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**Article** *in* Photomedicine and Laser Surgery · January 2006

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Photomedicine and Laser Surgery
Volume 23, Number 6, 2005

Pp. 549–555

# Influence of Visible Light and Ultraviolet Irradiation on Motility and Fertility of Mammalian and Fish Sperm

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## ABSTRACT

***Objective:*** **The effects of visible light irradiation on sperm motility, fertility, and reactive oxygen species (ROS) formation were investigated and compared in ram and fish (tilapia).** ***Background Data:*** **Low-energy visible light has previously been found to modulate various processes in different biological systems. In the literature, it is accepted that the first step following visible light irradiation is the formation of ROS by endogenous cellular photosensitizers.** ***Methods:*** **Sperm of ram and tilapia were irradiated with various light sources (400–800 nm white light, 660 nm red light, 360 nm blue light, 294 nm UV), and their motility and fertility rates were measured. The amount of ROS generated by irradiation was estimated using electron paramagnetic resonance (EPR) technique.** ***Results:*** **Sperm taken from tilapia showed higher motility and fertility following red and white light irradiation. In contrast, the motility and fertility of ram sperm were slightly increased only by red light. A negative effect on motility and fertility of sperm of both species was obtained following irradiation with UV and blue light. The amount of ROS produced in irradiated tilapia sperm was much higher than that of ram sperm.** ***Conclusions:*** **The results show that different wavelengths differentially affect tilapia and ram sperm motility and fertilization. The difference in response to the various light sources might be explained by the different amounts of ROS formation by ram and tilapia, which are in agreement with the physiology of fertilization appropriate to each of these species. Based on these results, it is suggested that *in vitro* fertilization in mammals should be performed in darkness or at least under red light.**

## INTRODUCTION

THE THERAPEUTIC EFFECTS of visible light have been long known. Low-energy visible light (LEVL) increases the healing rate of diabetic wounds[1] and defective bones[2]; there is also evidence of the promotion of restoration of severely injured peripheral nerves following visible light irradiation.[3] *In vitro* experiments show that LEVL increases cell proliferation,[4–6] induces respiratory burst in neutrophils,[7] and enhances the fertilizing capability of sperm cells.[8]

The cellular mechanisms underlying visible light–tissue interactions are also under extensive study.[7,9,10] Though no single mechanism has been unequivocally established, most researchers agree that the first step following visible-light irradiation is the formation of reactive oxygen species (ROS) by endogenous cellular photosensitizers.[9,11,12] Possible endogenous photosensitizers in the visible range can be porphyrins,[13–15] mitochondrial cytochromes,[16] pyridine cofactors, NADPH/NADH,[17] Fe-S clusters,[18] and flavoproteins/riboflavin.[19–22]

Low ROS fluxes (in contrast to high fluxes which destroy the cell) have recently been shown to act as messengers which activate cell processes such as transcription factor release, gene expression, muscle contraction, and cell growth,[23–26] whereas in sperm cells, there is evidence that minute amounts of ROS are involved in capacitation and acrosome reaction.[27]

The tight link between ROS and intracellular calcium ions ($[Ca^{2+}]$) led to the study of changes in $[Ca^{2+}]_i$ following illumination. For example, we have shown that an increase in $[Ca^{2+}]_i$ is detected in illuminated cardiomyocytes[28] and sperm cells,[8] following generation of $O_2^{\cdot -}$ or $H_2O_2$ in LEVL illuminated cardiomyocytes[28] and sperm cells.[8]

The influence of x-ray and UV radiation on sperm motility and fertilization has been extensively studied.[29–31] As for the

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influence of visible-light irradiation on sperm motility and fertility, only a few works are mentioned in the literature.

Singer et al.[32] found that human sperm motility could be improved by near IR irradiation at 940 nm, and Lenzi et al.[33] showed that progressive motility of human sperm could be enhanced following red-light irradiation at 647 nm. Our group[34,35] found that exposure of both ram and bull sperm to 632 nm laser irradiation changed their intracellular calcium concentration ($[Ca^{2+}]$), which is essential for capacitation and acrosomal reaction, and therefore, successful fertilization.

