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FACTORS AFFECTING SPERM MOTILITY. III. INFLUENCE OF VISIBLE LIGHT AND OTHER ELECTROMAGNETIC RADIATIONS ON HUMAN SPERM VELOCITY AND SURVIVAL*

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Specimens of semen from fertile and infertile patients were exposed to different electromagnetic radiations, including visible light, ultraviolet (UV) light, x-rays, and high-frequency radio waves. Sperm motility was analyzed before, during, and after irradiation by the multiple exposure photography (MEP) method. No significant difference was found between controls and specimens exposed to various doses of visible and UV light and x-rays either immediately or several hours after exposure. In contrast to spermatozoa of other species that were reported to be adversely affected by visible and UV light and and x-rays, human spermatozoa seem to be highly resistant to similar doses of these radiations. A deleterious influence was observed when high-frequency radio waves were applied to human spermatozoa. This may be attributed to an intracellular diathermic effect.

The informative value of this study in relation to routine semen analyses and experimental studies in the physiology and comparative anatomy of spermatozoa is discussed. Fertil Steril 33:439, 1980

Spermatozoa of most species, including man, are not exposed to visible light throughout their lifespans. Passage from the male reproductive tract into the female genitalia and transportation to the site where fertilization takes place occur in complete darkness. Visible light can therefore be considered unnatural or foreign with respect to semen and spermatozoa. However, during routine semen analysis and in vitro experiments, specimens are exposed to visible light while kept in transparent jars and test tubes or when illuminated under microscopic observation. It is therefore important to establish whether spermatozoal motility is affected by exposure to light in order to avoid erroneous results in sperm studies.

No studies have been reported on the effects of light on human spermatozoa, probably because the usual subjective methods for motility evaluation[1, 2] are not sensitive enough for this purpose. With the aid of the recently developed multiple exposure photography (MEP) method,[3] small variations in sperm velocity and percentage of motility can now be measured objectively and accurately.

In this study the MEP method was used to investigate the possible influence of light on sperm motility. To determine whether the presence or absence of such an effect in specific to visible light, additional experiments were performed in which other electromagnetic radiations such as ultraviolet (UV) light, x-rays, and high-frequency radio waves were used to irradiate semen specimens.

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MATERIALS AND METHODS

Semen Specimens

The effects of the various radiations on speci-

mens with a high motility rate as well as on specimens with a low motility rate were investigated. According to standards established from the results of our previous objective measurements, a motility rate of 40% to 45% defines the borderline between the two groups.[4] Specimens of both kinds, obtained from fertile and infertile patients attending our clinic, were incorporated in each experiment. No specific etiology was encountered in any patient. Specimens from men with varicocele, men with genital tract infection, men with a history of mumps, and those with mainly idiopathic asthenospermia were included. All specimens were collected by masturbation and the experiments were performed within 1 to 2 hours, at room temperature. Prior to each experiment, each specimen was divided into two equal portions. One part was irradiated and the other part served as a control.

The following experiments were performed, using a separate group of specimens for each.

Experiment 1: Visible Light. Specimens in glass test tubes were kept in the dark from the moment of ejaculation. The experimental samples were illuminated for 24 hours by a standard 40-watt fluorescent light bulb at a distance of 10 cm (estimated intensity 4000 footcandles). Spermatozoal motility was evaluated periodically for the illuminated and control samples during the 24-hour period.

Experiment 2: Ultraviolet Light. Because UV light does not penetrate glass, the specimens were transferred to shallow plastic containers of 2-cm diameter and 1-cm depth and irradiated by a lamp located 10 cm *above* the specimens. The nominal wavelength was 366 nm and the intensity at the sample was approximately 1 mwatt/sq cm. One group of specimens was exposed for 30 minutes and a second group was exposed for 4 hours. Spermatozoal motility was evaluated periodically for both groups and for their nonexposed controls kept in similar containers.

Experiment 3: X-Rays. Two groups of specimens in glass test tubes were irradiated for 30 minutes by 100-kV x-rays (HVT = 0.6 mm Al). One group received an average of 150 rads and another group received an average of 6000 rads. Spermatozoal motility was evaluated periodically for both groups and their controls during the 4 hours after the irradiations.

