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Laser-assisted intracytoplasmic sperm injection

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Objective: To improve oocyte survival and fertilization rates after intracytoplasmic sperm injection (ICSI) in patients with inherent oocyte fragility.

Design: Pilot feasibility study and case report.

Setting: Private hospital.

Patient(s): Infertile couple with repeated failures of ICSI caused by oocyte degeneration.

Intervention(s): Laser-assisted drilling of the zona pellucida followed by ICSI.

Main Outcome Measure(s): Oocyte survival, fertilization, and pregnancy.

Results: In a couple with four previous ICSI failures because of poor oocyte survival (33.3%, 0%, 20%, and 18%), a fifth attempt using laser-assisted ICSI resulted in the survival of 8 oocytes out of 13 injected. Normal fertilization occurred in 5 oocytes, and a clinical pregnancy was established.

Conclusion(s): Performing ICSI through a laser-drilled hole in the zona pellucida reduces the risk of oocyte damage related to deformation during the initial phase of the microinjection procedure. This modification of ICSI appears to be suitable for patients whose oocyte show inherent fragility and high degeneration rates after the standard ICSI procedure. (Fertil Steril® 2001;76:1045–7. ©2001 by American Society for Reproductive Medicine.)

Key Words: Oocytes, human, embryos, degeneration, intracytoplasmic sperm injection (ICSI), laser-assisted drilling, zona pellucida

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After the first experience with fertilization of mammalian (hamster) oocytes by intracytoplasmic sperm injection (ICSI) was reported in 1976 (1), and the first report of fertilization and pregnancy after application of ICSI in human-assisted reproduction was published in 1992 (2); since that time, this technique has been widely used in different mammalian species with different success rates. In some species, such as the mouse, oocytes are easily damaged by the ICSI procedure; thus, special modifications, such as assistance via a piezo-driven opening of the zona pellucida (3, 4), are required to ensure acceptable success rates.

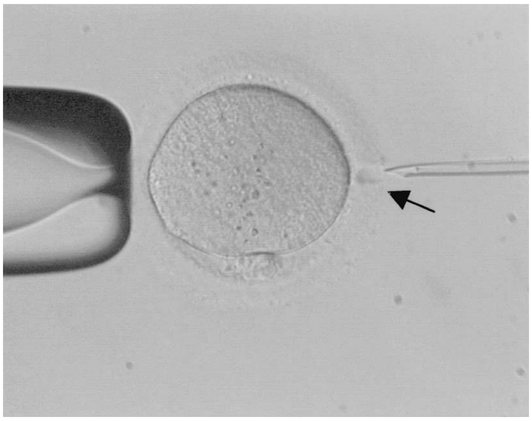
Human oocytes are generally resistant to damage caused by the ICSI procedure. However, low survival rates after ICSI have been reported for oocytes from certain individuals (5). In our assisted reproduction program we treated a couple for whom four previous ICSI failures had been characterized by a high oocyte mortality. During the last ICSI attempt (the only one performed in our center), we noted a very high resistance of the zona pellucida to penetration by the ICSI needle, resulting in a unusually extensive deformation of all oocytes in the initial phase of ICSI. We suspected that this situation was the cause of the high oocyte mortality. To avoid such deformations, our subsequent treatment attempt was performed with a modified ICSI technique: spermatozoa were injected into oocytes through preformed laser-drilled holes in the zona pellucida. This paper presents a description of the technique and the case in which it was first employed.

MATERIALS AND METHODS

Ovarian stimulation, oocyte recovery, sperm recovery, and sperm and oocyte preparation for ICSI were performed according to standard protocols. Just before ICSI each oocyte was immobilized by gentle aspiration on a holding pipette with the first polar body in the 6-o'clock position, and a hole approximately

FIGURE 1

Metaphase II oocytes with a hole of approximately 10 μm in diameter (*arrow*) created in the zona pellucida by laser equipment.



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10 μm in diameter (Fig. 1) was drilled in the zona pellucida with the use of a Fertilase 1.48 diode microsurgical laser device (Medical Technology, Montreux, Switzerland). The ICSI needle containing an immobilized spermatozoon was subsequently introduced through the laser-drilled hole in the zona pellucida into the oocyte. Because the zona pellucida, representing the main mechanical barrier to oocyte penetration, did not have to be negotiated by the ICSI needle, this modification of the ICSI procedure produced only a minimal deformation of the oocyte (Fig. 2B) as compared to oocyte penetration with standard ICSI without laser assistance (Fig. 2A). After ICSI oocytes were cultured under standard conditions, embryos developing from normally fertilized oocytes were transferred to the patient's uterus 2 days later.

CASE REPORT

A couple (36-year-old man and 47-year-old woman) presented to our center with primary male factor infertility (3 years). No apparent female pathology was detected. The couple had undergone four previous assisted reproduction attempts using ICSI, of which only the last was performed at our center. All these attempts were unsuccessful and were marked by a very low oocyte survival rate after ICSI due to difficulties with zona pellucida penetration (Table 1). No viable embryo resulted from the three first attempts. In the fourth attempt, only 2 of 11 metaphase II oocytes injected with spermatozoa survived; only one of them fertilized normally and developed into an embryo that could be transferred to the patient's uterus (see Table 1). Pictures taken during ICSI in that attempt showed an excessive deformation of oocytes, apparently due to the increased resistance of the zona pellucida to penetration by the ICSI needle (see Fig. 2B).

