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SHORT REPORT

Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte

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Intracytoplasmic sperm injection (ICSI) is a promising assisted-fertilisation technique that may benefit women who have not become pregnant by in-vitro fertilisation (IVF) or subzonal insemination (SUZI) of oocytes. We have used ICSI to treat couples with infertility because of severely impaired sperm characteristics, and in whom IVF and SUZI had failed. Direct injection of a single spermatozoon into the ooplasm was done in 47 metaphase-II oocytes: 38 oocytes remained intact after injection, 31 became fertilised, and 15 embryos were replaced in utero. Four pregnancies occurred after eight treatment cycles—two singleton and one twin pregnancy, and a preclinical abortion. Two healthy boys have been delivered from the singleton pregnancies and a healthy boy and girl from the twin pregnancy.

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Assisted-fertilisation methods—eg, partial zona dissection (PZD)[1] and subzonal insemination (SUZI)[2]—have been successful in some couples with severe male-factor infertility who could not be helped by in-vitro fertilisation (IVF). Pregnancies and births have been reported, but despite use of these methods some women still do not conceive. Intracytoplasmic sperm injection (ICSI) is a promising assisted-fertilisation technique. In rabbits and cattle, embryos obtained by such injections have been transferred to recipient mothers and live offspring have resulted.[3] In three recent reports,[4–6] two-pronuclear zygotes were seen in 30 oocytes after ICSI of 143 oocytes; placement of 4 zygotes in 2 women and of 11 embryos in 7 women did not result in pregnancy. We describe successful intracytoplasmic injection into metaphase-II oocytes of single spermatozoa from men with severely impaired spermatozoa characteristics after IVF and SUZI had failed.

The four couples (A, B, C, and D aged [female/male] 37/36, 31/32, 29/31, and 34/34, respectively) treated by assisted fertilisation had had primary infertility for between 3 and 13 years due to oligoasthenoteratospermia (couples A, B, and C) or asthenoteratospermia (couple D). Couples A, B, and D had undergone a total of 8 IVF treatment cycles, during which only 1 of 103 inseminated preovulatory oocytes had become fertilised. This lack of fertilisation persisted even after increasing the numbers of motile spermatozoa added to each oocyte. Couple C was not accepted for IVF because the spermatozoa count of the male partner was too low. During our study, couples were treated by both ICSI and SUZI.

Before treatment, women were superovulated by gonadotropin-releasing-hormone agonist then stimulated with human menopausal gonadotropins and chorionic gonadotropin (HCG). Oocytes were retrieved and cultured as described previously.[7] Men were asked to produce a semen sample 1 day before and on the day of oocyte retrieval. A combination of sperm-preparation techniques was used to retain as many spermatozoa as possible. The percentage of acrosome-reacted spermatozoa in the sperm suspension was increased by incubating the spermatozoa for 24 h in T6 medium and then exposing them to an electrical field of 1250 V/cm for 2·5 ms, followed by washing and incubation in T6 medium supplemented with 3·5 mmol/l pentoxifylline.[7,8]

Shortly after oocyte retrieval, cumulus cells and corona radiata were removed by transferring oocytes into M2 medium with 1 mg/ml hyaluronidase for up to 1 min. Only intact oocytes that had extruded the first polar body were microinjected. The holding and injection pipettes were made by drawing glass capillary tubes with a pipette puller. The injection pipette was opened and sharpened by

TABLE I—SEMEN CHARACTERISTICS BEFORE AND AFTER SPERM SELECTION

Average semen characteristics	Couple			
	A	B	C	D
Initial				
Volume (ml)	5·8	1·5	2·3	4·1
Concentration (× 10^6/ml)	2·2	0·7	0·1	57
Percent progressive motility	20	13	ND	7
Percent normal morphology	20	8	ND	13
After selection				
Volume (ml)	0·2	0·2	0·2	0·2
Concentration (× 10^6/ml)	2·95	0·27	ND	3·8
Percent total motility	46	88	ND	100
Percent normal morphology	37	ND	ND	50

