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Detection of Ultra-Weak Photon Emissions from Mouse Embryos with Implications for Assisted Reproduction

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ABSTRACT

Background: In the past four decades, *In Vitro* Fertilization (IVF) has benefited from substantial advancements and become a routine medical procedure. Embryo development can be moderated with time lapse systems, but such systems use visible light that can harm cells. Living cells have spontaneous Ultraweak Photon Emissions (UPEs) that are generated by metabolic reactions and influenced by physiological conditions.

Methods: Embryo-emitted photons were detected with a custom in-house ORCA-Quest CMOS camera and microscope incubator system. Images were taken in the dark. Negative control measures were taken for an empty vessel and a vessel with only oil and media. Optimal data were collected with all software filters off.

Findings: Reference measurements showed only negligible differences between empty and incubation medium filled samples. When four cell embryos were removed from their culture incubators for examination in laboratory air, light and temperature conditions, degenerated two cell stage embryos were observed to have lower UPE levels than cleaving embryos. Fresh embryos had significantly greater UPE levels than previously frozen and then thawed embryos.

Interpretation: UPE detection in mouse embryos can provide a foundation for the development of a photon emission embryo control system.

Keywords: Photon emission; Mice embryo; Spectral fingerprint; IVF; EW-SFD

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INTRODUCTION

Fertilization outside of the body has become a routine gynecological procedure in recent decades. In 1978, the fundamental assisted reproduction technique known as In Vitro Fertilization (IVF) lead to the birth of the first so-called test-tube baby, establishing the possibility for couples struggling with infertility to produce biological children. Assisted reproduction treatments have enabled more than 8 million children to be born worldwide. Nowadays, each year, some 1·5 million IVF cycles are performed worldwide resulting in approximately 350 thousand children. In Hungary, 1·5%-2% of babies are born due to IVF treatment.

There has been substantial advancement in IVF procedures since IVF was first introduced. Typically, stimulated cycles yield several oocytes, which allows multiple embryos to develop, increasing the chance of pregnancy. Generally, even if several embryos are available, patients expect only one or two to be transferred at once. This situation raises important ethical and practical questions regarding how one should select the embryo or embryos to be transferred. The most widely used selection method is based on an examination of embryo morphology, which can be done by simple microscopic viewing or by time lapse techniques that provide additional information about the dynamics of embryo development. Time-lapse technology introduces the concept of stable culture conditions and it enables the continuous observation of embryos throughout development albeit with a requirement for visible light [1].

Early embryonic development is characterized by rapid cell division and the activation of embryonic genes. These processes make embryos extremely vulnerable and sensitive to environmental influences. The female genital tract organs produce eggs and provide a safe environment for gametes and embryos while providing protection against visible light and radiation exposure. During IVF and especially Intracytoplasmic Sperm Injection (ICSI), light can harm oocytes and sperm during preparation and can harm embryos during incubation, microscopic examination and embryo transfer [2-4].

Although toxic effects of Ultraviolet (UV) light on cells are well established, the effects of visible light (400~700 nm) are less well known. Harmful effects of light may be related to the hydrogen peroxide formed in peroxisomes and mitochondria. Light can trigger stress gene activation and DNA damage in embryos. Within the visible range of light wavelengths, blue light (400~500 nm) is orders of magnitude more dangerous than longer-wavelength visible light [5-7].

Fertilization, blastocyst development and pregnancy rates are higher for embryos handled in light-protected conditions than for those handled in conventional light conditions, suggesting that a dark environment and light filters can reduce harmful environmental effects on embryos in IVF laboratories [8]. Thus, to protect gametes and embryos from light exposure, IVF practitioners can conduct laboratory procedures in a dark environment, which we produce by covering IVF/ICSI devices with aluminum foil and placing red filters on laboratory light sources as well as on the built-in light sources of microscopes and IVF workstations.

