Light exposure of the ovum and preimplantation embryo during ART procedures

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Abstract Exposure to visible light (400–700 nm wavelengths) is an unnatural stress factor to preimplantation embryos cultured *in vitro*. This study investigated the spectral composition and intensity of light during IVF procedures, and calculated radiation doses reaching the embryo during handling and manipulation. The study shows that normal IVF procedure may result in stressing radiation doses, unless filters are applied. This is at present not sufficiently recognised. No Danish IVF clinics use filters to protect embryos against visible light. 95% of the radiation was from microscopes. Ambient light, in contrast, was not a significant contributor to light stress and the use of dark laboratories is not justified.

Keywords Blue light filter · Light energy exposure · *In vitro* culture · Preimplantation embryo · Visible light

Introduction

An IVF procedure implies that embryos are exposed to light during inspection and transportation in the laboratory. This unnatural exposure adds to the number of culture-induced stresses which may compromise pre-implantation development *in vitro*. The possible detrimental effect of light has been subject for several different studies (Table 1). The light intensity is however often reported in lux only, and this makes a direct comparison of radiation energy used in the studies and conditions in your own lab very imprecise. Direct com-

parison of radiation energy requires an irradiance (W/m^2) measure.

A body of literature (Table 1) shows the general negative effect of light exposure on embryo development. The detrimental effect of visible light is not directly related to the intensity and exposure time, but is also a function of the spectral composition of the light. Blue light (400–500 nm), and near blue light, appears to be orders of magnitude more harmful than longer wavelength visible light [2, 8–10]. It has been suggested that the harmful effect of blue light is associated with generation of H_2O_2 and specific absorption by enzymes applied in the respiratory chain [8, 10–12]. H_2O_2 and its metabolites, hydroxyl radicals, are known to cause cellular damage and result in impaired mitochondria function through reaction with amino acids, phospholipids, nucleotides, organic acids [13]. Two recent studies report harmful effects at blue light radiation doses in the interval 1–10 kJ/m^2 [10, 14]. One way to exclude blue light is the application of blue light filters (look yellow and cuts off around <500 nm), as recognised by [15, 16].

The harmful effect of visible light is well documented, and practitioners generally seem to acknowledge the problem. However, in our experience, actions to protect embryos against visible light rarely exceed loose recommendations about keeping the embryos outside the incubator as short as possible, and it is simply not possible to guess if your lab procedures still suffer from inadequate protection against visible light. A survey among 15 Danish IVF clinics, with 14 adequate replies, showed that 0 out of 14 used blue light filters on their microscopes. In continuation of our questionnaire, the clinics asked for recommendations on the subject. This leads us to believe that the lacking use of filters to a large extent is due to unawareness of the relevance and not based upon an evidence based decision. A usable analysis of actual radiation doses requires information about spectral

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Table 1 Published investigations on the impact of visible light, an overview

Author	Light source	Object	Parameter	Conclusion
Takahashi et al. 1999 [1]	Visible light 380–780 nm, 400 lux	Hamster embryos	DNA fragmentation, evaluated by comet assay	Significant effect after ≥ 5 min illumination
Umaoka et al. 1992 [2]	Visible fluorescent light, 2400 lux	Hamster embryos	Development of hamster 1 cell embryos to the 4 and 8 cell stage	Significant reduction after 5 min illumination. 30 min completely detrimental for the first cell cleavage
Schumacher and Fischer 1988 [3]	Visible fluorescent light 400–700 nm, 1600 lux	Rabbit embryos, day 1 post coitum	Cell proliferation by thymidin incorporation	Significant reduction after 30 min exposure
Barlow et al. 1992 [4]	Halogen incandescent light, 4000 lux	Fertilized mouse oocytes	First cleavage, fertilized mouse oocytes	Significant reduction after 60 min illumination
Daniel 1964 [5]	Visible (UV and IR shielded) fluorescent light 2600 lux	Rabbit embryos	Number of cleavages per time	Significant reduction after 4 hr illumination
Schumacher et al. 1998 [6]	Visible fluorescent light <1600 lux	Rabbit day 3 and 4 embryos	DNA ploidy abnormalities	No increase in number after 24 hr illumination
Kruger and Stander 1985 [7]	Incandescent/ fluorescent visible light, 2900 lux	Mouse embryos	Development of 2-cell embryos to blastocysts	No effect after 30 min illumination
Squirrel et al. 2001 [8]	Monochromatic laser light 514, 532 and 568 nm, 115 KJ/m ²	Hamster embryos	Development of 2-cell embryos to morula / blastocysts	Significant reduction at irradiance 115 KJ/m ²

composition of various light sources, intensity and direct exposure time. Therefore, we carefully examined light sources in the lab, timed typical procedures with respect to light exposure, and by calculation linked the data to the existing knowledge about the sensitivity of embryos and cells to the exposure to light.