A new attempt was thus performed with laser-assisted ICSI. Thirteen metaphase II oocytes were available for this attempt. When the ICSI needle was introduced into the oocytes through the predrilled hole in the zona pellucida, oocyte deformation was minimal (see Fig. 2). Eight oocytes survived the procedure, and five of them were fertilized normally. Of the remaining three surviving oocytes, one failed to fertilize, and two developed three and four pronuclei, respectively. Two days after laser-assisted ICSI, three of the normally fertilized oocytes reached the four-cell stage and one reached the six-cell stage. All of these cleaving embryos showed an excellent morphology (equal-sized blastomeres and $<$10% of volume occupied by cell fragments). Four embryos were transferred to the patient's uterus 2 days after laser-assisted ICSI. Determination of serum β-hCG 12

FIGURE 2

Introduction of the injection pipette through the zona pellucida of human oocytes: (**A**), Standard intracytoplasmic sperm injection of an oocyte into a particularly hard zona pellucida. (**B**), Laser-assisted intracytoplasmic sperm injection.

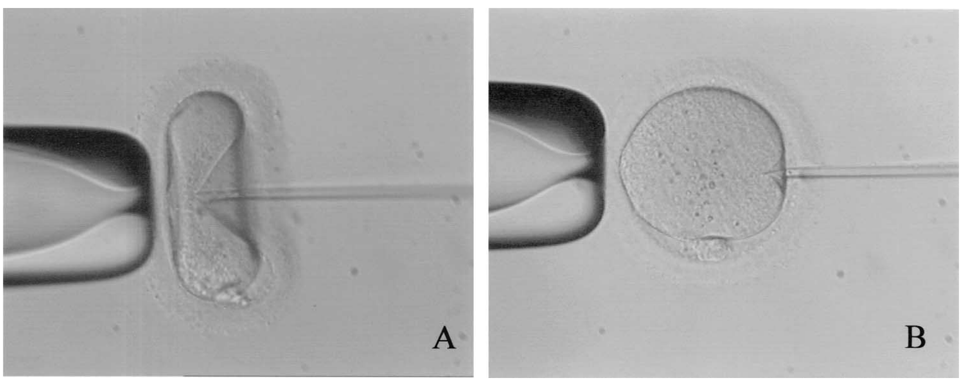

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TABLE 1

Summary of previous assisted reproduction attempts using standard ICSI procedure.

	Oocytes			Embryos			
Attempt	Total	Mature and injected	Survived	Fertilized	Cleaved	Transferred	Implanted
1	13	9	3	0	0	0	0
2	9	6	0	0	0	0	0
3	13	10	2	1	0	0	0
4	14	11	2	1	1	1	0

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days after embryo transfer signaled a pregnancy, which was subsequently confirmed by an ultrasound examination showing the presence of three embryonic sacs within the uterus. The pregnancy ended with a pre-term delivery at 33 weeks of three healthy babies, one boy and two girls, 2000 g, 1820 g, and 1890 g, respectively.

DISCUSSION

It has been shown previously that oocyte survival after ICSI is compromised by abnormal patterns of oolemma breakage during the penetration of the microinjection needle into the oocyte and that this situation is related to particular features of the ovarian stimulation response (5). Unlike mouse oocytes, in which fragile oolemma is a common finding (6), this condition usually affects only a minority of human oocytes available for ICSI in a given couple. On rare occasions, however, most or even all of the oocytes recovered for an assisted reproduction attempt show an inherent fragility, leading to degeneration and death after ICSI. In the case reported in this study, oocyte fragility was the source four previous ICSI failures. In contrast, the application of laser-assisted ICSI in the fifth attempt allowed better oocyte survival, resulting in the occurrence of normal fertilizations and the formation of viable embryos. This outcome was apparently related to the minimization of oocyte deformation during the initial phase of the microinjection procedure when the mechanical resistance of the zona pellucida did not need to be negotiated.

Oocyte survival rate after ICSI, particularly in humans, is always related to the characteristics of the oolemma, which change according to the hormonal milieu (5). Moreover, the thickness of the zona pellucida, another factor determining the ease of the ICSI procedure, is also associated with the hormonal environment and the cause of infertility (7). Therefore, there may be a possibility to manipulate the resistance of the oolemma and of the zona pellucida by adjusting ovarian stimulation and/or changing in vitro culture conditions. These possibilities should be further investigated to better understand the impact of ovarian stimulation and gamete culture on the oolemma and zona pellucida properties. However, the way ovarian stimulation or gamete culture should be modified in cases of fragile oolemma or resistant zona pellucida is unknown at present. Thus, modification of the ICSI technique is currently the only immediately available solution.

Even though deformity of the zona pellucida during ICSI can be reduced by using a sharp injection tool (8), the most efficient way of preventing oocyte damage in mouse ICSI (3) and in cases of human ICSI with fragile oolemma (9) is the use of a blunt injection needle operated by a piezo-electric device. Piezo-assisted ICSI may thus have a similar advantage to laser-assisted ICSI in a selected population of patients characterized by increased oocyte fragility. For practical reasons, however, laser-assisted ICSI will be more convenient than piezo-assisted ICSI for all those laboratories that already are equipped with a microsurgical laser device or are considering its acquisition for other purposes. In fact, performing laser-assisted ICSI widens the potential applications for this multipurpose device, which is currently being used in several other human reproductive technologies such as assisted hatching or embryo and polar body biopsy for preimplantation genetic diagnosis. The usefulness of laser-assisted ICSI in other mammalian species whose oocytes are easily damaged by the conventional ICSI procedure, such as the mouse, remains to be evaluated.

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