Beam me up, baby: exploring the use of lasers in assisted reproductive technologies

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Assisted reproductive technology (ART) has been rapidly expanding since the birth of Louise Brown, the first test tube baby, in 1978. Although an increasingly complex array of laboratory skills and procedures have been developed for infertility treatments, the success rate of ART hovers around 30%. Even in successful cases, the incidence of multiple births, due to multiple embryo transfer, and their associated morbidity, is dangerously high. In an attempt to make ART safer and more efficient, international medical practice is trending towards single embryo transfers and the use of innovative, sophisticated technologies to identify promising gametes and embryos with the highest potential to generate a pregnancy. Laser technology is increasingly being used to accomplish these aims and improve the efficiency of ART. The use of laser technology reduces procedure times and increases consistency and reproducibility of traditional ART techniques such as sperm immobilization, embryo biopsies, and assisted embryo hatching. Herein we discuss how the use of laser technology in ART is working to improve traditional laboratory techniques in order to overcome infertility.

Introduction

An estimated one in seven couples experience difficulty conceiving (NICE, 2013). There are different reasons why conception may not occur naturally. Causes of infertility can range from age and polycystic ovarian disease in women to poor sperm count and quality in men. A combination of factors within the male, female or both, can contribute to infertility problems, such as a lack of eggs, reduced egg quality, the inability of sperm to meet and fertilise the egg or the inability of fertilised embryos to implant or grow in the uterus. In around 15-30% of cases, however, a clear cause is never established (Gelbaya et al., 2014). Assisted reproductive technology (ART) is the collective term for the reproductive technologies and procedures performed on sperm, eggs, and embryos in the laboratory to help sub-fertile couples potentially achieve a pregnancy.

In vitro fertilisation (IVF) is one of several treatment options used in ART. The IVF process involves an interplay of diagnostic tests, hormonal supplementation, surgery and laboratory techniques to help generate a healthy pregnancy. First, tests are performed to try and ascertain the cause of sub-fertility for the couple in question to determine the optimal ART treatment for their clinical situation. In a typical IVF procedure, the ovaries are first stimulated by hormones in order to permit the collection of multiple eggs from the woman, and a sperm sample is obtained from the male. Following this, the eggs are incubated in a petri dish with 200 000 sperm to allow for natural fertilisation. For fertilisation to occur, the sperm must penetrate through the egg's protective outer layer, called the 'zona pellucida'. In some cases, instead of incubating the oocyte with sperm in a dish, a single sperm is manually injected into the egg to aid fertilisation. If fertilisation is successful, a 2-cell zygote forms and undergoes cell cleavages to form an embryo, which depending on the ART intervention, grows over a 2-6 day laboratory culture period (depending on the clinical strategy) before transfer to the patient's uterus. The zona pellucida shell protects the developing embryo until it is large enough to break free at around day 5 post-fertilisation in a process known as 'hatching'. A hatched day-5 embryo is fully prepared to implant into the uterine wall to generate a pregnancy. When possible, multiple embryos are cultured simultaneously in the laboratory and monitored throughout their growth. The healthiest and strongest embryos (routinely assessed by morphology) are selected and 1-3 embryos are transferred to the uterus at day 3 or day 5 post-fertilisation. Any additional embryos that have developed healthily are frozen for possible transfer in a future cycle (Craig & Turner, 2013).

ART has undergone tremendous and rapid expansion over the past decades, largely owing to revolutionary techniques such as IVF, genetic screening, and cryopreservation. The increasing number of couples seeking infertility treatment, together with a medical imperative towards single rather than multiple embryo transfers, makes it vital that embryologists are able to identify the healthiest and strongest gametes (sperm and eggs) for procedural intervention. Making this identification and subsequently choosing the most viable embryo for re-implantation is crucial in the improvement of ART efficiency (Shivhare et al., 2011).