Living cells have spontaneous Ultraweak Photon Emissions (UPEs), mainly in the spectral range of 200 nm-800 nm and UPEs have been associated with reactive oxygen species [9,10]. UPE intensity varies from a few photons to several hundred photons per second per square centimeter, with variations being associated with physiological and pathological conditions, such as the presence of thermal, chemical and mechanical stressors and biological activities, such as those of the mitochondrial respiratory chain, cell cycle and cancerous growths [11-13].

IVF practitioners have an ethical obligation to use evidence-based state-of-the art methods. Cellular photon emissions might be harnessed to provide protection from visible light. Given the extremely low energy of UPEs, it is unknown whether it is realistic to detect embryo photon emissions under the applied conditions of embryo development. We examined this question in the present study by detecting photon emissions in mouse embryos.

Theoretical Considerations

The second law of thermodynamics states that the total entropy of a closed system is static (Clausius' formulation). In the context of living beings with self-replicating properties, including embryos, structures have dissipative adaptations that enable the absorption, use and emission of energy. Energy emission can be measured in the form of image data [14-21]. We developed a unique algorithm to separate such data from a single delimited region called the Entropy Weighted Spectral Fractal Dimension (EW-SFD) algorithm [22-25]. We submit the data produced to a structural analysis procedure derived from the general fractal dimension employing a novel application of fractals that is described in detail elsewhere [26].

MATERIALS AND METHODS

Animals Used for Retrieval of the Embryos

Thirty 7-week-old CD1 female mice and 20 9-week-old male mice (Charles River, Germany) were housed in Uniprotect Ng/M cabinets (Zoonlab Gmbh, Germany), which provided controlled temperature (24°C), day/night lighting (12/12) and humidity (50%). The animals were given at least 2 weeks to acclimate to the environment before being subjected to experiments.

Superovulation Treatment, Embryo Retrieval and Culture

At 8-12 weeks old, CD1 female mice were injected with 5 IU of follicle stimulating hormone (Merional, IBSA Pharma, Switzerland); 48 hours later, each of these female mice were treated with 5 IU of luteinizing hormone (Chloragon, Ferring, Hungary) and placed in shared housing with a CD1 male. Two days after co-housing cross-sex mice, at ~1·5 dpc (days post-

coitum), two and four-cell stage embryos were collected by flushing the mice's fallopian tubes and cultured en masse (10–14 embryos/droplet) in 50-µl droplets of KSOM medium (Millipore, England) supplemented with 0·4% bovine serum albumin under mineral oil at 37°C, 5% CO_2 in the air. Culture media was replaced after 2 d. These fresh embryos were subjected to UPE measurements immediately (2-cell stage embryos) or after a 24-hour culture (4-8 cell stage). Only high-quality embryos were investigated.

Frozen Embryos

Good-quality 6-8-cell-stage embryos were frozen. For measurements, frozen embryos were vitrificated and warmed with Rapid-i vitrification and warming sets (Vitrolife AG Gothenburg, Sweden) and allowed to acclimate to the culture media for 1-2 h of culture. The rate of live embryos after warming was >90%.

Photon Emission Detection

During UPE detection, embryos were cultured in EmbrioSlide culture dishes prepared according to the recommendation of the manufacturer (Vitrolife, Gothenburg, Sweden). The microwells of the equipment made it possible to keep embryos still during data collection. UPEs were detected using an ORCA-Quest CMOS camera (Hamamatsu Photonics®, Japan), which offers single-photon detection sensitivity and the ability to track time-dependent photon emission intensity. The system was equipped with a quantitative CMOS image sensor with maximum spectral response in the 300 nm-1000 nm range at -20°C. The sensor featured an effective pixel count of 4096 × 2304 (horizontal × vertical; pixel size 4.6 µm × 4.6 µm) and a quantum efficiency of 90% at 475 nm and 33% at 900 nm. A microscope incubator (Olympus®) provided ideal conditions for embryo incubation, enabling photons emitted by embryos to be detected by excluding visible light in completely dark conditions (**Figure 1**).

