Intracytoplasmic sperm injection: a state of the art technique

Ragaa Mansour

The Egyptian IVF-ET Center, 3–B Rd 161, Hadaek El-Maadi, Maadi, Cairo 11431, Egypt

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Of the micromanipulation techniques developed in the twentieth century, intracytoplasmic sperm injection (ICSI) has been the major breakthrough in the field of assisted fertilization. This article reviews the indications for the use of ICSI, its clinical application, the establishment of an ICSI programme including protocol and the results obtained since the introduction of ICSI and the potential risks. In addition, intracytoplasmic spermatid injection is briefly discussed.

Key words: intracytoplasmic sperm injection/in-vitro fertilization

Introduction

This year is the twentieth anniversary of the first human baby resulting from in-vitro fertilization (IVF) and embryo transfer (Steptoe and Edwards, 1978). During the past 20 years, many evolutionary changes have occurred in ovulation induction, the shift of oocyte retrieval from the laparoscopic to the transvaginal route, oocyte and embryo culture techniques, the cryopreservation of embryos, and the technique of embryo transfer. A notable event in the field of assisted reproduction has been the development of different techniques to assist fertilization.

Working with human gametes and IVF for the past 15 years, no technique has fascinated me more than intracytoplasmic sperm injection (ICSI; Palermo et al., 1992), which was a real breakthrough in the field of assisted fertilization, especially with regard to the problem of male factor infertility. Moreover, it brought the embryologist into closer contact with the oocyte and the spermatozoon, as each gamete is manipulated individually. It also gave the embryologist the confidence to work within the cytoplasm of the oocyte, which is not necessary during conventional IVF work. This has opened the door to a wide field of research on the oocyte. This article is a review of ICSI with special emphasis on indications for its use, its technique, and results.

History

The advent of IVF–embryo transfer enabled patients who were irremediably infertile due to absolute tubal factor to conceive (Edwards et al., 1980). However, a considerable number of patients remained unable to achieve pregnancy through IVF mainly due to failure of fertilization. Therefore, various micromanipulation techniques have been developed to assist fertilization. These techniques originated from a pre-existing technology of micromanipulation that was developed for various purposes other than assisted fertilization.

From the beginning of the twentieth century scientists have been trying to develop various techniques for direct manipulation of living cells under the microscope. In 1911, Kite discussed a nuclear dissection experiment in which he used micro-needles to separate the pronuclei in newly fertilized zygotes (Chambers, 1940). In 1928, Emmerson described the first micromanipulator with a joystick design that could directly transmit the movements required by the operator (El Badry, 1963). In 1934, de Fonbrune described hydraulic principles that could be used for the joystick manipulators. de Fonbrune (1934) also developed the first microforge and microtool-making techniques that are still in use today. Micromanipulation techniques have gradually developed over the century and have reached such a precision that skilled scientists are able to
microdissect one single chromosome. The micromanipulation techniques that were developed to assist fertilization include partial zona dissection (PZD), subzonal sperm injection (SUZI) and intracytoplasmic sperm injection (ICSI).

The principal breakthrough in the field of assisted fertilization was ICSI. Interestingly enough, this technique was developed as early as 1966 for experiments with non-mammalian gametes. At that time, it was not performed for the purpose of assisted fertilization, but to prove that sperm nuclear decondensation and male pronuclear formation did not require prior interaction between the spermatozoon and the oocyte membranes (Hiramoto, 1966). Several experiments followed, and improvements in microinjection using a rabbit model not only led to pronuclear formation following ICSI-induced fertilization, but also resulted in further embryonic cleavage and the birth of normal, live offspring (Hosoi et al., 1988; Iritani, 1989). Direct ICSI to human oocytes was first applied by Lanzendorf et al. (1988a). Their experiments demonstrated that human oocytes were capable of surviving microinjection and were subsequently able to support the formation of male and female pronuclei. Finally, the report by Palermo et al. (1992) of the first human pregnancies following ICSI represented a major breakthrough in the field of assisted fertilization.

Clinical application of ICSI

ICSI has been widely and successfully used to treat infertility due to severe oligoasthenozoospermia (Palermo et al., 1993; Van Steirteghem et al., 1993b; Payne et al., 1994; Redgment et al., 1994; Mansour et al., 1995). It enabled men with very few living spermatozoa (even occult azoospermia) to achieve fertilization and parenthood, and it has proven to be the most efficient treatment for male factor infertility (Van Steirteghem et al., 1993b; Palermo et al., 1995).

The use of ICSI in patients with borderline semen was studied by Aboulghar et al. (1995). A significantly higher fertilization rate was achieved with ICSI than with conventional IVF using sibling oocytes and spermatozoa from patients with borderline semen or suspected male factor infertility. The study found that 45% of patients with borderline semen would have lost their chance of embryo transfer because of total fertilization failure had ICSI not been performed on some sibling oocytes. Use of ICSI in patients with suspected male factor infertility was also previously recommended by Payne et al. (1994).

Unexplained infertility treated with conventional IVF may result in total failure of fertilization. Aboulghar et al. (1996a) achieved a significantly higher fertilization rate using ICSI, compared to conventional IVF, with sibling oocytes in cases of unexplained infertility. Their study showed that ~23% of patients with unexplained infertility would have had total fertilization failure if ICSI had not been performed in some sibling oocytes.

