Review Article

# Intracytoplasmic Sperm Injection – An Overview

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## Introduction

Two decades ago, at 11.47 p.m., on Tuesday, July 27, 1978, Louise Brown, was born – the world's first baby to be conceived in the laboratory. Her birth marked the beginning of a new era in the management of infertility. It generated a flurry of clinical and laboratory research that changed every aspect of infertility management and, within the short span of 20 years, has led to the development of a number of new procedures based on the principle of in vitro fertilization. The most exotic of these – cloning – is still far removed from human application, but IntraCytoplasmic Sperm Injection (ICSI) – a technique that is almost as radical – is already in widespread use. The possibility of injecting a single sperm – and now, even sperm precursor cells – into an oocyte, and achieving a viable pregnancy has allowed us to bypass many of the processes involved in natural fertilization and has made possible that which was impossible yesterday.

As a result, there has been a radical change in the treatment options available to the infertile couple. However, there is much that is still unknown about the long-term consequences of ICSI, especially when used for severe male infertility cases. Hence, it is essential that this enthusiasm be guided by a proper understanding of what really is possible and what risks are involved. This review seeks to answer these issues by providing a broad perspective on all aspects of ICSI – its evolution , clinical indications for its use, results, effect on offspring, genetic concerns and future applications.

## Evolution of ICSI

In vitro fertilization was originally developed as a treatment for female tubal factor infertility. However, once the technique was standardized, and steady successes obtained, the indications were soon expanded to include endometriosis, unexplained infertility, and even male factor infertility since a much smaller number of sperms are required for IVF as compared to natural conception. However, though IVF proved useful in achieving pregnancies in many couples with male infertility where all else has failed, fertilization rates were usually low and failure of fertilization occurred in about one third of the cycles (Tournaye et al.,1992). It was clear that the technique of IVF would have to evolve if it was to be of value in treating the challenging problem of severe male factor infertility.

Micromanipulation of gametes to achieve fertilization grew out of this compulsion. Interestingly, the procedure was not new. In 1966, Graham showed that sperm nuclei could transform into pronuclei when live frog sperms were injected into unfertilized frog eggs. Uchare and Yanagimachi (1977) went a step further and showed that nuclei of human sperm developed into pronuclei when injected into hamster oocytes, thus demonstrating that cytoplasmic regulation of pronucleus formation was not species specific. Equally impressively, Markert (1983) showed that even sperm heads, immotile sperm and

grossly defective sperm could induce the same oocyte events as did normal sperm. Finally, in 1985, Hosia et al (unpublished, quoted in Iritani, 1991) achieved delivery of viable young after transfer of microinjected rabbit eggs. This success was soon replicated by other scientists with Keefer (1989) reporting fetal development from microinjected rabbit eggs, and Goto et al. (quoted in Iritani, 1991) achieving birth of a calf in 1990. However, human application of this technique proceeded slowly with only a preclinical study by Lanzendorf et al. in 1988, and two tentative, and unsuccessful, attempts by Veeck et al. (1990) and Ng SC et al. (1991).

There were two main reasons for this reluctance to apply ICSI clinically. First, was the fear that the injection procedure could damage some vital intra-cellular organelle, with subsequent consequences on the embryo. Second, was the concern that since ICSI bypassed many of the natural barriers to fertilization – which were presumed to act as biological filters allowing only the best sperm to fertilize – there may be problems in the offspring due to fertilization by "unfit" sperm. Hence, when Gordon et al. (1986, 1988) presented zona drilling as a new micromanipulation technique for facilitating fertilization while partially preserving natural biological barriers, most centers switched to this, and related, techniques. Three methods were used:

Zona Drilling (ZD) – a hole was created in the zona using acid tyrode; the purpose was to create a path for the sperm to reach the oolemma (which was still intact), without having to struggle through the zona.

Partial Zona Dissection (PZD) – a slit was created in the zona by mechanically disrupting it using a micro-needle; this achieved the same purpose as zone drilling without the damage caused by the acid tyrode.

