Novel use of laser to assist ICSI for patients with fragile oocytes: a case report

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Abstract

An inadvertent consequence of intracytoplasmic sperm injection (ICSI) is the degeneration of some of the microinjected oocytes. Most patients may not suffer any disadvantage through losing oocyte(s) during micromanipulation; however, in some circumstances, this can result in a reduction of the chances for pregnancy. This study reports a clinical pregnancy obtained by a novel approach using laser-assisted micro-opening of the zona pellucida prior to ICSI to secure a non-traumatic micro-injection that avoids degeneration of oocytes. A total of 12 oocytes were obtained from the 36 year old patient in her third IVF treatment cycle, following two previously failed attempts where very high degeneration rates of oocytes after ICSI were recorded, together with suboptimal embryo quality. Five of the 11 matured (MII) oocytes were submitted to conventional ICSI and the other six MII oocytes first underwent laser-assisted opening of the zona pellucida (5–7 μm hole size was created with a 1.48 μm diode laser) before microinjection (LA-ICSI). Three of the five conventionally microinjected oocytes degenerated while one oocyte fertilized normally and developed to a good quality embryo. After the LA-ICSI procedure, one of the six oocytes degenerated and four oocytes fertilized normally; of these, two developed to excellent quality embryos, one to a good quality embryo and one to a poor quality embryo. The three best embryos (LA-ICSI group) were transferred to the patient on day 3. Rising serum human chorionic gonadotrophin concentrations were measured 12 days after transfer and on week 7 two implantation sites were detected, together with regular heart activity. The results of the present report suggest that laser-assisted ICSI may provide a safer approach to non-traumatic microinjection of oocytes than conventional ICSI, thereby minimizing the risk of degeneration and possibly also improving embryo quality. Therefore, it is suggested that laser-assisted ICSI might be applied in all cases associated with difficult zona pellucida penetration or/and fragile oolemma, or where patients have very few oocytes available, to improve the chances for pregnancy.

Keywords: degeneration, embryo, intracytoplasmic sperm injection, laser, oocyte, pregnancy

Introduction

Intracytoplasmic sperm injection (ICSI) has become the most widely applied assisted fertilization treatment procedure throughout the world in the last decade (Palermo et al., 1992). ICSI is highly efficient in achieving high normal fertilization rates in most circumstances, independently from oocyte and sperm factors (Van Steirteghem et al., 1993; Nagy et al., 1995a; Devroey et al., 1996). However, ICSI has also been associated with degeneration of some of the microinjected oocytes. This is partly due to technical conditions that can be improved; nevertheless, degeneration of oocytes occurs even when maximum care is exercised. This is particularly evident when the oolemma is very fragile and/or the zona pellucida is very resistant, resulting in sudden breakage of the membrane during ICSI (Nagy et al., 1995b; Palermo et al., 1996). It is also inadvisable to perform injection without breaking the oolemma, because one of the key elements in the success of ICSI is to break the membrane of the oocyte to ensure the deposition of a spermatozoon inside the ooplasm after passing the zona pellucida (Vanderzwalmen et al., 1996). This rather traumatic action usually does not harm the oocyte; however, an
average 10% of oocytes may be lost because of degeneration as a result of the procedure (Van Steirteghem et al., 1993). It has been shown in two studies (Nagy et al., 1995b; Palermo et al., 1996) that degeneration does not affect all oocytes equally, but depends on oolemna characteristics. A typical oolemna reaction associated with high degeneration rate is sudden breakage of the membrane when pushing the injection needle into the oocyte initially, or a little deeper into the oocyte before the tip of the injection needle arrives at the point of sperm deposition (Nagy et al., 1995b; Palermo et al., 1996). When many or all of the patient’s oocytes have fragile membranes, most, if not all, of the oocytes may suffer degeneration. This probably does not affect the results in most ICSI cycles, but occasionally may cause cancellation of the cycle, especially if there are very few oocytes available (Liu et al., 1995). So far, no real possibilities have existed to alleviate this kind of difficulty.

In the present study, a new approach is described that prevents traumatic injection of oocytes independent of the characteristics of the zona pellucida and the oolemma. Micro-opening of the zona pellucida was performed with a laser micro-beam just prior to ICSI, using the oocytes of a patient who in two previously failed attempts had a high rate of oocyte degeneration.

