A SIMPLE METHOD TO MEASURE DRUG EFFECTS ON HUMAN SPERM MOTILITY

It is likely that sperm motility is an important determinant of male fertility. The lack of progress in pharmacological investigation into this field is due in part to the absence of a simple and objective method of measuring drug effects on sperm motility. Most seminology laboratories use simple microscopic examination (Zaneveld & Polakoski, 1977) which is subjective and not accurate enough for research purposes. Recently developed photographic (Makler, Makler, Itzkovitz & Brandes, 1980), spectrophotometric (Atherton, 1979), turbidimetric (Levin, Shofer & Greenberg, 1980) and laser light scattering (Lee & Blandau, 1979) techniques are either too complicated or subject to interference by crystals, tissue debris and inflammatory cells which are often present in the semen. Because human semen volume is small and motility varies greatly both between and within individuals, an ideal pharmacological method for studying drug effects on human sperm motility should use sufficiently small volumes to make possible multiple comparisons on a single semen sample. Few currently available methods fulfill this requirement.

We have developed a trans-membrane migration method using two chambers separated by a membrane in which there are many evenly distributed 5 μm capillary pores (Nucleopore Co.). The upper chamber is made from the plunger of a 2 ml Sabre syringe (Gillette Ltd). To the lower end of it a sheet of membrane 13 mm in diameter is bonded. Fresh human semen 100 μl is pipetted into this chamber which is then inserted into a siliconized glass bottle forming the lower chamber and containing 2 ml of phosphate buffered saline at pH 7.3 (Dulbecco 'A', Oxoid Ltd.). The relative position of the chambers is fixed so that the fluid levels are equal (Figure 1).

The apparatus is then incubated in a water bath for 2 h at 37°C. The upper chamber is then removed and the sperms in the lower chamber killed with 50 μl of formalin solution. The number of sperms in the upper and lower chambers is counted in a haemocytometer. The proportion of sperms that moves across the membrane into the lower chamber is called the trans-membrane migration ratio (TMMR).

When drug effects are studied, 100 μl of fresh human semen is mixed with 50 μl of drug solution, then 100 μl of this semen-drug mixture is pipetted into upper chamber. All drugs are dissolved in the phosphate buffered saline and the pH is readjusted to 7.3. A 2:1 semen-buffer mixture is used as the control.

Figure 2 shows the correlation between TMMR and the percentage of sperms which show progressive,

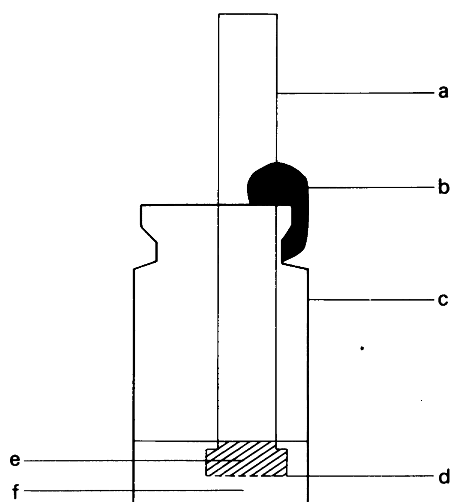

Figure 1 Diagram of the apparatus for measuring trans-membrane migration of sperms. A Nucleopore membrane (d) is bonded to the lower end of the upper chamber (a) which contains semen (e). A glass bottle forms the lower chamber (c) and contains buffered saline (f). The relative position of the chambers is fixed with a small piece of plasticine (b).

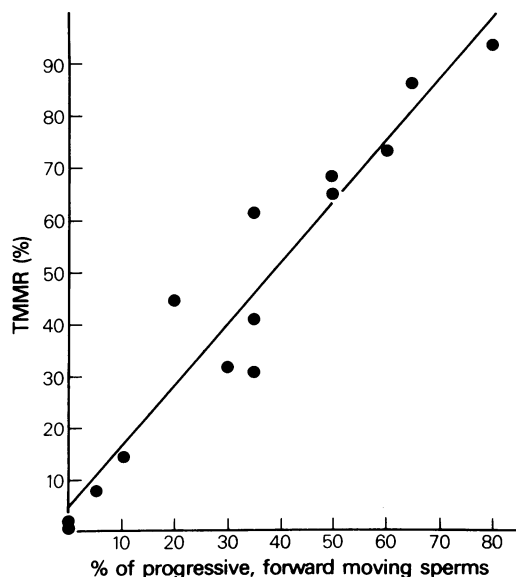

Figure 2 Correlation between trans-membrane migration method (TMMR) and microscopic examination, $r = 0.9613$.

forward movement under microscopic examination. The correlation coefficient derived from fourteen samples is 0.9613. The regression equation is $y = 1.19x + 3.41$. The graph shows that our method consistently overestimates the motility when compared to the microscopic examination. This may be due to the difference in temperature between these two methods. Specimens are incubated only in the trans-membrane migration method and this has been shown to increase sperm motility (Milligan, Harris & Dennis, 1978).

The coefficients of variation in five replicates of seven samples with mean TMMR of 0.05, 10.8, 14.1, 20.1, 32.4, 44.4 and 51.0 were 100, 23.0, 10.5, 4.5, 6.2, 8.7 and 8.3% respectively. They were calculated from arc-sine transformations of the original data points. This transformation was used to stabilize the variance.