Materials and methods

Spectral composition was measured using a radiospectrometer GER2600 (Geophysical and Environmental Research Corp., One Bennet Common, Millbrook, NY 12545). Illuminance was measured with a lux meter Tektronix J17 (Tektronix inc., P.O. Box 1000 Wilsonville, OR 97070), and total irradiance was measured with a Li-Cor LI-250 Light Meter (LI-COR, 4421 Superior Street Lincoln, Nebraska USA 68504-0425). Measurements were conducted under true *in vitro* conditions, in order to measure the light as experienced by the developing embryo. Light reaching the embryo during *in vitro* manipulation was evaluated for intensity and spectral composition. Background illumination (ambient light, other light sources than microscopes) was measured during daytime in an IVF laboratory with windows; with no direct sunlight reaching the laboratory (always excluded using window blinds). Ambient light was also evaluated in a laboratory without windows. Ambient light was in all cases of the fluorescent type (1 × 120 cm neon tube each 5–7 m² room area), mixed with daylight if windows were present. Ambient light will be subject to vari-

ation from day to day and from lab to lab, but was in this study measured under conditions which, based on our experience, commonly is found in IVF labs: A subdued, yet comfortable intensity without direct sunlight. In order to establish the typical microscope light intensity range prevailing in the IVF lab, light from different microscopes was measured at three different intensities. 1) Low light intensity, adjusted individually by 5 technicians to a level considered the lowest acceptable light intensity without compromising visual perception, 2) Preferred intensity and 3) High light intensity, adjusted to the highest intensity possible without annoying the technician and giving him/her a desire to reduce intensity. Intensity was measured for each setting and was believed to cover endpoints of the general interval experienced by the developing embryo during handling and inspection in a majority of IVF laboratories. Measurements were repeated after mounting a short wavelength cut off optical filter, Lee filter 101 yellow, cutting of 100% at <450 nm and 60% <500 nm in order to evaluate the fraction of energy cut off by the filter. The typical time extension of light exposure at different situations was estimated by examining standard laboratory routines.

Results

Light intensity

Light was typically emitted from inspection microscopes at an intensity of 20–30 W/m² (2500 to 5000 lux). Ambient light intensity in the laboratory was generally much lower

Fig. 1 1A: Illuminance (Lux) levels from microscopes, set by skilled technicians. Low: Lowest acceptable intensity where further reduction would compromise visual inspection. Preferred: preferred intensity. High: Highest intensity not giving a desire to reduce intensity. Ambi-

ent: background intensity from ceiling light and windows. Intensities are shown with and without SW cut off filter. 1B: As 1A except unit on y-axis in irradiance (W/m^2)

at an intensity of 0.1 to 0.5 w/m^2 (200–400 lux), (Table 1 a and b, Fig. 1). Usual microscope work light intensity was evaluated by 5 technicians asked to adjust the intensity to their individual preferred intensity interval, on two different microscopes. Lowest and highest intensities, which one could expect to be used, was found by asking technicians to adjust intensity to lowest respectively highest level within what was defined as normal working range. This was carried out in two different laboratories. Furthermore, on another occasion, light intensity of 5 different microscopes used for inspection of ova and embryos was measured independently as left by the technicians after a regular work day (Irradiance $\text{w}/\text{m}^2 = 24$ SEM = 10.1; Lux : 2343 SEM = 1128). This number was close to the “preferred” level adjusted intendedly by the technicians (Irradiance $\text{w}/\text{m}^2 = 19.3$ SEM = 1.9. Lux : 3026 SEM = 365). Spectral composition of the light sources: Fig. 2A shows a typical spectrum for halogen light. Mounting a blue light filter efficiently eliminates wavelength below ≈ 490 nm (Fig. 2B). Culture dishes made of Polystyrene PS absorbed a small equally distributed proportion of the light (Fig. 2C). ID show fluorescent light from ceiling mounted light tubes shows a much different spectrum, with a low intensity in the shorter wavelength interval relative to the microscope light.

Total energy (irradiance_{preferred level} * exposure time plus low and high endpoints) was calculated for typical ET procedures, IVF, ICSI and ICSI + PGD (Table 2a and b). From aspiration to transfer, the embryos in this study received at total energy of approximately 3.2 to 17.4 KJ/m^2 , depending on procedure, where roughly 0.2–1 KJ/m^2 was from wavelengths in the interval 400–500 nm.

More than 90% of the radiation energy from the halogen incandescent light was from wavelengths > 500 nm (Table 2c). Halogen incandescent light from the microscopes composed more than 95% of the total radiation energy reaching the embryo.

