Journal of Reproductive Immunology 132 (2019) 21–28

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

The effect of light exposure on the cleavage rate and implantation capacity of preimplantation murine embryos

Zoltan Bognar[a,b,c], Timea Judit Csabai[a,b,c], Eva Pallinger[e], Timea Balassa[a,b,c], Nelli Farkas[f], Janos Schmidt[g], Eva Görgey[a], Gergely Berta[a], Julia Szekeres-Bartho[a,b,c,d,h,*], Jozsef Bodis[b,c,d,h]

[a] Department of Medical Biology and Central Electron Microscope Laboratory, Medical School, Pecs University, Pecs, Hungary
[b] János Szentágothai Research Centre, Pecs University, Pecs, Hungary
[c] Endocrine Studies, Centre of Excellence, Pecs University, Pecs, Hungary
[d] Department of Obstetrics and Gynaecology, Medical School, Pecs University, Pecs, Hungary
[e] Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary
[f] Department of Bioanalysis, Medical School, Pecs University, Pecs, Hungary
[g] Department of Biochemistry and Medical Chemistry, Medical School, Pecs University, Pecs, Hungary
[h] MTA - PTE Human Reproduction Research Group, Pecs, Hungary

ARTICLE INFO

Keywords:
Murine embryos
Light stress
Implantation rate
DNA fragmentation

ABSTRACT

During assisted reproduction the embryos are subjected to light. We investigated the relationship between light exposure and the developmental- and implantation capacity of mouse embryos. In vitro cultured embryos were exposed to white or red filtered light, then transferred to the uteri of pseudo-pregnant females. The mice were sacrificed on day 8.5 and implantation sites were counted. The number of nucleic acid containing (PI+) extracellular vesicles (EVs) in culture media of light-exposed and control embryos, as well as, the effect of the EVs on IL-10 production of CD8+ spleen cells was determined by flow cytometry. DNA fragmentation in control and light exposed embryos was detected in a TUNEL assay. The effect of light on the expression of apoptosis-related molecules was assessed in an apoptosis array.

Light exposure significantly reduced the implantation capacity of the embryos. The harmful effect was related to the wavelength, rather than to the brightness of the light. Culture media of light exposed groups contained significantly higher number of PI + EVs than those of the control embryos, and failed to induce IL-10 production of spleen cells. The number of nuclei with fragmented DNA, was significantly higher in embryos treated with white light, than in the other two groups.

In conclusion exposure to white light impairs the implantation potential of in vitro cultured mouse embryos. These effects are partly corrected by using a red filter. Since there is no information on the light sensitivity of human embryos, embryo manipulation during IVF and ICSI should be performed with caution.

1. Introduction

Among physiological conditions in mammals, the preimplantation embryo develops to blastocyst in the fallopian tubes, whereas during assisted reproduction, the embryo is removed from its natural environment.

Transition from the 2 cell embryo to the blastocyst stage is characterized by rapid cell division and activation of a large number of genes that control embryonic and extra embryonic differentiation (Gardner, 1998). The high-speed development renders the embryo extremely vulnerable to environmental changes[1], and being removed from its natural environment, represents an array of stress factors for the embryo (Khosla et al., 2001; Mann et al., 2004; Rinaudo et al., 2006; Hamdoun and Epel, 2007; Schultz, 2007).

While undergoing the procedures of assisted reproduction, the gametes, zygotes and the embryos are subjected to a variable spectrum of light from different sources, including; ambient light, illumination of the safety cabinet, light emitted from the microscope or from the time-lapse imaging cameras (Ottosen et al., 2007; Pomeroy and Reed, 2013).

In some species, e.g. fish and amphibians embryonic development takes place close to the water surface, exposing the embryos to strong visible and UV light, (Schultz, 2007) and in response, these species have developed mechanisms that protect the embryos from the harmful effects of light (Schultz, 2007). However, these defence mechanisms are

* Corresponding author at: Department of Medical Biology, Medical School, Pecs University, 12 Szigeti Street, Pecs, Hungary.
E-mail address: Szekeres.julia@pte.hu (J. Szekeres-Bartho).

https://doi.org/10.1016/j.jri.2019.02.003
Received 14 December 2018; Received in revised form 18 February 2019; Accepted 22 February 2019

possibly absent in mammals (Hamdoun and Epel, 2007; Schultz, 2007).

Light affects the embryo either directly, or indirectly, via increased production of reactive oxygen radicals, inducing gene transcription orincreasing the amount of breakdown products in the culture medium (Pomeroy and Reed, 2013).

The duration of light exposure adds up to 25 min during IVF and 50 min in case of intracytoplasmic sperm injection (ICSI) (Pomeroy and Reed, 2013; Li et al., 2014). The light load–depending on the frequency of images captured- might be lower during time lapse imaging (Takenaka et al., 2007; Li et al., 2014).

The harmful effects of light on embryo development are already known in mammals. The extent of the damage depends on the intensity and wavelength of the light, the length of exposure and the light sensitivity of the embryos (Kruger and Stander, 1985; Oh et al., 2007; Takenaka et al., 2007; Nakahara et al., 2010). Based on these data, the revised ESHRE Guideline for good practice in IVF laboratories, recommends minimizing the light exposure of the embryos during all phases of manipulation (ESHRE Guideline Group, 2015).

For ethical reasons, it is not possible to perform light stress experiments on human embryos, therefore there is no information about their light sensitivity (Schultz, 2007; Pomeroy and Reed, 2013). Hamster and pig embryos are very sensitive to light, rabbit embryos are relatively light resistant, and a somewhat less resistant model is the mouse embryo (Takenaka et al., 2007). Light exposure has been shown to affect cell division, production of reactive oxygen radicals (ROS), and to cause DNA damage (Takahashi et al., 1999, 2000; Oh et al., 2007; Takenaka et al., 2007) yet the evidence on implantation capacity of light stressed embryos is sparse (Schultz, 2007; Takenaka et al., 2007).