In cases of previous total failure of fertilization with conventional IVF, the first line of treatment is ICSI. It has been shown that a couple who fails to achieve fertilization following a single IVF treatment has a <25% chance of fertilization when conventional IVF is attempted again (Cohen et al., 1994). In contrast, when Palermo et al. (1993) performed ICSI for 38 couples with previous total failure of fertilization in conventional IVF, they achieved a high pregnancy rate. Also, one study (Lundin et al., 1996) reported that ICSI can be applied on 1-day-old oocytes in cases of fertilization failure after IVF, leading to two pregnancies and deliveries.

ICSI has also proved to be of value in the treatment of patients with male immunological infertility (Nagy et al., 1995a) or with acrosomeless spermatozoa (Lanzendorf et al., 1988b; Lundin et al., 1994; Bourne et al., 1995a). Although pregnancy and birth have been reported following fertilization with totally immotile spermatozoa (Stulf et al., 1995; Kahraman et al., 1996a; Nijs et al., 1996), the fertilization rate is very low. Microinjection of completely immotile spermatozoa is an adverse prognostic factor (Nagy et al., 1995c). The use of hypo-osmotic tests (Casper et al., 1996) or fertilization with testicular spermatozoa (Tournaye et al., 1996) have been applied to this problem.

The introduction of ICSI has made fertilization efficient and precise, to the extent that a very low number of spermatozoa, regardless of their motility pattern, is required to inject all the oocytes retrieved for the procedure. Therefore, ICSI is the technique of choice in cases of obstructive azoospermia since it enables the best use of microsurgically retrieved spermatozoa from the epididymis or testis (Schoysman et al., 1993a,b; Silber et al., 1994, 1995a; Tournaye et al., 1994; Mansour et al., 1996a).

Microsurgical epididymal sperm aspiration (MESA) was the method first described for surgical retrieval of spermatozoa in cases of obstructive azoospermia (Temple-Smith et al., 1985). High fertilization and pregnancy rates have been reported after the combination of MESA and ICSI (Silber et al., 1994; Tournaye et al., 1994; Hovatta et al., 1995; Mansour et al., 1996a). However, the technique is lengthy, requires general anaesthesia, special skills and equipment, and results in a certain amount of trauma and postoperative morbidity. For these reasons, percutaneous epididymal sperm aspiration
(PESA) was introduced (Craft et al., 1995). Compared to MESA, this procedure is very simple and can be performed under local anaesthesia. However, the necessary blind puncturing of the delicate and very vascular epididymis has been strongly criticized as a potentially mutilating procedure (Schlegel et al., 1994). In cases of congenital absence of the vas deferens and in some cases of acquired obstruction, PESA can be attempted (Craft et al., 1995; Collins et al., 1996; Tsirigotis et al., 1996; Mansour et al., 1997a), or a modified PESA technique (Cha et al., 1997).

When the epididymis is absent or fibrosed, a testicular biopsy can be performed. Until recently, the fertilizing capacity of testicular spermatozoa was unexplored. However, it was reported in 1993 that the use of testicular spermatozoa in ICSI for cases of obstructive azoospermia could achieve fertilization and pregnancy (Craft et al., 1993; Schoysman et al., 1993a,b). Following that, other different programmes reported the use of testicular spermatozoa with ICSI (Devroey et al., 1994; Bourne et al., 1995b; Craft and Tsirigotis, 1995; Nagy et al., 1995b; Silber et al., 1995a; Fahmy et al., 1996; Mansour et al., 1996a). Recently, the indications for testicular sperm extraction (TESE) and ICSI have been expanded to include cases with non-obstructive azoospermia due to severe impairment of spermatogenesis (Devroey et al., 1995; Tournaye et al., 1995; Gil-Salom et al., 1995; Kahraman et al., 1996b; Silber et al., 1996; Mansour et al. 1997a; Tournaye et al., 1997).

Because sperm retrieval from either the epididymis or the testis is a complex procedure, it is important to cryopreserve supernumerary spermatozoa for future use. Pregnancies were first reported from the use of cryo-thawed epididymal sperm by Lacham-Kaplan and Trounson (1994). Other cases of successful ICSI using cryo-thawed epididymal spermatozoa have also been reported recently (Nagy et al., 1995b; Kamal et al., 1997). However, freezing of testicular spermatozoa is generally difficult because of the low number of spermatozoa and the very poor motility (Silber et al., 1994). Methods of testicular recovery, processing and cryopreservation have been refined and successful fertilization and pregnancies have resulted from the use of cryo-thawed testicular spermatozoa with ICSI (Nagy et al., 1995b; Gil-Salom et al., 1996; Podsiadly et al., 1996; Kamal et al., 1997). Recently, it has been reported that a low number of spermatozoa can be successfully recovered after thawing by cryopreserving them inside an empty zona pellucida (Cohen et al., 1997).

In cases of failure of semen collection on the day of ovum retrieval and when no cryopreserved semen sample is available, testicular sperm aspiration and ICSI is a possible solution (Watkins et al., 1996).

ICSI has also been used to fertilize cryo-thawed mature oocytes. Gook et al. (1995) and Kazem et al. (1996) suggested that fertilization and cleavage rates of cryo-thawed oocytes can be enhanced significantly using ICSI. Also, their data suggest that the cryopreservation technique can affect the zona pellucida deleteriously, hindering normal sperm attachment and penetration, and hence ICSI is recommended.

