Research in Molecular Medicine (RMM)
Volume 6, Issue no. 3, DOI 10.18502/rmm.v6i3.4607
Production and Hosting by Knowledge E

Research Article

# The Influence of Light on Apoptosis in Preimplantation Mouse Embryos *In Vitro*

**Sepideh Khalili-Savadkouhi[1], Abbasali Karimpour Malekshah[2,3], Mehri Mirhoseini[2,4], Mahmood Moosazadeh[5], and Maryam shahidi[6]**

[1]Molecular and Cell Biology Research Center, Hemoglobinopathy Institue, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[2]Molecular and Cell Biology Research Center, Hemoglobinopathy Institue, Mazandaran University of Medical Sciences, Sari, Iran
[3]Department of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[4]Amol Faculty of Nursing and Midwifery, Mazandaran University of Medical Sciences, Sari, Iran
[5]Health Sciences Research Center, Addiction Institute, Mazandaran University of Medical Sciences, Sari, Iran
[6]Department of Biophysics and Biochemistry, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

## Abstract

**Background:** *In vitro* culture of mammalian embryos can slow or stop growth completely. This may be due to the medium used, pH, temperature, or light. There is considerable concern about the harmful effect of light in the laboratory environment. Cell number and apoptosis are useful parameters that indicate embryonic development and health. In this study, we assessed these two factors in the blastocyst. **Materials and methods:** A total of 128 embryos were extracted from NMRI mice at the 2-cell stage and were divided into 4 groups. The embryos were exposed to light for 0, 5, 15, and 30 min, and then cultured for 96 h. The degree of embryonic development were recorded every 24 h. Furthermore, several morphologically normal blastocysts were evaluated using the TUNEL assay. **Results:** There was no significant difference in developmental stages between the experimental and control groups. An evaluation of the percentage of blastomeres and apoptotic cells revealed significant differences among the four groups. The maximum number of apoptotic blastomeres was observed in the group exposed to light for 30 minutes. **Conclusion:** Up to thirty minutes of white fluorescent light can induce apoptosis in blastomeres, but it does not prevent embryo development.

**Keywords:** apoptosis, blastocyst, mice, light, embryonic structures

Corresponding Author:
Mehri Mirhoseini;
email:
m.mirhoseini@mazums.ac.ir

Production and Hosting by Knowledge E



Editor-in-Chief:
Dr. Alireza Rafiei

## 1. Introduction

Many *in vitro* maturation culture media for preimplantation embryos have been developed in recent years. However, there is still room to develop more optimal conditions (1).

Preimplantation mammalian embryos have high plasticity. Nevertheless, suboptimal conditions may affect the gene expression and even the development of embryos (2-4). Mammalian embryos usually experience slow growth or even stop growing *in vitro*, which does not happen in the oviduct. In addition to the contents of the culture medium, other factors such as pH fluctuations, high or low laboratory temperatures, changes in $CO_2$ and $O_2$ levels, and light may affect growth *in vitro*. Amongst all these environmental factors, the harmful effects of light from microscopes and the laboratory environment have been previously studied (1, 5).

In lower organisms that live at the surface of water, there is a protective mechanism against light, especially UV radiation (6). In contrast, mammalian embryos do not have these defense mechanisms because they develop in the lightless reproductive tract of the mother during the preimplantation phase. Because the cell number in embryos is low, they are expected to be sensitive to light-induced damage (6), and light during *in vitro* manipulation may damage the embryo (7).

Light exposure leads to subnormal stress in the embryo; therefore, it might modify embryonic development. Light is the reason of delay in the cleavage of mouse and rabbit embryos. It is also one of the main physical factors in the embryonic environment, and its effect on embryos should not be ignored (8).

Studies show that hamster embryos are quite sensitive to light, and that at least 5 minutes of light exposure inhibits 2 and 8-cell embryo development. Visible light causes dullness in the cleavage of rabbit ova. (5). Cell death in embryos that were developed *in vitro* is greater than in embryos that were developed *in vivo* (9). Light may cause DNA damage, such as breakage, not only through direct effects, but also indirectly by producing reactive oxygen species (5, 10, 11). In contrast, another report showed that exposure to light with an intensity of 1200 lux for 15 minutes did not have any effect on embryonic development (1, 7).