We investigated the relationship between exposure to white or red filtered light, and developmental- as well as implantation capacity of mouse embryos. A further aim of this study was to determine, whether the possible harmful effects of light are associated with brightness, or rather the wavelength of the light. The duration of light exposure was similar to, what human embryos suffer during IVF and ICSI.

2. Materials and methods

2.1. Embryo retrieval and culture

Eight to 12 weeks old CD1 female mice (Charles River, Germany) were injected with 5 IU of FSH (Merional, IBSA Pharma, Switzerland). Forty eight hours later the mice were treated with 5 IU LH (Chloragon, Ferring, Hungary), and directly placed to CD1 males. Two days later two cell stage embryos were flushed from the fallopian tubes, and cultured in groups in 50 µl droplets in KSOM medium (Millipore, England), supplemented with 0.4% of BSA, under mineral oil at 37 C°, 5% CO2 in air. The number of embryos in droplets varied between 10 and 14. Culture media were replaced every 24 h. During light treatment, the embryos were placed in M2 manipulation medium.

2.2. Light treatment of embryos

Light treatment was performed in a dark room. The culture dishes containing the embryos were placed under a polystyrene hood, which was either covered with a red foil, or nothing. Two compact bulbs were used as a light source. The intensity of the emitted light was measured with a digital Lux meter (Hold Peak- HP 881B, accuracy ≤10,000 lx is ± 4%) on the top of the dishes. The intensity of white light was 1130 lx and 450 lx of the red filtered light. In spectral analysis of the emitted light (with a STS-VIS Miniature Spectrometer, Ocean Optics, USA) two major peaks were seen; a lower peak at the 450 nm wavelength and the higher one at the 600–620 nm. Red filter covering of the culture dishes increased the wave length (peak at 620 nm), and diminished the intensity of the light to 450 lx (Supplementary Fig. 1).

The dishes containing the control embryos were handled under the usual conditions, and were incubated in the dark, at room temperature during light treatment of the test embryos.

Four cell stage (at the 2.5 post coital day) mouse embryos in M2 medium (Millipore England), were exposed to light for 25 min or 50 min at room temperature. The embryos were than cultured for 24 h, and the light treatment was repeated under the same conditions.

In order to determine, whether it is the wave length or the light intensity that matters, a group of embryos received 1130 lx of red filtered light.

2.3. Grouping

The following groups were formed;

1 Control embryos (N = 652).
   a 2 × 50 min incubation at room temperature (N = 456)
   b 2 × 25 min incubation at room temperature (N = 196)
2 Embryos subjected to white light (1130 lx) for 2 × 25 min (N = 81).
3 Embryos subjected to white light (1130 lx) for 2 × 50 min (N = 520).
4 Embryos subjected to red filtered light (450 lx) for 2 × 25 min (N = 81).
5 Embryos subjected to red filtered light (450 lx) for 2 × 50 min (No = 207)
6 Embryos subjected to red filtered light (1130 lx) for 2 × 50 min (N = 394).

Three hours after the 2nd light exposure, a part of viable embryos were transferred to the uteri of pseudo-pregnant females.

The rest of the embryos were examined under invert microscope, and cultured for 24 h, when division potential and the number of degenerated embryos were assessed under the microscope. Non-viable embryos were excluded from further examinations.

2.4. Microscopic evaluation of developmental potential of the embryos

After 24 h of culture, following the light stress, the embryos were scored for developmental stage. Embryos showing signs of degeneration and those which did not divide during the 24 h culture were considered non-viable. More developed (expanded and hatching) blastocysts and less developed blastocysts were considered as distinct groups.

The development and the viability of the embryos was checked a) before, b) 24 h after the 1st, and c) 24 h after the 2nd light exposure. The development of control embryos was evaluated at the same time points.

Four cell stage embryos were subjected to the first light treatment. Twenty four hours later the embryos reached the morula or the cavitating blastocyst stage, when the 2nd light treatment was performed.

Twenty four h after the 2nd light treatment viable embryos reached the blastocyst stage (non-expanded, expanded, or hatching blastocyst). In the statistical analysis, expanded and hatching blastocysts were considered in the same group.

2.5. Embryo transfer

Light stressed and control (day 3.5) embryos were transferred to 2.5 day pseudo pregnant B6CBA F1 (Charles-River Germany) females. Surgical, two sided embryo transfers were performed. Embryos exposed to red filtered light were transferred to the right horn, while those illuminated with white light to the left horn of the same female, in order to provide similar uterine environment for the different groups of embryos. Six to eight embryos were transferred to each side. The mice were sacrificed on day 8.5, and the implantation sites in the uteri were counted. Altogether 611 embryos were transferred to 44 females.

2.6. In situ detection of DNA fragmentation in control and light exposed embryos

The TUNEL assay (Thermo Fisher Scientific) was performed according to the instructions of the manufacturer. Briefly; the embryos were fixed in 4% paraformaldehyde for 25 min at room temperature, and stored in PBS at 4 °C until used. The cells were permeabilized with 0.5% Triton X-100 in PBS for 60 min at room temperature. After washing, the cells were incubated with the DNA labelling solution for 3 h at 37 °C. The embryos were then rinsed for 2 × 5 min, and reacted with Alexa488 labelled anti BrdU antibody for 30 min at room temperature in the dark. Nuclei were counterstained with RNAse containing propidium idodide for 30 min at room temperature in the dark. The cells were examined under an Olympus FV-1000 laser scanning confocal system (Olympus Europa, Hamburg, Germany). The number of double labelled blastomeres was expressed as the % of all blastomeres.

