THE LATEST ARMAMENROTIUM IN INFERTILITY MANAGEMENT-INTRA CYTOPLASMIC SPERM INJECTION (ICSI)

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SUMMARY

During July, 1994 to August 1995, 40 infertile couples (Phase I-19 and Phase II-21) were included in our micro-manipulation programme for assisted fertilisation to treat mainly male factor infertility and cases where previous attempts of IVF had failed. In Phase-I, 96 metaphase-II oocytes were micro-injected with single spermatozoon where 25 oocytes (31.6%) were fertilised of which 14 (56%) had normal cleavage.11 patients had embryo transfer where 3 had zygote transfer through fallopian tube (ZIFT). One of these three ZIFT procedures conceived and continued to term giving birth to a healthy female baby on 30.4.1995. To the best of our knowledge this is the first ICSI-ZIFT term delivery to be reported from India. In the Phase-II of study, out of 124 metaphase II oocytes micro-injected, 73 fertilised and in 16 patients at least one embryo was transferred and 4 pregnancies were achieved (25%). 3 pregnancies at 19, 27 and 12 weeks of gestation are continuing uneventfully. We conclude that ICSI is an efficient technique to treat male factor infertility as well as cases where an intrinsic oocyte factor probably inhibits fertilisation with conventional IVF.

INTRODUCTION

Micro manipulation of living cells was practised to inoculate them nearly nine decades ago. Chang and Bedford (1962) first reported that hyaluronidase enzyme treated rabit eggs were still fertilisable.

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Lanzendorf et al (1988) showed that human oocytes are capable of surviving the mechanical insertion of spermatozoa directly to the ooplasm. Currently Subzonal insemination (SUZI), (Ng. Sc et al 1988) and Intra Cytoplasmic Sperm Injection (ICSI) (Palermo 1992) are practiced with reasonable rate of normal fertilisation, subsequent cleavage and pregnancy. Steirthghem et al (1993), have reported higher success rate by ICSI than SUZI in a series of 300 treatment cycles. We started micromanipulation along with our existing IVF programme and chose ICSI to treat several varieties of male infertility as well as cases of failed fertilisation in previous IVF attempts in otherwise normal subjects.

MATERIALS AND METHODS

19 couples in Phase I (July, 1994 - November, 1994), and 21 couples during Phase II (February, 1995 - August, 1995), were included for micro manipulation procedure (ICSI), 16 out of 19 males in Phase I (Table I) and 17 out of 21 in Phase II (Table II) had varying degrees of semen defects. The rest of the male partners (Phase I & II) had normal semen parameters and here indication for ICSI was total failure of fertilisation in previous attempts of IVF.

OVARIAN STIMULATION PROTOCOL & OOCYTE RECOVERY

In short long GnRH analogue (Suprefact, Hoechst) down regulation followed by

Table I
(n = 16) (PHASE I)

No.	Parameters	Mean	Range
1.	Total Count per ml. ($X\ 10^6$)	5.6	3.1 - 8.1
2.	Motility (%)	12.2	8 - 18.0
3.	Normal Morphology (%)	16.3	14 - 24

Table II
(n = 17) (PHASE II)

No.	Parameters	Mean	Range
1.	Total Count per ml. ($X\ 10^6$)	5.2	2.9 - 7.6
2.	Motility (%)	11.9	7.2 - 19.1
3.	Normal Morphology (%)	17.1	4.0 - 24

stimulation by Gonadotropins (hMG, Humegon, Infar India Ltd.) was done. Combined pelvic vaginal sonography and serum estradiol levels were used to monitor follicular maturation. Ovulation was finally induced with 10,000 I.U. of hCG when at least 3 follicles measured transverse diameter of 18mm or more. Oocytes were recovered transvaginally 36 hrs. following hCG administration.

PREPARATION OF OOCYTES FOR ICSI

Soon after recovery, the oocytes were graded for maturity as described by Veek (1986). The cumulus and corona cells surrounding the oocytes were removed by incubation of oocytes for about 30 sec. in Hepes Buffered Earle's medium with 80 IU. hyaluronidase per ml. (Type VIII Sigma Chemical Co., St. Louis, USA) and subsequent flushing of the oocyte complexes in and out of hand drawn glass pipette with an opening of about 200 microns. Approximately after 30 seconds of this treatment, the oocytes, free of cumulus corona cells, were washed twice in Earle's medium and then placed into droplets of equibrated B2 medium (INRA MENZO B2 Medium Cat. No. ZA 127) under paraffin oil (International Medical, Brussels_ and incubated for 3-4 hours and subsequently examined to observe the first polar body in the perivitelline space. Oocytes with first polar body were used in ICSI procedure.