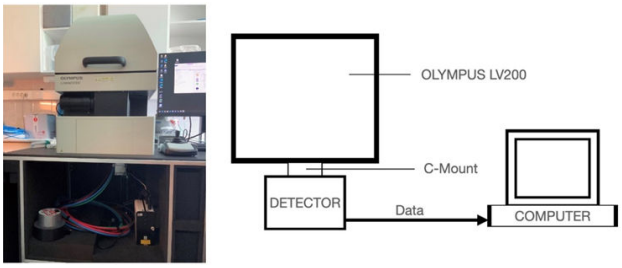

Figure 1: Schematic illustration of CMOS camera, microscope incubator and computer instrument complex.

RESULTS

In the first series of biological experiments, embryos cultured in an incubator were removed from an incubator and examined in laboratory air, light and temperature conditions. The samples included live cleaving embryos (primarily four and eight cell stage) and degenerated two-cell stage embryos. The degenerated two cell stage embryos were observed to have significantly decreased photon emission levels compared to the photon emission levels observed for their live cleaving counterparts (**Figure 2**).

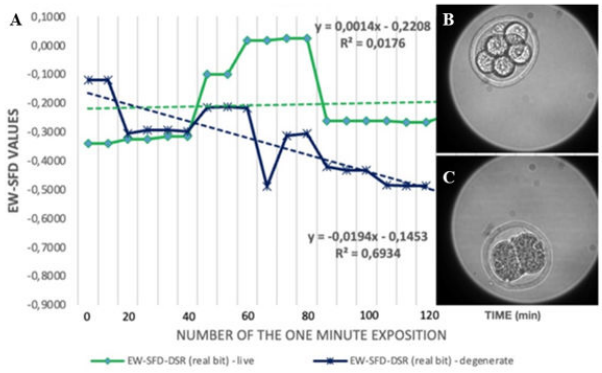

Figure 2: Photon emissions of a normally developing (green) and a degenerated (black) embryo. EW-SFD values (A) obtained for cleaving four-cell to blastocyst stage embryos (B) and for two cell-stage degenerated embryos (C).

In a second series of experiments, we obtained continuous recordings with a 1-min integration time. The photon emission measurement data were evaluated based on entropy, a spectral fractal dimension and an entropy weighted spectral fractal dimension (**Figure 3**) and it was demonstrated that the blank control, fresh and frozen-thawed samples could be distinguished from one another based on analyses of entropy-weighted spectral fractal dimension data representing 50 h of time-compressed continuous time-lapse data collected in the dark across 1 min exposures with the embryos held in a microscope incubator (**Figure 4**). The photon emission levels observed for the fresh embryos were significantly higher than those of embryos that had been frozen and thawed.

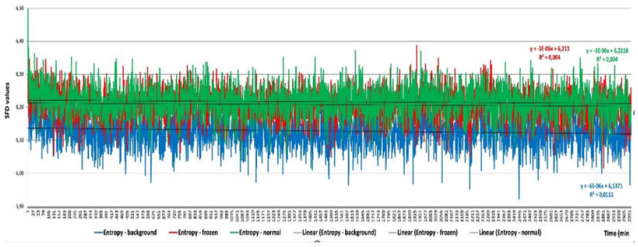

Figure 3: Entropy-based evaluation of frozen embryos, normal embryos, and background for 50 h of time-compressed data with a 1-min integration time.

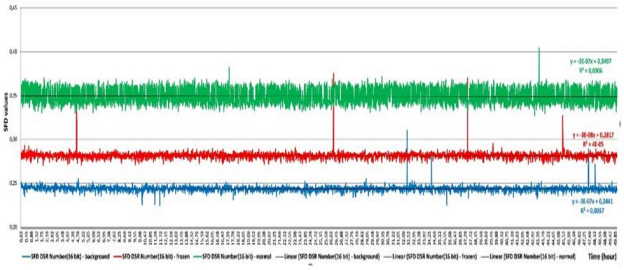

Figure 4: Spectral Fractal Dimension (SFD)-based assessment of frozen embryos, normal embryos, and background for 50 h of time-compressed data with 1-min integration time.