ND = not done—ie, too few spermatozoa to allow assessment

TABLE II—OUTCOME OF ASSISTED FERTILISATION

Couple	Cycle	Metaphase-II oocytes	Fertilised/injected oocytes		Embryos		Pregnancy
			Subzonal	Intracytoplasmic	Transferred	Frozen	
A	1	15	2/14	0/1	0	0	. .
	2	12	0/11	1/1	1	0	Yes (singleton)
B	1	17	1/16	1/1	2	0	No
	2	26	0/6	16/20	3	3	Yes (twin)
C	1	15	0/4	5/11	3	1	Yes (singleton)
D	1	10	0/8	2/2	2	0	No
	2	10	0/6	1/4	1	0	No
	3	12	0/5	5/7	3	0	Yes (miscarriage)

a micro-grinder directly after being drawn. The outer and inner diameters of the holding and injection pipettes were, respectively, 60 and 20 μm and 7 and 5 μm. The injection pipette had a bevel angle of 50° and a sharp spike to assist penetration through the oolemma. An immotile spermatozoon was aspirated tail-first into the tip of the microinjection pipette. The oocyte was held by the holding pipette and the microinjection needle was introduced across the zona pellucida. For SUZI, up to five spermatozoa were injected into the perivitelline space in a region far from the polar body. For ICSI, the micropipette was pushed through the zona pellucida and into the ooplasm. The injection pipette was withdrawn gently and the oocyte was released from the holding pipette.

16–18 h after injection, the state of fertilisation of oocytes was assessed by looking for presence of pronuclei, and 24 h later the state of embryo cleavage was recorded. If embryos had been produced, about 48 h after sperm injection, up to three embryos were placed into the uterine cavity and any extra embryos of good morphological quality were cryopreserved.[9] Pregnancy was confirmed by detecting rising serum HCG concentrations on not less than two occasions at least 10 days after embryo transfer. Clinical pregnancy was established by observing a gestational sac with echocardiographic screening at 7 weeks. A prospective follow-up of the children born after assisted conception is planned.

Semen characteristics at the time of collection and after Percoll treatment are shown in table I. Induction of the acrosome reaction was done on semen samples provided by the male partner of couples A, B, and D, but the low spermatozoa concentration of semen provided by male partner C prevented induction.

From a total of 139 oocyte-cumulus complexes, 4 germinal-vesicle-bearing, 9 metaphase-I, and 117 metaphase-II oocytes were retrieved after cumulus removal. Only 3 of 70 (4·3%) oocytes became fertilised after SUZI (table II). 81% (38 of 47) of oocytes survived the injection of sperm into the ooplasm. 31 of 47 (66%) oocytes were fertilised after ICSI. 18 of these fertilised oocytes developed into embryos that were of a good enough quality to be transferred. Embryo replacement was done in seven of eight assisted-fertilisation cycles, resulting in four pregnancies (table II).

The pregnancy and preclinical abortion in patient D was shown by increasing serum HCG concentrations during days 11 to 22 after transfer followed by a decrease in serum HCG. The four fetal karyotypes obtained by amniocentesis at 16 weeks' gestation were normal (three 46, XY, one 46, XX). Patient A delivered a healthy boy vaginally at 40 weeks' gestation after an uneventful pregnancy. Patient B delivered a healthy boy and girl vaginally at 38 weeks' gestation, and patient C delivered a healthy boy vaginally at 38 weeks' gestation.

We report successful use of ICSI of oocytes to achieve pregnancy in four women who had not benefitted from IVF and SUZI treatment. Previously, we treated 56 women by SUZI and ICSI—187 oocytes underwent ICSI, 160 remained intact after injection, 102 were fertilised, and 13 embryo transfers were done; no pregnancies were obtained.

ICSI bypasses many steps in the fertilisation process—ie, the spermatozoon's binding to and penetration into the zona pellucida and its fusion with the oolemma. ICSI may allow fertilisation by spermatozoa with deficient kinetic properties or anomalies of the acrosome. Lanzendorf et al[4] showed that human oocytes are capable of surviving the mechanical insertion of a spermatozoon directly into the ooplasm. However, mechanical damage to the oocyte may still occur, the likelihood of which may be influenced by the characteristics of the injection pipette or of the micromanipulation technique. Further studies are necessary to confirm the high survival and fertilisation rates obtained after ICSI in 47 oocytes. Mechanical damage to the ooplasmic structures by the injection procedures may also influence development of the fertilised oocytes into embryos that can be transferred or frozen. In this study, 65% (20/31) of fertilised oocytes cleaved to good-quality embryos.

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