Sub Zonal Insemination (SUZI) – a microneedle was introduced through the zona and sperms were injected into the peri-vitelline space; this method bypassed the zona entirely, while maintaining the integrity of the oolemma.

Clinical pregnancies were soon established by these methods (Cohen et al., 1988, Ng et al., 1988). However, despite some successes, the fertilization and pregnancy rates remained low (Levren 1993). The next development occurred by chance.

During SUZI, it is possible to accidentally puncture the oolemma and inject the sperm intra-cytoplasmically. Working at the Dutch-speaking Brussels Free University, Palermo noticed that while many of the eggs thus accidentally injected underwent immediate lysis, a few survived and cleaved into good embryos, even when sibling eggs that underwent the planned SUZI failed to fertilize. Inspired by this chance observation, Palermo et al (1992) performed deliberate ICSI in some of the eggs of patients undergoing SUZI.

In 1992, Palermo et al. reported the first 4 pregnancies obtained through ICSI. All 4 patients had severe oligoasthenozoospermia, and 3 had failed a total of 4 IVF cycles (only 1 of 103 oocytes fertilized). Results of SUZI and ICSI were performed on sibling oocytes. Of 50 oocytes were subjected to SUZI, only 3 (4%) fertilized. 47 oocytes were injected with a single non-motile sperm: 31 (66%) fertilized and 18 developed into good quality embryos. This resulted in 2 singleton and 1 twin pregnancies, and 1 miscarriage.

This was soon followed by a report of their results with 300 cycles in 202 couples, performed between October 1990 and January 1992 (Palermo et al., 1993), again confirming the superiority of ICSI over SUZI. However, despite these successes the overall fertilization rates of approximately 35% were still rather low.

Then in the second half of 1992, this changed. Thanks to three significant modifications in the technique of ICSI – use of a lower concentration of hyaluronidase to remove cumulus, selection of motile instead of immotile sperm, and aspiration of the oolemma to ensure rupture – the fertilization rates soared to 65%, matching those achieved during conventional IVF for tubal factor (Joris et al., 1998). Finally, ICSI had "arrived".

With standardization of techniques, and the availability of commercially prepared high quality injection needles

ICSI rapidly became a routine procedure that could be offered by any established IVF laboratory. Thus, the number of ESHRE registered centres offering ICSI increased from 35 in 1993 to 101 in 1995 and today, in the city of Mumbai alone, there are almost a dozen centres offering ICSI !

With this widespread enthusiasm for ICSI, its applications were rapidly expanded. It was soon realized that when sperm aspirated from the epididymis had to be used (as in cases of vas aplasia or obstructive azoospermia) much higher fertilization and pregnancy rates were achieved when ICSI was used instead of conventional IVF Silber et al., 1994). It was further found that with ICSI even frozen-thawed epididymal sperm had the same fertilization rate as fresh sperm (Devroey et al., 1994 a). This meant that one sperm retrieval procedure could yield sperm for many ICSI cycles.

Meanwhile, Jow et al. (1993) showed that motile sperm could be recovered directly from a normal testis, and Devroey et al. (1994 b) reported fertilization and development of good embryos after ICSI with testicular sperm. Soon, pregnancies with testicular sperm were being obtained with the same success rate as with ejaculated and epididymal sperm. Spurred by this success in obstructive azoospermia, attempts were made to recover sperm from the testes of men with non-obstructive azoospermia.

Reviewing his earlier work on testicular biopsies, Silber (1995) pointed out that many biopsies showing maturation arrest, or even Sertoli cell syndrome only, had an occasional tubule that showed presence of sperm. He suggested that by doing multiple testicular biopsies it would be possible to access these tiny focal areas of spermatogenesis and retrieve these sperm for ICSI. Shortly thereafter, Devroey et al. (1995) reported success in this previously hopeless group of patients. In 15 patients with non-obstructive azoospermia, treated between May and August 1994, they were able to recover sperm from the testes of 13 men. These sperm achieved a 47.8% fertilization rate, and 3 pregnancies were produced.