Figure 1: Infra-red Saturn 5 Active laser objective (40X, shown in red) alongside regular objectives for inverted microscopes, as manufactured by Research Instruments Ltd (UK). Image courtesy of Research Instruments Ltd (reproduced with permission)

Figure 2: Full ART micromanipulator and laser system, featuring an inverted microscope fitted with a micro-manipulation stage, micro-manipulation tools, and infra-red laser. The stage, laser, and laser software shown here are manufactured by Research Instruments Ltd (UK). Image courtesy of Research Instruments Ltd (reproduced with permission)

Laser manipulation of gametes and embryos represents a powerful advancement in ART, making procedures more efficient by reducing tissue exposure time to sub-optimal culture conditions. The use of lasers in ART techniques have been successfully employed in clinical practice to improve the efficiency of techniques and are continually the subject of investigative research in order to generate new methods to improve applications. Laser assisted procedures are applied in the clinic for sperm selection to aid fertilisation, as well as assisted hatching to create a controlled opening through the zona pellucida and facilitate embryo implantation. Similarly, creating this opening through the zona pellucida allows embryologists to extract cells from the embryo for genetic screening and the diagnosis of genetic diseases, as well as for cellular microsurgery. The application of lasers is also being researched for uses to facilitate intracytoplasmic sperm injection (ICSI), although this is not yet adopted in routine clinic procedures. Herein, we discuss the benefits of laser applications and their improvements on traditional ART techniques.

Application of lasers to benefit ART and clinical research

During ART manipulations, the embryo's culture environment must remain constant so as to

Figure 3: Precise embryo micromanipulation tools fitted onto an Integra micromanipulation stage (Research Instruments Ltd UK). Image courtesy of Research Instruments Ltd (reproduced with permission)

optimise potential for a viable pregnancy. Therefore, to provide consistent, reliable, and reproducible results, the lasers used for ART must integrate well and efficiently into the equipment required for routine laboratory procedures. Construction of the laser is such that the beam travels through a specialised microscope objective of 40X or 20X magnification. These objectives are placed into inverted microscopes with brightfield, phase contrast and Hoffman modulation optics, which are used in routine ART. This set up facilitates the application of laser pulses during normal manipulations [Figure 2.tif]. The inverted microscope is a versatile instrument used in ART. It allows precise modifications to the stage to facilitate the incorporation and control of precision micro-tools, which are fundamental requirements for the micromanipulation of sperm, eggs, and embryos. In addition to being fitted with lasers and micromanipulation tools, these systems can also be equipped with heated or cooled stages to allow cells and embryos to be maintained at constant physiological temperature throughout manipulation (Kashir et al., 2012).

Current IVF clinics utilise lasers which use infra-red light wavelengths to prevent any ultraviolet (UV) damage to DNA, and non-contact lasers to prevent any unnecessary embryo manipulation contact. The power and pulse lengths of the lasers are optimised in a way that prevents any detrimental thermal

effects upon the embryo and surrounding culture media. The integration of lasers that are fast and easy to use helps to reduce procedure times, thus minimising exposure of the embryo to a sub-optimal culture environment outside of the incubator.

Intra-cytoplasmic sperm injection (ICSI)

For fertilisation to occur, the sperm must penetrate through the outer protective layer of the egg, called the zona pellucida, a matrix of glycoproteins surrounding the egg which contains proteins to specifically bind sperm. The zona pellucida is a strong and thick polymeric structure, which exhibits elasticity, stability, and resistance to mechanical force. When there is a case of male factor infertility with extremely low sperm count or motility, there is often a failure of natural sperm entry from incubation with the egg alone. In these cases, a technique called ICSI can be performed, where the embryologist selects a single sperm and manually injects it through the zona pellucida into the egg using a fine glass injection needle in order to facilitate fertilisation. Over one million ICSI treatments are performed globally each year, accounting for up to 7% of all births in some developed countries (Guner et al., 2010; Shivhare et al., 2011).

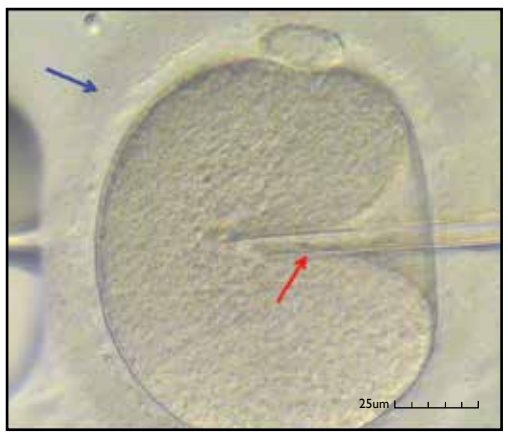

Figure 4: Brightfield image of a human egg undergoing an intra-cytoplasmic sperm injection (ICSI) procedure. The blue arrow indicates the surrounding zona pellucida. The red arrow indicates a single sperm isolated inside the glass injection pipette ready to be injected into the egg to initiate fertilisation. Image courtesy of Tracey Griffiths (reproduced with permission)

Mechanical puncturing of the zona pellucida is the current standard for conventional ICSI and is performed using a sharp glass needle (Ross, 2013). However, due to the fragility of the egg and the force required for the needle to transverse the zona pellucida, the egg can be strained and deformed during the injection. This can potentially compromise embryo development by causing damage to structures inside the egg or by inducing egg degeneration. The use of lasers to minimise this damage and improve ICSI is currently being investigated for possible future implementation in regular clinical practice.

