

Intracytoplasmic Sperm Injection: The New Frontier in Male Infertility Treatment

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For many cultures fertility is associated with magic or religion. Dancing around the maypole was a fertility ritual. Dances, dolls, and food such as bull horn, mandrake root, and oysters have long been associated with potency and fertility. In the Bible, Ruth, Job, Hannah, and others were infertile and were “cured” by a miracle. Pregnancy was a gift from God—or the gods—and thus part of a natural cycle. As long ago as 1800 BC, the ancient Egyptian Kahun papers described 17 prescriptions for the assessment of sterility and pregnancy.¹

The modern era of infertility diagnosis and treatment began with Antonie van Leeuwenhoek, who in 1674 discovered spermatozoa with his microscope. However, it was not until 1928 that the relationship between sperm count and fertility was established.² Subsequently, Spellanzini discovered that frozen stallion semen would reactivate with thawing. Hunter performed the first artificial insemination by collecting the semen of a hypospadiac male,

sucking it up into a quill, and inserting it into the wife's vagina.¹ Thanks to the good doctor, the wife became pregnant. Although hormonal manipulation of the female ovulatory cycle coupled with optimization of sperm-washing techniques made intrauterine insemination more effective, no real advances in infertility were realized until development of the assisted reproductive technologies.

In 1978 the first "test tube baby" was born and created a furor in the scientific and lay press about in vitro fertilization (IVF). Now, only 20 years later, the process of removing the seeds of life from their natural environment and producing babies fills multiple journals and is an internationally accepted treatment for infertility. Transvaginal ultrasound has now replaced laparoscopy because it is a much less invasive oocyte-harvesting technique. The availability of the inverted microscope, hydroptic precision instruments, and veterinary studies for improving livestock has led to the widespread clinical application of micromanipulation. Micromanipulation began as an attempt to create conditions under which poorly functioning sperm could penetrate oocytes more easily. Efforts began with zona drilling, which used acidic Tyrode's medium to chemically thin and open the zona, and progressed to partial zona dissection, in which the zona was actually perforated. Both techniques proved ineffective because of low pregnancy rates, high polyspermy rates, and abnormal cleavage of embryos.³ Mann subsequently developed subzonal insertion (SUZI or SZI) in mice. As its name implies, spermatozoa were injected directly into the perivitelline space, between the egg and the zona. Although this greatly improved the pregnancy rates for male-factor patients, the risk of polyspermy was still approximately 50% of the monospermic rate.³ The pregnancy rate was directly correlated with patient selection, with poor sperm quality resulting in approximately a 20% fertilization rate and relatively few subsequent pregnancies.⁴

Intracytoplasmic sperm injection (ICSI) has been practiced on animals since Lin⁵ first described injecting medium into mouse oocytes. However, numerous subsequent animal experiments resulted only in disappointment, with a high incidence of oocyte damage and frequent implantation failure. In retrospect, we now know that many animal oocytes (especially those of the mouse) are a poor choice for ICSI because membrane characteristics cause them to be intolerant of puncture. Veeck attempted ICSI in humans in 1989, also with disappointing results. It had appeared that ICSI was not suitable for humans until Dr. Palermo experienced a fortunate accident while performing SUZI. A sperm was injected into a human egg during SZI and two pronuclei were subsequently observed.

Thus in 1992 the first successful human ICSI was reported.^{6, 7} In 1993 Drs. Palermo and Van Steirteghem then introduced the technique clinically.⁸ With experience, tools and techniques have improved, and presently, ICSI has replaced all its predecessors. For many patients, ICSI offers the only chance for fathering a child.

THE METHOD

Because of both the cost and the potential for genetic errors, ICSI should be reserved for specific subsets of infertile couples: those with vasal obstruction from congenital absence of the vas, severe oligospermia, asthenospermia and teratospermia, failed vasovasotomy or trauma, severe antisperm antibodies, primary testicular failure resulting in less than 0.5×10^6 sperm, and idiopathic failure of IVF in which both partners appear normal by all testing parameters yet have low fertilization and no pregnancy and thus a presumed cryptic defect.