Experiment 4: High-Frequency Radio Waves. Specimens were placed in specially designed flat Plexiglas containers which were inserted between two parallel metal plates. The plates were connected to a 27-MHz high-frequency generator normally used for diathermy. The field strength within the specimens was estimated to be about 0.6 volt/cm. The irradiation time was 30 minutes and the spermatozoal motility was evaluated periodically during the following 4 hours.

In all experiments no significant change in the temperature of the samples was observed at the end of the irradiation period.

Motility Evaluation

Motility was measured with the aid of the MEP method, which has been described in detail elsewhere.[3, 5] For each measurement a drop of semen from a well-mixed specimen was placed in a special 10-μm chamber,[6] and 8 to 12 fields containing 200 to 400 spermatozoa were photographed. Each film was exposed for 1 second, during which time the sample was illuminated by six light pulses. Images of the photographed spermatozoa were projected onto sheets of paper from which the percentage of motile sperm, sperm velocity, and sperm concentration were calculated.

The results of the experiments were analyzed

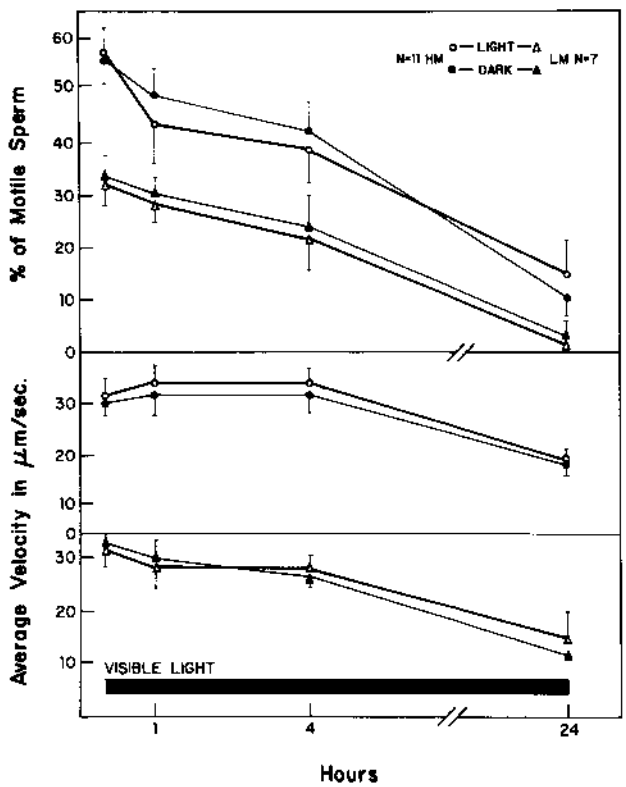

FIG. 1. Change in sperm motility with time in groups of specimens with high motility (*HM*) and low motility (*LM*) exposed to a visible light of intensity 4000 footcandles for 24 hours. Each *point* represents the mean value; the standard deviation is indicated by *bars*.