In laser-assisted ICSI, a precise controlled hole can be drilled into the zona pellucida, leaving the innermost layer of the zona intact, thereby providing embryologists with a very small opening through which the injection pipette (containing a single sperm) can be easily carried into the egg with minimal force. Multiple studies have shown that creating a micro-hole on the zona pellucida of the egg by a laser beam prior to ICSI results in less traumatic penetration of the injection needle. This results in lower egg and embryo degeneration, as well as higher fertilisation, embryonic development, and pregnancy rates compared to conventional ICSI for patients with fragile eggs (Abdelmassih et al., 2002; Rienzi et al., 2004; Choi et al., 2011). Laser-assisted ICSI however is not widely adopted in clinics yet, as there is some debate regarding the overall benefit of the procedure. The damage rate of conventional mechanical ICSI on eggs is only around 4%, and it is believed that the oolema (a membranous layer underneath the zona pellucida closest to the embryo cells) is the actual structure that causes difficulties during mechanical injection,

Before a sperm can be injected into an egg for ICSI, the sperm tail must also be immobilised – this ensures that a beating tail does not cause damage inside the egg. Traditionally, this procedure is performed using a fine-tipped micro-tool which is brought down and across the tail, causing the tail to break (Ross, 2013). This represents a highly challenging but critical skill for embryologists to acquire for effective sperm capture. Immobilisation of sperm can be simplified by using a low level laser pulse on the sperm tail to incapacitate its beating without affecting viability (Ebner et al., 2002).

In severe cases where sperm are seen to be non-motile, it is difficult to select a sperm for ICSI as it is unknown whether any of the sperm are alive or not. In such cases, laser pulses can be used to assess sperm viability. A single pulse to the tip of a sperm tail will distinguish dead from non-motile living sperm, since a non-motile but living sperm's tail will curl up in response to the laser, whereas the tail of a dead sperm will not respond (Nordhoff et al., 2013). Lasers are thus a very useful tool to facilitate sperm selection and injection during ICSI and increase the likelihood of successful fertilisation, all while exercising minimal force upon the egg.

Embryo biopsies for genetic testing

Pre-implantation genetic diagnosis and screening (PGD and PGS) are processes in which genetic material from an embryo or egg is genetically analysed prior to transferring an embryo into the patient's uterus for implantation. Once genetic material is obtained, it can be analysed for specific hereditary genetic mutations (PGD) or for abnormal chromosomal number (PGS). Genetic testing can be performed on the egg by extracting a polar body (a waste product of meiosis), on an 8-cell embryo by extracting a single cell called a blastomere, or on a blastocyst stage embryo by extracting 5 embryo cells from the area called the trophectoderm. In the egg, the polar body can provide key information by reflecting the maternal genetic material contained within to ensure abnormal eggs are excluded from treatments (Griffiths, 2013). Examining the polar body has the drawback of only providing genetic information from the maternal genome and not the paternal genome. However, this is an attractive option for couples who do not wish to discard fertilised embryos for personal, cultural or religious reasons, since the procedure is carried out prior to fertilisation. If genetic information from both parents is required, a single blastomere from the 8-cell stage embryo, or 5 trophectoderm cells from the blastocyst stage embryo can be extracted and analysed. Blastomere and trophectoderm biopsies are often opted for when both parents exhibit a hereditary genetic defect they wish to avoid passing onto their children, if the woman is of advanced maternal age with a high chance of producing embryos with an abnormal number of chromosomes, if the patient has a history of recurrent miscarriage or previous abnormal pregnancies, or just to increase chances of success.