DISCUSSION

Spontaneous photon emissions of developing mouse embryos were detected successfully under ideal incubation conditions, without external stimulation, using a custom in-house instrument created from an Olympus® microscope incubator and a hamamatsu photonics® photon camera with single photon detection sensitivity. This approach differs from prior embryo photon emission studies in that it obviates the need for embryo-stressing stimulation (light, laser or chemical). The goal of this line of research is to provide maximal protection for gametes and embryos against all physical, chemical and biological factors during IVF procedures and assisted reproductive technology research.

Bioluminescence is a well-known phenomenon seen across phylogenetically diverse organisms. A new field of research focused on biophotons is emerging with the aim of quantifying cell-emitted photons at quantities too small to be seen. For example, Esmaeilpour et al. investigated UPEs from neural stem cells and observed UPE intensity was greater before cell differentiation than after cell differentiation and suggested that UPE measurement may be useful for assessing nanoparticle effects on living cells. Subsequently, in a study examining how internal factors influence human-cell UPEs, Zapata et al. found that UPE levels varied in response to disease states (including diabetes, hemiparesis, protoporphyria or a typical cold) and brain activity changes. They suggested that UPEs represent a natural and promising non-invasive spectroscopic variable that may have broad diagnostic applications [29].

Importantly, cell-to-cell communication *via* biophotons has been demonstrated in plants, bacteria, animal neutrophil granulocytes, kidney cells and neurons [29]. Thus, biophotons enable cells to interact without a molecular signal, indicating that there are inter-cellular processes that are not reliant on molecule-receptor recognition [30-34]. Indeed, Potapovich, et al., obtained strong evidence of non-chemical intercellular signaling leading to biological cellular responses [35]. They found that cells of various types appear to generate death signals under oxidative stress that can affect target cells over long distances through non-aquatic environments, resulting in morphological alterations and viability loss. These findings strongly support the supposition that biophotons may have biological significance.

In a study conducted in the dark, Mayburov found that biophotons emitted by older loach (fish) eggs inhibit the growth of immature loach eggs [36]. Previously, such radiation has been found to enable synchronization of development across distant samples. He recognized a pattern of binary biophoton data that resembled the exchange of binary-coded information over noisy communication channels in computer networks, including periodic bursts of photons that yielded responses in recipient cells suggestive of biophoton mediated encoding of information that is similar to that of an analog number time algorithm.

Popp, et al., postulated that UPE biophotons may present a wide variety of frequencies that originate from DNA and obtained data showing that biophotons are coherent and thus hypothesized that such emissions may regulate biological processes within organisms [33].

However, it is not yet known whether UPEs are a mere byproduct of biological metabolism or are mediating an informational or functional role, such as a spectral fingerprint of an embryo. More research is needed to determine how cells produce, sense and react to photons. In this regard, it is interesting to note that Adee, et al., described a bioelectric phenomenon wherein cells appear to crackle with electrical signals that may act to guide embryonic development and wound healing [37]. If so, then it may be possible to harness and modify such a bioelectric code for oncological and regenerative medicine applications.

CONCLUSION

This study provided a demonstration of the feasibility of UPE detection in embryos. UPE detection analysis has the potential to be used in an embryo-monitoring system that would allow close observation of the developmental, physiological and energetic processes of embryos under ideal incubation conditions without perturbation from external physical or chemical stimulation. We hope that this approach may mature into a photon emission embryo control system that can provide full metabolic and energetic control of early-stage embryos and their environments, respectively, together with tight control of time lapse photon emission analyses.

LIMITATIONS OF THE STUDY

This study mainly focused on health workers' perceptions in a hard-to-reach area who had been trained in iCCM and used their basic phones, which they had bought for their everyday use. The study can, therefore, not advise in totality recommendations for application in areas that are not hard to reach and the rural context like Nyaguda sub-location.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the declaration of Helsinki and approved by the regional and local research ethics committee of the university of Pecs, Pecs, Hungary (#PTE KK 7072-2018). All methods were carried out in accordance with relevant guidelines and regulations of the animal health committee of baranya.

DATA AVAILABILITY STATEMENT

Data collected during this study is available on request from the corresponding author.

DECLARATION OF INTEREST

The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

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