The aim of the present study was to investigate whether visible light has a different effect on motility and fertility of fish (tilapia) and ram sperm, as fish sperm are directly exposed to light during underwater extra-corporeal fertilization, while mammalian sperm fertilization is accomplished internally, without exposure to any source of light.

We increased the motility and fertility of tilapia sperm using red and white lights, whereas the motility and fertility of ram sperm were slightly increased only by red light. As expected, UV and blue light strongly decreased the motility of both fish and ram sperm. We also found that production of light-induced reactive oxygen species (ROS) in fish sperm is much higher than in ram, which might explain their different responses to the various light sources.

## MATERIALS AND METHODS

### *Ram sperm preparation*

Fresh sperm ejaculates from rams were supplied by the animal facilities at Bar-Ilan University. After ejaculation, semen was diluted 1:1 with ringer glucose phosphate (pH = 7.8), as described by Mann.[36] The semen was washed twice at 400g maximum for 15 min. Sperm cells were brought to a concentration of $50 \times 10^6$ sperm cells/mL (this concentration was found optimal for sperm motility measurements with a spermeter.[37,38]

### *Tilapia sperm preparation*

After urine drainage of male tilapia, the sperm was withdrawn by manual stripping of the lower abdomen. A pool of semen taken from three to five males was diluted 1:2 with filtered aquarium water, and 1 mL of it was added to a 24-mm-diameter tissue culture Petri dish that was irradiated by the selected light source. Motility was then determined using a spermeter (Gammeta-3, developed in Bar-Ilan University).[37,38]

### *Sperm motility and fertility*

A hematocrit glass capillary (Supe-Rior, Germany) was filled with sperm, and motility was measured using a spermeter.[38] Motility was measured three times for each sample and the average motility ± SD was calculated.

Fertility of tilapia sperm was examined using *in vitro* fertilization of *O. niloticus* eggs with *O. aureus* sperm. Eggs and sperm were stripped from adult fish a few minutes before spawning. Eggs were washed and fertilized with sperm that had been exposed to light. After 2–3 min, the fertilized eggs were put in Zuger bottles at 28°C. Seven days later, embryo viability was determined at the swimming stage[39] in order to define the fertility rate. The fertility of ram sperm was tested as previously described.[37,40] Eggs were taken from ewe ovary immediately after slaughter, washed and fertilized with sperm as detailed above.

### *Irradiation tools*

Sperm samples received various doses of intermittent light for 1–5 min every 2 or 5 min. The following light sources were used: white light 400–800 nm, 40 mW/cm$^2$; red light, 660 nm light-emitting diode, 10 mW/cm$^2$; blue light, 360 nm, 1.5 mW/cm$^2$; UV, 294 nm, 0.1 mW/cm$^2$.

### *ROS measurement*

The ROS measurements were performed as previously reported[41] using electron paramagnetic resonance (EPR) coupled with the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). In brief, DMPO (0.02 M, Sigma) was added to ram sperm suspension at $10^9$ or $10^7$ cells/mL tilapia sperm cells. Then, 100 µL of the sperm suspension were drawn into a gas-permeable teflon capillary and inserted into a narrow quartz tube that was open at both ends (to ensure the presence of sufficient oxygen during irradiation). The quartz tube was then inserted into the EPR cavity, and the ESR spectra recorded on an x-band spectrometer.

The microwave of the EPR was set at 9.7 GHz and power at 20 mW. Modulation frequency and modulation amplitude were 100 KHz and 1 G, respectively. Receiver gain was $2 \times 10^5$, time constant (TC) was 655 msec, conversion time 82 msec, and measurement time 168 sec. After acquisition, the spectra were processed by Bruker WIN-EPR software, version 2.11, for peak integration.

Trapping of the OH radical by DMPO results in DMPO-OH spin adduct, characterized by a typical quartet signal. DMPO also traps super oxide anion radical to give DMPO-OOH, which decomposes to DMPO. Assuming a linear dependence of the generated hydroxyl and super oxide anion radicals on cell number and fluency of the light source, we normalized the first peak area (of the DMPO-OH quartet) to one cell/cm$^2$ illuminated with an energy dose of 1 J/cm$^2$. The normalized area for each wavelength is shown in Figure 5.