All these amazing advances are just the beginning of the revolution that has overtaken infertility therapy. Already human pregnancies have been achieved after intracytoplasmic injection of spermatids (Fishel et al.,1995; Tesarik et al., 1996) and the indefatigable Yanagimachi (1998) has been able to achieve pregnancies and fertile offspring in mice after injection of nuclei of round spermatids and secondary spermatocytes. Much more is yet to come.

**Indications**

ICSI needs just as many sperm as there are eggs. Further, the occurrence of fertilization and pregnancy are independent of sperm count, motility (as long as they are viable), morphology (Nagy et al.,1995a), or the source of sperm (epididymal or testicular) (Nagy et al.,1995b). Hence, ICSI is useful in all those cases where the sperm quality is too poor for fertilization to occur in any other way. These indications are discussed in detail below:

(1) Very low sperm counts. If the sperm harvest yields <500,000 motile sperm, routine IVF is likely to fail: on the other hand ICSI can succeed even if the sperm count is so low that sperm can be found only after centrifuging the semen sample.

(2) Severe asthenozoospermia. If the ejaculate shows less than 20% motile sperm, routine IVF is not likely to succeed. For ICSI, motility is unimportant : as long as there is some movement that indicates that the sperm is viable, ICSI can succeed.

(3) Total asthenozoospermia. A totally immotile sperm may be viable but immotile (immotile sperm syndrome), or it may be dead and therefore non-motile (necrozoospermia). It is important to distinguish between these two situations since ICSI with viable immotile sperm will achieve fertilization while ICSI with non-viable sperm will fail. Hence semen samples with total asthenozoospermia should undergo supravital staining. If most of the sperm are viable then ICSI can be done with ejaculated sperm; if most of the sperm are found to be dead then it is preferable

to use testicular sperm instead of ejaculated sperm, since it is more likely that the testicular sperm, though immotile, will be viable. The hypo-osmotic swelling test can also be used to identify which immotile sperms are viable and hence suitable for ICSI (Casper et al., 1996).

(4) Severe teratozoospermia. If critical morphological evaluation of the sperm reveals less than 4% normal sperm, IVF will show poor fertilization. However, this does not pose a barrier to fertilization when ICSI is used.

(5) Globozoospermia These are sperms with no acrosomes and therefore unable to fertilize. Since ICSI does not need the acrosomal enzymes, ICSI can achieve fertilization in these cases but the rates are low, and pregnancies rare. (Liu et al., 1995).

(6) Frozen-thawed sperm with poor recovery – when a semen sample of impaired quality is cryopreserved the harvest is usually too poor to be used for IUI or even IVF e.g. when semen is cryopreserved in young men with testicular cancer prior to therapy. In these cases ICSI offers the only hope.

(7) Electro-ejaculated sperm with poor motility – If a man is unable to ejaculate due to neurogenic or psychogenic causes, electro-ejaculation may be required. Often the sample obtained shows excellent count but poor motility, necessitating ICSI.

(8) Failed IVF in two or more cycles – A study of couples who had failed, or poor, fertilization at their first attempt at IVF found that two-thirds of them will have satisfactory fertilization at the next attempt at IVF (Hamberger et al., 1998). The remaining one-third will fertilize only when ICSI is used. Thus one IVF failure need not mandate ICSI but most centres would prefer not to take a chance and would do ICSI.

(9) When epididymal or testicular sperm are used – Both epididymal and testicular sperm are capable of fertilizing eggs by conventional IVF, but fertilization rates are very low since these sperm are immature, and frequently immotile. ICSI offers a much higher success rate.

**Epididymal sperm** are retrieved by percutaneous aspiration using an insulin syringe or butterfly scalp vein needle (PESA). Use of epididymal sperm is indicated in the following situations:

(a) Bilateral congenital absence of the vasa deferentia Sperm production is normal but reconstructive surgery is not possible. The older procedure of creating a spermatocele and aspirating this to retrieve sperm for IUI has been given up due to very low success rates.