**Materials and methods**

The couple had a history of 1.5 years of primary infertility due to male factor. No significant illnesses or any medical interventions appeared in the records of the 36 year old woman. Physical and gynaecological examination of the woman did not reveal any abnormalities. Laboratory test results were also in the normal range. The 39 year old man also had an uneventful medical history. At the infertility check-up, analysis of his semen revealed poor sperm parameters as the cause of the infertility. Sperm concentration at initial investigation was 2.2 × 10⁶/µl and total motility was <20%. The proportion of spermatozoa with normal morphology was 11% according to Kruger’s criteria (Kruger et al., 1988). After a diagnosis of andrological infertility, the couple entered the ICSI programme.

Ovarian stimulation was carried out on each occasion by a desensitizing long protocol using a gonadotrophin-releasing hormone (GnRH) agonist (leuprolide acetate, Lupron; Abbott Laboratorios do Brasil Ltda, São Paulo, Brazil) and recombinant FSH (Gonal-F; Serono Laboratorios). Follicular development was monitored periodically by measuring serum oestradiol and performing vaginal ultrasound. Human chorionic gonadotrophin (HCG) 10,000 IU (Profasi; Serono Laboratorios) was administered when two follicles reached a mean diameter of 19–20 mm and oocyte retrieval was performed by ultrasound guided transvaginal aspiration 34 h later.

Cumulus–corona–oocyte complexes were aspirated in follicular fluid and sent to the adjacent laboratory, where they were identified using a dissecting microscope at ×50 magnification.

Cumulus and corona cells were removed from oocytes by incubating them for ~30 s in IVF culture medium (IVF Science Scandinavian, Gothenburg, Sweden) containing 40 IU/ml of hyaluronidase (Hyay-1 IVF; Science Scandinavian) and after pipetting in and out using a 125 µm gauge pipette, connected to a stripper (Mid Atlantic Diagnostics Inc., Medford, NJ, USA). After denudation, nuclear maturity of the oocytes was assessed under an inverted microscope at x200 magnification and the oocytes were classified as metaphase-II (MII), metaphase-I (MI) or prophase-I (germinal vesicle; GV). Cleaned oocytes were incubated for 2–4 h at 37°C in a 5% CO₂ atmosphere. Metaphase-II (MII) oocytes were microinjected within a period of 2–4 h of collection.

Semen treatment consisted of two washing steps in Sperm Rinse medium (IVF Science Scandinavian) with centrifugation for 15 min at 800 g. The pellet was kept and was resuspended in IVF-100 medium.

Conventional ICSI was performed as described earlier (Van Steirteghem et al., 1993; Nagy et al., 1995b).

Laser-assisted ICSI (LA-ICSI) was performed in the following manner. Oocytes and spermatozoa were placed into an injection dish as usual and a single but immobilized spermatozoon was aspirated into the injection pipette. Prior to microinjection, the oocyte to be injected was secured on the holding pipette in such a way that a small space was present between the inner surface of the zona pellucida and the oolemma (perivitelline space) at the 3 o’clock position (where the injection needle would penetrate into the oocyte). Using a laser beam generated by a 1.48 µm diode laser (Fertilase, MTG; Medical Technology, Aldorf, Germany), a channel with small diameter (5–6 µm) was drilled with three to five pulses (depending on the characteristics of the zona pellucida). The injection pipette was introduced through this channel and microinjection was performed as usual.

Type of membrane breakage during microinjection was also recorded as described earlier (Nagy et al., 1995b). To summarize briefly: (i) sudden breakage of the membrane (type A) was noted when the oolemma was broken during the course of pushing the injection pipette inside the ooplasm (A1 when breakage occurred at the beginning of the procedure and A2 when breakage of membrane occurred more deeply in the ooplasm); (ii) normal breakage (type B when the oolemma was broken after pushing the pipette deep inside the ooplasm and applying minimal aspiration); and (iii) difficult breakage (type C, type D and type E when strong aspiration and/or repositioning of injection needle was required to break the membrane) (Nagy et al.,1995b; Palermo et al., 1996).

After microinjection, oocytes were incubated in 1 ml of IVF-100 medium (IVF Science Scandinavian) and covered with mineral oil for 16–18 h. Oocytes were then observed for the presence or absence of pronuclei and polar bodies. Fertilization was considered normal when two clearly distinct pronuclei containing nucleoli were present together with two polar bodies in the perivitelline space.

Normally fertilized oocytes containing two pronuclei were transferred into another Petri dish containing droplets of 30 µl of IVF-100 medium for culturing until day 3. The embryos were observed at ~72 h after injection and classified according to the following criteria: (i) number of blastomeres; (ii) fragmentation; (iii) equality of the size and shape of the
blastomeres; (iv) multinucleation of blastomeres. Embryos with >50% fragmentation and/or with fewer than four cells were not transferred (day 3).