EGG PREPARATION

In vitro fertilization requires a much greater number of eggs than are naturally produced in unstimulated cycles to achieve reasonable pregnancy rates. Therefore, the most effective way to begin assisted reproduction is to hyperstimulate the female partner to produce an excess of mature oocytes. There are a number of different stimulatory protocols, all requiring hormonal manipulation with injections of exogenous luteinizing hormone/follicle-stimulating hormone, human menopausal gonadotropin, and human chorionic gonadotropin. The injections, painful and embarrassing tests, and resulting hormonal imbalance, along with the pervasive psychological difficulties inherent in infertility, can understandably be difficult for both partners.

Oocytes are then harvested by transvaginal ultrasound-guided aspiration with a 17 F, 33-cm needle. The oocytes are aspirated with the cumulus oophorus intact. The aspirated oocytes are incubated in medium in 5% CO₂ in air for 2 to 4 hours. Before the ICSI procedure, the cumulus oophorus must be removed by briefly washing the oocyte with hyaluronidase and drawing it through a fine-bore pipette. Once clean, the oocyte is examined under an inverted microscope for its degree of maturation. Only those oocytes with the first polar body extruded (indicating metaphase II) are microinjected with the sperm. The eggs are graded on the basis of the appearance of the oocyte-corona-cumulus complex, nuclear membrane, and polar body and the condition of the ooplasm and corona. Noting the degree of immaturity and atresia will help physi-

cians improve the timing of future cycles, weed out poor-quality oocytes, and predict fertilization success.⁹

SPERM PREPARATION

Semen preparation begins with ejaculation into a sterile, nonspermicidal container after 48 hours of abstinence. Alternately, it may be necessary to obtain the sperm through microsurgical epididymal sperm aspiration (MESA), electroejaculation, or testis biopsy or from a previously frozen specimen. The optimal washing technique remains a topic of debate inasmuch as the quality of the specimen is usually poor. However, because ICSI only requires approximately 20 moving sperm, procedures are rarely canceled because the number of sperm is inadequate.

MICROINJECTION

The microinjection is performed on an inverted microscope with two glass capillary tubes fashioned into pipettes, one for holding the oocyte and one for catching and injecting the sperm. Although these pipettes can be produced in the laboratory, they may be purchased (Humagen Fertility Diagnostics, Charlottesville, Va). The tip of the holding pipette has outer and inner diameters of 80 and 10 μm , respectively. The injection pipette has an 8- μm outer and 5- μm inner diameter. For comparison, the diameter of a red blood cell is 3 μm . The pipettes can aspirate as well as expel fluid and cells. They are connected to hydraulic micromanipulators that control movement to a few microns and reduce tremor. An air table reduces vibration as well.

The procedure is performed on the heated stage of the microscope in a microinjection chamber. The male and female gametes are in separate droplets of medium covered with mineral oil to prevent evaporation. Four microliters of polyvinylpyrrolidone is added to the sperm suspension medium to increase the viscosity and facilitate sperm capture. The injection pipette is placed in the semen droplet, and motile sperm are immobilized, captured, and aspirated into the pipette. Immobilization is accomplished by compressing and rolling the tail of the sperm with the injection pipette. This destabilizes the sperm membrane; the sperm must be alive but immobile for successful injection.¹⁰ Both pipettes are then brought into a droplet containing oocytes. The holding pipette approximates an oocyte at an angle perpendicular to the polar body and mitotic plate, and then with gentle suction the holding pipette grasps and holds the oocyte. Medium is flushed from the injection pipette until a single sperm is near the tip. Then the microinjec-

tion pipette is lined up opposite the holding pipette, and the oocyte is gently pierced. The oocyte membrane and cytoplasm are sucked into the injection pipette until it is clear that the membrane is broken; then a single sperm is injected and the injection pipette withdrawn. A channel will be present in the oocyte for a few minutes but will close. The oocytes are incubated at 37°C in 5% CO₂ and examined in 17 hours for fertilization.