In-vitro maturation of immature oocytes was recently applied clinically and it was found that ICSI was the technique of choice to achieve fertilization of these oocytes (Cha et al., 1996; Russell et al., 1996; Jones and Trounson, 1997).

Another possible indication for the use of ICSI may be abnormal oocytes. The technique of ICSI has revealed an under-estimated ‘oocyte factor’ as a cause of infertility. Complete removal of cumulus–corona cells from around the oocyte and its close observation under ×400 magnification during the ICSI procedure allow a better estimation of oocyte quality. In a review of 1000 ICSI cycles, it was found that ~6.6% of all cycles were diagnosed to have abnormal morphology of all retrieved oocytes, representing 5.7% of all oocytes injected (Mansour et al., 1997b). It was observed that some oocytes were morphologically mature but had a structurally abnormal zona pellucida: either the sperm receptor was defective or the zona matrix was hindering sperm passage (Cohen, 1992). High fertilization and pregnancy rates were achieved by ICSI in patients with persistent failure of IVF–embryo transfer associated with disordered zona pellucida-induced acrosome reaction (Liu et al., 1997). However, oocytes with abnormalities in the cytoplasm may not benefit from the technique of ICSI: a study of ICSI in dysmorphic oocytes (Alikani et al., 1995) concluded that aberrations in the morphology of human oocytes (most probably a product of controlled ovarian stimulation) were of little or no consequence to fertilization or early cleavage after ICSI, but it is possible that these embryos had reduced potential for implantation and future development (Alikani et al., 1995).

It has even been suggested that ICSI should completely replace conventional IVF in the future, as pregnancy rates will be improved if normal spermatozoa are injected. However, it is currently recommended that, until more data are available, ICSI should be restricted to cases of male factor infertility (Hamberger et al., 1995). To investigate the results of conventional IVF versus ICSI using normal semen, Aboulghar et al. (1996b) carried out a prospective randomized study on 116 infertile patients with tubal factor infertility (and normal semen) that were randomly divided to receive ICSI or IVF. The results demonstrated that ICSI does not achieve a higher pregnancy rate compared with IVF in the treatment of cases of tubal factor infertility with
normal semen. ICSI is a technique which is more expensive and time consuming, requires more equipment, extra skills, and is also an invasive procedure.

**Establishing a laboratory for ICSI**

An already existing successful IVF programme is a prerequisite for the success of any ICSI programme, as all the equipment, environmental requirements and skills in gamete handling that are needed for oocyte and embryo culture *in vitro* are essential to the establishment of an ICSI programme. H.D. Schmidt (1859) made a highly relevant comment on the use of his microscopic dissector (a very early version of a micromanipulator): ‘To manage the instrument successfully, delicacy of touch and a great deal of patience are required; but it is only the latter, combined with perseverance, energy, and close observations that scientific facts have, or ever will be established’.

The equipment required for ICSI comprises an inverted phase microscope and a micromanipulation set. Microscopes used for gamete micromanipulation should be equipped with ×10, ×20 and ×40 objectives, and for precise procedures such as ICSI, ×400 magnification is required. A steady stage is essential for micromanipulation, rendering inverted microscopes the most suitable. Also, the microscope should have a long working distance condenser. In general, the microscope used for micromanipulation should be of the highest available quality. The pseudo-three-dimensional image provided by the Normarski or Hoffman optical systems offers special image enhancement that is of great value in these intricate procedures (Malter, 1992). The Normarski system, designed for an optical path through glass only, does not produce clear images when plastic containers are used. The Hoffman system, however, is designed to compensate for the effect of plastic.

Most of the micromanipulation systems currently in use today combine some of the best design elements from previous micromanipulators. Based on de Fonbrune’s system, three hydraulic cylinders can achieve simultaneous three-dimensional remote-controlled positioning, and the convenient three-dimensional control of the joystick is combined with the precise linear control of a separate single-axis unit. The hydraulic drive is mounted on separate micromanipulators that provide three-dimensional coarse positioning. There are two options for coarse positioning, the first being a simple mechanical device and the second an electric joystick remote-control to drive high-precision electric motors. This provides a very convenient and versatile system in which all the necessary controls are at the operator’s fingertips (Malter, 1992).

The basic design for the microinjection unit has changed very little since Chamber’s original device (Chambers and Chambers, 1961), which involves the use of airtight glass syringes under the control of different microdrives. The performance may be enhanced by substituting high-quality syringes that are completely airtight. For connection to the micropipette holder, flexible plastic tubing with airtight fitting is required.

It is important to fit the microscope stage with a form of temperature control. Ambient temperature is of vital importance for the survival of the oocyte, which should not be exposed to temperature change for even a brief period during micromanipulation.

Microtools are very sensitive to ambient vibration, and therefore a heavy balance table is required. Bolting the table to the floor or wall, or using massive support, such as a granite balance table, may be sufficient.

Several companies manufacture equipment for fabrication of microtools (micropipettes for injection and holding), making work much easier and saving a lot of effort and time.