In mammalian development, there is a quality control mechanism to remove abnormal and nonfunctional cells (12). This mechanism, known as apoptosis, is observed in many cell types, such as cumulus cells, bovine oocytes, and preimplantation embryos (13).

There is a paucity of information on the effects of light on *in vitro* embryos, and the results are contradictory. Therefore, the present study was designed to investigate the effect of light, based on the intensity used in laboratories (800 lux for 30 min), on the *in vitro* maturation of embryos. Cell number and apoptosis are two important factors that show embryonic development and health (14); therefore, this study used these two factors for embryo assessment.

## 2. Materials and Methods

### 2.1. Animals

An experimental study was carried out in which 12 NMRI female mice (6–8-week-old) were obtained from the Experimental Animal Research Center affiliated with the Mazandaran University of Medical Sciences. This study was performed following the guidelines of the ethics committee of the Mazandaran University of Medical Sciences (code: 88-127).

The mice were given ad libitum access to food and water under standard conditions (12 h light/12 h dark cycle and 21 ± 2°C). To induce superovulation, animals were injected with 7 IU PMSG, and then with 7 IU HCG after 48 h, intraperitoneally, and then mated with males from the same strain with proven fertility overnight. After vaginal plug confirmation the next morning, pregnant mice were sacrificed 44–46 h after HCG-injection.

## 2.2. Embryo culture and assessment of preimplantation development

Embryos were extracted at the 2-cell stage by flushing the oviduct with HTF medium containing HEPES under a light microscope. Embryos were washed with HTF containing HEPES droplets (20 µl), overlaid with washing mineral oil 3 times, and then incubated in HTF medium containing 10% human serum albumin (HSA) and incubated under humidified air with 5% $CO_2$ at 37°C (MCO- 18AIC; Sanyo, Sakata, Japan) for 96 h. Levels of embryonic development were assessed and recorded every 24 h using a light microscope. Moreover, embryonic development was photographed at different stages (Figure 1). Oil washing and preparation of microdrops was performed using standard protocols (7).

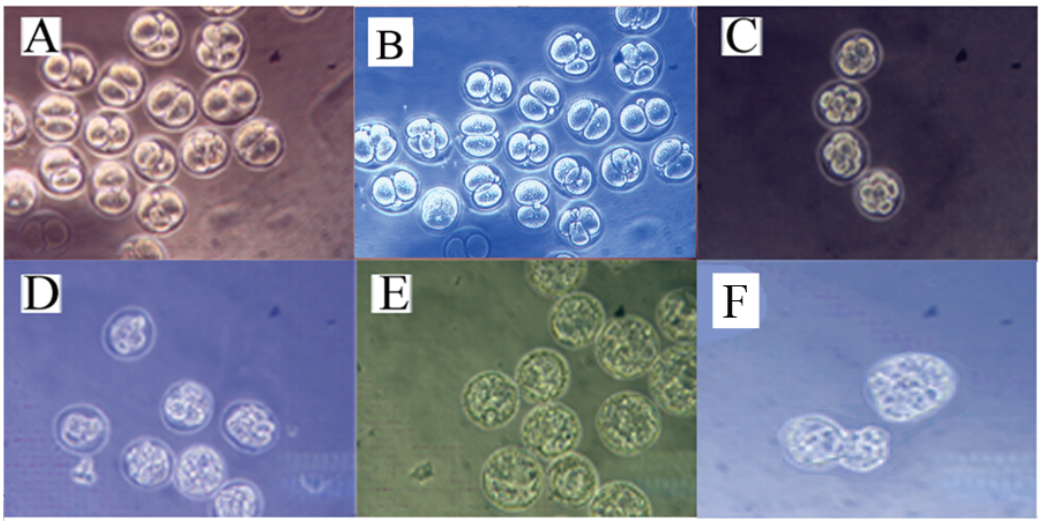


**Figure** 1: Levels of embryonic development up until the blastocyst stage. A; 2-cell embryos, B; 4-cell embryos, C; 8-cell embryos, D; morula stage embryos, E; early and expanded blastocysts, F; hatched blastocysts.