TECHNICAL PROBLEMS

The possibility of complications is easy to understand. If the oocyte is lined up incorrectly, the internal architecture might be damaged. Rough handling could damage the cell membrane itself. Too much sperm medium or too many sperm might be injected, or the oocyte membrane might be torn. The egg loss rate ranges from 5% to 20% depending on the experience of the technician and criteria of the reporting center. An egg damage rate of 15% or less is acceptable.

RESULTS

In general, fertilization rates with standard IVF are directly proportional to the sperm count.¹¹ Men with a total motile sperm count of less than 500,000 have a chance of approximately 8% per IVF cycle of initiating a pregnancy. This increases to 22% as the count normalizes. The reason for the popularity of ICSI is clear. Table 1 shows the results reported from a number of centers. Although the results are comparable, the table reveals one of the problems in evaluating success. Results may be reported in terms of either fertilization or pregnancy and measured per cycle or per transfer. The pregnancy rates may be reported as a percentage per embryos transferred, per oocyte fertilized, or per ICSI attempt. Pregnancy may be established by either an elevated basic human chorionic gonadotropin level or by ultrasound evidence of a fetal heartbeat. The results are consequently influenced by numerous variables, including the treatment of poor-quality and damaged embryos, the number of embryos transferred (varying from 2 to 8), treatment of twins as one or two pregnancies, the age and fertility status of the female patients, and the definition of pregnancy. For patients undergoing ICSI, an approximation of a typical cycle might be 12 eggs aspirated, 8 of which are mature, with 1 damaged during ICSI. Of the remaining 7, 2 fail to progress, which leaves 5 embryos of variable quality for implantation or freezing, depending on the age of the patient and the judgment of the attending physician.

TABLE 1.
Results of Intracytoplasmic Sperm Injection

Outcome	Van Steirteghem, 1994 ³²	Lipshultz and Lamb, unpublished data, 1996	Van Steirteghem, 1993 ³³	Svalander, 1995 ³⁴	Harari, 1995 ³⁵
No. oocytes injected	5,906	5,341	1,821	1,499	1,185
Damaged	9.5%	12.8%	13.5%	10%	1,073
Fertilized	67%	65%		629 (45%)	718
Embryos	44%		51%	553	662
Pregnancies	36%/T	29%/F	43.5%/T	24.5%/T	20%/ICSI

Abbreviations: T, transfer; F, fertilization; ICSI, intracytoplasmic sperm injection.

Essentially there are no differences between pregnancy rates when sperm are fresh or frozen or when they are obtained from ejaculate or taken directly from the epididymis (MESA).¹² What remains to be assessed is whether the pregnancies achieved with these sperm proceed to term or miscarry at a higher rate than those achieved with normal IVF. Until recently, when Dr. Michael Tucker (Atlanta) reported a live normal birth, no pregnancy achieved with testicular sperm had been reported to reach term; all had miscarried. Sofeketis reported a few births with injection of round spermatid nuclei (ROSNI), but his success has not been repeated elsewhere. A large number of children have already been born via ICSI. The initial fear that bypassing natural selection would increase the incidence of birth defects has not been borne out. Birth defects do occur, but at a frequency no greater than that associated with natural conception.¹³

Logically, ICSI removes all the work for the spermatozoa. Yet despite bypassing the mechanical factors, ICSI pregnancy rates remain 25% to 50% depending on the IVF center. Obviously there are other factors that influence the initiation of a pregnancy.

With this in mind, we will now look more closely at the factors required for successful outcome of ICSI: mature prepared oocytes, adequate sperm, and the laboratory environment.

THE MALE FACTOR

Sperm have few defining characteristics. They are classified on the basis of number, motility, morphology, and place of origin. Except in severe cases, semen analysis does not predict ICSI success.^{7, 14, 15} Other functional and biochemical studies can suggest a sperm sample's potential for fertilization but cannot absolutely predict the results of IVF in an individual patient.^{9, 15} Immobility and therefore possibly senescence or mortality and severe combined defects¹⁴ are the only exceptions, and even some patients with these severe defects have achieved pregnancies.