**Table I. Overall intracytoplasmic sperm injection results (from Tarlatzis, 1996, and de Mouzon and Lancaster, 1997)**

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cycles</th>
<th>Pregnancy rate per retrieval (%)</th>
<th>Take-home baby rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>3 157</td>
<td>23.6</td>
<td>–</td>
</tr>
<tr>
<td>1994</td>
<td>12 586</td>
<td>21.8</td>
<td>–</td>
</tr>
<tr>
<td>1995</td>
<td>47 654</td>
<td>21.7</td>
<td>15.9</td>
</tr>
</tbody>
</table>

**The technique of ICSI**

**Preparation of the oocytes**

To enable retrieval of sufficiently mature oocytes, an interval of 36 h between human chorionic gonadotrophin administration and ovum retrieval has been shown to be optimum (Mansour *et al.*, 1994). For better visualization and precise intracytoplasmic injection of the oocyte, the surrounding cumulus granulosa cells must be removed. Usually, this is done ~3 h after oocyte retrieval. The oocyte cumulus–corona complex is placed in 80 mIU/ml hyaluronidase in HEPES-buffered tissue culture medium for a maximum of 10–15 s, during which it is repeatedly pipetted in a standard pipette. The oocytes are then transferred to tissue culture medium for complete removal of the corona cells by repeated aspiration in a finely pulled pipette. The oocytes are then rinsed and incubated till the time of microinjection and those that have extruded their first polar body are chosen for the injection.
Sperm processing

Ejaculated semen is processed for ICSI using different techniques. Samples that are not severely oligoasthenozoospermic are usually processed with a simple washing and swim-up technique. A method based on the migration sedimentation phenomenon of spermatozoa, using a Tea tube (Lucena et al., 1989), has also been used successfully. Severe oligoasthenozoospermic samples have to be handled with care, and a second semen sample should be obtained where possible in most cases of severe oligoasthenozoospermia. In our experience, even very poor samples can be processed using a Tea tube, and the few motile spermatozoa can be collected by aspirating a microdroplet from the bottom of the central cone if sufficient time (4–5 h) is available (Mansour et al., 1995). If no motile spermatozoa are found in the central cone, a microdroplet from the resuspended pellet is placed in the injection dish near the polyvinylpyrrolidone (PVP) droplet and motile spermatozoa are transferred with the injecting pipette to the PVP droplet. Similarly, 1–2 µl of the resuspended pellet could be placed with care inside the PVP droplet at the periphery (at the side where the holding pipette goes) without producing turbulence. The debris and immotile spermatozoa will remain in place while the motile spermatozoa will spread through the PVP droplet and can be picked up easily with the injecting pipette. Fujii et al. (1997) have described another simple method for recovering motile spermatozoa from extremely low quality sperm samples, in which the sperm pellet is deposited into a 3% PVP solution, allowing the motile sperm to swim out, away from the debris and immotile spermatozoa. We have avoided the use of Percoll for semen processing, hence avoiding injection of silica particles inside the oocytes. However, the majority of ICSI centres use Percoll for sperm processing.

When testicular spermatozoa are to be used, the testicular biopsy is macerated in a petri dish under the dissecting microscope using either sterile glass slides or two needles. The macerated tissue is then incubated in tissue culture medium prior to use. The contents of the tube are then mixed and allowed to settle for 1 min so that the large particles can settle and be removed. When samples are from cases of obstructive azoospermia, the tube is allowed to settle for an additional 10 min, after which sufficient spermatozoa can be aspirated from the bottom of the tube. When samples are from cases of non-obstructive azoospermia, the tubes are centrifuged at 400 g for 7–10 min and the pellet is resuspended in a very small volume. Any excess testicular spermatozoa should be cryopreserved for possible future use.

Preparation of the microinjection dish

Several microdroplets (5–10 µl) of HEPES-buffered medium are placed in the injection dish and covered with mineral oil. For one microdroplet, this medium is replaced by PVP (molecular weight 360 000), a very viscous solution that permits the spermatozoon to be controlled inside the microinjection pipette, and also prevents its adhesion to the pipette. The use of PVP also allows the three-dimensional motility patterns of the spermatozoon to be carefully observed (Cohen et al., 1994). However, the injection can be performed without PVP. Kuczynski et al. (1996) compared the use of PVP and HEPES-buffered media for sperm immobilization in a prospective controlled study and concluded that PVP may have a negative impact on fertilization rate, embryo quality and blastocyst formation. Other investigators have also raised concerns about the injection into the oocyte of such a potentially harmful agent (Feichtinger et al., 1995; Jean et al., 1996). However, the majority of ICSI programmes continue to use PVP.

One microdroplet of sperm suspension is placed at the periphery of the PVP droplet and the oocytes are positioned individually in the surrounding droplets. The number of oocytes to be manipulated at one time in the injection dish varies according to the experience of the ICSI operator, to minimize the period of their exposure to unsuitable conditions outside the incubator.

