Of diamond surfaces, red light photobiomodulation and fertility: lessons from the laboratory

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There is hardly a day that goes by in my various professional roles as scientist, science watcher or clinician without encountering new information or a new scenario that highlights the complexity of biology and biological systems. Such occurrences warrant careful evaluation and oftentimes lead to new management strategies or form the basis for further scientific investigation. This is inevitable as the knowledge base expands and our understanding of the variables potentially impacting our strategies and outcomes increases. We continually learn, re-learn and refine what we do and how we do it. The survival and performance of human spermatozoa *in vitro* is one evolving story that has far-reaching implications in numerous fields in addition to human reproduction.

Infertility is a problem that affects 15% of couples. Male reproductive issues account for one third of infertility cases with another third caused by combined male and female reproductive issues or by unknown etiologies (1). Several strategies, including in vitro fertilization (IVF) techniques are employed clinically in an effort to assist infertile couples in their quest of a successful pregnancy. Viable, strong and normally motile sperm are critical to the success of IVF. It is well-known that spermatozoa in standard culture weaken and lose viability and motility at 12 hours and that by 42 hours, only about 52% remain viable (2,3). Fewer strong and motile sperm reduce the probability of a successful IVF cycle. Protocols that could improve viability and performance of sperm *in vitro* would be of great interest to clinicians and patients alike.

Reactive oxygen species (ROS) are toxic to both spermatozoa and oocytes, with the former being more sensitive to high ROS concentrations (3-10). MacLeod demonstrated that oxidative stress was responsible for decreases in sperm motility and that the adverse effects of increased oxygen concentrations could be mitigated by adding catalase to cultures (5).

At low concentrations, ROS act as second messengers that regulate increases in cyclic adenosine monophosphate (cAMP), the activation of protein kinase A (PKA), the phosphorylation of PKA substrates of the arginine-X-X-(serine/threonine) motif, the phosphorylation of extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MEK) proteins and the threonine-glutamate tyrosine motif, as well as fibrous sheath protein tyrosine phosphorylation (4,5,10). These functions are involved in sperm capacitation, acrosome reaction and oocyte fertilization (4,5,10).

Sperm plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) whereas their cytoplasm contains low concentrations of enzymes that scavenge ROS (4,5,10). High concentrations of ROS overwhelm the endogenous antioxidant defenses of gametes, causing multiple derangements. High concentrations of ROS cause peroxidative damage to plasma membrane PUFA, DNA damage, the depletion of mitochondrial adenosine triphosphate (ATP), apoptosis and the loss of sperm motility (4,5,10).

ROS are generally short-lived *in vivo* due to a number of antioxidant pathways and compounds at play (4,5,9,10). However, they are known to accumulate in cultures of both oocytes and spermatozoa, both of which have the capacity to generate ROS in small quantities as are required for the fertilization process (5,8,9,10).

Sommer *et al.* posited that polystyrene softens in the presence of aqueous solutions and that this creates conditions that would in turn cause a nanoscopic layer of ROS to become established in plastic Petri dishes in common laboratory use (6,8-10). This hypothesis was confirmed by evaluating cell performance of ROS-sensitive cell lines cultured in both polystyrene and ultrasmooth nanodiamond coated Petri dishes. The cell lines tested included mouse P19 embryonal carcinoma cells, murine-derived L929 cells and HeLa cells derived from human cervical cancer (8-10). The nanomechanical softening was demonstrated in subsequent work by this group and others (8-13).

The use of nanodiamond surface coating of culture dishes was based on the knowledge that this material is both chemically and biologically inert, with a capacity to bind a nanoscopic layer of water to its surface (6,8,9-13). Sommer demonstrated that the material and this nanolayer were for practical purposes, ROS-free (9,13,14). They subsequently reported that culturing human sperm cells in diamond-coated Petri dishes rather than the polystyrene dishes typically used for IVF resulted in approximately 20% greater cell survival at 42 hours in the nanodiamond coated cultures (9,10,13,14). This confirmed that the culture dishes themselves play a role in sperm survival *in vitro* and that accumulation of ROS on the polystyrene surface are a causative factor in the decrease in viability over time.

Sommer *et al.* went further by exposing the cultured spermatozoa to red light at 670 nm (10,15). Light at this wavelength is known to be absorbed by cytochrome C oxidase and other molecules, stimulating ATP synthesis and affecting ROS production, among numerous other activities at the cellular, tissue and whole organism level (16). The caveat however is that the light dose and dose rate are important and that all cell and tissues are not equally responsive to photoradiation (16-22).