To harvest the polar body or blastomere, the zona pellucida must be breached to reach the material inside. Historically, to allow the extraction pipette to traverse through the zona pellucida, a hole was created in the outer layer by degradation with Tyrode's acid. The embryo was immobilised while acid in a microcapillary tube was gently blown over the embryo until a localised region of the zona pellucida began to dissolve. The use of acid presented many technical challenges, exposed the embryo to abnormal pH levels, and required a change of culture media afterwards to remove excess acid, potentially introducing greater risk of contamination. Another method used for breaching the zona pellucida for biopsy is to mechanically carve a hole using sharp glass pipettes (Griffiths, 2013). However, both of these techniques are highly variable, lead to inconsistent hole sizes, and could easily result in damage or loss of cells.

In current clinical practice, laser embryo biopsy is the preferred method, it is used to simplify the process and reduce damage to the egg or embryo. Laser pulses are directed at the zona pellucida, creating a uniform hole through which a blunt glass capillary can be inserted to extract target cells. The ability to use a blunt capillary, rather than a sharp glass needle to puncture the egg, avoids damage such as mechanical strain and deformation of the embryo. A capillary can be inserted to suck out a single blastomere cells from an 8-cell embryo, or it can

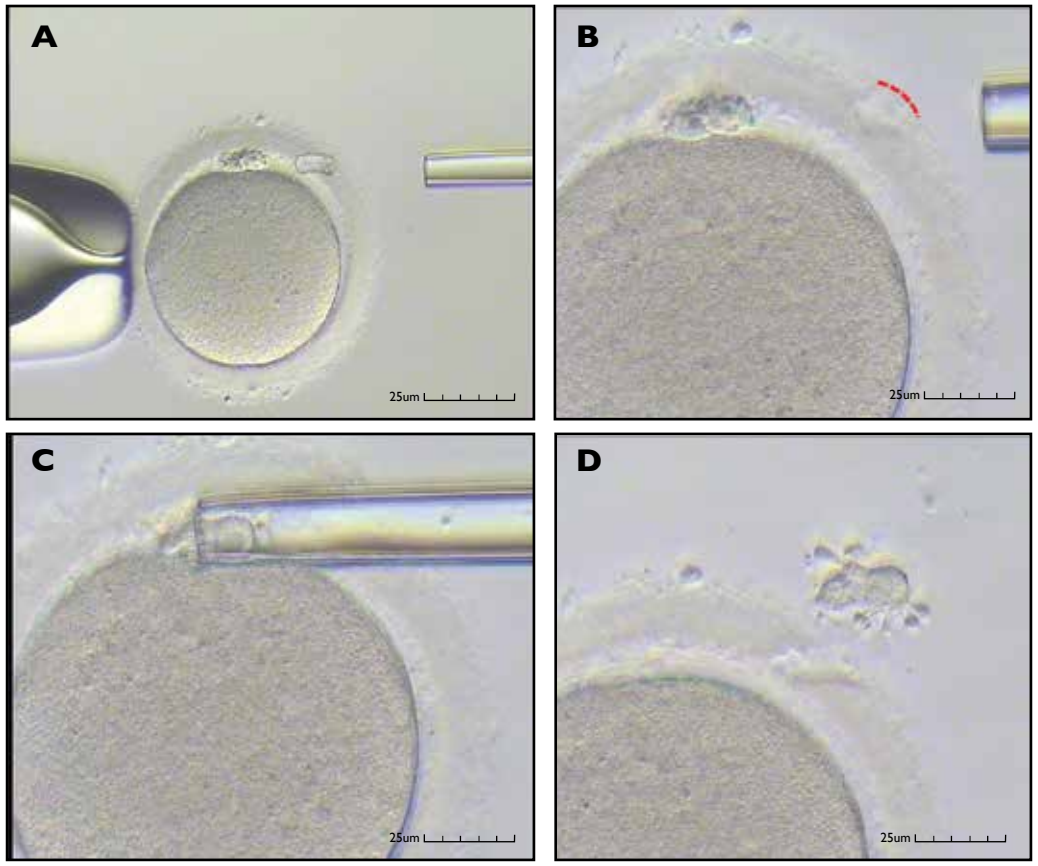

Figure 5: Images showing a polar body biopsy for genetic screening. (A) Laser pulse path alignment. (B) Red line indicating where the zona pellucida has been treated with laser pulses. (C) Insertion of a blunt capillary tube to reach the polar body cell. (D) Extracted polar body outside of the zona pellucida ready for collection and genetic analysis. Image courtesy of Tracey Griffiths (reproduced with permission)