Sperm selection and immobilization before injection

The tip of the microinjection pipette is lowered into the PVP solution, which is then aspirated. The pressure is controlled to be in a neutral position. A single living spermatozoon is chosen on the basis of its morphology. At a magnification of ×400 this selection is difficult to perform on motile spermatozoa. However, selection of normal-appearing spermatozoon can be achieved to a certain extent by observing shape, light reaction and motion patterns (Cohen et al., 1994). The type of motility does not matter as long as the spermatozoon demonstrates slight twitching movements, a sign of viability. Other sperm parameters do not affect fertilization and pregnancy rates, or the outcome of pregnancy, providing a motile spermatozoon that appears morphologically normal is used for the injection (Mansour et al., 1995; Nagy et al., 1995). The spermatozoon should be completely immobilized by touching the tail near the mid piece, and rubbing the tail with the pipette against the bottom of the dish. This step is believed to be important for oocyte activation, as damage of the sperm plasma membrane is important for the release of sperm cytolytic components, factors involved in oocyte activation (Fishel et al., 1995a; Palermo et al., 1996a). Fishel et al. (1995a) demon-
strated a highly significant increase in fertilization rate following ICSI when the sperm tail plasma membrane was damaged. Palermo et al. (1996) also reported a significantly higher fertilization rate following aggressive damage to the sperm tail. The immobilized spermatozoon is aspirated tail first into the injection pipette and the pressure is controlled so that the spermatozoon advances very slowly and is finally positioned near the tip of the pipette. Differences in the consistency of PVP and culture medium must be taken into account when this procedure is performed.

**Positioning of the oocyte for the injection**

The oocyte is held in position by applying minimal suction on the holding pipette. It is first rotated gently using the injecting and holding pipettes to locate the polar body and position it at 6 or 12 o’clock. This position has been used since the original work on ICSI (Palermo et al., 1992) since it enables avoidance of damage to the spindle. A recent study by Blake et al. (1996) demonstrated that the best result was obtained when the polar body was at the 8 o’clock position. They concluded that the spermatozoon should be adjacent to, but not inside, the spindle area after injection. Further, the distal region of the spermatozoon should not face the contralateral oolemma of the polar body.

**Injection of the oocyte**

Before microinjection, the pipette is advanced towards the zona. It is very important at this point to ensure that the equatorial plane of the oocyte, the internal opening of the holding pipette and the tip of the microinjection pipette are all in the same focal point. The microinjection pipette, which contains the spermatozoon near the tip, is introduced slowly into the oocyte after piercing the zona pellucida, which is usually very easy to traverse. The oolemmal membrane is stretched by the advancing microinjection pipette. Great care must be taken when piercing the oolemma to avoid releasing the spermatozoon into the furrow made by the microinjection pipette, outside the cytoplasm. Piercing of the oolemmal membrane is observed when the pulled membrane suddenly yields, accompanied by a kinetic turbulence of the cytoplasm around the pipette (Mansour et al., 1996b). Occasionally, the oolemmal membrane is not pierced, even when the tip of the injection pipette is near the 9 o’clock position. In this situation, the injecting pipette can be withdrawn slowly to the centre of the oocyte and then readvanced in a slightly upwards or downwards direction. The technique performed by most centres to ensure piercing of the membrane involves the application of minimal suction which is stopped as soon as the cytoplasm is observed flowing at the tip of the pipette. Then the spermatozoon is deposited slowly, in the least possible volume, and the pipette is withdrawn gradually. After injection, the oocytes are rinsed and incubated under oil.

Different patterns of oolemmal response to the injecting pipette have been described by Palermo et al. (1996b) and include normal breakage when the injection needle creates an invagination that ruptures at the approximate centre of the egg, sudden breakage when the membrane breaks without creating a funnel, and difficult breakage when the membrane does not break or breaks only after several attempts. These recorded patterns appeared to be predictive of the survival and fertilization ability of the injected oocytes, as well as of the incidence of digyny.

Cytoplasmic aspiration has been considered to be an integral part of the ICSI procedure and an essential step for oocyte activation (Palermo et al., 1995). Vigorous aspiration of the oocyte cytoplasm has been reported to be a crucial factor for the success of ICSI (Tesarik and Sousa, 1995). However, in a controlled prospective study on sibling oocytes, the effects of cytoplasmic aspiration and non-aspiration on the rate of oocyte damage, fertilization, and embryo quality were compared (Mansour et al., 1996b). Cytoplasmic aspiration before sperm injection was not essential for oocyte activation, as it increased the rate of oocyte damage and it did not improve the fertilization rate.

**Results of ICSI**

Thanks to the effort of both the International Working Groups for Registers on Assisted Reproduction, and the ESHRE Task Force on ICSI, we now have enough available data to evaluate the efficacy of this newly introduced line of treatment. Unfortunately this data may have potential limitations, due to its heterogeneity, as there were differences between centres with regard to experience, technique, the number of cycles performed and the amount of information recorded (Tarlatzis, 1996). However, the data are of utmost importance in providing information to evaluate the efficacy of the treatment at an early stage.

The number of ICSI cycles using ejaculated, epididymal and testicular spermatozoa increased significantly from 3157 cycles in 1993, to 12 586 cycles in 1994, and to 47 654 cycles in 1995 (Tarlatzis, 1996; de Mouzon and Lancaster, 1997). This represents a significant increase in interest worldwide in the use of this new technique in the field of male infertility. The overall clinical pregnancy rates per oocyte retrieval were 23.6% for 1993, 21.8% for 1994 and 21.7% for 1995 (Tarlatzis, 1996; de Mouzon and Lancaster, 1997). The take-home baby rate was 15.9% in 1995 (de Mouzon and Lancaster, 1997; Table I).
The results of ICSI, using either ejaculated, epididymal, or testicular spermatozoa, did not differ significantly. It is of interest to mention that, when ejaculated spermatozoa were used, different sperm parameters did not affect the fertilization rate (Mansour et al., 1995; Nagy et al., 1995c; Oehninger et al., 1995; Palermo et al., 1995). The fertilization rate after ICSI using ejaculated spermatozoa ranged from 60.7 to 76% (Cohen et al., 1994; Payne et al., 1994; Nagy et al., 1995b; Oehninger et al., 1995; Mansour et al., 1996d; Palermo et al., 1996a; Schoolcraft et al., 1996; Vanderzwalmen et al., 1996: Table II).