Even patients with severe oligospermia or teratospermia usually have the 20 or so normal-appearing, moving sperm needed for ICSI. All that is required to initiate IVF-ICSI is a number of sperm equal to the number of eggs. Yet some patients cannot produce even this small number. Patients with congenital absence of the vas, failed vasectomy reversal, and idiopathic azoospermia have no visible sperm in their ejaculate. The only option is to proceed to the epididymis or testis. Patients with obstruction can be treated with MESA. This microsurgical technique requires exposure of the epididymides, incision of a tubule, and aspiration of the efflux. Sometimes the quality of spermatozoa obtained by MESA is quite poor,

but again the only requirement for successful ICSI is merely 20 moving sperm. Percutaneous epididymal aspiration has also been described but is quite difficult in a scarred epididymis with distorted anatomy. Active forward progression is not mandatory as long as enough motion is present to indicate vitality. Immotile and therefore possibly senescent or dead sperm are used when needed and have induced pregnancy, but the fertilization rate is low and the miscarriage rate high.^{9, 16}

Because of the small numbers of sperm required for ICSI, we often freeze aliquots of MESA-extracted sperm for use in future IVF cycles if needed, thus reducing the number of surgical procedures required. Nevertheless, MESA can be repeated multiple times if necessary. When MESA fails to procure adequate sperm, the urologist then moves to testicular biopsy. The biopsy tissue is gently teased and the spermatozoa removed. The numbers and quality found vary with the cause of infertility. Motile sperm can be difficult to find in testis biopsy tissue, and the difficulties associated with immobility apply to testicular sperm as well.

THE FEMALE FACTOR

In spite of a technically perfect microinjection procedure, fertilization may not occur. Other than immobility and probably abnormal morphology, overall sperm quality has little effect on ICSI success. The question then is what causes one egg and not another to become fertilized. First we must understand the difference between fertilization and embryo development inasmuch as one does not necessarily follow the other. Whether the correct physiologic changes are occurring can be determined only if the cells continue to divide, thus forming an embryo. Fertilization may begin and then cease because of an unfavorable laboratory environment or genetic abnormalities in either parent. Polyploidies, monosomies, and mosaics are more common than one would expect. Polyploidy or mosaicism is present in 40% of arrested embryos and 2% to 14% of those presumed normal. These abnormalities are not related to maternal age.¹⁷ In contradistinction, aneuploidy increases in normal-appearing embryos from 4% in patients 20 to 34 years old to 37% in women 40 or older. Of embryos that do not implant, 70% to 95% are thought to have genetic abnormalities.¹⁸ Abnormal fertilization can also occur simply because of exposure to hyaluronidase or parthenogenic stimulation,¹⁶ which is known to happen with ICSI.

To determine whether failure to develop results from defects in the sperm or in the egg, a number of studies have been

performed. We know that it is more difficult to stimulate ovulation in older females. They produce fewer oocytes, which are more brittle and less likely to become fertilized.^{19, 20} We know that decondensation of chromatin is dependent on factors in the oocyte cytoplasm.²¹ We also know that when the cytosolic fraction of the male gamete is injected into an oocyte, cell division is induced.³ Electron microscopic studies of arrested embryos have also found both intact sperm heads and partially decondensed sperm chromatin. Analysis of fertilization failures, e.g., eggs that begin dividing and then arrest (as determined by both serial section and electron microscopy), implies activation failure.²² All these studies suggest that either the sperm or the egg may be defective.

Once the embryo is obtained, the final step is also female dependent. Experiments with donor oocytes and older females both exonerate and implicate uterine and implantation difficulties as causes of pregnancy failure.^{19, 20, 23, 31} These studies suggest that both partners play a role in the initiation of a pregnancy and that we need much more scientific information to understand these respective roles.