## 2.3. Groups

In total, 128 embryos were randomly divided into four groups as follows:

Group 1 (control group): Embryos were in the same condition as other embryos except they were not exposed to light. n= 32

Group 2: Embryos were exposed to light for 5 min. n = 32

Group 3: Embryos were exposed to light for 15 min. n = 32

Group 4: Embryos were exposed to light for 30 min. n = 32

## 2.4. Conditions for lighting

Two fluorescent lamps (23 W, 230 V, 50 HZ, 6400 K, 190 MA) were placed in the vicinity of plates so that 800 lux was shined onto the plates. The quality of light was measured using a lux meter. Plates were covered with foil during times when they were not to receive light, and were preserved in the same position. After light exposure, the embryos were transferred to T6 culture medium and incubated in a $CO_2$ incubator.

Embryos were cultured for 96 h and their development was observed every 24 h. Developmental stages including the 4 and 8 cell stages (24 h), the morula stage (48 h), the blastocyst stage (72 h), and the hatching stage (96 h) were assessed. Degeneration cases were also recorded. Development of embryos was seen by a stereo microscope (SMZ-3 Japan). Among the embryos that reached the blastocyst stage (72 h), 28 morphologically normal blastocysts were selected for assessment by TUNEL assay.

## 2.5. TUNEL assay (Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling)

The TUNEL assay (Roche Diagnostics, East Sussex, U.K.) was performed according to the manufacturer's instructions. After fixation in paraformaldehyde (4% in PBS, pH 7.4, freshly prepared), the embryos were washed in washing buffer (PBS/PVP solution: Phosphate buffered saline with 0.3% PVP) 3–4 times for 30 minutes. Then the embryos were transferred to permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate, freshly prepared) for 50 minutes at 4°C. After washing with buffer solution, the embryos were transferred to the TUNEL reaction mixture for 1 h at 37°C in an incubator. After washing with buffer solution, all blastocyst nuclei were stained with RNase A and propidium iodide (PI) solution. The blastocysts were incubated in RNase A for 60 minutes, and then transferred into PI solution for 20 minutes at room temperature. After washing, the embryos were transferred to slides and were observed with a fluorescent microscope (Nikon eclipse 50i/Ds-$Fi_1$) by an excitation wavelength ranging from 450 to 500 nm and a detection wavelength ranging from 515 to 565 nm. Total nuclei and TUNEL-positive nuclei were counted in each embryo. Each TUNEL-labeled nucleus was designated as an apoptotic cell. Apoptotic cell nuclei shown by the TUNEL assay are yellow (Figure 2). The rate of apoptosis was calculated as the percentage of apoptotic blastomeres compared to total blastomeres (7).

## 2.6. Stastistics

Statistical analysis was performed by applying the chi-square test in SPSS version 16. All P-values less than 0.05 were regarded as statistically significant.