Moreover, the pregnancy outcome does not seem to be influenced by the source of spermatozoa, with most pregnancies (67–73%) being viable (Tarlatzis, 1996). The incidence of multiple pregnancies has also increased, from 29 to 35% (Tarlatzis et al., 1996), denoting the production of good quality embryos and indicating the need to reduce the number of embryos per transfer. The incidence of ectopic pregnancies reported after ICSI was 1.7% (Tarlatzis, 1996) and 1.2% (de Mouzon and Lancaster, 1997), significantly lower than the rates of 4.3 and 3.6% reported after regular IVF (de Mouzon and Lancaster, 1995, 1997). This can be explained by the fact that most women undergoing ICSI have normal Fallopian tubes.

It has been shown that, in assisted reproductive technology, ongoing pregnancy rates decrease significantly with advancing age (FIVNAT, 1990). In women older than 40 years, the delivery rate is extremely low (Greenhall and Vessey, 1990; Lansac, 1995). Most probably, the decrease in implantation rates with advancing age is related more to poor oocyte quality rather than to uterine function (Navot et al., 1991). Also, the number of good quality embryos is significantly higher in women aged <40 years, and more embryos are available for replacement (Devroey et al., 1996). A number of studies have demonstrated clearly that advancing female age is a negative prognostic factor (Oehninger et al., 1995; Rosenwaks et al., 1995; Mansour et al., 1996d).

### Potential risks of ICSI

The fact that intracytoplasmic injection is an invasive procedure should always be borne in mind. The potential risks of many factors have been debated: (i) since the natural selection of spermatozoa is bypassed, a defective spermatozoon may be injected, and Cummins and Jequier (1994) have warned of the existence of cryptic genetic defects in cases of severe male factor infertility and the consequent risks of micro-assisted fertilization; (ii) there is a possibility of introduction of unknown foreign material into the cytoplasm during the injection, e.g. PVP, debris, oil, traces of Percoll; (iii) there is a possibility of oocyte damage during the injection due to meiotic spindle damage.

The frequency of chromosomal anomalies has been shown to be increased in infertile males (Kjessler, 1974; Chandley, 1995; Yoshida et al., 1995). It has been suggested that there is an association between male infertility, ICSI using aneuploid spermatozoa and the development of offspring with aneuploidy, especially aneuploidy of the sex chromosomes (Persson et al., 1996). With the increasing use of ICSI with testicular spermatozoa in cases of non-obstructive azoospermia, there might be a greater risk of sex chromosome aneuploidy because of the chromosomal nature of the underlying pathology. Another concern about ICSI is the use of epididymal or testicular spermatozoa from men with bilateral congenital absent vas deferens (BCAVD). This has major genetic implications because ICSI using spermatozoa from BCAVD males is likely to result in an increased frequency of cystic fibrosis in the offspring, in comparison with the frequency within the population at large (Chillon et al., 1995; Silber et al., 1995; Persson et al., 1996). It has also been documented that a significant number of azoospermic men have translocation defects affecting the long arm of the Y chromosome (Hendry et al., 1976). Therefore, clinicians and scientists providing ICSI for infertile men must be aware of the genetic implications of male factor infertility.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cycles</th>
<th>Fertilization injected oocyte (%)</th>
<th>Pregnancy rate (%) per embryo transfer</th>
</tr>
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<tbody>
<tr>
<td>Palermo et al. (1996a)</td>
<td>756</td>
<td>71.5</td>
<td>43.9</td>
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<tr>
<td>Schoolcraft et al. (1996)</td>
<td>71</td>
<td>60.7</td>
<td>56</td>
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<tr>
<td>Vanderzwalmen et al. (1996)</td>
<td>740</td>
<td>63</td>
<td>29</td>
</tr>
<tr>
<td>Mansour et al. (1996d)</td>
<td>650</td>
<td>61</td>
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<tr>
<td>Nagy et al. (1995b)</td>
<td>965</td>
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<td>30</td>
</tr>
<tr>
<td>Oehninger et al. (1995)</td>
<td>102</td>
<td>60.9</td>
<td>31.9</td>
</tr>
<tr>
<td>Cohen et al. (1994)</td>
<td>227</td>
<td>59.6</td>
<td>44.1</td>
</tr>
<tr>
<td>Payne et al. (1994)</td>
<td>100</td>
<td>67</td>
<td>32</td>
</tr>
</tbody>
</table>
Table III. Results of intracytoplasmic sperm injection using fresh epididymal spermatozoa

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cycles</th>
<th>2PN fertilization rate per injected oocyte (%)</th>
<th>Pregnancy rate (%) per retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palermo et al. (1996a)</td>
<td>52</td>
<td>65</td>
<td>66.9</td>
</tr>
<tr>
<td>Nagy et al. (1995b)</td>
<td>43</td>
<td>56</td>
<td>30</td>
</tr>
<tr>
<td>Craft et al. (1995)</td>
<td>42(^a)</td>
<td>32.8 ± 1.9</td>
<td>28.6</td>
</tr>
<tr>
<td>Silber et al. (1995b)</td>
<td>52(^b)</td>
<td>43</td>
<td>56</td>
</tr>
<tr>
<td>Mansour et al. (1997a)</td>
<td>44</td>
<td>59.5</td>
<td>27</td>
</tr>
</tbody>
</table>

\(^a\)Retrieved through percutaneous epididymal sperm aspiration.  
\(^b\)All from patients with congenital absence of the vas deferens.