2.7. Apoptosis array

Lysates of mouse embryos were subjected to an apoptosis array (proteome profiler mouse apoptosis array kit, R&D Systems). All procedures were performed according to the suggestions of the manufacturer. The kit contains capture- and control antibodies, specific for 21 apoptosis-related molecules, spotted in duplicate on nitrocellulose membranes. The membranes were blocked for 1 h at room temperature, than the embryo lysates were incubated overnight with the membranes at 4 °C. The array was then washed to remove unbound proteins, and incubated with a cocktail of biotinylated detection antibodies for 60 min at room temperature. Streptavidin-HRP and chemiluminescent detection reagents were than applied for 30 min at room temperature, and chemiluminescent detection reagent was added. A signal produced at each capture spot corresponding to the amount of protein bound was detected.

The images of the membranes were analysed with Image Software.

2.8. Determining the number of nucleic acid containing extracellular vesicles (EVs) in culture media of light-exposed and control embryos

Propidium iodide (PI) was used for labelling of the nucleic acid content of embryo-derived EVs as described earlier (Pallinger et al., 2017). PI is a fluorescent intercalating agent which is commonly used as a DNA stain for flow cytometry. Twenty five μl of conditioned embryo culture media were incubated for 15 min at room temperature with 100 μl of 4% formaldehyde (from paraformaldehyde (PFA) solution. At the end of the incubation, 150 μl of filtered PBS, 1 μl of PI solution (50 μg/ml) and 50 μl of Count Check beads (Sysmex Partec GmbH) were added to the sample. FACS analysis was carried out within 30 min after PI staining. Unstained samples were used for the detection of auto fluorescence. A FACSCalibur flow cytometer (Beckton Dickinson) was used for measurments, and data were analysed by CellQuestPro software. Gating strategy and controls are shown in Supplementary Figs. 2 and 3.

Microbeads-based absolute counting was used for calculating the absolute counts of EVs in IVF medium. Count Check Microbeads (SysmexPartec GmbH) was used as an internal standard. The number of PI + EVS was calculated according to the formula: number of PI + vesicles in sample = (detected PI + events in the EV gate minus PI + events in staining control/ number of events in bead gate) x absolute count of Count Check beads in the tube.

2.9. Isolation of CD4 and CD8 positive splenocytes

Mononuclear cells were separated from the spleens of female CD1 mice by density gradient centrifugation (Hystopaque 1083; Sigma, St Louis USA). Then CD4+ or CD8+ T cells were isolated from this single-cell suspension using the CD4+ T Cell Isolation Kit or the CD8+ T Cell isolation Kit of Miltenyi Biotec (Bergisch Gladbach, Germany) and LS Columns by negative separation. The purity and also the viability of isolated T cell subsets were characterized by flow cytometry.

Table 1A
Development of embryos after the 1st (*) and 2nd (**) 25 min light exposure (brightness of the red and white light are 450 lx and 1130 lx respectively).

Stage of embryo development	White light No of embryos (%)	Red light No of embryos (%)	Control No of embryos (%)
4 cell*	81 (100)	81 (100)	196 (100)
Compacting morula	62 (77)	66 (81.5)	161 (82.1)
Early blastocyst	11 (13.6)	12 (14.8)	24 (12.2)
Non-viable	8 (9.8)	3 (3.7)	11 (5.6)
Compacting morula + Early blastocyst**	48 (100)	48 (100)	124(100)
blastocyst	7 (14.6)	7 (14.6)	5 (4)
Expanded blastocyst + hatching blastocyst	30 (62.5)	34 (70.8)	102 (82.3)
Non-viable	11 (22.9)	7 (14.6)	17(13.7)

Table 1B
Development of embryos after the 1st (*) and 2nd (**) 50 min light exposure (brightness of the red and white light are 450 lx and 1130 lx respectively).

Stage of embryo development	White light No of embryos (%)	Red light No of embryos (%)	Control No of embryos (%)
4 cell*	520 (100)	207 (100)	456 (100)
Compacting morula	425 (81.7)	177 (85.5)	382 (83.8)
Early blastocyst	68 (13.1)	23 (11.1)	55 (12)
Non-viable	27 (5.2)	7 (3.4)	19 (4.2)
Compacting morula + Early blastocyst**	293 (100)	112 (100)	360 (100)
blastocyst	39 (13.3)	26 (23.2)	16 (4.4)
Expanded blastocyst + hatching blastocyst	201 (68.6)	72 (64.3)	304 (84.5)
Non-viable	53 (18.1)	14 (12.5)	40 (11.1)

Table 1C
Development of embryos after the 1st (*) and 2nd (**) 50 min light exposure (brightness of the red and white light are both 1130 lx).