THE LABORATORY

The part played by the laboratory in IVF success is obvious. The gamete environment must be meticulously controlled to maximize the number and quality of embryos. Sterility, warmth, pH, oxygen tension, proper nutrients, etc., are mandatory. Something as simple as the wrong type of plasticware or water can kill carefully harvested gametes. In vitro fertilization and ICSI technicians require careful training before clinical practice. With ICSI the ova kill rates are decreased, and pregnancy rates increase in direct correlation with the skill of the technician.²⁴ Moreover, great care must be taken to avoid confusion or contamination in a busy laboratory. There are frightening stories of misuse or carelessness that range from implanting another couple's frozen embryos without consent²⁵ to inadvertently contaminating one semen specimen with another.²⁶ The latter resulted in one mother delivering twins of different races. Obviously these occurrences are rare, but they are devastating to the patients nonetheless and can be avoided if laboratory procedures are in place to maximize quality control.

THE FUTURE

Attempts have been made to decrease IVF and ICSI failures. Oocytes that appear unfertilized by IVF, along with those that were immature when harvested and have matured 24 hours later, un-

dergo ICSI about 24 hours after aspiration. A lower fertilization rate (38%) with an 84% cleavage rate has been reported by two centers performing day 2 ICSI.¹ The pregnancy rate could not be determined because both initial and "day 2" embryos were transferred together. Intracytoplasmic sperm injection after failed IVF has resulted in a 42% fertilization rate. We perform this at Baylor with a 66% fertilization rate.

A very productive outgrowth of the advent of micromanipulation is preimplantation genetic diagnosis, or the ability to define the genetic information of the embryo before returning it to the mother. When an embryo reaches the six- to eight-cell stage, a single cell can be extracted and screened for worrisome genetic abnormalities. The F508 site has been used as a marker for cystic fibrosis and has been identified in this manner. Those embryos homozygous for cystic fibrosis are not replaced. Couples have thus been able to deliver healthy infants without the risk of cystic fibrosis.²⁷ Previously they would have been counseled about donor insemination, amniocentesis, and abortion, if necessary, or adoption.

Sperm may have the capacity to carry DNA, as found in viruses, on their surfaces that they can deposit into a mouse blastocyst.²⁸ If sperm could be loaded with the appropriate missing genetic material, known diseases like muscular dystrophy or cystic fibrosis could be prevented prenatally. This unique potential also harbors a potential problem. Many men with AIDS would still like to be fathers, yet protect their wives and future children. The capacity of sperm to carry viruses makes sperm washing and insemination a dangerous proposal for HIV-positive men, even though it is being done in some centers in Italy.²⁹

Since the use of IVF began, critics have voiced concern that we were creating a new generation of infertile patients. Some semblance of natural selection takes place in IVF in that the sperm must still capacitate, acrosome-react, bind to and penetrate the zona, and fuse with the oocyte membrane. However, with ICSI the only selection techniques are motility and reasonable morphology. We assume that a morphologically normal sperm would be genetically normal. The fact is that to date we know little about the genetics of infertility in either sex. Recent work on the Y chromosome has revealed an association between loss of the *DAZ* gene and azoospermia. Some men with this abnormality would be candidates for ICSI, MESA, or ROSNI. The question arises as to whether professionals in the field of reproductive medicine have the responsibility to prevent these men from having male children, who in turn would most

likely be missing the same "fertility gene" and thus possibly be infertile themselves. How many generations would it take for us to double or triple the number of infertile men? Moreover, as we unravel the genetic mysteries of reproduction, we will undoubtedly find genes on the X chromosome, as well as autosomes that are also necessary for fertility whose absence is compatible with a seemingly normal phenotype.

However, increased infertility is not the only issue. One must consider the possibility of linked or related genes. For example Kovacs et al.³⁰ identified a relationship between Y chromosome deletions and papillary renal cell cancer. We seldom monitor the children of our patients to adulthood to watch for the occurrence of diseases that may have stemmed from our prenatal intervention. As with treatment of prostate cancer, we must wait many years to see the effects of what we are doing today. Yet despite the possible dire results in the future, we cannot deny that there are now thousands of phenotypically normal children who would not be alive without ICSI. It remains an important "treatment" for otherwise untreatable male-factor infertility, a means of producing biologic children for couples who in the past would have required donor sperm or adoption.

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