be used to suck 5 cells from the trophectoderm of a blastocyst embryo. Once the blastocyst cells are sucked out of the zona pellucida, a laser can be used to separate them from the rest of the trophectoderm. Trophectoderm cell biopsy from blastocyst embryos is often the preferred method of genetic testing as the testing of multiple cells can provide the most reliable results, however for trophectoderm biopsy, the embryos need to be healthy and strong enough to develop up to the blastocyst stage in laboratory culture (around 5 days). Studies comparing the use of Tyrode's acid with laser zona drilling for embryo biopsy have shown that laser drilling is an easier and faster procedure, resulting in a greater number of intact blastomere cells post-procedure (Joris, et al., 2003; Wang et al., 2008). Prior to laser-assisted biopsy, the technique was limited by operator skill and non-standardised tunnel size. Furthermore, the embryos were subjected to a drastic pH change due to the introduction of acid to the environment. Now, while still requiring skilled embryologists with a high level of operator skill, consistent holes can be

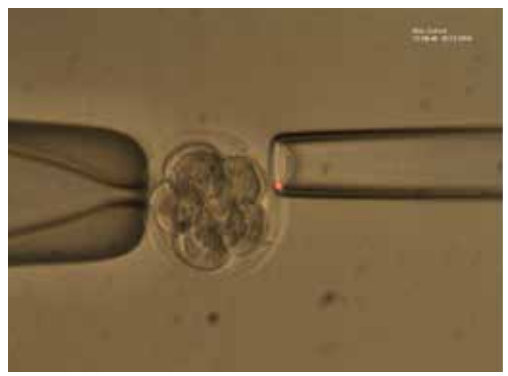

Figure 6: Brightfield image showing a pilot (guide) laser beam alongside the zona pellucida of an 8-cell human embryo in preparation for blastomere biopsy for genetic screening. Image courtesy of Celine Jones (reproduced with permission)