Stage of embryo development	White light No of embryos (%)	Red light No of embryos (%)	Control No of embryos (%)
4 cell*	520 (100)	207 (100)	456 (100)
Compacting morula	425 (81.7)	355(90.1)	382 (83.8)
Early blastocyst	68 (13.1)	17(4.3))	55 (12)
Non-viable	27 (5.2)	22 (5.)	19 (4.2)
Compacting morula + Early blastocyst**	293 (100)	203 (100)	360 (100)
blastocyst	39 (13.3)	47 (23.2)	16 (4.4)
Expanded blastocyst + hatching blastocyst	201 (68.6)	118 (58.1)	304 (84.5)
Non-viable	53 (18.1)	38 (18.7)	40 (11.1)

2.10. Intracellular IL-10 staining of splenocytes

One hundred thousand CD4+ or CD8+ T cells were stimulated with embryo-derived EVs for 4 h in the presence of the protein transport inhibitor brefeldin A (Sigma, St Louis, USA). Pooled embryo culture media were used for EV separation (5 culture media/treatment). Stimulated cells were fixed (4% paraformaldehyde solution for 10 min, at room temperature), permeabilized with 0.1% saponin and incubated in 50 μl of staining buffer (PBS containing 1% BSA) with a pre-titrated optimal concentration of phycoerythrin-conjugated anti-mouse IL-10 monoclonal antibody (PE Rat Anti-Mouse IL-10 Clone JES5-16E3 (RUO) 561060; BD BioSciences, USA) for 15–30 min, at room temperature. The cells were washed in 2 ml of 0.1% saponin solution and centrifuged

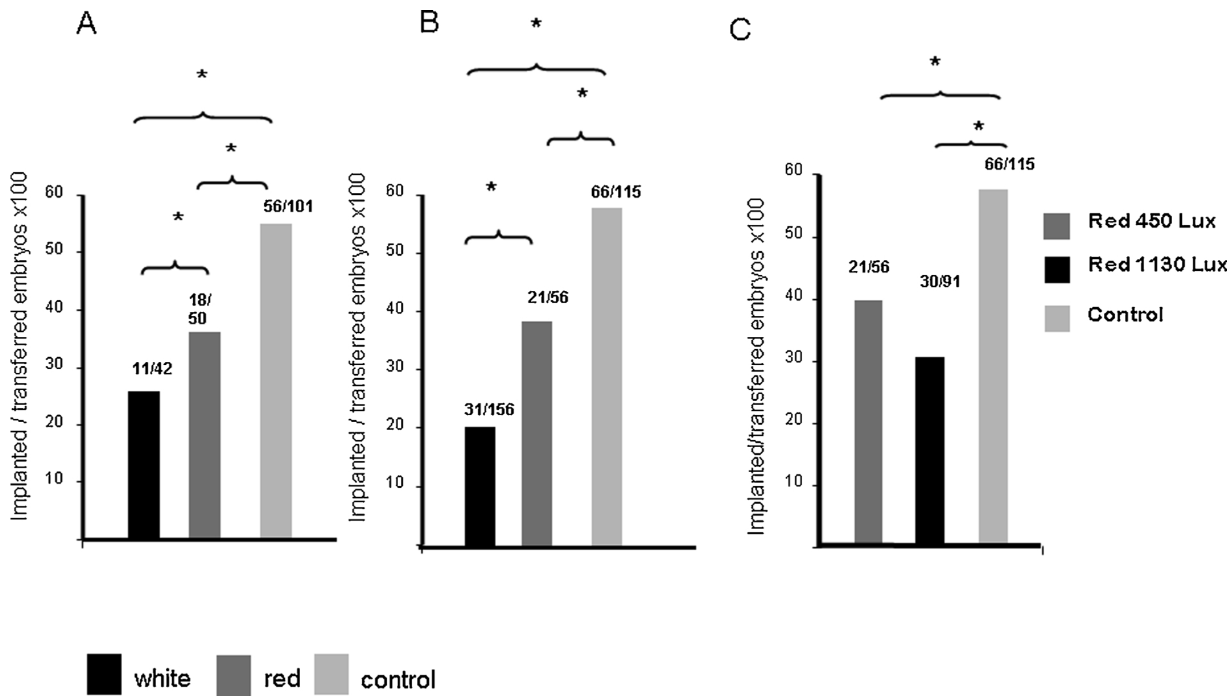

Fig. 1. Implantation rates of light exposed and control embryos.
A. The embryos were subjected to white light (1130 lx) or red filtered light (450 lx) for 2 × 25 min.
B. The embryos were subjected to white light (1130 lx) or red filtered light (450 lx) for 2 × 50 min.
C. The embryos were subjected to red filtered light (450 lx) or (1130 lx) for 2 × 50 min.
The numbers of implanted/transferred embryos are shown on the top of the bars.
*$p < 0.001$.

for 5 min at 300 g, and fixed with 300 µl of 2% paraformaldehyde (Sigma, St Louis, USA). Stained cells were stored at 4 °C in dark before analysis. Tests were carried out by measuring, 5×10^4 cells/tube, on the day of the staining. All the FACS data were analyzed with CellQuestPro software (Becton Dickinson, CA, USA).

2.11. Statistical analysis

To analyse the effect of light exposure on the development and implantation capacity of the embryos as well as DNA fragmentation we used the Chi-square-Test. To avoid the decision error we adjusted the p-values with Bonferroni correction, p-value under 0.05 was considered as significant. The two tailed *t*-test was used to compare PI + EV counts in embryo culture media. For all calculations we used the IBM-SPSS Version 22 software package.

2.12. Ethical approval

All methods were carried out in accordance with relevant guidelines and regulations.

All experimental protocols were approved by the Animal Health Committee of Baranya County.

3. Results

3.1. Development and viability of light stressed embryos

The frequency of the following developmental forms were compared;

- morula stage embryos, cavitating blastocysts and non-viable embryos, 24 h after the first light treatment
- blastocysts, expanded + hatching blastocyst and non-viable embryos, 24 h after the 2nd light treatment.

Seventy seven per cent of embryos exposed to white light, and 81% of those exposed to red light for 2 × 25 min reached the blastocyst stage. Eighty one per cent of embryos exposed to white light and 88% of those exposed to red light for 2 × 50 min developed to blastocysts. Similarly to the white light treated embryos, 81% of embryos subjected to red light of 1130 lx reached the blastocyst stage, while 88% of control embryos developed to blastocysts.