Discussion

A typical IVF procedure implies 400–500 nm irradiance doses of roughly 0.2 to 1 kJ/m^2 without filtering the light and at typical light intensities (Table 2). Such irradiation doses contribute to the number of non lethal stress factors related to *in vitro* culture, according to the data from [10, 14], possibly through stress on the respiratory chain. In a review [17] it is discussed how cultured embryos exhibit reduced oxidative capacity during *in vitro* culture, and hypothesized that the mitochondria are damaged by free radicals at ambient oxygen concentration. It is an interesting hypothesis that blue light and high ambient oxygen act in concert on the mitochondria and result in a reduced oxidative capacity of the developing pre-implantation embryo during *in vitro* culture. Reducing stress on the mitochondria during *in vitro* culture, in order to increase oxidative capacity, could be helped by a general reduction of light exposure and exclusion of blue light in concert with reduced oxygen concentration during culture inspection and handling.

The evaluation of radiation energy can not rely on a lux measurement alone although this has been the sole measure in several studies (Table 1). In future studies, an adequate description of a light source should include a spectroradiogram

Fig. 2 2A: Spectral composition of microscope halogen light. 2B: As 2A with short wavelength (sw) cut off yellow filter. 2C: Light absorption by polystyrene typically used for culture dishes. 2D: Spectral compo-

sition of background light from neon fluorescent light and daylight through windows, with and without filter

showing the irradiance of each wavelength of the emission spectrum. However, tungsten halogen incandescent light is a very common light source in microscopes and exhibits a rather constant emission spectrum. It is therefore possible to roughly convert a lux measurement to a blue light irradiance measure using numbers from this study where $150 \text{ lux} \approx 1 \text{ W/m}^2$ ($W = \text{J/s}$) and approximately 7% of the energy is 4–500 nm light. With these numbers, the blue light energy transmission to the embryo from the microscope can be calculated very roughly with a lux meter and a stop watch.

Conclusions

During typical IVF lab procedures, which do not differ much globally, embryos receive radiation energy from light within a range that has been shown to stress biological systems.

A literature review suggests that the damaging effect of visible light primarily concentrates to the 400–500 nm range. The stress upon the embryos can be reduced by mounting filters on the inspection microscopes such that radiation energy in the 400–500 nm range is excluded, without compromising visual inspection.

Concern should be directed towards the light microscopes. It is worth to reduce inspection microscope light as much as possible without compromising visual inspection and further to keep the exposure time as short as possible, e.g. by narrowing the illuminated area of the microscope table, such that only the embryo being inspected is present there.

It is not worth bothering about ambient light when kept subdued, yet comfortable. This is a nice thing to know for the lab practitioner as walking around in yellow or red light would be annoying.

This study furthermore gives a rule of thumb which allows the practitioner to evaluate his own lab using a simple lux meter, which is usually quite available.

Table 2 Recording of typical extension of total light exposure during IVF routines and energy distribution as a function of wavelength interval, derived from analysis of spectroradiograms as shown in figure

a) Total irradiation					
Operation (<i>n</i> = embryos timed)	Handling time microscope light seconds (SD)	Handling time ambient light seconds	Microscope light irradiance W/m ² preferred intensity (PI) (low-high)	Microscope light energy exposure kJ/m ² , PI (low-high)	Ambient light exposure interval kJ/m ²
IVF (<i>n</i> = 12)	167 (35)	400–600	19.3 (6.1–36.1)	3.2 (1.0–6.0)	0.04–0.3
ICSI (<i>n</i> = 20)	507 (125)	400–600	19.3 (6.1–36.1)	9.8 (3.1–18.3)	0.04–0.3
ICSI + PGD (<i>n</i> = 18)	904 (27)	400–600	19.3 (6.1–36.1)	17.4 (5.5–32.6)	0.04–0.3
b) Irradiation < 500 nm					
Operation (<i>n</i> = embryos timed)	Handling time microscope light seconds (SD)	Handling time ambient light seconds	Irradiance W/m ² interval (at PI)	Microscope light exposure interval kJ/m ² (at PI)	Ambient light exposure interval kJ/m ²
IVF (<i>n</i> = 12)	167 (35)	400–600	0.9 (0.4–1.7)	0.15(0.07–0.28)	0.0054–0.04
ICSI (<i>n</i> = 20)	507 (125)	400–600	0.9 (0.4–1.7)	0.46(0.2–0.87)	0.0054–0.04
ICSI + PGD (<i>n</i> = 18)	904 (27)	400–600	0.9 (0.4–1.7)	0.82(0.36–1.5)	0.0054–0.04
c)					
Wavelength interval	Light source	Radiation energy %	Potential effects		
300–400	Halogen	0.8%	Nucleic acid lesions		
	Fluorescent	< 2%* (Not detectable)			
400–500	Halogen	6.5 %	Mitochondrial		
	Fluorescent	13–14%	respiration damage		
> 500	Halogen	92–93%	Heating of medium		
	Fluorescent	85–86%			

*Literature value.

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