Table IV. Results of intracytoplasmic sperm injection using fresh testicular spermatozoa in cases of azoospermia

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cycles</th>
<th>2PN fertilization rate per injected oocyte (%)</th>
<th>Clinical pregnancy rate (%) per retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstructive azoospermia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silber et al. (1996)</td>
<td>47</td>
<td>47</td>
<td>36</td>
</tr>
<tr>
<td>Tournaye et al. (1996b)</td>
<td>70</td>
<td>62.5</td>
<td>43.5</td>
</tr>
<tr>
<td>Kahraman et al. (1996c)</td>
<td>16</td>
<td>65.3</td>
<td>62.5</td>
</tr>
<tr>
<td>Mansour et al. (1997)</td>
<td>135</td>
<td>56</td>
<td>30.4</td>
</tr>
<tr>
<td>Non-obstructive azoospermia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Devroey et al. (1995)</td>
<td>15</td>
<td>47.8</td>
<td>20</td>
</tr>
<tr>
<td>Silber et al. (1996)</td>
<td>25</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>Kahraman et al. (1996b)</td>
<td>29</td>
<td>38.6</td>
<td>21</td>
</tr>
<tr>
<td>Tournaye et al. (1996b)</td>
<td>54</td>
<td>52.5</td>
<td>47.2</td>
</tr>
<tr>
<td>Mansour et al. (1997a)</td>
<td>106</td>
<td>39</td>
<td>11.3</td>
</tr>
</tbody>
</table>

ICSI using surgically retrieved spermatozoa has achieved good fertilization and pregnancy rates that are comparable to those for ICSI with ejaculated spermatozoa (Craft et al., 1993; FIVNAT, 1993; Silber et al., 1994; Tournaye et al., 1994; Hovatta et al., 1995; Fahmy et al., 1996; Tarlatzis, 1996; Mansour et al., 1997a). Different researchers have studied fertilization and pregnancy rates after ICSI using epididymal versus testicular spermatozoa (Hovatta et al., 1995; Nagy et al., 1995b; Silber et al., 1995a; Mansour et al., 1997a; Tarlatzis, 1996). The results showed that epididymal and testicular spermatozoa yield similar fertilization and pregnancy rates in cases of obstructive azoospermia. Table III shows the results of ICSI using epididymal spermatozoa. The fertilization and pregnancy rates using testicular spermatozoa are shown in Table IV. The fertilization and pregnancy rates were significantly higher in cases of obstructive azoospermia compared to non-obstructive cases (Kahraman et al., 1996c; Mansour et al., 1997a). This may be due to the fact that non-obstructive azoospermic patients may suffer from a genetic defect or a genetically determined barrier to reproduction (Smith et al., 1979; Vogt et al., 1992; Vogt, 1995; Martin-du-Pain and Casanpana, 1993; Chandley, 1995).

To determine whether a newly introduced technique such as ICSI is associated with any increased risks of birth defects or other complications, a careful follow up of babies, and notification of information to registers is essential. A great effort has been made both by the ESHRE Task Force on Intracytoplasmic Sperm Injection and the International Working Group for Registers on Assisted Reproduction to collect information on ICSI. A report on 3325 ICSI babies showed an incidence of 2.7% of malformation, a rate which is comparable to those observed in natural conception (de Mouzon and Lancaster, 1997). The ESHRE Task Force Report (Tarlatzis, 1997) on congenital malformations in children born after ICSI using ejaculated, epididymal and testicular spermatozoa for the years 1993–1995 showed that the incidence of major or minor malformations was not greater than those following IVF or natural conception (2.2%), whereas the sex chromosomal aberration rate was slightly increased (2%). This increase was attributed to the inheritance of paternally derived chro-
mosomal abnormalities, but it may also be due to de-novo defects of both the autosomes and the sex chromosomes.

An excellent follow up of children conceived by ICSI was reported by the Center for Reproductive Medicine in Brussels (Bonduelle et al., 1995, 1996; Liebaers et al., 1995; Van Steirteghem and Bonduelle, 1997), which published detailed information on >1500 children born after ICSI. So far, their results have indicated a slight increase (~1%) in de-novo, mainly sex chromosomal, aberrations, which are probably derived from the infertile men. The percentage of major congenital malformations was comparable to those recorded by most registers of children born in the general population or after assisted reproduction.

In another large study of 578 children born after ICSI (Palermo et al., 1996c), 15 (2.6%) were found to have congenital anomalies (nine major and six minor).