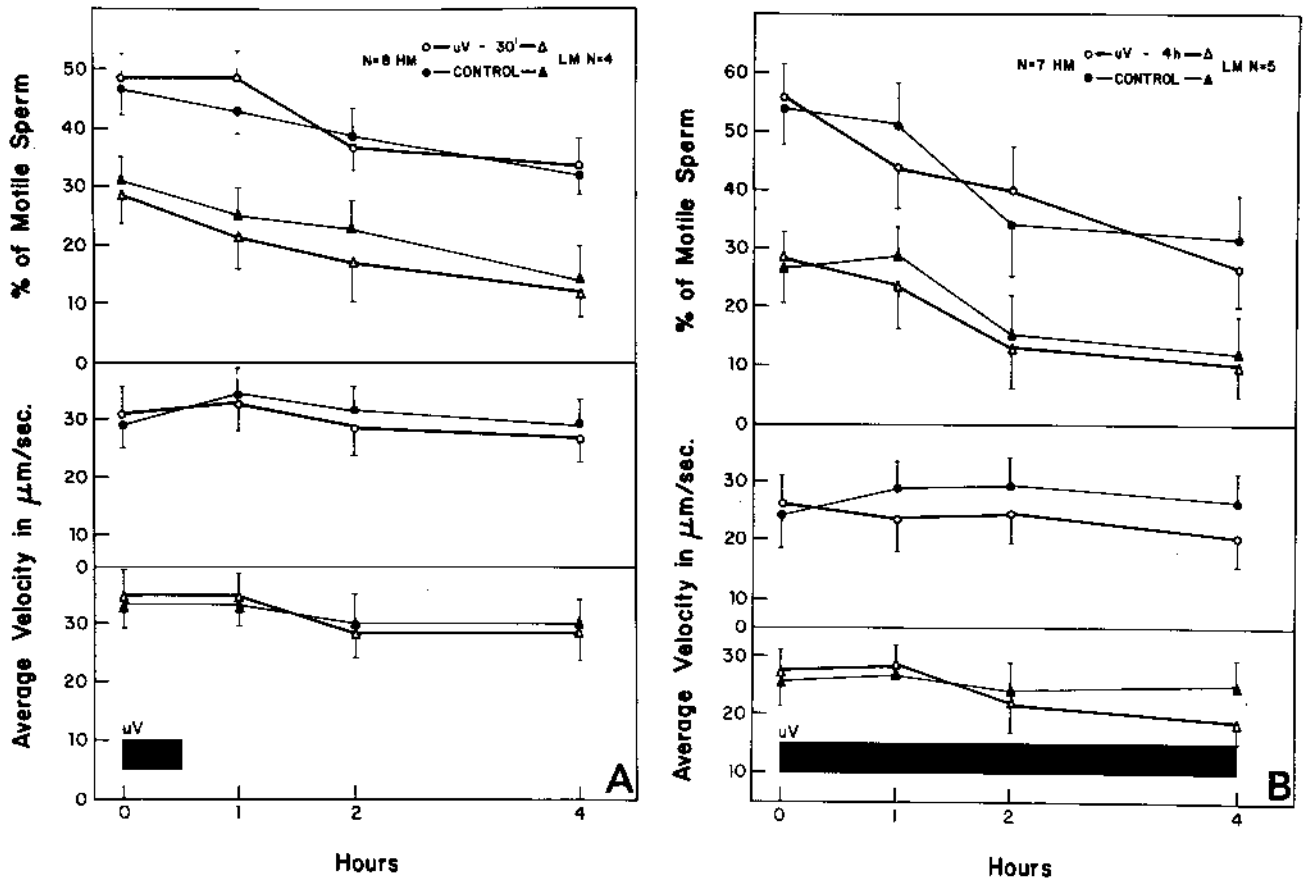

FIG. 2. *A*, Change in sperm motility with time in groups of specimens with high motility (*HM*) and low motility (*LM*) exposed to a UV light of intensity of 1 mwatt/sq cm for 30 minutes. *B*, Change in sperm motility with time in groups of specimens with high motility (*HM*) and low motility (*LM*) exposed to UV light of intensity 1 mwatt/sq cm for 4 hours.

TABLE 1. *Sperm Velocity and Percentage of Motility in Six Groups of Specimens Under Various Kinds of Electromagnetic Radiation*[a]

Type of irradiation	Time after exposure									
	0		1 hr		2 hr		4 hr		24 hr	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Visible light (N = 18)										
Percentage motile sperm	48	48	42	40			37	35	6	9
Average velocity (nm/second)	30.3	30.2	31.3	32.4			30.0	31.6	15.1	16.6
UV light for 30 minutes (N = 12)										
Percentage motile sperm	40	40	35	36	32	28	24	24		
Average velocity (nm/second)	30.2	33.1	34.7	33.5	31.6	28.4	29.1	27.6		
UV light for 4 hours (N = 12)										
Percentage motile sperm	37	38	38	39	22	23	20	16		
Average velocity (nm/second)	26.2	26.4	27.2	25.8	26.2	23.5	25.1	19.8		
X-ray 150 rads (N = 13)										
Percentage motile sperm	45	45	40	39	36	34	30	29		
Average velocity (nm/second)	30.1	29.3	32.4	32.8	31.7	29.8	29.1	30.0		
X-ray 6000 rads (N = 11)										
Percentage motile sperm	37	36	29	31	24	26	23	19		
Average velocity (nm/second)	25.6	26.0	24.8	23.9	25.7	22.8	23.2	21.7		
High-frequency radio wave (N = 11)										
Percentage motile sperm	53	54	50	36[b]			41	26[c]		
Average velocity (nm/second)	32.1	31.7	33.6	25.1[b]			33.6	23.2[c]		

[a]Values are means of each experimental and control group analyzed periodically with the MEP method.
[b]$P < 0.05$.
[c]$P < 0.01$.