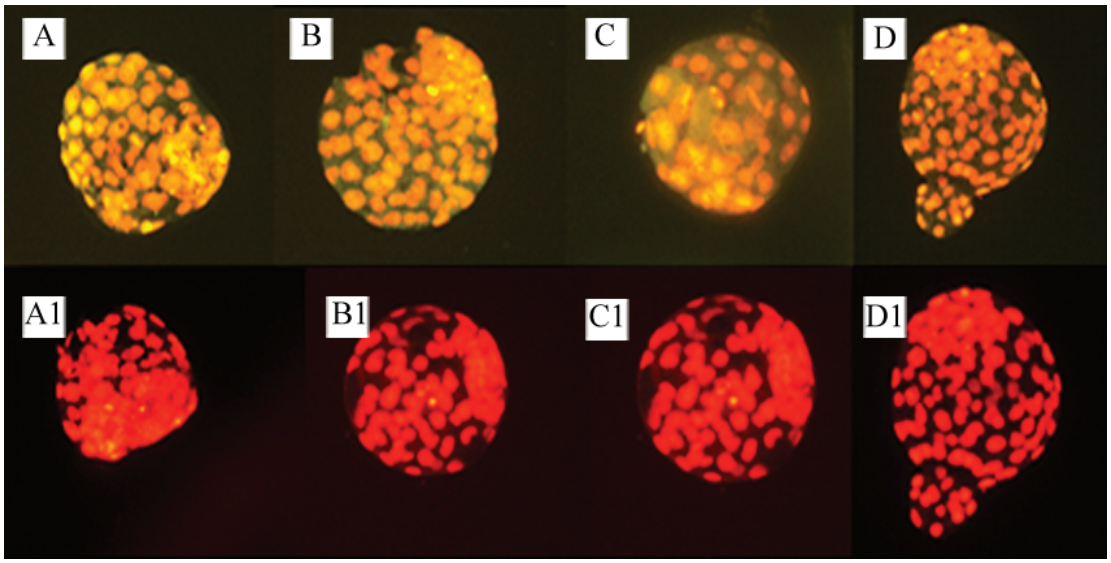


**Figure** 2: Detection of apoptotic cells in mouse blastocysts using TUNEL (fluoroscein isothiocyanate-conjugated dUTP; green channel) (A-D) and propidium iodide (red channel) (A1-D1) assays. Blastocysts, which were developed from the 2-cell stage, were exposed to fluorescent light (800 lx) for 0 (A, A1), 5(B, B1), 15 (C, C1), and 30 (D, D1) minutes. Apoptotic cell nuclei are shown as yellow points.

## 3. Results

The percentage of embryos that developed up until the blastocyst stage was calculated in each group and compared between groups (Figure 1). The results showed no statistically significant difference in the degeneration ratio between the experimental groups and the control group (1, 2: P=0.802; 1, 3: P=1.000; 1,4: P=0.802). The chi-square test showed that differences in degeneration rates were not significant among the 4 groups (P=0.969) (Table 1).

Table 1: Comparison of embryonic development up to the blastocyst stage after 72 h of culture.

| Group | 2-cell embryos N | Blastocysts N (%) | Degenerated embryos N (%) | P value (chi-square) |
|---|---|---|---|---|
| 1 | 32 | 15(46.8%) | 17(53.1%) | 0.969 |
| 2 | 32 | 14(43.7%) | 18(56.2%) | |
| 3 | 32 | 15(46.8%) | 17(53.1%) | |
| 4 | 32 | 16(50%) | 16(50%) | |

Among the embryos that reached the blastocyst stage, 28 morphologically normal expanded blastocysts were selected for assessment by TUNEL assay. The number of blastomeres and the number of apoptotic cells in each group were observed. Comparison among the 4 groups (1, 2, 3, 4) revealed significant differences: Group 4 (14.6%) > Group 1 (11.4%) > Group 2 (8.5%) > Group 3 (5.6%), P<0.001 (Table 2).

Table 2: Comparison of the number of blastomeres and the number of apoptotic cells among groups.

| Group | Blastocyst N | Apoptotic cells N (%) | Normal Blastomeres N (%) | P value |
|---|---|---|---|---|
| 1 | 6 | 43(11.4%) | 334(88.6%) | <0.001 |
| 2 | 8 | 62(8.5%) | 664(91.5%) | |
| 3 | 7 | 40(5.6%) | 673(94.4%) | |
| 4 | 7 | 84(14.6%) | 490(85.4%) | |

## 4. Discussion

In this study, we used fluorescent light, which is routinely used in infertility treatment centers.

We found that visible light does not stop the development of embryos to the blastocyst stage. As shown in our previous study, 800 lux of light exposure for up to 30 minutes did not have remarkable harmful morphological effects on mouse embryo development to the blastocyst stage (3). Similarly, Takenaka et al. observed that emission of 1200 lux of white fluorescent light for 15 minutes did not prevent embryo development to the blastocyst stage (9).

Another study reported that embryos developed to the blastocyst stage after exposure to 2900 lux of light for 30 minutes (15). Our findings are in agreement with these results.

Conversely, Nematollahi et al. found that 30–45 minutes of 1600 lux of light could damage embryos and prevent them from reaching the blastocyst stage. Aside from differences in the intensity and type of light (halogen lamp), this difference might also be due to different conditions in this study. Specifically, light radiation was performed above culture dishes inside a $CO_2$ incubator while their lids were open; in contrast, in the current study, the embryos were directly exposed to light, similarly to what is done in IVF laboratories (1).