We have used this method to measure the effect of caffeine on sperm motility. The stimulatory effect of caffeine was first demonstrated in epididymal (Drevius, 1971) and ejaculated (Garbers, First, Sullivan & Lardy, 1971) bovine sperms. Several studies (Bunge, 1973; Haesungcharern & Chulavatnatol, 1973; Schoenfeld, Amelar & Dubin, 1973; Levin *et al.*, 1980; Makler *et al.*, 1980) have shown a similar response in human sperms, although one study (Dougherty, Cockett & Urry, 1976) failed to confirm it. These studies differ in the concentrations of caffeine, the methods of evaluation and the control used for comparison. None of these methods was able to establish the relationship between dose and response, possibly because of the difficulty in performing within sample comparison.

Caeffine in final concentrations of 10^{-4}, 5×10^{-4}, 10^{-3}, 5×10^{-3} and 10^{-2} mol/l were tested on each sample. Figure 3 shows the maximal stimulatory effect on nine specimens. The numbers of specimens that reach their maximal response at concentrations of 10^{-3}, 5×10^{-3} and 10^{-2} mol/l are 3, 4 and 1 respectively. One specimen with a control TMMR of 1.1 showed no response at all. Once attaining their maximal stimulation, the size of response decreased in four samples despite the further increased caffeine concentration; however, a plateau was maintained in the other samples. A representative dose-response curve is shown in Figure 4. The mean ± s.e. mean of maximal increment in TMMR was 22.3 ± 3.7 for the eight responsive specimens.

Gassner, Goldzieher, Masken & Hopwood (1959) reported a method based on the ability of spermatozoa to migrate through the 10 μm wide spaces of a wire mesh, into centrifuged sperm-free semen plasma. They diluted the semen samples with phosphate buffer in order to make the volume large enough for centrifugation. The result of that study was disappointing, possibly because 10 μm is twice the diameter of average sperm head and sperm motility can be changed by dilution and centrifugation. 'Good motility' specimens graded by microscopic criteria showed a wide range of migration ratio using their method, sometimes worse than poorer specimens.

In conclusion, we have developed a trans-membrane migration method which is simple, quantitative and reproducible and which uses a small aliquot of human semen to evaluate drug effects on sperm

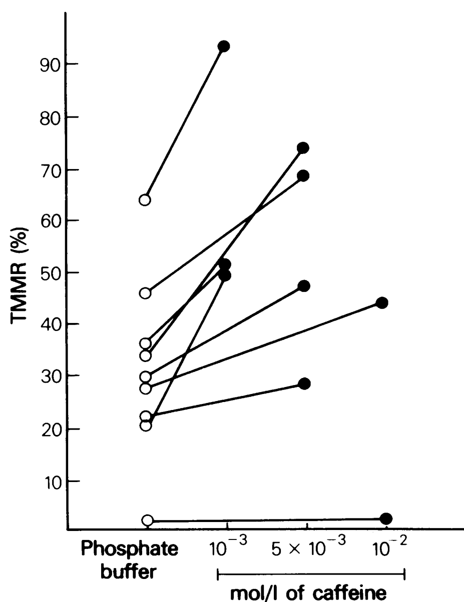

Figure 3 Maximal increase (●) in sperm motility after caffeine stimulation measured by trans-membrane migration method. Semen-buffer mixture (○) is used as the control.

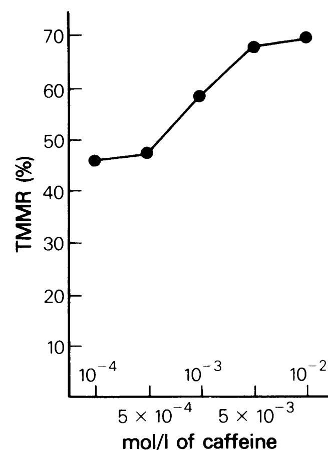

Figure 4 Representative dose-response curve for caffeine-stimulated sperm motility measured by trans-membrane migration method.

motility. With this method, we have demonstrated that the maximal stimulatory concentration of caffeine ranges from 10^{-3} to 10^{-2} mol/l and the response varies from sample to sample.

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ORAL SUSTAINED-RELEASE AMINOPHYLLINE IN MEDICAL INPATIENTS

We read with interest the paper by Ramsay, Mackay, Eppel & Oliver (1980) on the use of oral sustained-release aminophylline in medical inpatients. It has been previously reported that 5–15% of patients may experience gastrointestinal toxicity at plasma levels below 20 μg/ml (Ogilvie, 1978).

Only two patients (patients 12 and 16) included in the study had uncomplicated asthma.

We were surprised that patients 2 and 3 had their dose increased when they had already exhibited clinical toxicity at the lower dose; this was particularly disturbing in patient 3 whose plasma level was already above 20 μg/ml at the lower dose and rose to 50 μg/ml on the higher dose.

Five patients (Nos. 2, 3, 8, 14 and 15) experienced toxicity at the lower dose of 450 mg/day. Two of these patients (Nos. 3 and 15) had plasma levels in excess of 20 μg/ml. In the other three patients, all of whom were over 60 years in age, heart failure was a major clinical problem. All of them were on diuretics and two were also receiving digoxin.

A further three patients (Nos. 5, 7 and 9) experienced toxicity at the higher dose of 900 mg/day. These three patients also had the problems of age, cardiac failure and its therapy.

Although the authors were unable, in this small sample, to demonstrate a statistically significant relationship between age, heart failure or diuretic treatment and toxicity, the occurrence of nausea at levels lower than 20 μg/ml in patients with these complicating factors is not surprising. An interpretation of the findings in this study would be that