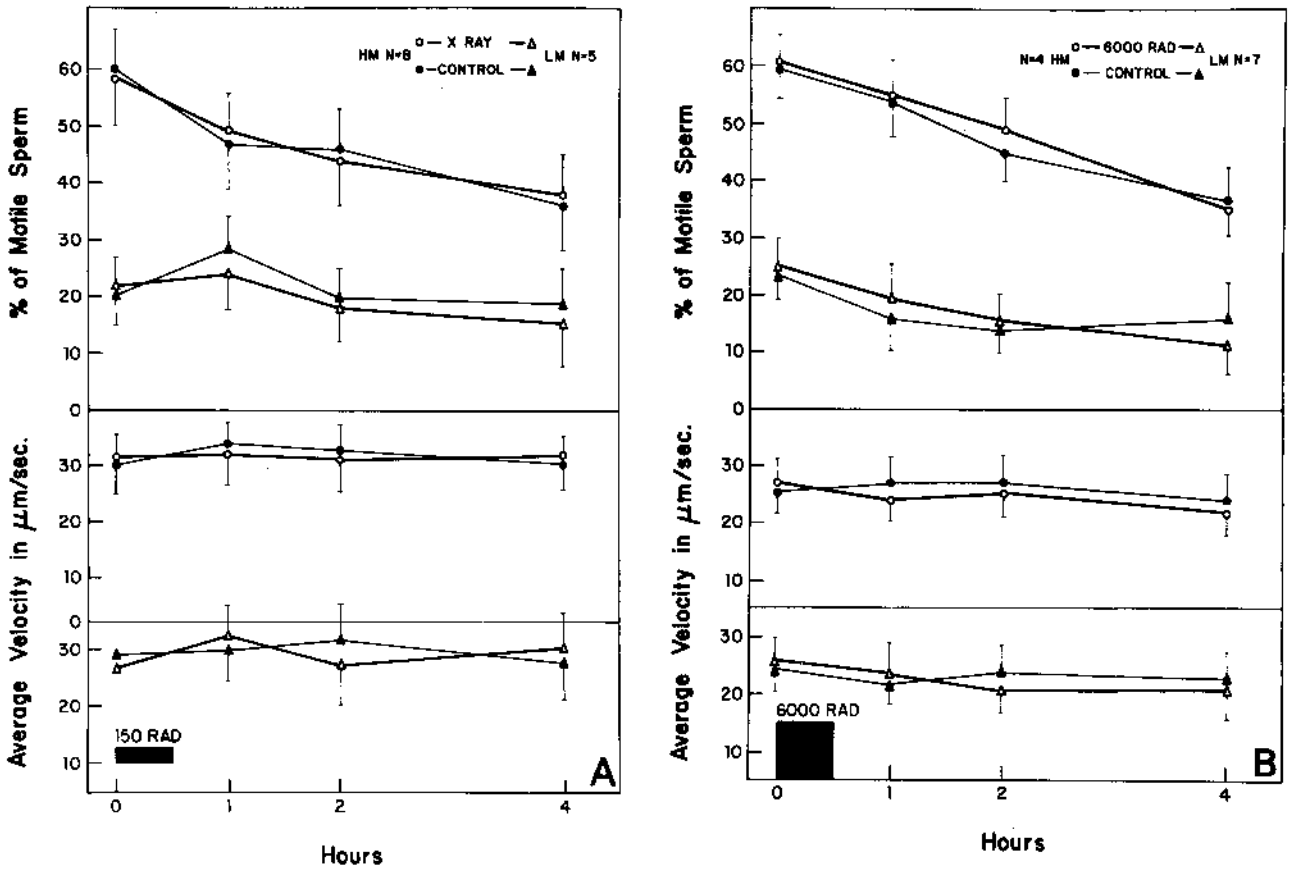

FIG. 3. *A*, Change in sperm motility with time in groups of specimens with high motility (*HM*) and low motility (*LM*) given a dose of 150 rads of x-rays. *B*, Change in sperm motility with time in groups of specimens with high motility (*HM*) and low motility (*LM*) given a dose of 6000 rads of x-rays.

statistically, and significance was determined by the pair *t*-test.