SPERM PREPARATION & PVP TREATMENT

Liquified semen samples were analysed using a Makler Chamber (SEFI MEDICAL, Isreal). To maximise the number of motile sperms specially in cases of low sperm density and motility, different combinations of sperm preparation procedures were adopted like the swim up procedure as described by Kerin et al (1984) and by mini-percoll technique as described by Ord et al, (1990). From the final suspension a sperm concentration of 1 million/ml. was prepared and incubated. To make the sperm immobile, sperm fraction was added to approximately 4 to 5 micro litres of Polyvinyl Pyrolidone (PVP) solution in Hepes buffered Earle's medium (W/V) filtered through 0.8 micron millipore filter as described by Steirtegham et al (1993). Five micro-litre of sperm PVP droplet was prepared and incubated under oil. The same petri dish contained 3-5 oocytes in Metaphase II in Hepis buffered Earle's medium and is ready for ICSI.

EGG HOLDING AND ICSI PIPETTES AND BASIC ST UP FOR ICSI

Commercially available micro-pipettes, both holding (Inner diameter, ID, 18.2 microns, Outer diameter, OD, 20.0 microns) and injection pipettes (ID 5.7 microns and O.D. 6 microns Embryo Tech Lab Inc., M.A., USA) was used. They were fitted with the tool holder attached with the micro manipulation system accompanied by teflon tubing to a microinjector (IM 188 Narishige, Japan). Mineral oil was used to fill the teflon tube and the micro injector (either negative suction to hold the oocyte or positive suction for injection of sperm) was controlled via 1 microlitre resolution, vernier micro meter using the syringe attached to the teflon tubing. Using the right sided joystick hydraulic remote control manipulator (MO 202, Narishige, Japan) the fine movements of the injection pipette was performed (Figure. 1). A Nikon

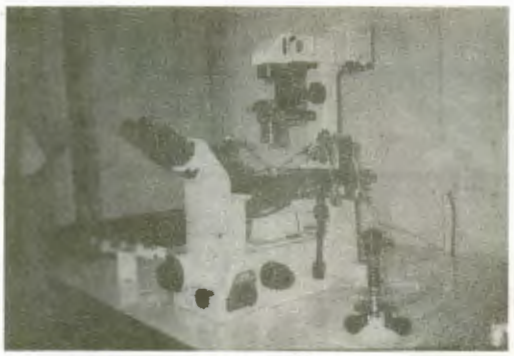

Fig. 1. Inverted microscope and micromanipulator for ICSI

inverted phase contrast microscope (Diaphot 300) fitted with joy stick manipulator (MN 151, Narishige, Japan) was used. This was fitted with "Sony CCTV" colour camera and a T.V. monitor.

TECHNIQUE OF ICSI

Using right sided joy stick hydraulic manipulator (MO-202) and injection micro-pippete, a single almost immotile spermatozoon was selected and aspirated from central sperm PVP droplet in the injection pipette. The tail of the sperm was drawn first followed by slow suction of this sperm completely within the lumen of the ICSI needle. The oocyte was immobilised using left sided joy stick manipulator (MN 151 Narishige, Japan) with egg holding pipette so that the polar body is at 12 o'clock or 6 o' clock position. The micro-injection pipette containing a single sperm was mobilised towards the oocyte. Intracytoplasmic sperm injection performed with a very small amount of medium and the injection pipette drawn out gently. The oocyte was then examined for its intactness and negative suction slowly released to free the oocyte within the droplet. Similar technique was followed in remaining metaphase-II oocytes for microinjection. Micro-injected oocytes were transferred to droplets of B2 medium for further culture. Oocytes were examined for presence of two distinct pronuclei confirming normal fertilisation, 14 to 16 hours following ICSI.

EMBRYO TRANSFER

A maximum of three morphologically normal embryos were transferred via cervical OS to the uterine fundus using Gynetics embryo transfer catheter (cat. no. 4219 set, Contech Devices, India). In four cases, (3 in Phase I and 1 in Phase II) two cell stage zygotes, were transferred to the fallopian tube (ZIFT) under laparoscopic guidance. Luteal phase support was provided by 50 mgm of progesterone in oil (Gestone, U.S.A.) injection intramuscularly from the day of transfer. Pregnancy was confirmed by serum BhCr 10 days following transfer. Transvaginal ultrasonographic recording of fetal cardiac activity was performed around six weeks of gestation.

RESULTS PHASE - I (TABLE II)

In 1994 (5 months) 105 Oocytes were retrieved and after the removal of the cumulus corona, 3 oocytes were found to be germinal-vesicle bearing (2.9%) and 6 oocytes were found to be of metaphase I (5.7%). A total of 96 metaphase II oocytes were subjected to ICSI procedure (Fig. 2). Following ICSI,

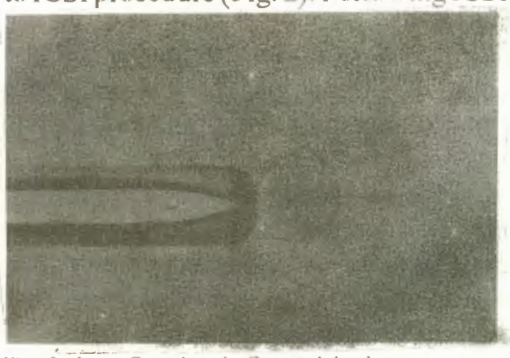

Fig. 2. Intra Cytoplasmic Sperm injection.