Based on the above data, patients can be counselled that there is a higher risk of transmitted chromosomal aberrations, a risk of de-novo, mainly sex chromosome aberrations, and a risk of transmitting fertility problems to the offspring. They can also be reassured that there seems to be no increased risk of congenital malformation (Bonduelle et al., 1996). Considering the potential benefits and risks of ICSI, many recommendations have been made (De Jonge and Pierce, 1995): (i) genetic screening of potential parents based on history and clinical examination, (ii) minimum standards for semen and genetic screening for severe defects, (iii) conservative use of ICSI, (iv) informed consent of the patients, (v) long-term follow up of children born after ICSI and (vi) increased research.

**Intracytoplasmic spermatid injection**

In some cases of non-obstructive azoospermia, no spermatozoa can be found in the testicular tissues even after hours of extensive search. These cases represent ~30–33% of patients undergoing ICSI and TESE for non-obstructive azoospermia (Mansour et al., 1997a; Mulhall et al., 1997). The only hope for these patients is to look for spermatids that can be used for intracytoplasmic injection. Spermatids are a unique source of a haploid number of chromosomes (Edwards et al., 1994). Normal fertilization has been reported following ICSI using round spermatids into hamster, mice and rabbit oocytes (Ogura and Yanagimachi, 1993; Ogura et al., 1993, 1994; Sofikitis et al., 1994, 1996). These animal experiments showed the potential of spermatids to achieve fertilization and pregnancy, and led to the suggestion of using spermatids for ICSI in humans when spermatozoa are completely absent (Edwards et al., 1994). Fertilization and pregnancies following the use of round spermatids (Tesarik et al., 1995, 1996) and elongated spermatids (Fishe et al., 1995b, 1996; Hannay et al., 1995; Vanderzwalemen et al., 1995; Chen et al., 1996; Mansour et al., 1996c; Tesarik and Mendoza, 1996; Antonori, 1997a; Araki et al., 1997) have been reported for humans. Frozen–thawed testicular spermatids have also been reported to result in an ongoing pregnancy (Antinori et al., 1997b).

There is a continuous debate about the certainty of identifying spermatids, in the live state in the absence of stain, to be used for ICSI. In our opinion, any experienced embryologist can easily identify round and elongated spermatids in a testicular sample from a case of obstructive azoospermia. Figure 1 is an excellent illustration by de Kretser and Kerr (1994) of electron microscopic appearance of round and elongated spermatids. Note the formation of the acrosomal vesicle, the dark condensed nucleus and the minimal cytoplasm of the round spermatids. Note also the darker and more condensed oblong nucleus of the elongated spermatid (Sb2) with the cytoplasm detached from around the nucleus.
which resembles the head of spermatozoa. Part of the cytoplasm has been shed from around the nucleus, leaving a very characteristic shape, which resembles an ice-cream cone. Figure 2 shows an elongated spermatid and can be compared with the illustration of the Sb2 stage in Figure 1.

The difficulty in identifying spermatids in cases of non-obstructive azoospermia is due to their rarity. We observed, in a series of 240 cycles of ICSI in cases of non-obstructive azoospermia, that many elongated spermatids (ice-cream cone shape; Figure 2) were present in only four cycles in which we found not a single spermatozoon. For the remaining cases for which spermatid injection was attempted in our programme, we found only a few elongated spermatids along with rare round spermatids and other spermatogenic cells. So far, not a single case has presented with only round spermatids. The two spermatid pregnancies in our programme resulted from the injection of elongated (ice-cream cone) spermatids, and resulted in the delivery of two healthy boys with normal karyotyping. There are still multiple areas to be explored in spermatid injection, since this is an immature cell that has not undergone histone–protamine transition. Also, there is the problem of the functional integration of the spermatid centrosome. Further research is needed before this technique can be widely adopted.

**Conclusion**

Micromanipulation techniques have been developed since the beginning of the twentieth century for a variety of purposes other than assisted fertilization. Various techniques of micromanipulation have been developed to assist fertilization, including PZD and SUZI, but the development of ICSI was the major breakthrough in the field of assisted fertilization.

ICSI using ejaculated, epididymal or testicular spermatozoa is a successful technique associated with very satisfactory fertilization and pregnancy rates. Men with oligoasthenozoospermia, obstructive or non-obstructive azoospermia, acrosomeless spermatozoa, immunological infertility, borderline semen, unexplained infertility, and previous fertilization failure in conventional IVF have achieved successful fertilization and parenthood with the use of ICSI.

So far, there seems to be no higher incidence of congenital malformation in babies born after ICSI than in the general population, but patients should be counselled about the higher risk of transmitted chromosomal aberrations, of sex chromosomal aberrations, and the risk of transmitting fertility problems to the offspring.

**References**


Aboulghar, M.A., Mansour, R.T., Serour, G.I. *et al.* (1996b) Prospective controlled randomized study of in vitro fertilization versus...


Blake, M., Garrisi, G.I., Sadowy, S. et al. (1996) Sperm head and spindle position during intracytoplasmic sperm injection determine fertilization and development outcome. 52nd Annual Meeting of the American Society for Reproductive Medicine, Boston, USA.


Mansour, R.T., Aboulghar, M.A., Serour, G.I. et al. (1997b) Abnormal oocytes: an underestimated infertility factor revealed by ICSI. 53rd Annual Meeting of the American Society for Reproductive Medicine, Cincinnati, OH, USA.


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