For transfer, embryos were loaded into 15 μl of IVF-100 medium always using a 23 cm Edwards Wallace catheter (Simcare Manufacturing Ltd., Hythe, Kent, UK).

The luteal phase was supplemented with daily administration of 800 mg vaginal progesterone (Utrogestan, Laboratoires Besins-Isvovesco- Paris, France). Serum β-HCG concentrations were measured 12 days after embryo transfer. Transvaginal ultrasound was performed only after the third treatment cycle when increasing β-HCG titres were observed; the presence and number of intrauterine gestational sacs were assessed, as well as the presence of a fetal pole and cardiac activity.

Results

The most important findings from the three treatment cycles are summarized in Table 1. Eleven MII oocytes were injected in the first cycle, of which 54% type A1 membrane breakage was observed (oolemma was broken at the very beginning of introducing the injection pipette). At the same time, six injected oocytes degenerated shortly after microinjection. Four oocytes fertilized normally; two developed to good quality embryos and the other two developed to poor quality embryos. No pregnancy was obtained.

In the second treatment cycle, seven MII oocytes were injected, of which three had type A1 membrane breakage and three oocytes degenerated soon after microinjection. Three oocytes fertilized normally; one developed to a good quality embryo and two developed to poor quality embryos by day 3. No pregnancy was obtained after transferring two embryos.

In the third cycle, five MII oocytes were injected in the usual way, of which three had type A1 membrane breakage; three oocytes degenerated soon after ICSI. At the same time, the other six MII oocytes gained from the same aspiration were injected after laser-assisted micro-hole drilling; one had type A1 membrane breakage and one oocyte degenerated after injection. In the conventional ICSI group, one oocyte fertilized normally and developed to a good quality embryo. In the LA-ICSI group, four oocytes fertilized normally; two developed to excellent quality embryos, one to a good quality embryo and the fourth to a poor quality embryo. Assisted hatching was performed on all embryos selected for transfer using the same laser equipment and creating an opening of 15–20 μm on the zona pellucida. An ongoing twin pregnancy was obtained, as confirmed by ultrasound detection of fetal heart activity after transferring the three best embryos from the LA-ICSI group.

Discussion

This case report demonstrates the possibility of using laser-assisted micro-hole drilling on the zona pellucida to facilitate the penetration of the ICSI injection pipette into the oocyte and to obtain non-traumatic injection. This approach appears to be important for some patients, for instance when oocytes are fragile, as in the present case.

Lasers were introduced in assisted reproduction some years ago; however, application has been restricted to manipulation

| Table 1. Results of two conventional ICSI treatment cycles and one mixed laser-assisted intracytoplasmic sperm injection (LA-ICSI) and conventional ICSI treatment cycle of the same patient. |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Retrieved cumulus–corona–oocyte complexes        | 14              | 9               | 12              |
| Injected metaphase-II oocytes                    | 11              | 7               | 5               |
| **Type of membrane breakage**                   |                 |                 |                 |
| Sudden (A1)                                     | 6               | 3               | 3               |
| Sudden (A2)                                     | 3               | 3               | 2               |
| Normal (B)                                      | 2               | 1               | 0               |
| Difficult (C/D/E)                               | 0               | 0               | 0               |
| Degenerated oocytes (%)                         | 6 (54)          | 3 (43)          | 3 (60)          |
| Normally fertilized oocytes (%)                 | 4 (36)          | 3 (43)          | 1 (20)          |
| **Embryo quality**                              |                 |                 |                 |
| Excellent                                       | 0               | 0               | 0               |
| Good                                            | 2               | 1               | 1               |
| Poor                                            | 2               | 2               | 0               |
| Transferred embryos                             | 2               | 2               | 0               |
| Fetal heart beat                                | 0               | 0               | –               |
of the zona pellucida of the embryos (Strohmer and Feichtinger, 1992), with the primary purpose to perform assisted hatching (Antinori et al., 1996). Assisted hatching of human embryos to facilitate evacuation from the zona pellucida, which may be hardened in in-vitro culture (De Vos and Van Steirteghem, 2000), was described more than a decade ago, initially using acid Tyrode’s solution (Cohen et al., 1988). So far, however, there has been no agreement that it offers any benefit (Cohen et al., 1990; Bider et al., 1997; Chao et al., 1997; Magli et al., 1998; Lanzendorf et al., 1998) in this regard.

