Of light and mouse embryos: Less is more

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Although light is essential for life on this planet, Hamlet's sun pun, "Not so, my lord, I am too much in the sun" (*Hamlet*, Act I, Scene 2, line 68), points to the detrimental effects of light on cells by means of light's ability to generate DNA damage by promoting thymidine-dimer formation as well as reactive oxygen species (ROS) that can damage DNA, proteins, and lipids. Such damage can lead to premature cell death or cancer. The small number of cells of the early developing embryo makes it particularly vulnerable to light-induced damage. In lower species, such as marine organisms that develop at or near the water's surface, mechanisms have evolved to protect the developing embryo from light, in particular high-energy UV radiation. These mechanisms typically entail the egg's producing UV-absorbing compounds (1). The work reported by Takenaka *et al.* (2) in a recent issue of PNAS describes the effect of light on mouse zygotes.

In contrast to the development of lower species, early mammalian development, i.e., preimplantation development, occurs within the lightless reproductive tract of the mother. This fact likely accounts for the absence of a mechanism(s) protecting from light, be it UV or visible light, in mammalian zygotes and embryos. Over the course of the past 4–5 decades, culture systems have been developed that support oocyte maturation and fertilization *in vitro*, and development of zygotes to become developmentally competent blastocysts, i.e., blastocysts that, after embryo transfer, develop to term. The ability to recapitulate preimplantation development *in vitro* paved the way for molecular, cell biological, and biochemical studies addressed at understanding basic molecular and cellular processes that govern early development. These, in turn, have led to advances in treatment of human infertility, cloning, and stem cell biology.

What became apparent during the course of developing these culture conditions is that preimplantation embryos respond to the culture conditions by mounting a stress response. For example, embryo culture results in shifts in energy metabolism (3, 4). Mouse blastocysts that develop *in vivo* convert 40–50% of the glucose to lactate, whereas blastocysts that develop *in vitro* from the morula stage convert ≈100% of the glucose to lactate. Early cleavage-stage embryos also exhibit an increase in glycolysis in response to culture that is associated with reduced implantation and development after embryo transfer. Culture of preimplantation mouse embryos can lead to changes in gene expression (5, 6), including changes in expression of imprinted genes, e.g., *H19* (7, 8). Moreover, these changes in expression of imprinted genes persist after implantation and are readily observed in placental tissue (8, 9). It is noteworthy that adults derived from cultured embryos exhibit specific behavioral alterations in the elevated zero maze and Morris water maze tasks, stressing how early and subtle perturbations that occur during preimplantation development can have a long-term impact on offspring (10).

In vitro manipulation of mammalian gametes and preimplantation embryos inherently entails their being exposed to light, the period of exposure ranging from just a few minutes to many minutes, e.g., micromanipulation procedures, before their being returned to an incubator and hence shielded from light. Light's detrimental effects on preimplantation development have been reported previously, but the effects have not been systematically studied. The work reported by Takenaka *et al.* (2) carefully examines the effects of visible light on development of preimplantation mouse and hamster embryos, in particular by examining the effects of warm and cool fluorescent light; cool fluorescent lights are commonly used in laboratories. The emission spectrum for both types of light is in the 400- to 700-nm range, with cool fluorescent light displaying a higher relative light intensity in the 400- to 500-nm range.

Sensitivity of Zygotes to Light

Consistent with previous reports that hamster zygotes are extremely sensitive to light exposure, Takenaka *et al.* (2) noted that none of the hamster zygotes exposed to cool fluorescent light (1,200 lux for 15 min) developed beyond the two-cell stage, whereas all of the mouse zygotes developed to the blastocyst stage. Genome activation occurs during the two-cell stage in hamster and mouse embryos and entails a dramatic reprogramming of gene expression that is essential for further development. To minimize the effects of light during the course of zygote manipulation on the microscope stage, the incandescent light source was covered with a piece of red cellophane, resulting in the zygotes being exposed to a light intensity of 80 lux. The experiments were conducted in a windowless room equipped with nine fluorescent ceiling lamps (100 V, 40 W each) that emitted either cool or warm light. In the absence of ceiling light, the room was illuminated with a single incandescent lamp (100 V, 20 W). Under these conditions, light intensity on the laboratory bench and microscope stage was 8 and 250 lux, respectively.