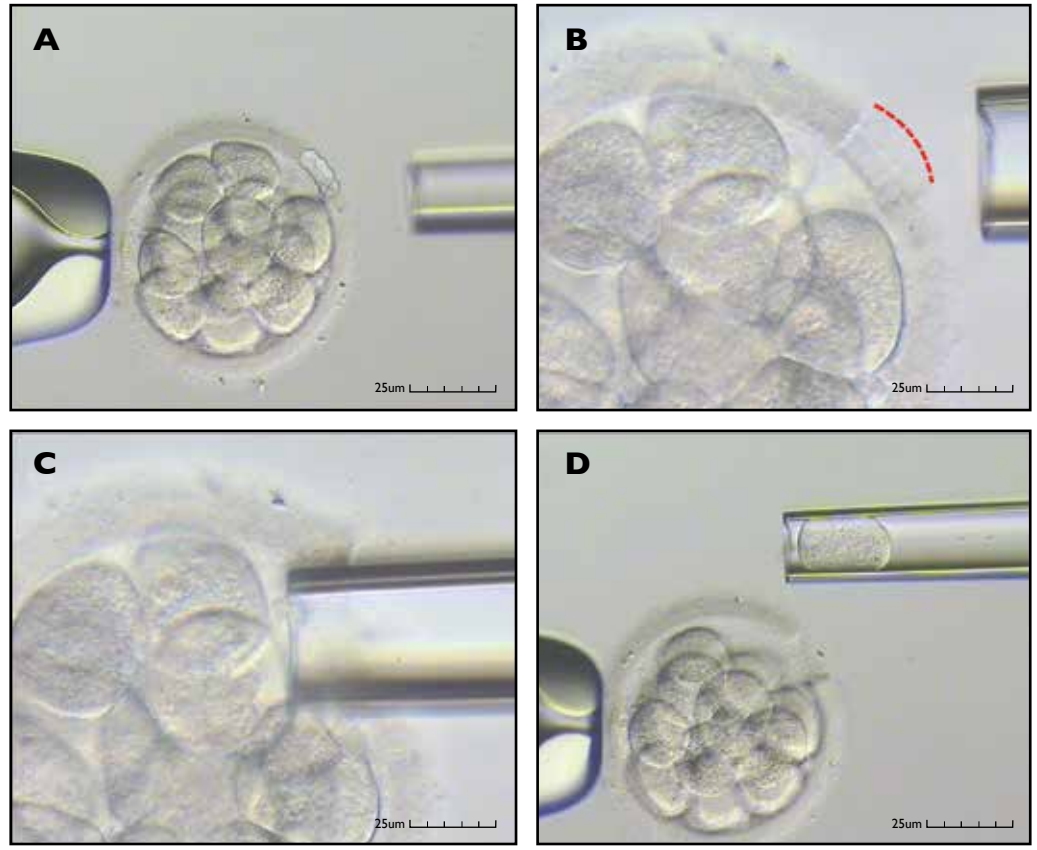

Figure 7: Images showing a blastomere biopsy from a day 3 embryo for genetic screening. (A) Laser pulse path alignment along the zona pellucida. (B) Red line indicating where the zona pellucida has been treated with laser pulses. (C) Insertion of a blunt capillary tube to reach a blastomere near the periphery of the embryo. (D). Capillary tube containing the extracted blastomere ready for genetic analysis. Image courtesy of Tracey Griffiths (reproduced with permission)

created in embryos and the biopsy procedure takes far less time, thus minimizing the time the embryo spends outside of the incubator.

Assisted embryo hatching

For a successful pregnancy to occur, the embryo must hatch out of the zona pellucida, around day 5 or 6 following fertilisation, to make contact with the endometrium and begin to grow, a process known as implantation. In some instances the embryo is unable to hatch from the zona pellucida, therefore implantation cannot occur and a pregnancy will not develop. The exact causes of failed implantation are unknown, but increased maternal age (which is linked to increased chances of abnormal chromosome number), reduced egg quality, poor embryo morphology, and the presence of a thicker zona pellucida from *in vitro* culture, are all known to be contributing factors. An ART process known as 'assisted hatching' can create a controlled opening in the zona pellucida to facilitate embryo implantation after transfer to the patient's uterus. Assisted hatching is often offered to patients who have had two or more failed IVF cycles, patients of advanced maternal age, or patients who are using embryos that had previously been frozen (Martins et al., 2011; Kissin et al., 2014).

Initial methods used to thin the zona pellucida for assisted hatching involved dissolving the shell with Tyrode's acid or with a special enzyme known to induce degradation. Another method involves mechanical thinning using a mechanical dissection pipette to slice off thin layers of the zona pellucida. However, these methods are limited by inter-operator variability, differing depths of

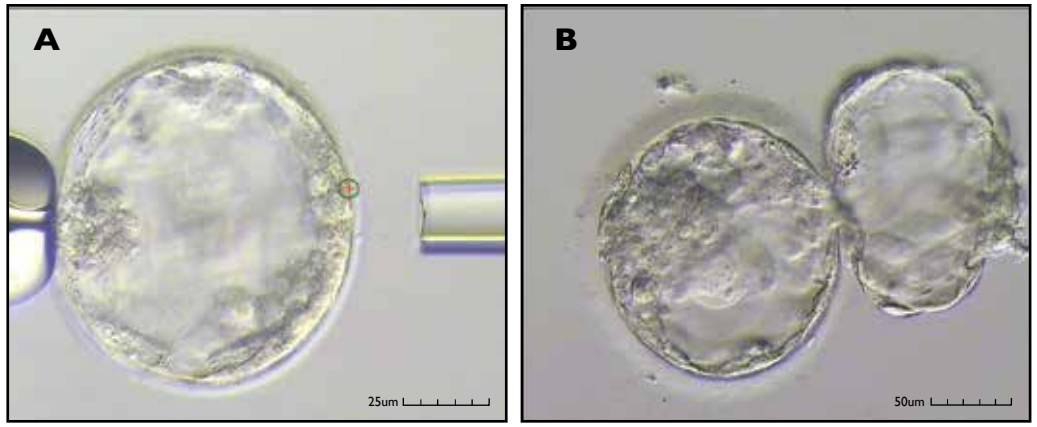

Figure 8: Laser-assisted hatching. (A) Laser pulse alignment along the zona pellucida of a blastocyst stage embryo. (B) Blastocyst in the midst of hatching out from the zona pellucida through a laser induced hole. Image courtesy of Tracey Griffiths (reproduced with permission)

zona penetration, and disruptive mechanical force upon the embryo. Laser assisted embryo hatching is currently offered in clinics in addition to the two older methods, and provides an alternative method for zona pellucida thinning that is faster, easier, and more consistent, ultimately resulting in a more efficient and reproducible process without compromising embryo quality (Jones et al., 2006; Zhou et al., 2014). A recent Cochrane review demonstrated that assisted hatching using all three methods offers a significantly increased chance of achieving a clinical pregnancy (Carney et al., 2012).