High-intensity light (4000 lux) was found to have significant adverse effects on mouse embryonic development (15). Studies show that light has adverse effects on mammalian embryos, but the levels of sensitivity, tolerance, and the ability to resist probable injuries differs in various species (5, 9).

We used mouse embryos in this study. Although mouse and hamster models may not be the best for assessing the effect of light on human oocytes or embryos, other mammalian species could be more sensitive than expected. Compared with mouse embryos, hamster embryos are reported to be more sensitive to light (9). Despite the vital role of light in living organisms, studies show that both visible and invisible light may have adverse effects on cells. Visible light induces reactive oxygen species (ROS) formation, and consequently cell damage (16, 17), such as base oxidation and DNA strand breakage (5, 18).

In the current study, 5 and 15 minutes of visible light did not induce mouse embryonic cell apoptosis. Apoptosis is a natural phenomenon used to eliminate damaged cells and cells with defective developmental potential. Suboptimal conditions also increase

apoptosis in cultured embryos (9). Even though the role of cell death in the early embryo is unknown, it may participate in the elimination of abnormal cells or cells with impaired potential (14).

It seems that mouse embryos can bear 5 and 15 minutes of visible light with an intensity that is typically found in laboratories. Among the different light exposure times, 30 minutes was the most stressful for the blastocyst, and therefore the maximum rate of apoptosis was seen in group 4.

Excessive apoptosis is believed to decrease the number of inner cell mass cells in blastocysts, consequently damaging the embryonic developmental process (9). According to current findings, the rate of apoptosis observed in this study is not enough to disturb embryonic development, and does not prevent the embryo from reaching the blastocyst stage.

Takenaka et al. exposed mouse zygotes to 1,200 lux of cool white fluorescent light for 5, 15, and 30 min. They observed that all of the mouse zygotes exposed to light developed into blastocysts, but zygotes that were exposed to light for 15 and 30 min demonstrated considerably high levels of apoptosis. They concluded that this light is harmful to mouse zygotes (9). The discrepancy between this result and our findings is probably due to differences in experimental conditions, such as the amount of light and the stage of embryonic growth studied. Additionally, they implanted cultured embryos into the mother's uterus after light exposure, but we studied embryos only *in vitro*.

DNA repair, which is performed by repair genes, is very active in preimplantation embryos (19). The repair genes in mice are expressed in oocytes and preimplantation embryos from the 2-cell stage to the 4-cell stage (20). Embryos exposed to light may compensate for DNA damage by using repair phenomena and compensation mechanisms that are very active in these embryos (21).

In inappropriate conditions, cells make proteins in their cytoplasm that increase cell tolerance and cell adaptation against stress. Such mechanisms are reported in different cells after heat shock exposure (22, 23). Early embryos may use this mechanism to adapt to and survive inappropriate conditions.

It is likely that the variety and complexity of these compensation and adaptation mechanisms can explain the differences between the various reports discussing the effect of light on early embryos.

In this study, the amount of ROS production and its effect on changes in gene expression were not measured. Further studies are recommended to determine the optimal time for handling embryos *in vitro*, specifically by performing the same experiment and measuring the amount of ROS production and gene expression under the same conditions.

## 5. Conclusion

This study showed that brief light exposure (5 to 15 min) is safe for blastocysts. Use of a dark room is impossible and unnecessary. On the other hand, long exposure to white

fluorescent light can induce apoptosis in blastomeres. Although, blastocysts develop under these conditions, caution is advisable because of an increased rate of apoptosis.

## Acknowledgments

The authors are thankful for the support provided by the Vice-chancellor for Research at the Mazandaran University of Medical Sciences.

## Conflicts of Interest

None declared.

## References

[1] Nematollahi-mahani SN, Pahang H, Moshkdanian G, Nematollahi-mahani A. Effect of embryonic fibroblast cell co-culture on development of mouse embryos following exposure to visible light. J Assist Reprod Genet. 2009;26(2-3):129-35.

[2] Fernández-Gonzalez R, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez MA, et al. Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. Proc Natl Acad Sci USA. 2004;101(16):5880-5.