The effect of cool and warm fluorescent light exposure on ROS production, as assayed by hydrogen peroxide formation, was then assessed. For both hamster and mouse zygotes, a 15-min exposure to cool fluorescent light generated a significant increase in ROS production, the increase being more prominent in the more sensitive hamster zygotes. Warm fluorescent light also produced a significant increase in ROS in hamster but not mouse zygotes. Although a 15-min exposure to cool fluorescent light did not inhibit development of mouse zygotes to the blastocyst stage, this exposure did result in a marked increase in the incidence of apoptosis as assessed by TUNEL, an effect inhibited by including polyphenol, an antioxidant, in the culture medium during the period of light exposure. Exposing mouse zygotes to warm light for 15 min did not result in any increase in the incidence of apoptosis. In contrast, brief exposure of zygotes to sunlight (1–60 s, >20,000 lux) resulted in a marked increase in apoptosis in the resulting blastocysts.

Development to the blastocyst stage is no longer considered an adequate means to assess developmental competence. For example, cloned embryos develop to the blastocyst stage and appear morphologically normal, but many of these clones misexpress genes (11) and exhibit a high incidence of apoptosis (12). The accepted gold standard to assess developmental competence is to conduct embryo transfer experiments and measure either late stages of fetal development or development to term. Consistent with the effects of cool and warm fluorescent light on apoptosis, the

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authors noted that developmental competence of blastocysts derived from zygotes exposed to cool light for 15 min was markedly reduced when compared with their counterparts exposed to warm fluorescent light. Only 44% of the transferred blastocysts exposed to cool light developed to live fetuses at 19 days postcoitum, in contrast to 73% of the control embryos. Transfer of blastocysts that developed from zygotes exposed to warm light resulted in 58% developing to live fetuses at 19 days postcoitum, a difference that was not statistically significant. The incidence of resorption (≈20%) was the same in control and experimental groups. A 1-min exposure to sunlight was catastrophic, with only 25% of the embryos developing and 35% being resorbed. Of great interest will be to assess the long-term effects of light exposure on development and behavior of the offspring.

Ramifications

Results of these studies, although simple in design and outcome, have profound clinical ramifications. Although much media attention focuses on problems associated with a growing world population, infertility is quite common among couples in developed countries; it is estimated that some 10–15% of such couples are infertile. This translates worldwide to up to 70 million couples (13). These couples have turned to assisted reproductive technology (ART) to treat their infertility, and it is apparent that use of ART will continue to grow as women in developed countries postpone the time of having their first child.

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Since the birth of Louise Brown in 1978, it is estimated that ≈3 million children have been conceived by ART. In developed countries, it is estimated that ≈2–3% of children born are conceived by ART, and in Denmark this figure is ≈6%.

Intrinsic to most ART procedures is manipulation of gametes and embryos, and this in turn entails their being exposed to light. A recent study from Denmark (14) noted that normal ART procedures used result in human zygotes being exposed to stressful dosages of visible light, and yet no Danish *in vitro* fertilization program seems to take preventive measures to protect the embryos from light. A similar situation likely exists in many ART clinics throughout the world. The results reported by Takenaka *et al.* (2) reinforce the need to assess critically every step involved in ART to assess its short- and long-term impact (15). For example, recent studies demonstrate that merely pipetting embryos can result in activating stress-induced kinases (16). This need for critical assessment is highlighted by the now accepted finding that results of recent retrospective studies revealed that children conceived by ART display a significantly higher incidence of Angelman syndrome (17) and Beckwith–Wiedemann syndrome because of loss of imprinting (specifically because of the loss of DNA methylation in critical regulatory regions that control expression of genes responsible for these syndromes) (18, 19), as well as retinoblastoma (20). ART-conceived children may also be at risk for Silver–Russell syndrome (21).

Children have never had a say regarding who are their parents and now do not even have a say as to how they are conceived. The results of experiments reported by Takenaka *et al.* (2) suggest that perhaps the children should be kept in the dark.

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