(b) Epididymal Obstruction – Traditionally this is treated by microsurgical vaso-epididymal anastomosis (VEA) that bypasses the epididymal block. However, in certain situations ICSI is preferable to VEA:

(1) Epididymal obstruction following hydrocele surgery is very common in our patients; due to adhesions caused by the previous surgery VEA is extremely difficult and usually fails.
(2) Aging wife – It usually takes one year after surgery for good quality sperm to appear; if the wife is over 35 years, then every year counts; ICSI would help save time and allow for an early pregnancy.
(3) Failed VEA – Redo VEA has a low success rate; hence ICSI is preferred.

(c) Ejaculatory duct obstruction can sometimes be treated by transurethral resection of the block. If this fails, sperm can be aspirated from the vas or epididymis and used for IUI / IVF / ICSI depending on sperm quality.

**Testicular sperm** can be obtained by testicular aspiration, needle biopsy, or open biopsy. Testicular sperm are used for ICSI in the following situations:

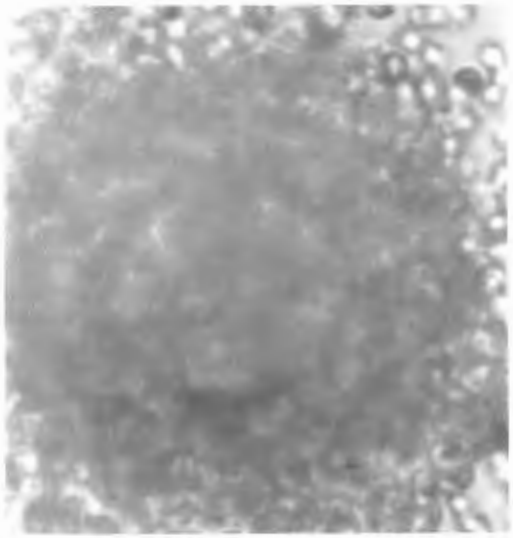

Fig 1: Mature oocyte with expanded cumulus at the time of ovum pick up.

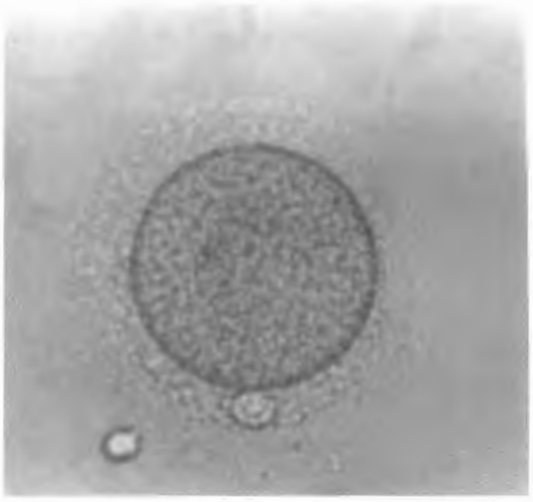

Fig 2: Enzymatically denuded oocyte showing First Polar body at 6' O Clock position.

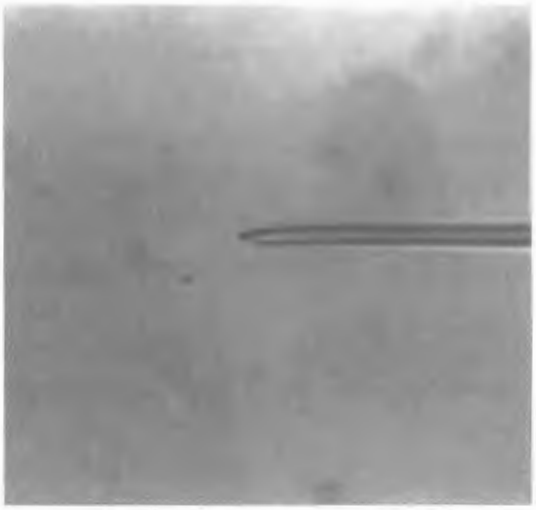

Fig 3. Immobilization of morphologically normal sperm

Fig 4. Fixing denuded metaphase II oocyte using holding pipette and bringing injecting pipette-loaded with immobilized sperm - in the same plane.