Lasers have also been used in preimplantation genetic diagnosis (PGD), when breaching the zona pellucida is performed to remove the polar body(ies) or to remove blastomere(s) (Veiga et al., 1997; Montag et al., 1998). However, lasers are not used today in routine assisted reproductive techniques except for the above-mentioned applications, even though some early publications suggested their use to assist partial zona dissection insemination (PZD; Strohmer and Feichtinger, 1992) or even to perform subzonal insemination (SUZI) (Schutze et al., 1994) or to manipulate the spermatozoon along with an optical tweezer (Tadir et al., 1989).

Although different laser systems are available, including contact type (Strohmer and Feichtinger, 1992) and non-contact but emitting at different wave length (UV), the use of a 1.48 μm infrared diode laser system in the routine work of assisted reproduction offers a fast, efficient, and reliable way for opening of the zona pellucida to assist ICSI. This high accuracy of the laser (even in the non-contact mode) enabled us to create a channel on the zona pellucida with a very narrow opening just wide enough to permit the passage of the ICSI pipette. The diameter of the channel in the zona pellucida probably has considerable significance, since it was observed in mice (after assisted hatching) that when the diameter of the channel was not large enough, a higher proportion of hatching embryos were trapped (Malter and Cohen, 1989); this might contribute to an increased chance of monzygotic twinning (Nijss et al., 1993; da Costa et al., 2001). Therefore, in this case the thinnest possible channel was created that still permitted passage of the ICSI needle. Additionally, assisted hatching was performed on all embryos selected for transfer, creating a relatively large opening in the zona to ensure that when hatching did occur, it would happen through the larger opening.

The possible consequences of LA-ICSI in regard to cryopreservation have yet to be clarified. It has been reported that manipulations of the zona pellucida (such as SUZI, ICSI or drilling of the zona) when the result is only a small opening do not affect adversely the cryosurvival of embryos (Deppere et al., 1991; Al-Hasani et al., 1996). On the other hand, larger sized holes in the zona pellucida, as created for embryo biopsy for blastomere retrieval, are negatively associated with the outcome of embryo cryopreservation (Joris et al., 1999). At this point, it is difficult to suppose any disadvantage of the LA-ICSI technique on the cryosurvival of embryos because the size of the hole is relatively small; however, further experimental data are needed regarding practical performance.

Although total fertilization failure is not frequent after ICSI, it does still occur (Liu et al., 1995) and is often caused by the complete or nearly complete degeneration of all oocytes. This can happen also when a patient has a relatively large number of oocytes, but it is more abrupt when there are only a few oocytes available. However, a much larger number of patients experience only partial degeneration of oocytes. This may not lead directly to cancellation of the cycle but does impair the chances of pregnancy by reducing the number of surviving oocytes, thus lowering the number of zygotes and embryos and consequently reducing the possibility of selecting the best embryos for transfer. Even more, it might also be conceivable that when conventional ICSI is performed on more sensitive oocytes, not only do more oocytes degenerate, but the developing embryos may be of poorer quality as a result of the sublethal damaging force of the traumatic injection, which may affect the cytoskeleton and other structures of the oocyte. This might be an explanation for the observation in our patient that embryo quality was poorer after conventional ICSI and was better after LA-ICSI.

When analysing the three consecutive treatment cycles of the patient presented in this report, no significant difference was observed in the response of the ovary after applying the same stimulation regimen. A similar number of cumulus–corona–oocyte complexes were recovered each time with the same proportion of matured (MII) oocytes. On every occasion after conventional ICSI, a similarly high proportion of oocytes degenerated (about half of the injected oocytes). Similar to this, about half of the oocytes had a type A1 membrane breakage, signifying that the oolemma was very fragile because it was broken at the beginning of introduction of the injection pipette (Nagy et al., 1995b). Therefore, a lower number of oocytes survived and fertilized. Embryo development following conventional ICSI was relatively poor. Most embryos showed unequal size of blastomeres with much fragmentation, though high enough cell number by day 3 after conventional ICSI. On the other hand, following laser-assisted ICSI, only one oocyte had a sudden (type A1) membrane breakage, although another three still had early breakage of the oolemma. However, only one oocyte degenerated, permitting more zygotes to develop by day 3 after laser-assisted injection. It was also observed that these embryos had no or few fragmentations, an even greater contrast when compared with the embryos that developed after conventional ICSI.

In conclusion, laser-assisted ICSI seems to provide the possibility of non-traumatic microinjection of oocytes and in this way minimizes the risk of degeneration; it might also be associated with improved embryo quality.

**References**