## RESULTS

### *Influence of UV and visible light on motility of ram and tilapia sperm*

In general, IOMs (which represent an average of the sperm motility index) of ram sperm in the dark (~3000), were found to be almost five fold higher than those of tilapia (~550; Fig. 1a,b). The IOM profile following blue light (360 nm) showed a continuous decline both in ram and fish sperm, as the irradiation energy dose increased (Fig. 1a,b). However, different IOM profiles were obtained in tilapia and ram sperm in response to red and white light. IOM profiles obtained with white and red light in fish sperm peaked at 5 and 10 min of ir-

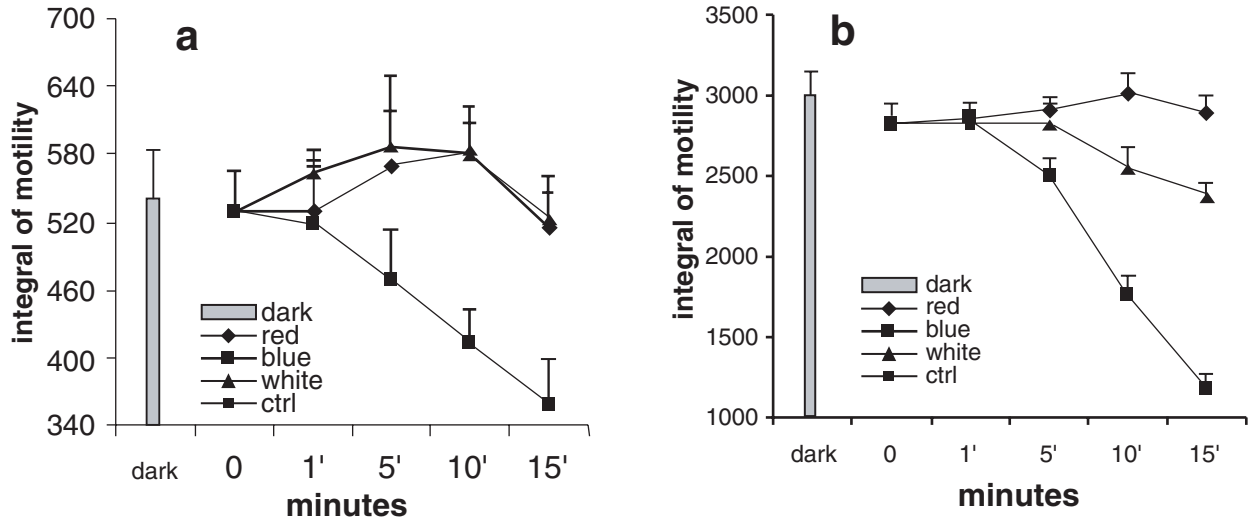


**FIG. 1.** Integral of motility of tilapia (**a**) and ram (**b**) sperm. The sperm were exposed to red (660 nm), blue (360 nm), and white light (400–800 nm) for different time durations compared to darkness.

radiation, respectively, before showing significant decrease (Fig. 1a), whereas ram sperm motility showed a slight insignificant increase with red light and a decrease with white light following more than 5 min of irradiation (Fig.1b).

Low intensity UV (294 nm) irradiation of tilapia sperm resulted in a significant decrease as early as the first 10 sec, followed by slight IOM recovery at 20 and 30 sec (Fig. 2a), before dropping completely at 75 seconds. Ram sperm maintained its initial decreased motility up to 30 sec, before exhibiting a strong decrease at 40 sec (Fig. 2b). The white-light irradiation of UV-treated (UV+, 10″) tilapia sperm resulted in significantly increased motility compared to the UV-irradiated sperm ($p = 0.001$) or to the dark ($p = 0.006$) controls. The white light also had a significant ($p = 0.02$) pro-motility effect on the control UV group (Fig. 3a). In contrast, white-light irradiation of UV-treated ram sperm, sperm resulted in a highly significant decrease of motility ($p = 0.001$ and $p = 0.002$, respectively) as compared to dark control (Fig. 3b).