These differences between the groups in either development or the number of non-viable embryos were not statistically significant (Tables 1A–1C).

3.2. Implantation capacity of light stressed embryos

Implantation capacity of embryos subjected to either red or white light for 2 × 25 min was significantly ($p < 0.001$) reduced, compared to the controls. The implantation rate of the embryos treated with the red light was significantly ($p < 0.01$) higher that of those exposed to white light (Fig. 1a).

The implantation rates of embryos subjected to white light for 2 × 50 min, were significantly ($p < 0.01$) lower than of those, treated with the red light, and both groups implanted at a significantly lower ($p < 0.001$) rate than the controls (Fig. 1b).

In order to investigate, whether the wavelength, or rather the intensity of the light was responsible, for the harmful effect, we illuminated embryos with 1130 lx of red light.

There was no significant difference ($p = 0.058$) between the implantation rates of embryos subjected to 450 lx or 1130 lx red light (Fig. 1c), suggesting, that the wavelength of the light is responsible for the impaired implantation capacity of the embryos.

3.3. Nucleic acid containing extracellular vesicles (EVs) in culture media of light stressed and control embryos

In an earlier study we showed that competent human embryos

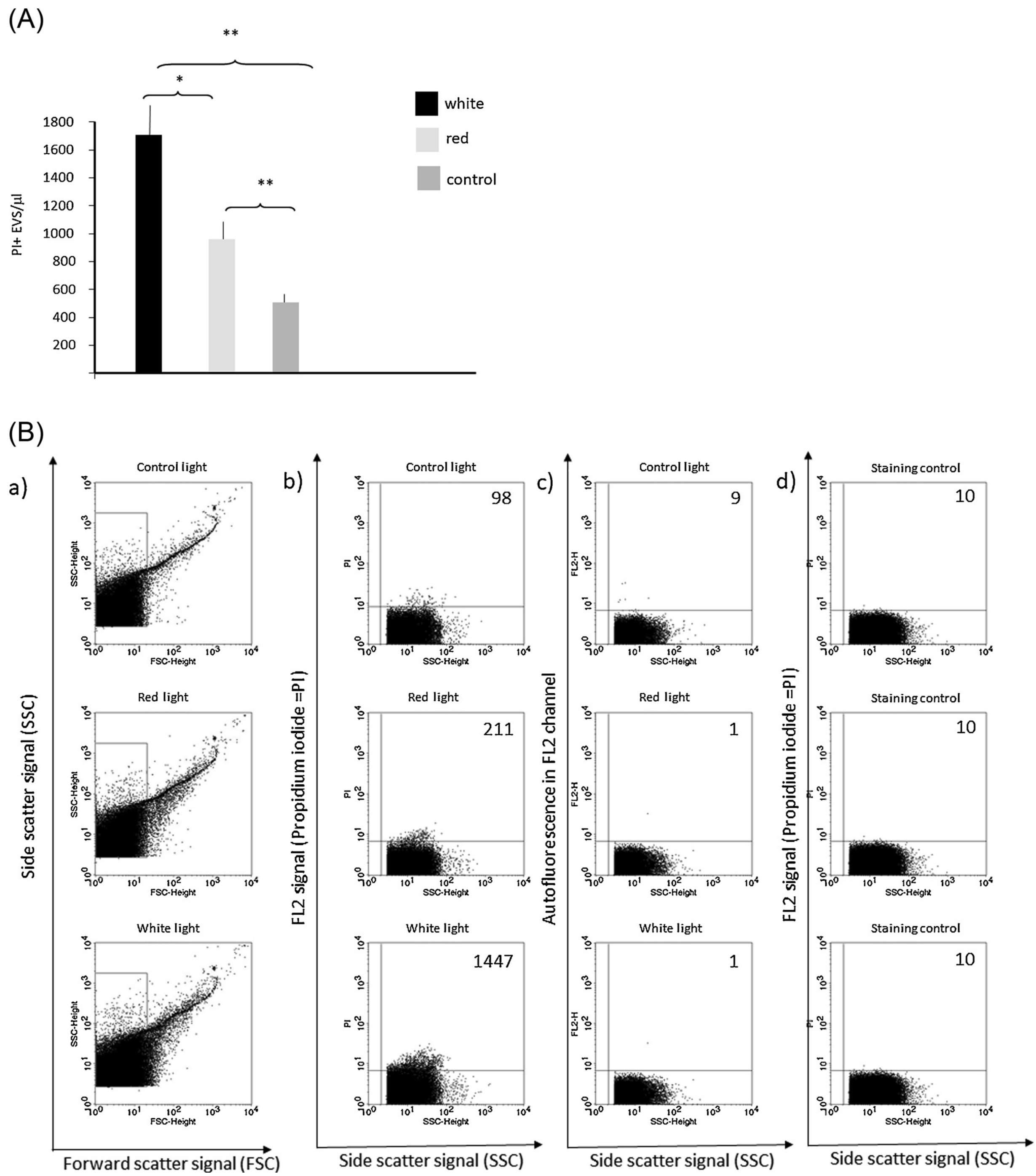

Fig. 2. PI + EVs in culture media of light exposed and control embryos.
Four cell stage embryos were illuminated with white (1130 lx) or red filtered (450 lx) light for 50 min.
A) The bars represent the mean +/− SEM of 31 (white), 12 (red filtered) and 38 (control) measurements.
*$p < 0.01$.
**$p < 0.001$.
B) A representative dot plot showing the numbers of PI + EVs in the culture media of embryos exposed to white or red filtered light, as well as of controls.

released less nucleic acid containing EVs in the culture medium than those that failed to implant (Pallinger et al., 2017). This prompted us to investigate the nucleic acid containing EVs in culture media of light exposed and control embryos.