There are three different laser-assisted embryo hatching methods of varying invasiveness, with the optimal method still being debated. First, the laser can be used to create a hole completely penetrating through the whole zona pellucida layer. Secondly, a small hole through most of the zona pellucida can be created to partially hatch the embryo. Thirdly, the laser can be used to thin a large inner or outer area of the zona pellucida around the embryo to facilitate natural hatching. It has been shown that the least invasive method, zona pellucida thinning, increases pregnancy rates compared to the other laser methods in patients with repeated implantation failure (Ma et al., 2014). Studies have reported that laser-assisted hatching particularly improves success rates of achieving a clinical pregnancy for patients using frozen embryos (Hiraoka et al., 2008; Wan et al., 2014).

Blastocyst collapsing for cryopreservation of embryos

In ART, many eggs are harvested and fertilised at once. When many embryos are successfully matured to the transfer stage, any good quality surplus embryos can be cryopreserved for potential transfer in a future cycle. One method of cryopreservation, called vitrification, uses high concentrations of cryoprotectants and rapid cooling at a rate of -20,000°C per minute to help prevent ice crystal formation in cells (Craig & Turner, 2013). With the application of cryopreservation, it was found that well-developed embryos successfully grown to the blastocyst stage (day 5) had lower survival rates after freezing compared to embryos frozen at earlier stages (day 3). The primary structural difference between the blastocyst stage and earlier stages is the presence of a fluid filled cavity called the blastocoele. Artificial shrinkage of the blastocoele cavity to reduce fluid volume prior to cryopreservation has been shown to increase embryo survival after the freezing process. Shrinking of the blastocoele can be performed in three different ways. First, via mechanical puncture with a sharp glass needle, where the blastocyst collapses over a period of 30 seconds to 2 minutes. Secondly, by osmotic shock which uses high sucrose concentrations to induce the transport of water out of cells and dehydrate the embryo (Iwayama et al., 2011). Thirdly, a short laser pulse can be delivered to the embryo, shrinking the cavity immediately without any other manipulation. The laser pulse can be directed towards an area of the blastocyst that develops into the placenta far away from the cells that become the developing foetus. Moreover, the application of a laser pulse is much shorter, faster and imparts less mechanical force and strain upon the embryo. Studies have shown that human and mouse blastocysts vitrified after mechanical and laser collapse show fewer damaged cells and have a higher rate of re-expansion upon thawing (Iwayami et al., 2011). The Oxford Fertility Unit (Oxford, UK) found that embryo survival rates following thawing improved 98% when laser assisted blastocyst collapsing was employed rather than mechanical collapse (Griffiths *et al*., Unpublished).

Cellular microsurgery

Material from embryos that might be detrimental to subsequent embryo development, such as cellular fragments from uneven division or dying cells, can be removed with the help of laser technology. Fragments can appear and disrupt normal embryo development because they block the natural planes on which embryos divide. Embryos with higher levels of fragmentation are associated with lower implantation and pregnancy rates. Necrotic blastomeres are often observed following thawing from cryopreserved embryos. These dying cells can release toxic metabolites that can interfere with other cells and thus cause harmful effects upon embryo development or implantation. In such situations, a small hole in the zona pellucida can be created by a laser through which the dying cells or fragments can be extracted using a blunt capillary tube similar to the procedure for embryo biopsies (Rienzi et al., 2002).

Safety of laser use in clinical intervention

There were concerns about safety when the use of lasers for ART was first proposed. Since it is applied at such a delicate stage of human development, most concerns centered upon questions of DNA damage, failed embryo development and possible congenital disorders. Such concerns were primarily founded on laser wavelength, heat generation and the amount of manipulation required by fragile embryos.

The Food and Drug Administration (FDA) currently classifies lasers as class II devices, requiring them to undergo extensive measures for marketing and safety standards. The UV wavelength historically used in lasers posed significant concern, given their reputation to be potentially mutagenic to DNA. As a result, laser technology adapted to operate in the infra-red or near infra-red wavelength. Thermal damage by the laser was another cause for debate, as it could potentially heat and damage surrounding cells. To assess the effects of laser induced thermal damage on embryos, one study looked at the levels of heat-shock proteins produced in embryonic cells following laser treatment, as cells produce these proteins as a rescue mechanism when exposed to heat or environmental stress. The study investigated the effects of elevated laser pulses, higher than those applied for clinical procedures, and found that when a laser was used to create holes in the zona pellucida, there was no increase in the level of heat-shock protein produced in nearby cells. This suggested that no excessive heating of the cells occurred even using these higher energy pulses. It was therefore concluded that the lower energy pulses used in ART applications did not cause heat stress to the embryonic cells (Hartshorne et al., 2005).