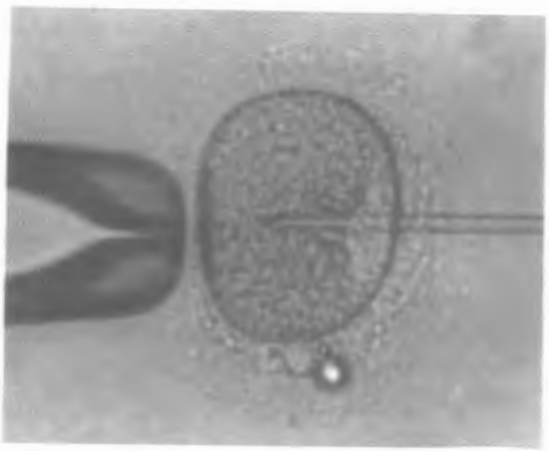

Fig 5 - Inserting injecting pipette through zona pellucida and breaking oolema to inject sperm in the ooplasm

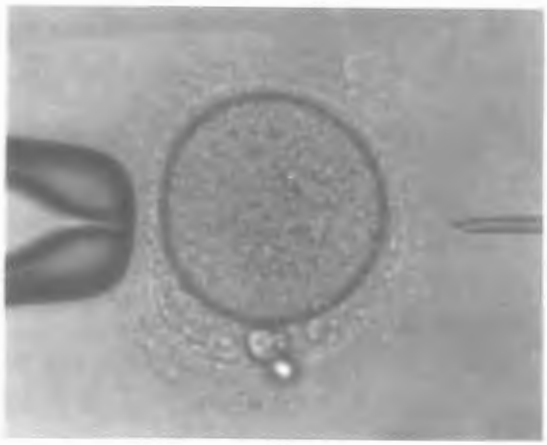

Fig 6: A healthy 4-cell stage embryo, 40 hours after ICSI.

(a) Obstructive azoospermia when the epididymis cannot be located due to previous surgery or complete agenesis

(b) Non-obstructive azoospermia In 30% to 50% of men with azoospermia due to primary testicular failure, with small testes, elevated FSH and maturation arrest on biopsy, it is possible to obtain a few sperm by taking multiple biopsies from all over the testes to tap pockets of scanty spermatogenesis. These sperm are usually immotile, and have lower fertilization rates following ICSI, but the final pregnancy rates in those in whom sperm were found are similar to other groups.

**Some important points to note:**

-Motile epididymal sperm are preferred to testicular sperm.

Testicular sperm are preferred if epididymal sperm are completely immotile.

-Cryopreserved epididymal sperm are comparable to fresh epididymal sperm.

-Significantly higher fertilization rates are obtained with testicular sperm from men with obstructive azoospermia as compared to men with non-obstructive azoospermia.

(c) Necrozoospermia in men with all non-viable and immotile sperm in the semen, testicular sperm are used in the belief that they are more likely to be viable.

(10). Pre-implantation genetic diagnosis when fertilization is obtained by routine IVF extra sperm stick into the zona and can contaminate the blastomere biopsy, thus interfering with the genetic probes. Hence, ICSI is preferred.

Since ICSI virtually eliminates the risk of failed fertilization some clinicians have suggested the routine use of ICSI for all cases of IVF. However, most experts agree that ICSI should be used only when routine IVF will not work. The reason for this caution is the fact that the genetic consequences and long term development of the ICSI offspring are yet unknown. Also, ICSI is a more complex procedure than routine IVF and places additional technical demands on the centre and an extra financial burden on the patient.

Sometimes, it is uncertain whether routine IVF will suffice or whether ICSI will be required. In such cases, if an adequate number of eggs are available, half can be subjected to IVF and half to ICSI. If IVF achieves adequate fertilization those embryos are used first and in future only IVF is done; if IVF fails then the cycle will not be wasted since ICSI fertilized embryos will be available. This approach is particularly useful in men with severe OATS and borderline harvest and in couples who have failed one cycle of IVF.