### *Influence of visible light on ram and tilapia sperm fertility*

Red-light and white-light irradiated tilapia sperm resulted in higher embryo viability (swimming stage) of 45% (red) and 42% (white), as compared to 30% obtained from non-irradiated control sperm. In contrast, blue-light irradiation resulted in a significant ($p = 0.05$) decrease of viability to 20% (Fig. 4a).

Red-light irradiation of ram sperm resulted in an embryo viability of 65%. In contrast, white light and blue light decreased this viability to 32% and 19%, respectively. Ram sperm kept in darkness consistently produced an embryo viability of 56% (Fig. 4b).

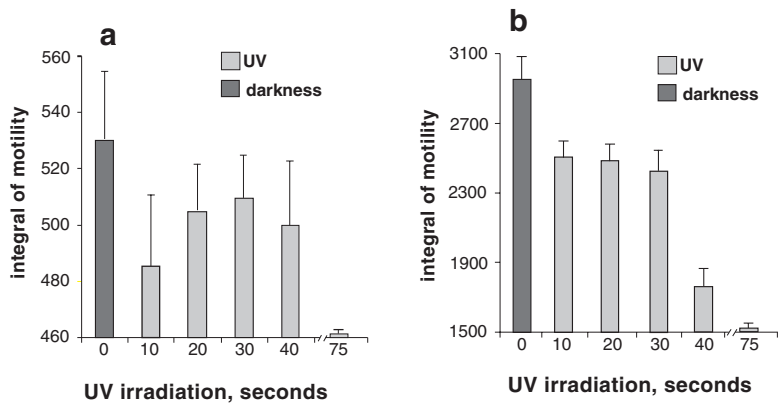


**FIG. 2.** Integral of motility of tilapia (**a**) ram (**b**) sperm. The sperm were exposed to different durations of UV or maintained in darkness.

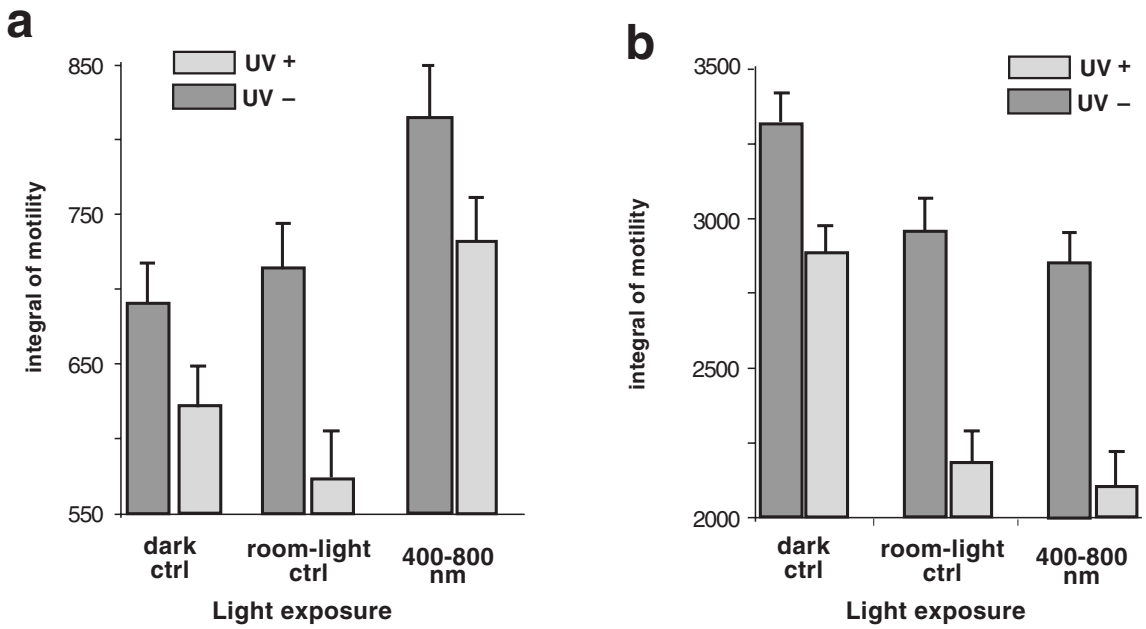


**FIG. 3.** Integral of motility of tilapia (**a**) and ram (**b**) sperm exposed to white light for 6 min following UV irradiation, compared to sperm, which received only UV irradiation.