In 31 culture media of embryos treated with white light for 50 min, the number of nucleic acid containing EVs was 1684 +/−260/µl, significantly higher ($p < 0.01$), than the 691 +/− 113/µl measured in 12 culture media from embryos treated with 450 lx of red light.

The culture media of both light exposed groups contained significantly higher ($p < 0.001$) number of PI + EVs than that measured in 38 culture media from control embryos (438+/−60/µl) (Fig. 2A and B). There was no difference in either the number of AnnexinV + Evs, or the size of the EVs between the groups.

3.4. Embryo-derived EVs induce IL-10 production in CD8+ spleen cells

CD8+ cells were isolated from mouse spleen and incubated with EVs from untreated, or white light exposed embryos for 30 min. at

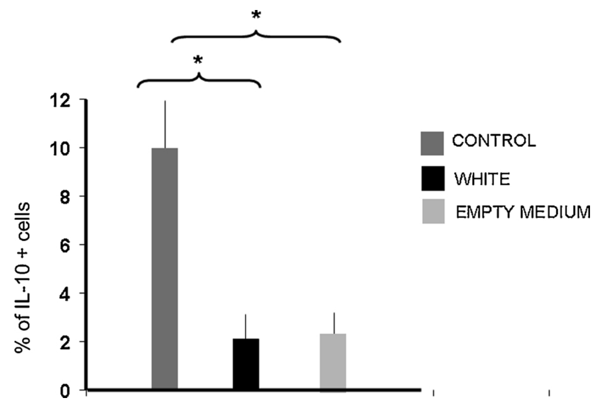

Fig. 3. IL-10 positive cells among CD8+ murine spleen cells incubated with EVs from control and white light exposed embryos.
The bars represent the mean+/− SEM of 7 experiments.
*p = 0.02.

37 °C. Than the cells were labelled with anti- IL-10 antibody, and the number of IL-10 positive cells among the subpopulations was determined by flow cytometry.

Compared to the controls (CD8+ spleen cells incubated with empty medium), the percentage of IL-10+ CD8+ cells significantly increased ($p < 0.02$) in the presence of EVS from untreated embryos, but not in those from white light exposed ones (Fig. 3). The presence of EVs did not alter IL-10 production in CD4+ cells (data not shown).

3.5. DNA fragmentation and apoptotic markers in control and light exposed embryos

DNA fragmentation in the nuclei of light exposed (for 50 min) and control embryos was tested in a TUNEL assay. The results were evaluated under a confocal microscope, by comparing the number of TUNEL positive nuclei to all nuclei.

Six hundred and forty one nuclei from 32 embryos were counted. Sixty two out of 202 (31.3%) nuclei from embryos exposed to white light, 23 of 213 (10.8%) nuclei from embryos exposed to red light and 23 of 226 (10.2%)nuclei from control embryos were TUNEL positive (Fig. 4).

Table 2
Altered expression of apoptosis-related molecules in the lysates of embryos subjected to white light for 2 × 50 min.

Apoptosis regulator	Increase compared to control
Bad {BBC6,BCL2L8}	23%
Bcl-2	47%
BcL-X {BCL2L1}	33%

The number of nuclei with fragmented DNA, was significantly ($p < 0.0001$) higher in embryos treated with white light, than in the other two groups. There was no significant difference in this respect between the red and the control group.

An apoptosis array was performed with lysates of untreated and red light exposed embryos. Compared to the controls, the expression of two anti-apoptotic molecules Bcl-2 and Bcl-x increased by 47% and 33% respectively, in white light exposed embryos (Table 2).

4. Discussion

During IVF laboratory procedures, the gametes and embryos are exposed to potential stressors. These include a lower CO2 atmosphere, and light exposure during manipulation and microscopic evaluation of the embryos.

The aim of this study was to investigate the effect of light exposure on the developmental and implantation potential of in vitro cultured murine embryos.

In attempt to simulate normal laboratory conditions during IVF, the length of light treatments corresponded to the cumulated light exposure during conventional (25 min) IVF and ICSI (50 min).

The embryos were in the morula - blastocyst stage, when transferred after the 2nd light treatment. Though embryos illuminated for 50 min had received the intended light load during the 1st treatment, another 50 min light treatment was performed before transfer. This allowed us to check the development of the embryos during a further 24 h of culture (corresponding to the in vivo development of transferred embryos).

Exposure to 25, or 50 min of 1130 lx warm light did not significantly affect embryo development or the number of non-viable cells,