Table 2

	Phase I		Phase II	
	n	%	n	%
No. of Oocytes retrieved	105		137	
No. of Oocytes micro-injected	96		124	
No. of intact Oocytes follwing ICSI	79	82.2	112	90.3
Fertilisation Rate	25	31.6	73	65.2
Cleaving Embryos	14	56.0	62	84.9
No. of Patients who had at least one embryo transferred	11	58.0	16	76.2
No. of Pregnancies	2		4	
Pregnancy rate (per transfer)		18.2		25.0
Early abortion	1		1	
Ingoing pregnancies			3*	
Term Delivery	1 (Following ICSI-ZIFT, Female baby Weighing 2,800 Kg.)		*(19, 27 and 12 weeks of gestation)	

79 injected oocytes remained intact (82.2%), of which 25 oocytes showed normal fertilisation with two distinct pronucleii (31.6%), 56% (i.e. 14) of the pronucleated eggs cleaved normally to the 2-4 cell stage and were used for embryo transfer. 11 patients underwent embryo transfer (8 uterine transfer and 3 ZIFT) giving a successful transfer rate of 58%, of patients attempted in Phase I. Out of 11 patients who had undergone embryo transfer, two pregnancies were achieved (18.2%). Of these two pregnancies, one pregnancy was achieved following ZIFT and a female baby, weighing 2 Kg. 800 gms was born at term on 30th april, 1995 by LSCS. In this couple male factor was the indication for ICSI.

Though successful ICSI programme has been earlier reported from Jaslok Hospital Group (Parikh et al, 1994) Bombay, to the best of our knowledge, ours is the first ICSI-ZIFT delivery to be reported from India.

PHASE II (Table II)

During the period from February, 1995 to August, 1995 137 oocytes were retrieved and after the removal of the cumulus corona, 4 oocytes were found to be germinal-vesicle bearing (2.9%) and 9 occytes were found to be of metaphase I (6.6%). A total of 124 metaphase II oocytes were subjected to ICSI procedure. Following ICSI, 112 injected oocytes remained intact (90.3%) of which 73 fertilised showing two distinct

pronucleii (65.2%), 84.9% (i.e. 62) prouncleated eggs cleaved normally were used for embryo transfer in 16 patients (15 within uterine transfer and ZIFT in one case) giving a successful transfer rate of 76.2% of patients attempted in Phase II. Out of 16 embryo transfer, four pregnancies were achieved (25.0%). At the time of this report there are 3 ongoing pregnancies at 19,27 and 12 weeks of gestation. One patient aborted during 8th Week of gestation in phase II.

DISCUSSION

The fertilisation rate in phase II was 65.2% compared to 31.6% in phase I. The percentage of cleaving embryos was much improved in phase II, i.e. 84.9% compared to 56% in phase I. We think that with increasing experience further refinement of this technique is possible. For example, while mocroinjection procedure per oocyte took approximately 4 to 6 minutes in the first phase, currently this timing has been reduced to nearly 2-3 minutes.

Since the first report (Palermo et al 1992) describing successful "Intra Cytoplasmic Sperm Injection" giving rise to full term delivery in otherwise infertile couples having extreme degree of male defects, ICSI has become the most popular amongstall micro manipulation techniques. Until recently, these patients were either treated by artificial insemination by donor semen or adoption was offered as the only alternative. Recently Brussels group have reported 2846 ICSI cycles showing that two third of successfully injected oocytes were normally fertilised and 70% of the pronucleated oocytes developed into embryos for transfer; resulting in 600 children born after this novel technique (Steriteghen et al 1995). Their study does not reveal a significant increase in major congenital anomalies. Pregnancy has also been reported by spermatozoa having no motility as in 'Kartezener Syndrome" (immotile cilia syndrome) and in cases of congenital absence of Vas (Levran et al, 93).

The other two promising areas of application of micro manipulation techniques appear to be, (a) assisted hatching (Cohen, 1993) and (b) blastomere biopsy for genetic counselling (Kontogianni et al 1993) using polymerised chain reaction (PCR).

CONCLUSION

Our initial results indicated reasonably good prospect of ICSI in the treatment of severe male factor infertility or where conventional IVF technique has failed. At present, it is not clarified as to what extent micro-manipulation will result in fertilisation by genetically defective spermatozoa since in ICSI, many steps of fertilisation are bypassed which could presumably restrict fertilisation by abnormal sperm. However, genetic aberrations will undoubtedly inhibit development beyond the pre-implantation stage and possibilities of abnormal conceptions are limited. With more number of centres throughout the world practising ICSI, the resulting off-springs will hopefully confirm its long term safety in near future. Finally, introduction of pre-transfer genetic counselling using the novel technique 'Blastomere Biopsy' appears to open a new horizon in the field of reproductive medicine in the near future.

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