RESULTS

The results of the four experiments are shown in Table 1 and Figures 1 to 4. Table 1 summarizes the mean change in spermatozoal velocity and percentage motility as a function of time in irradiated samples as compared with control samples. Figures 1 to 4 describe graphically the changes in these parameters for samples divided into groups showing high and low motility rates. From these results it can be seen that samples exposed to visible light, UV light, and x-rays did not differ significantly from their controls.

On the other hand, the samples exposed to high-frequency radio waves revealed a significant decrease in percentage motility and spermatozoal velocity for specimens from both high- and low-motility groups. This effect is demonstrated in photomicrographs of an experimental sample (Fig. 5*A*) and its control (Fig. 5*B*) 1 hour after the sample was exposed to the radio waves. The decrease in motility and spermatozoal velocity of the experimental sample is evident from the small number and shorter lengths of the six-ringed chains representing motile spermatozoa.

DISCUSSION

Most of our results are contrary to those reported by others on studies of animal sperm in which spermatozoa of bull, ram, mouse, rabbit, and cock were adversely affected by exposure to visible light, UV light, and x-rays. Norman and Goldberg[7] found that bull spermatozoa irradiated by a 40-watt fluorescent bulb at a distance of 33 cm during 10 to 24 hours decreased their oxygen consumption and motility rate. Van Duijn[8-10] and Van Duijn and Van Lierop,[11] who investigated the photodynamic effect of visible light on bull and cock spermatozoa, found that there was a decrease in motility and that a relationship existed between spermatozoal photosensitivity and the pH of the irradiated medium. Wales and Choong[12] showed

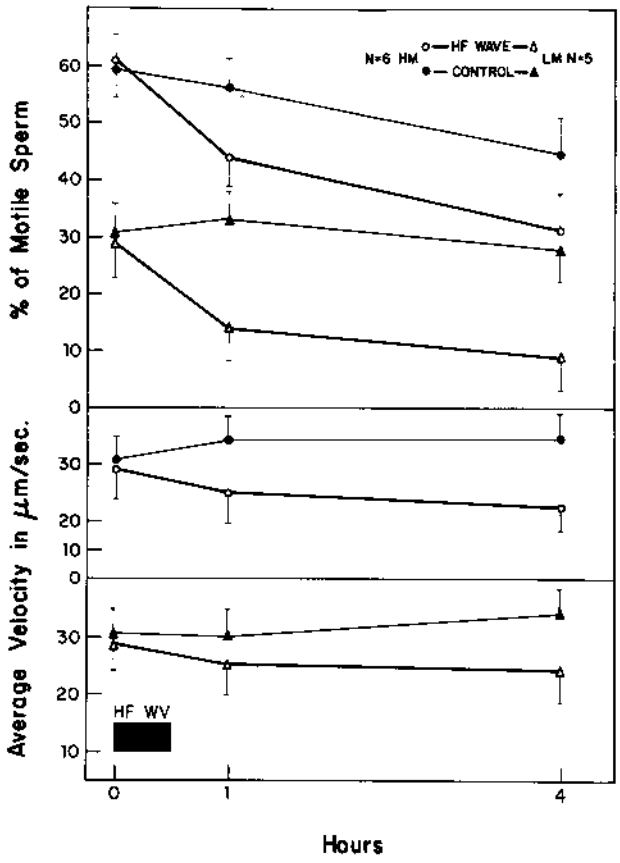

FIG. 4. Change in sperm motility with time in groups of specimens with high motility (*HM*) and low motility (*LM*) exposed to high-frequency radio waves of field strength 0.6 volt/cm for 30 minutes.

that irradiation of ram and bull spermatozoa for 60 minutes by UV light of 366-nm wavelength, produced by a 125-watt bulb at a distance of 30 cm, decreased sperm motility from 48% to 3%. Edwards[13] reported a decrease in activity and regularity of mouse spermatozoal movement caused by irradiation by UV light of 210- to 320-nm wavelength for 30 minutes. Hamner and Williams,[14] however, reported a stimulating effect of light on rabbit spermatozoa. The harmful effect of both kinds of light radiation was attributed by these authors to the production of hydrogen peroxide within sperm cells or semen which is highly spermicidic. The addition of catalase protected spermatozoa from these effects.