They found that the number of sperm cells demonstrating grade A motility was enhanced by nearly 300% after 1-hour contact with the nanodiamond coated quartz Petri dishes as compared to the counts obtained for spermatozoa in the polystyrene Petri dishes (10). They also observed that sperm motility was significantly different after contact with polystyrene and nanodiamond when longer periods of photoirradiation were applied. A 3× higher light dose was detrimental to motility of sperm in polystyrene plate cultures, resulting in a reduction of counts to those of the control group at 45 and 60 minutes post exposure. The same light dose delivered to spermatozoa cultured in nanodiamond dishes produced a dramatic increase in progressive motility (10,13-15).

This series of experiments demonstrates that diamond Petri dishes and NIR light delivered at specific parameters energize sperm cells in a complimentary fashion whereas polystyrene Petri dishes exhaust them. The red light counteracts effects of internal oxidative stress due to ROS production in mitochondria by suppressing ROS accumulation and enhancing ATP synthesis, while the diamond substrate prevents the build-up of a layer of interfacial ROS between the sperm cell and surface of the culture plate.

Photobiomodulation (PBM) describes the ability to stimulate or inhibit cellular functions by using light at specific wavelengths, intensities and dosing regimens. The classically described PBM treatment window is between 600 and 1,200 nm (16-22). Light in this portion of the spectrum readily penetrates skin and tissues via the so-called optical window. Light is absorbed by various structures and molecules, and primarily molecules that are instrumental in energy production and oxygen delivery.

PBM effects depend upon timing, site of treatment and treatment parameters (dose). PBM has shown efficacy clinically in accelerating wound healing, reducing pain and inflammation, as well as benefit in other applications, including the treatment of neurologic disorders and injuries (19-22).

The mechanistic basis for the outcomes observed after using photobiomodulation therapy (PBMT) are a result of the upregulation of intracellular metabolism by increasing production of ATP, augmenting other metabolic pathways, and the induction or reduction of ROS and other free radical production (16,19-22).

The interaction of photons with cells and cellular structures is a necessary condition for PBM. We have learned that all cells and tissues don't respond to PBM and that one size does not fit all when determining the dose or course of treatment (18-22). Different photobiomodulatory effects have been described depending upon the specific cell lines and species being investigated. Our laboratory demonstrated that cell proliferation and metabolism *in vitro* can be influenced by varying the dose frequency or treatment interval of the PBMT (17). We have also

demonstrated this same phenomenon as regards wound healing in a murine pressure ulcer model (18). These investigations underscore the concept that a unique dose frequency combination exists for tissues and cell lines and that this specific treatment paradigm must be determined to optimize outcomes and maximally stimulate cellular metabolism and proliferation. Our work also demonstrated that using other treatment strategies will paradoxically cause bioinhibition, despite the delivery of the same total energy (17,18,21,22).

It is becoming increasingly apparent that biological systems are quite complex, and that they contain numerous pathways that are poised to work in concert with, or in opposition to, one another, depending upon the current needs of the organism. We are beginning to understand that these systems utilize several common denominator substances and reactions and that these can be manipulated using a number of forces, including light (20-24).

As scientists and clinicians, we apply what we have gleaned from the laboratory to solve clinical problems and to form the basis for further investigations. We often base these decisions on results obtained by using various cell, tissue and whole animal models, presuming that these models are translatable to our specific applications. Careful *in vitro* studies can be powerful tools that guide the design of whole animal and human trials. They facilitate the efficient and reproducible screening of a matrix of treatment parameters. We presume that the animal models we develop accurately reflect the actual biology and physiology found in nature.

Abolins *et al.* recently demonstrated that the serological, cellular and functional immune responses of laboratory and wild mice differ and that wild type mice have a population of highly activated myeloid cells that are not found in their laboratory counterparts (25). The point here is that laboratory models and laboratory conditions in all likelihood do not entirely replicate nature.

Sommers' work demonstrates that PBM with red light at 670 nm improves spermatic function and viability *in vitro* and this effect augments the beneficial effects of using nanodiamond coated culture dishes (2,3,6,8-11). It also demonstrates that various cell lines respond differently to similar manipulations. This body of work also highlights the fact that the ubiquitous polystyrene culture dish can have a deleterious effect on outcomes. We would do well to recognize that the seemingly innocuous may not be and that we should remain cautious as we interpret experimental results and attempt to apply them. Every detail matters, even the seemingly mundane.

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Footnote

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