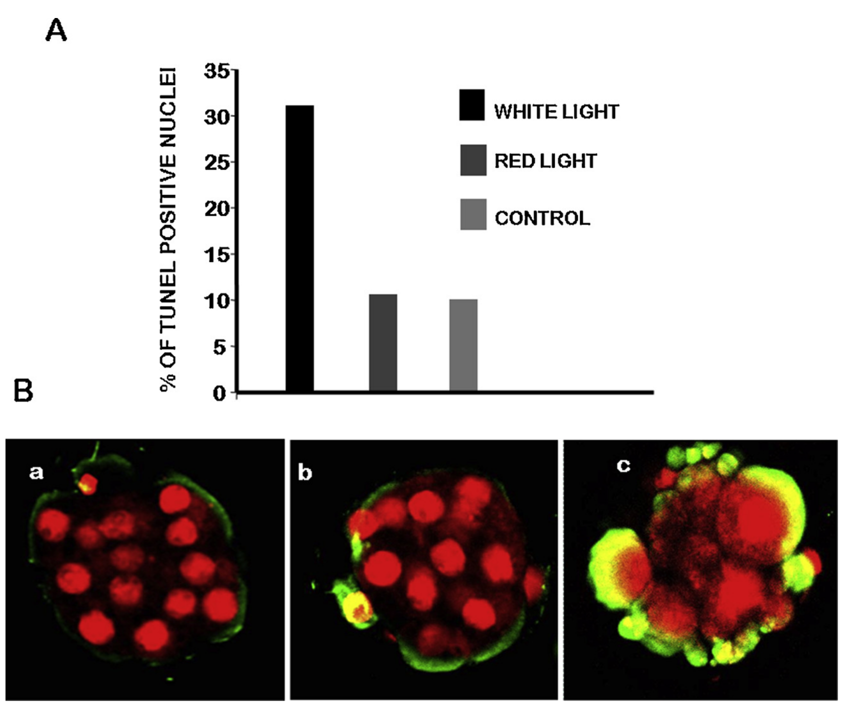

Fig. 4. DNA fragmentation in nuclei of light stressed embryos.
A. The percentage of TUNEL positive nuclei in embryos subjected to white light, red filtered light and in untreated controls.
*$p < 0.001$.
B. Representative images of light exposed and control embryos.
a. Representative image of a morula stage control embryo. One of the 30 counted nuclei were TUNEL positive.
b. Representative image of a morula stage embryo, subjected to red filtered light (450 lx, for 50 min, 24 h before fixation). Two out of 16 nuclei were TUNEL positive.
c. Representative image of a morula stage embryo, subjected to white light (1130 lx, 50 min). Fourteen out of the 20 nuclei were TUNEL positive.

compared to controls, or to red light (450 lx) exposed embryos.

There is a considerable difference in the light sensitivity of the zygotes from different species. Even a fraction of the above light load on the zygotes from hamsters, results in 2 cell block, while light treatment of hamster embryos inhibits cell division, and significantly reduces the number of embryos that reach the blastocyst stage (Oh et al., 2007; Takenaka et al., 2007).

In our hands, statistical analysis of altogether 970 light treated embryos revealed no significant alterations in developmental potential of the treated groups, suggesting that morphological evaluation of the embryos does not reveal the potential damage caused by light stress.

Our negative results can partly be explained by the lower light sensitivity of mouse embryos, and by the fact that the embryos in our setup were at a higher stage of development (four cell, or morula stage), when illuminated.

Nevertheless light stress must have had a deleterious effect on the embryos, since light exposure – even if for 2 × 25 min only – significantly impaired the implantation capacity of the embryos. The use of the red filter moderated this effect.

Red filtered light of 1130 lx was used to test, whether the protective effect was due to reduced brightness or to the different wavelength. The implantation rate of embryos treated with 1130 lx red filtered light for 2 × 50 min was still significantly higher, than of that of the embryos subjected to the white light under the same conditions, suggesting that the beneficial effect of red filtered light is due to the different wavelength, rather than to the reduced brightness.

Though embryo culture media are designed to create conditions as close to the natural environment as possible, still, removing the embryo from its natural conditions is stressful and affects the developmental potential of the embryo (Takahashi et al., 1999; Doherty et al., 2000; Takahashi et al., 2000). Furthermore, in vitro culturing might cause epigenetic modifications. Some of these are manifested in the phenotype, and, to what extent these induce genomic changes -is not known (Gardner, 1998; Cox et al., 2002; DeBaun et al., 2003; Maher et al., 2003).

Light stress causes in DNA damage in most mammalian embryos (Kruger and Stander, 1985; Takenaka et al., 2007; Nakahara et al., 2010).

Earlier we showed that the number of nucleic acid containing extracellular vesicles is significantly higher in culture media of embryos that fail to implant, than in those of competent embryos (Pallinger et al., 2017). The present study shows that in line with our previous findings, the number of PI + EVs in the culture medium was related to the well-being of the embryo. Culture media of white light exposed embryos that showed an impaired implantation capacity, contained significantly higher numbers of PI + EVs, than those of embryos exposed to red filtered light or of controls.

In line with this, the number of nuclei showing DNA fragmentation was higher in white light treated embryos, than in the other groups. On the other hand, in a apoptosis array we observed the increase of anti-rather than pro-apoptotic molecules, suggesting that the DNA fragmentation in this case is not connected with apoptotic damage.

A further indication of altered function in light exposed embryos is their impaired capacity to act on the immune system. Earlier we reported that mouse embryo-derived EVs induce IL-10 production in murine CD8+ spleen cells (Pallinger et al., 2018). Here, the EVs from white light exposed embryos failed to induce IL-10 in CD8+ murine spleen cells.

Both PI + EV counts and DNA fragmentation were investigated in embryos subjected to the light stress at four cell stage. They were further cultured for 24 h, before testing either the media for PI + EV counts, or the embryos for DNA fragmentation. This is the time point, when other embryos were transferred to the uteri of pseudo pregnant females. Therefore, this arrangement was likely to provide information about the changes occurring in the embryos prior to implantation.

Taken together; our results show that while light exposure did not affect the development of in vitro cultured mouse embryos, it clearly had a negative influence on their function. The implantation potential of light stresses embryos is significantly reduced, which might in part be due to DNA damage, and in the failure of the EVs from light-treated embryos to act on the immune system. Using a red optical filter significantly reduces the harmful effects of white light, both on DNA fragmentation and implantation capacity.

Data obtained on mouse embryos can of course, not be extrapolated directly to human. However, various aspects of preimplantation development are similar in mouse and human embryos. Not only the timing of development and the size of the embryos, but also, the metabolism, and therefore culture requirements of mouse and human embryos are comparable (Quinn and Horstman, 1998). Mouse embryos have for a long time been considered as a model for human IVF, because of the many similarities between the embryos from the two species, moreover several methodological considerations of human in vitro fertilization stem from earlier mouse studies.