Rikmenspoel and Van Harpen[15-17] extensively investigated the effect of various doses of x-rays on bull spermatozoa. They found a substantial decrease in percentage motility and sperm velocity after samples were irradiated with 5 to 10 kilorads. According to their explanation this was a result of the production of chemical poisons which inhibit oxidative mechanisms or induce damage to the contractile apparatus (spherical target organ) in the cells. This apparatus may be related to the developed centriole described by Fawcett and Phillips.[18] Overstreet and Adams[19] reported that a 6-kilorad dose of x-rays to rabbit spermatozoa prevented cleavage of fertilized eggs beyond four cells.

In our experiments the dosage and time durations of the irradiations by visible and UV light and x-rays were similar to those described in the experiments performed by the authors cited. Our finding that human sperm motility was not affected by these radiations can be explained in terms of species-specific differences between the spermatozoa of humans and those of other animals. It is known that human spermatozoa are more resistant than animal spermatozoa with regard to preservation at room temperature and the dilution effect.[20-24] Our conclusions do not imply

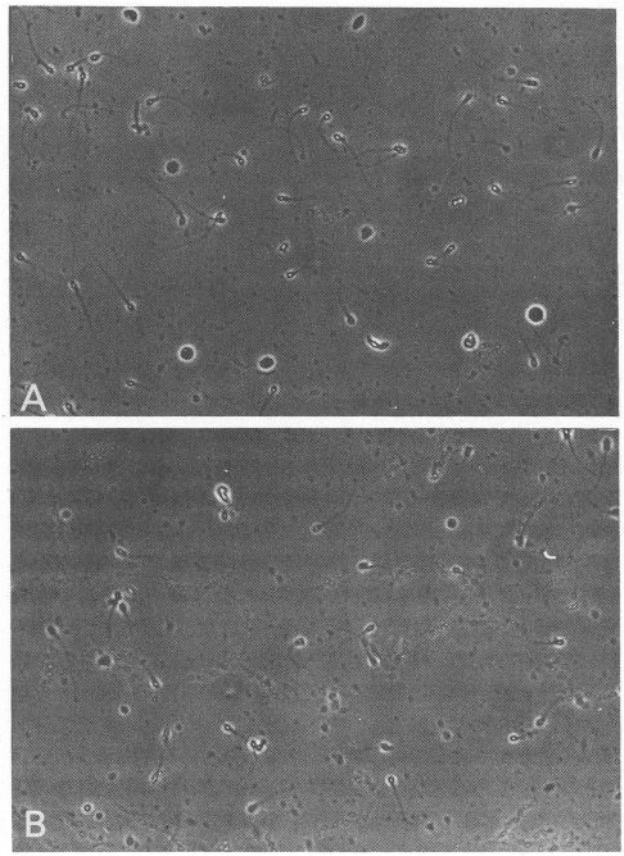

FIG. 5. Photomicrographs of two samples of the same specimen taken with the MEP method: *A*, 1 hour after exposure to high-frequency radio waves for 30 minutes; *B*, Unirradiated control photographed at the same time. Note that in the experimental sample the number of nonmotile spermatozoa is increased (accentuated images) whereas the number of motile spermatozoa is decreased and their path lengths are shorter (six-ringed chains).

that human spermatozoa are immune to the deleterious effects of radiation on their fertilizing capacity or to the induction of teratogenicity, especially in the case of x-rays.

The only electromagnetic radiation showing a depressant effect on human spermatozoal motility was the case of 27-MHz radio waves. The influence of high-frequency radio waves on animal and human spermatozoa has not been previously investigated. More study is required, especially on the dose-response relationship and on the mechanisms of possible physicochemical damage induced. The fact that no gross rise in the temperature of the samples occurred during our experiment does not exclude the possibility of an intracellular diathermic effect.

Obviously, the most important information provided by this study is that visible light has no stimulative or depressive effect on human spermatozoal motility. Semen samples may be kept in transparent containers during prolonged experimental studies with no harmful effects from exposure to ambient light. The absence of damage when samples are illuminated by the light source of the microscope for up to 20 minutes during routine semen analysis was demonstrated by us in a previous study.[25] Both of these observations may be of great value to investigators concerned with semen analyses.

From a practical standpoint, the absence of deleterious effects of UV light and x-rays is much less important. The incidence of such radiations on spermatozoa kept in vitro is rare, and this information should be of value mainly in the fields of pure physiology and the comparative anatomy of spermatozoa of different species.

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