*ROS production*

With the rough assumption that hydroxyl and super oxide anion radicals represent the amount of ROS in the irradiated cells, it can be seen that production of ROS in sperm was significantly higher in tilapia than in ram following irradiation with all wavelengths of visible light and with UV. White light induced the minimal ROS production ($2.4 \times 10^{-14}$ au in tilapia and $3.12 \times 10^{-15}$ au in ram); red light increased this production to $1.1 \times 10^{-13}$ and $8.4 \times 10^{-15}$, respectively, and blue light induced the greatest degree of ROS production ($4.4 \times 10^{-12}$ and $2.7 \times 10^{-13}$, respectively; Fig 5a). UV irradiation (2000 erg/mm$^2$) resulted in >50-fold higher ROS production in tilapia sperm than in ram sperm ($p = 0.001$; Fig. 5b).

## DISCUSSION

Our findings show that different wavelengths differentially modulate tilapia and ram sperm motility and fertilization. The effect of white light on sperm motility was evident in (1) the increase of tilapia sperm motility (Fig. 1a) and (2) restoration of the motility of UV-injured tilapia sperm to a level even

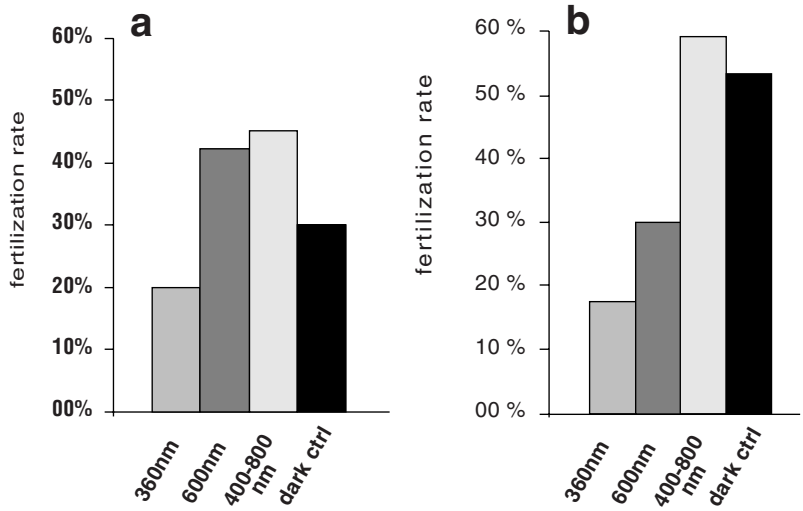


**FIG. 4.** Effect of different light sources (white, red, and blue, 6 min each) on fertilization of tilapia (**a**) and ram (**b**) sperm compared to non-irradiated samples.

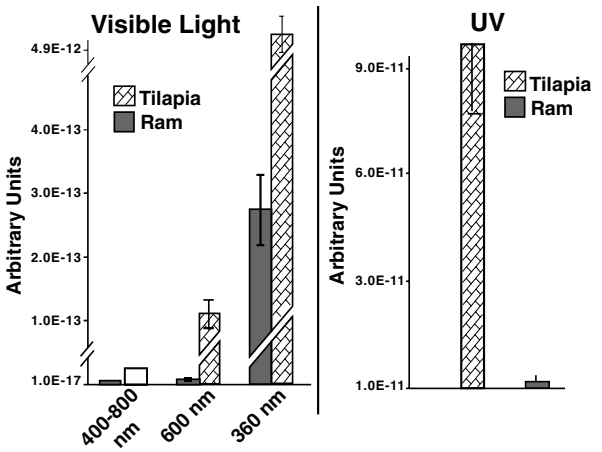


**FIG. 5.** ROS production (in arbitrary units) per 1 mW/cm$^2$ per sperm cell in tilapia and ram after exposure to blue, red, and white light (**a**) and UV irradiation (**b**).