Benefits of laser technology in research

Despite the rapidly advancing nature of ART, there are still many unknown factors which contribute to male and female infertility. The success rates of pregnancy and healthy delivery for IVF and ICSI rarely exceed 30% and 23% respectively (de Mouzon et al., 2010). While the evolution of laser use is contributing to the increased efficiency of ART and translating into increased clinical success rates, another exciting prospect of this technology lies in advancements that can be made in research

in the reproductive sciences. ART lasers are a powerful tool with which to help investigate and elucidate mechanisms underlying unknown or unexplained causes of infertility. Fine physiological processes in cells and organisms such as protein pathways, genetic modifications and mutations, or pathological disruptions to normal developmental biology pathways, can be investigated down to the molecular level using this technology.

In both human and animal embryo models, selective laser ablation of target tissues and cells can be used for regeneration studies. Specific cells and tissues can be purposefully injured in order to observe their repair and regeneration processes in different animal models (Otten & Abdelilah-Seyfried, 2013). In human blastocysts, embryonic cells begin to differentiate into 'trophectoderm' cells, which become the placenta, or 'inner cell mass' cells which become the developing fetus. These inner cell mass cells can be isolated and extracted using laser technology for the purpose of preparing and acquiring embryonic stem cells lines for research purposes. (Turetsky et al., 2008). These types of investigations can help to further understand the organised interactions and mechanisms involved in developmental biology to eventually help develop new treatments for infertility and reproductive pathologies.

Another exciting application of lasers in reproductive medical research is to assist in a process called 'somatic cell nuclear transfer' (SCNT), a method of reproductive cloning to create a viable embryo from an egg cell and a somatic cell (instead of sperm) for genetic research. This method, which was used to clone Dolly the sheep, is used for therapeutic and research purposes. The use of SCNT helps overcome many ethical issues concerning the use of embryonic stem cells, which naturally results in the destruction of viable embryos. The process consists of taking an egg cell and removing the nucleus, which contains the maternal genetic information, leaving a genetically 'empty' egg cell. Next, the nucleus from a regular body cell, which already has a full set of DNA from two parents, is extracted from the cell and inserted into the 'empty' egg which can then be cultured and grown into an embryo. Lasers can be used to assist in removing the nucleus from both cells in a faster and more efficient manner causing less mechanical stress on the embryo, and the process can be applied in both human models and animal models. These clones can be used to create transgenic organisms and to establish stem cell lines for research applications such as regenerative medicine and genetic reprogramming (Simerly et al., 2004).

A possible future clinical use for lasers in ART would be to facilitate nuclear transfer in the Three Parent *In Vitro* Fertilisation technique, which is used to treat women at risk of passing on mitochondrial diseases to their children. Mitochondrial disease is incurable and affects around 1 in 400 people. Symptoms include heart, liver, and kidney disease, neurological problems, blindness, and deafness (Poulton et al., 2009). Since mitochondria are only inherited from the mother, the technique involves using genetic material from a man and woman (the genetic parents) and cellular material from a third woman. The nuclear transfer process, similar to SCNT, is carried out by taking the nucleus containing maternal DNA out of the maternal egg and transferring it to a donor egg containing healthy mitochondria and which has had its original nucleus removed. Sperm can then fertilise the donor egg containing the transferred maternal DNA to create an embryo with the desired genetic parents, but free of mitochondrial disorder (Bredenoord et al., 2008). The use of lasers is likely to be employed for clinical application of nuclear transfer for three parent *in vitro* fertilisation.

Conclusion

It is clear that the continued success of ART lies in the dedication and skill of clinical embryologists and to the continued development and improvement of laboratory technologies. This is imperative to provide patients with the highest level of care and greatest chance of pregnancy. The manipulation of sperm, eggs, and embryos using lasers represents a

powerful tool to improve the efficiency and success rates of ART. Most notably, lasers are increasing the speed and efficiency of ART procedures by allowing embryologists to easily break through the zona pellucida with minimal force. This benefits embryo health by minimising mechanical strain, reducing manipulation times, and reducing the time required outside an incubator. Moreover, research in the reproductive sciences continues to benefit greatly from laser use as an investigative tool to elucidate mechanisms underlying infertility with a hope of eventually developing new treatment options.

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