**Technique of ICSI**

The technique of ICSI has been discussed extensively and is also demonstrated on CD-ROM (Van Steirteghem et al., 1995). The key steps have been illustrated in figures 1 to 6

**Results**

ICSI has established itself as the treatment of choice for severe male factor infertility with fertilization, implantation and pregnancy rates that match those obtained with conventional IVF for female factor infertility. The results of the ESHRE ICSI Task Force for 1995 (Tarlatzis and Bili, 1998), representing the experience of 101 centres, are summarized in Table-1.

**Table – 1**
**ICSI results using ejaculated, epididymal and testicular sperm - ESHRE ICSI Task Force Report for 1995**

| | Ejaculated Sperm | Epididymal Sperm | Testicular Sperm |
|---|---|---|---|
| Fertilization Rate (% of MII oocytes injected | 64% | 62.5% | 51.7% |
| Good Embryos (% of MII oocytes injected) | 43.5% | 45.7% | 38.9% |
| Fresh Embryo Transfers (% of started cycles) | 86% | 88.1% | 90% |
| Viable Pregnancy (>20 wks) (% of started cycles) | 21.3% | 21.8% | 18.7% |

- Egg damage rate is stable at around 10%
- Clinical abortion rate is 10 – 15%
- Viable pregnancy rate following transfer of frozen embryos is 5 – 10%.

**Table II**

**ICSI results as per etiology of infertility - Fertility Clinic (Mumbai) data from April 1994 to June 1998**

| | Severe OATS -Ejac. Sperm | Obstr. Azoo. -congenital | Obstr. Azoo. - acquired | Testicular failure |
|---|---|---|---|---|
| No. of cycles | 351 | 26 | 27 | 26 |
| (No. of patients) | (341) | (23) | (20) | (26) |
| Fresh Embryo Transfers | 348 | 24 | 25 | 15 |
| (% of started cycles) | (99.1%) | (85.7%) | (92.6%) | (57.7%) |
| Viable Pregnancy | 84 | 5 | 2 | 3 |
| (% of transfer cycles) | (24.1%) | (20.8%) | (8%) | (20%) |
| (% of started cycles) | (23.9%) | (19.2%) | (7.4%) | (11.5%) |

An analysis of results in terms of etiology is presented in table-II.

**Genetic Concerns**

ICSI is still a very new procedure. The explosion of clinical work has not been matched by a proportional number of pre clinical and experimental studies. Hence there is concern about the transmission, or induction, of genetic abnormalities to the offspring. Also, the long-term developmental consequences of ICSI on the offspring are as yet unknown. Hence, many countries have chosen a path of caution, bringing to a halt some of the more controversial applications of ICSI till more information is available. Thus, the Dutch have declared a moratorium on ICSI using epididymal and testicular sperm while the British have banned spermatid injections.

**Two types of risks are involved:**

(a) Known risks - transmission of a known paternal defect
(b) Unknown risks - unpredictable genetic consequences of the ICSI procedure

(a) Known risks - Several genetic abnormalities can cause infertility. By enabling such men to father children, ICSI allows the genetic defect to be transmitted to the offspring. Some of the common problems are:

(1) Vas Aplasia. Bilateral congenital absence of the vasa deferentia is now recognized as mild, localized form of cystic fibrosis (Chillon et al., 1995) due to mutations on the cystic fibrosis transmembrane conductance regulator (CFTR) gene located on chromosome 7. In western studies, 70% of these men have common cystic fibrosis mutations (usually △F508) on one allele, while 10% have mutations on both alleles (one mutation will be very mild). In the remaining 30% there is a splicing error on intron 8, called the T5 allele. If both husband and wife carry the △F508 mutation the child is at risk of developing cystic fibrosis; if they both have poly-T variants of intron 8 then the child be born with vas aplasia. Thus, genetic testing of a couple undergoing ICSI for vas aplasia is very important. Unfortunately, there is no information on the prevalence of CFTR gene mutations in the Indian population. Clinically, cystic fibrosis appears to be uncommon in Indian patients, but there is an urgent need to study the large number of vas aplasia patients who are seeking therapy, so as to understand their genetic status and the risk to the offspring produced through ICSI.