Because there is no available information on the light sensitivity of human embryos, it is advisable to be cautious during embryo manipulation in IVF.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by GINOP-2.3.2-15-201600021, PTE ÁOK-KA 2017–22, EFOP-3.6.1.-16-2016-00004 and EFOP-3.6.3-VEKOP-16-2017-00009 to J. SZ-B.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2019.02.003.

References

Cox, G.F., Burger, L., Lip, V., Mau, U., Sperling, K., Wu, B., 2002. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am. J. Hum. Genet. 71, 162–164.

DeBaun, M.R., Niemitz, E.L., Feinberg, A.P., 2003. Association of in vitro fertilization with Beckwith-Wiedemann Syndrome and epigenetic alterations of LIT1 and H19. Am. J. Hum. Genet. 72, 156–160.

Doherty, A.S., Mann, M.R.W., Tremblay, K.D., Bartolomei, M.S., Schultz, R.M., 2000. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biol. Reprod. 62, 1526–1535.

ESHRE Guideline Group on good practice in IVF labs, 2015. Revised guidelines for good practice in IVF laboratories. Guideline of the European Society of Human Reproduction and Endocrinology.

Gardner, D.K., 1998. Changes in requirements and utilization of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. Theriogenology 49, 83–102.

Hamdoun, A., Epel, D., 2007. Embryo stability and vulnerability in an always changing world. PNAS 104 (6), 1745–1750.

Khosla, S., Dean, W., Brown, D., Reik, W., Feil, R., 2001. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol. Reprod. 64, 918–926.

Kruger, T., Stander, F.S.H., 1985. The effect of fluorescent light on the cleavage of two-cell mouse embryos. South Afr. Med. J. 68, 744–745.

Li, R., Pedersen, K.S., Liu, Y., Pedersen, H.S., Lægdsmand, M., Rickelt, L.F., Kühl, M., Callesen, H., 2014. Effect of red light on the development and quality of mammalian embryos. Assist. Reprod. Genet. 31, 795–801.

Maher, E.R., Brueton, L.A., Bowdin, S.C., Luharia, A., Cooper, W., Cole, T.R., Macdonald, F., Sampson, J.R., Barratt, C.L., Reik, W., Hawkins, M.M., 2003. Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). J. Med. Genet. 40, 62–64.

Mann, M.R.W., Lee, S.S., Doherty, A.S., Verona, R.I., Nolen, L.D., Schultz, R.M., Bartolomei, M.S., 2004. Selective loss of imprinting in the placenta following preimplantation development in culture. Development 131, 3727–3735.

Nakahara, T., Iwase, A., Goto, M., Harata, T., Suzuki, M., Ienaga, M., Kobayashi, H., Takikawa, S., Manabe, S., Kikkawa, F., Ando, H., 2010. Evaluation of the safety of time-lapse observations for human embryos. J. Assist. Reprod. Genet. 27 (2–3), 93–96.

Oh, S.J., Gong, S.P., Lee, S.T., Lee, E.J., Lim, J.M., 2007. Light intensity and wavelength during embryo manipulation are important factors for maintaining viability of preimplantation embryos in vitro. Fertil. Steril. 88, 1150–1157.
Ottosen, L.D.M., Hindkjær, J., Ingerslev, J., 2007. Light exposure of the ovum and preimplantation embryo during ART procedures. J. Assist. Reprod. Genet. 24, 99–103.
Pallinger, E., Bognar, Z., Bodis, J., Csabai, T., Farkas, N., Godony, K., Varnagy, A., Buzas, E., Szekeres-Bartho, J., 2017. A simple and rapid flow cytometry-based assay to identify a competent embryo prior to embryo transfer. Sci. Rep. 6, 39927.
Pallinger, E., Bognar, Z., Bogdan, A., Csabai, T., Abraham, H., Szekeres-Bartho, J., 2018. PIBF+ extracellular vesicles from mouse embryos affect IL-10 production by CD8+ cells. Sci. Rep. 8, 4662.
Pomeroy, K.O., Reed, M.L., 2013. The effect of light on embryos and embryo culture. J. Reprod. Stem Cell Biotechnol. 3 (2), 46–54.
Quinn, P., Horstman, F.C., 1998. Is the mouse a good model for the human with respect to the development of the preimplantation embryo in vitro? Hum. Reprod. 13 (Suppl. 4).
Rinaudo, P.F., Giritharan, G., Said Talbi, S., Dobson, A.T., Schultz, R.M., 2006. Effects of oxygen tension on gene expression in preimplantation mouse embryos. Fertil. Steril. 86 (Suppl. 3), 1252–1265.
Schultz, R.M., 2007. Of light and mouse embryos: less is more. Proc. Natl. Acad. Sci. U. S. A. 104 (37), 14547–14548.
Takahashi, M., Saka, N., Takahashi, H., Kanai, Y., Schultz, R.M., Okano, A., 1999. Assessment of DNA damage in individual hamster embryos by comet assay. Mol. Reprod. Dev. 54 (1), 1–7.
Takahashi, M., Keicho, K., Takahashi, H., Ogawa, H., Schultz, R.M., Okano, A., 2000. Effect of oxidative stress on development and DNA damage in in-vitro cultured bovine embryos by Comet assay. Theriogenology 54, 137–145.
Takenaka, M., Horiuchi, T., Yanagimachi, R., 2007. Effects of light on development of mammalian zygotes. Proc. Natl. Acad. Sci. U. S. A. 104 (36), 14289–14293.