[3] Khalili-Savadkouhi S, Karimpour Malekshah A, Alizadeh A, Esmaeilnezhad Moghadam A, Shiravi A, Mirhosseini M. The effect of light on mouse preimplantation embryo development in vitro. J Mazandaran Univ Med Sci. 2011;20(80):17-24.

[4] Schultz RM. From egg to embryo: a peripatetic journey. Reproduction. 2005;130(6):825-8.

[5] Pomeroy KO, Reed ML. The effect of light on embryos and embryo culture. J Reprod Stem Cell Biotechnol. 2012;3(2):46-54.

[6] Schultz RM. Of light and mouse embryos: less is more. Proc Natl Acad Sci USA. 2007;104(37):14547-8.

[7] Oh SJ, Gong SP, Lee ST, Lee EJ, Lim JM. Light intensity and wavelength during embryo manipulation are important factors for maintaining viability of preimplantation embryos in vitro. Fertil Steril. 2007;88(4):1150-7.

[8] Nakahara T, Iwase A, Goto M, Harata T, Suzuki M, Ienaga M, et al. Evaluation of the safety of time-lapse observations for human embryos. J Assist Reprod Genet. 2010;27(2-3):93-6.

[9] Takenaka M, Horiuchi T, Yanagimachi R. Effects of light on development of mammalian zygotes. Proc Natl Acad Sci USA. 2007;104(36):14289-93.

[10] Kitagawa Y, Suzuki K, Yoneda A, Watanabe T. Effects of oxygen concentration and antioxidants on the in vitro developmental ability, production of reactive oxygen species (ROS), and DNA fragmentation in porcine embryos. Theriogenology. 2004;62(7):1186-97.

[11] Weissman L, de Souza-Pinto NC, Stevnsner T, Bohr V. DNA repair, mitochondria, and neurodegeneration. Neuroscience. 2007;145(4):1318-29.

[12] Mohn F, Schübeler D. Genetics and epigenetics: stability and plasticity during cellular differentiation. Trends Genet. 2009;25(3):129-36.

[13] Boelhauve M, Sinowatz F, Wolf E, Paula-Lopes FoF. Maturation of bovine oocytes in the presence of leptin improves development and reduces apoptosis of in vitro-produced blastocysts. Biol Reprod. 2005;73(4):737-44.

[14] Watson AJ, De Sousa P, Caveney A, Barcroft LC, Natale D, Urquhart J, et al. Impact of bovine oocyte maturation media on oocyte transcript levels, blastocyst development, cell number, and apoptosis. Biol Reprod. 2000;62(2):355-64.

[15] Ottosen LD, Hindkjær J, Ingerslev J. Light exposure of the ovum and preimplantation embryo during ART procedures. J Assist Reprod Genet. 2007;24(2-3):99-103.

[16] Dumollard R, Carroll J, Duchen M, Campbell K, Swann K, editors. Mitochondrial function and redox state in mammalian embryos. Semin Cell Dev Biol; 2009: Elsevier.

[17] Hernández-García D, Wood CD, Castro-Obregón S, Covarrubias L. Reactive oxygen species: a radical role in development? Free Rad Biol Med. 2010;49(2):130-43.

[18] Lesser MP, Kruse VA, Barry TM. Exposure to ultraviolet radiation causes apoptosis in developing sea urchin embryos. J Exp Biol. 2003;206(22):4097-103.

[19] Jaroudi S, SenGupta S. DNA repair in mammalian embryos. Mutat Res Rev Mutat Res. 2007;635(1):53-77.

[20] Zeng F, Baldwin DA, Schultz RM. Transcript profiling during preimplantation mouse development. Dev Biol. 2004;272(2):483-96.

[21] Artus J, Cohen-Tannoudji M. Cell cycle regulation during early mouse embryogenesis. Mol Cell Endocrinol. 2008;282(1):78-86.

[22] Latchman DS. Heat shock proteins and cardiac protection. Cardiovasc Res. 2001;51(4):637-46.

[23] Sørensen JG, Kristensen TN, Loeschcke V. The evolutionary and ecological role of heat shock proteins. Ecol Lett. 2003;6(11):1025-37.