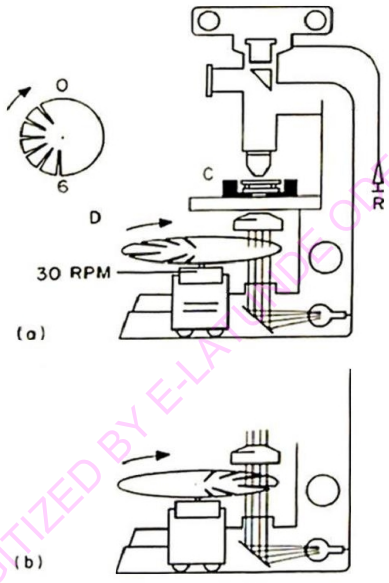

Fig. 2. Schematic description of the two basic positions of the rotating disc (D) in relation to the light source of the microscope. (a) The plunger of the cable release (R) is depressed to open the shutter but no light penetrates the camera. (b) While the slots are crossing the light source, six light pulses illuminate the specimen within the chamber (C) and penetrate the camera through the opened shutter (after Makler, 1980).

Result

Table 1 shows the distribution of the percentage sperm motility in the 41 semen specimens. Although a satisfactory correlation was observed in percentage sperm motility assessment using the two methods, the MEP provided a consistently more reliable result of percentage sperm motility (Fig. 3).

Discussion

The replacement of the common subjective method for sperm motility evaluation by an objective method has been the goal of several workers in modern andrology [10, 11, 12]. Of the methods suggested, MEP has been found easy to perform, highly accurate and relatively inexpensive in routine semen analysis [7, 9, 12].

Although a satisfactory correlation was obtained between ordinary microscopy and the multiple exposure photography (MEP) technique, the latter provided a more reliable estimation of the percentage sperm motility. This is in accordance with the findings of Makler [7, 9]. The findings in this study also indicated that the subjective estimation yielded a percentage motility rate higher than those determined objectively. This finding agrees with that of Makler *et al.* [12] that human judgement tends to overestimate motility because the eyes are

Table 1. Percentage sperm motility using MEP and ordinary microscopy method

Serial no. for the semen specimen	Mean percentage motility		Serial no. for the semen specimen	Mean percentage motility	
	Microscopy	MEP		Ordinary microscopy	MEP
1	60	48	22	80	86
2	40	44	23	40	52
3	75	47	24	80	34
4	70	34	25	90	39
5	30	31	26	55	45
6	80	30	27	40	87
7	60	52	28	70	73
8	90	73	29	40	29
9	90	62	30	75	36
10	80	25	31	70	62
11	84	34	32	40	53
12	78	39	33	30	67
13	40	43	34	50	55
14	70	50	35	80	62
15	80	70	36	65	10
16	50	50	37	60	75
17	60	37	38	65	48
18	40	49	39	65	64
19	50	33	40	80	69
20	80	17	41	65	26
21	75	66			

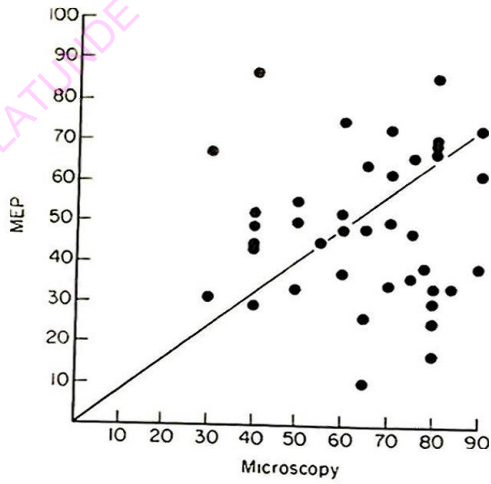

Fig. 3. Percentage motility distribution.

focussed more intently on motile, than on non-motile, sperm. Although MEP was used in this study to measure the percentage sperm motility, it could also determine the density, morphology and the speed of the spermatozoa in a semen specimen as recorded in other studies [7, 9, 12]. Like all objective methods, the MEP technique has an advantage of storage of information, re-examination of data and comparison of results.

In conclusion, the MEP technique, apart from providing a more reliable estimation of the percentage sperm motility in the semen sample than the ordinary subjective microscopy method, is simple to operate. In this regard, MEP has a place in the developing countries for male infertility investigation, in view of the limited financial resources and scarcity of skilled personnel. Also MEP, as an objective method, has the advantage of providing a permanent record that can be used to assess progress of therapy following re-examination of the patient.

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