(2) Chromosomal abnormalities. A meta-analysis of chromosomal studies in men with azoospermia or severe oligozoospermia (Van Assche et al., 1996) reported a 3.8% incidence of sex chromosomal and 1.3% incidence of autosomal chromosomal abnormalities. This is much higher than the corresponding incidence in newborns of 0.14% and 0.25% respectively. These chromosomal defects may result in a higher abortion rate. They may also lead to inherited chromosomal defects in the child or even dangerous unbalanced translocations.

(3) DAZ gene deletions - Micro-deletions in the distal portion of the euchromatic region of the long arm of the Y chromosome are identified in 13 % of patients with non-obstructive azoospermia or severe oligozoospermia (Reijo et al., 1995). Transmission of these deletions will result in infertility in the male offspring.

(b) Unknown risks - There are several theoretical reasons why ICSI may lead to genetic abnormalities. Points of concern are:

(1) The process of ICSI bypasses several of the cellular interactions which may be essential for proper egg activation.

(2) Aspiration of the cytoplasm (to confirm that the injection pipette has penetrated the oolemma) may damage the egg cytoplasm.

(3) ICSI may interfere with elimination of paternal mitochondria.

(4) ICSI bypasses the natural process of sperm selection and hence may allow genetically damaged sperm to achieve fertilization.

(5) Epididymal sperm obtained from men with obstructions may be aging sperm with a higher incidence of DNA damage.

(6) Immature testicular sperm and sperm precursors could have faulty genomic imprinting which may manifest later as growth retardation or functional impairment. This is discussed in the next section.

**Health of ICSI children**

In general, In-vitro fertilization leads to lower birth weight babies and increased perinatal risk, especially when there are multiple pregnancies.

In this section we will review whether the specific procedure of ICSI additionally compromises the wellbeing of children born through this procedure.

In a report (Tarlatzis and Bili, 1998) on 2486 children born after ICSI using ejaculated sperm, 1.9% major and 7.4% minor malformations were reported. No major and 2.5% minor malformations were observed in 119 babies born after use of epididymal sperm, and 4.8% major and 3.2% minor malformations were observed in 63 babies from testicular sperm. These rates are within the range observed in the general population.

Bonduelle et al., (1996) studied 486 karyotypes in 877 children born after ICSI and found a 1.2% incidence of de novo sex chromosome abnormalities. This is higher than expected in the general population. While sex chromosomal abnormalities usually pose no major health problems and there is no mental retardation, the individual will usually be infertile and major malformations do occur in Turner syndrome. The health implications of this need to be discussed with couples undergoing ICSI so that they may decide regarding the need for prenatal genetic diagnosis and whether they wish to undergo abortion if any abnormality is detected (Meschede and Horst, (1997).

Recetly, a report by Bowen et al. (1998), comparing ICSI and IVF babies born in Australia, suggested that ICSI babies fared lower in the Mental Development Index (MDI) scale; further, males fared poorer then females. However, this study has been criticized on grounds of inappropriate test methods and significant differences in the two patient populations. On the other hand, data from the Brussels group (Bonduelle et al, 1998) did not find any significant retardation in the psychomotor development, at the age of two years, in the ICSI babies studied by them.

**Thus, the current data suggests that:**

- ICSI does not lead to an increased incidence of congenital malformations
- There is a slightly increased risk of sex chromosomal abnormalities
- Further evaluation of the long-term psychomotor development of ICSI children is needed before its safety can be confidently asserted.

**Conclusion**

ICSI is a revolutionary new technique that has made it possible to treat most cases of severe male infertility. Even those men who are currently not treatable, will be

able to achieve fertilization in the future through the use of sperm precursor cells. However, the long-term consequences of ICSI are as yet unknown, and though the evidence so far suggests that ICSI does not lead to health risks to the offspring, further studies are needed before the safety of ICSI can be unequivocally asserted. The main limiting factor of ICSI is its cost and in our country donor insemination will continue to play an important role.

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