higher than in the control non-irradiated sperm (Fig. 3b). This palliative effect on the motility of UV pre-irradiated sperm is in line with that of Karu[42] who found that visible-light irradiation increased the survival of γ-irradiated HeLa cells. In contrast to tilapia, ram sperm was found susceptible to white light that caused a significant decrease in motility in both UV-irradiated and non-irradiated sperm when compared to sperm maintained in darkness (Fig. 3b). These findings are not surprising when we consider the natural conditions of fertilization of both tilapia and rams. Fish sperms are directly exposed to light during the underwater extra-corporeal fertilization, while mammalian sperm fertilization is accomplished internally without exposure to any source of light. Red light (660 nm) significantly improved only tilapia sperm motility but had minor positive effect on ram sperm. These results are in agreement with the finding of Lenzi et al.[33] who showed enhancement of the progressive-motility of human sperm following red-light irradiation at 647 nm. Both tilapia and ram sperm were found susceptible to UV and blue-light irradiation. The sperm motility level was reported to correlate with the fertility rate.[43] Al-Qarawi et al.[44] found a linear correlation between motility and fertility in the dromedary. Our results are consistent with this finding (Fig. 4). Based on these results, it seems reasonable to suggest that *in vitro* fertilization in mammals should be performed in darkness or even better, under conditions of red light, as red light slightly increased the *in vitro* fertilization rate of ram sperms (Fig. 4b). Red light was also previously found to stimulate the fertilizing capability of mouse.[8,45]

Although in male reproduction ROS are known mostly for their detrimental effects on sperm function, there is now increasing evidence to suggest that, as has been observed in other cell types (i.e., neutrophils[46]) and organelles (i.e., endoplasmic reticulum),[47] very low and controlled concentrations of ROS participate in signal transduction mechanisms. Sperm capacitation and acrosome reaction are complex processes also regulated by signal transduction mechanisms, and evidence that minute amounts of ROS are needed for these processes, is accumulating.[48]

To detect oxy radicals, we used the EPR-spin trapping technique coupled with the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). DMPO can trap radicals such as, $O_2^{\cdot -}$ and ·OH.

$$\text{DMPO} + \cdot\text{OH} \rightarrow \text{DMPO-OH} \quad (1)$$

$$\text{DMPO} + O_2^{\cdot -} \rightarrow \text{DMPO-OOH} \quad (2)$$

DMPO-OH quartet signal can arise either by addition of a genuine ·OH radical to the double bond of DMPO, or upon addition of $O_2^{\cdot -}$ to form DMPO-OOH, which decomposes to DMPO-OH.[49]

Assuming that the DMPO-OH signal monitors $O_2^{\cdot -}$ and OH radicals, which can represent cellular ROS production, our results show that tilapia sperm generates more ROS than ram upon red-light irradiation (Fig. 5), which can explain the positive effect of red light on tilapia sperm motility and fertility.

UV and blue-light irradiation, which were found to generate high levels of ROS, resulted in a significant decrease of motility and fertility in both tilapia and ram sperm.

In a previous report, UV irradiation of tilapia sperm with a dose of 11,000 erg/mm$^2$ was found to completely eliminate sperm fertility.[50] Exposure of ovules to UV-irradiated (7200 erg/mm$^2$) cattle sperm resulted in a significant ($p < 0.05$) decrease in fertility and in the embryonic development rate at the blastocyte stage.[51] In addition, Peer[52] showed that ROS at high concentrations (3.2–3.8 μm) caused a significant decrease in ram sperm motility.

Furthermore, our results showed that the longer the wavelength of the light used, the less ROS were produced. This result could be explained if ROS formation is attributed to specific cellular photosensitizers such as cytochromes, which absorb long wavelengths of light less effectively, and therefore also generate less ROS.[53]

In summary, our results suggest that visible light affects sperm motility and fertilization depending on the wavelength employed and the amount of ROS produced. UV or blue light generates high levels of ROS, resulting in a decrease in motility and fertility. On the other hand, low levels of ROS can increase motility and fertility. In tilapia sperm, red and white light, which induce low levels of ROS, were found to improve motility and fertilization, while in ram sperm, only red light slightly improved the motility to a small extent. The difference between the response to visible light of ram and tilapia is in agreement with the physiology of fertilization appropriate to each of these species. Based on these results it is suggested that *in vitro* fertilization in mammals should be performed in darkness or at least under red light.

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