

# Intracytoplasmic sperm injection (ICSI)

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IVF has become a well-established treatment for certain types of infertility, including long-standing infertility due to tubal disease, endometriosis, unexplained infertility or infertility involving a male factor. Soon it became clear that certain couples with severe male-factor infertility could not be helped by conventional IVF. Extremely low sperm counts, impaired motility and/or abnormal morphology represent the main causes of failed fertilization in conventional IVF. In order to tackle this problem, several procedures of assisted fertilization based on micromanipulation of oocytes and spermatozoa have been established. The evolution of these techniques started with partial zona dissection (PZD), followed by subzonal insemination (SUZI) and finally led to the procedure of intracytoplasmic sperm injection (ICSI). ICSI represents the injection of a single spermatozoon directly into the ooplasm, thereby crossing not only the zona pellucida but also the oolemma.

In 1992, the first human pregnancies and births after replacement of embryos generated by this novel procedure of assisted fertilization were reported (Palermo *et al.*, 1992). The use of PZD had become controversial and was subsequently abandoned by many workers. Fertilization rates after ICSI were reported to be significantly better than after SUZI (Van Steirteghem *et al.*, 1993a). Moreover, ICSI resulted in the production of more embryos with higher implantation rates, in comparison with SUZI (Van Steirteghem *et al.*, 1993b). As a result, ICSI has been used worldwide and successfully to treat infertility due to impaired testicular function or obstruction of the excretory ducts resulting in severe oligoasthenoteratozoospermia or even azoospermia in the ejaculate.

Successful IVF depends on the presence in the ejaculate of a certain number of spermatozoa with good motility and morphology. Riedel *et al.* (1989) reported minimum andrological requirements for in-vitro fertility by means of conventional IVF:  $5 \times 10^6$ /ml total count, 30% progressive motility and 30% normal morphology. Men with sperm parameters below these values were considered to have a poor prognosis. Today, however, the most efficient procedure to treat this type of male infertility is ICSI: only one motile (live) spermatozoon is required per mature metaphase II oocyte to be injected. A

summary of several years of ICSI practice indicated that similar results are achieved by ICSI with abnormal semen as by conventional IVF with normal semen parameters (Van Steirteghem *et al.*, 1995a; Staessen *et al.*, 1999).

The current indications for ICSI are as follows. ICSI with ejaculated spermatozoa can be used successfully in patients with fertilization failures after conventional IVF or other assisted fertilization procedures, and also in patients who cannot be accepted for these procedures because too few morphologically normal and progressively motile spermatozoa are present in the ejaculate (<500 000). High fertilization and pregnancy rates can be obtained when a motile spermatozoon is injected. Injection of only immotile or probably non-vital spermatozoa results in lower fertilization rates. Absolute asthenozoospermia is a sporadic rather than a permanent condition, except in cases of ultrastructural sperm defects. Thus, if the injection of ejaculated immotile spermatozoa in an initial cycle leads to poor results, performance of subsequent ICSI cycles with ejaculated spermatozoa is justified. Indeed, motile spermatozoa can be found in repetitive ejaculates. In cases where only immotile spermatozoa are available for microinjection, it is important to select vital spermatozoa, which can be done by using a hypo-osmotic swelling test. This test should be preceded by an eosin-Y staining test in order to exclude complete necrozoospermia. In the latter case where only non-vital sperm cells are present in the ejaculate, the use of testicular sperm is indicated. Other semen parameters, such as concentration, morphology (except for globozoospermia) and high titres of antisperm antibodies, do not influence the success rates of ICSI. Successful ICSI has also been described for patients with acrosomeless spermatozoa. Oocyte activation with  $\text{CaCl}_2$  and Ca-ionophore can be attempted in these cases.

Any form of infertility due to obstruction of the excretory ducts can be treated by ICSI with spermatozoa microsurgically recovered from either the epididymis or the testis. When no motile spermatozoa can be retrieved from the epididymis due to epididymal fibrosis, testicular spermatozoa can be isolated from a testicular biopsy specimen. Two approaches are used to obtain testicular tissue: an open excisional biopsy or fine needle aspiration. Testicular biopsy has also proven to be useful in some cases of non-obstructive azoospermia: in patients with severely impaired testicular function due to (incomplete) germ-cell aplasia (Sertoli cell-only syndrome), hypospermatogenesis or incomplete maturation arrest, spermatozoa may be recovered, sometimes only after taking multiple biopsies. Testicular sperm recovery may not always be successful in all azoospermic patients. Those factors correlated with a successful recovery procedure could allow objective counsel-

ling based on predictive factors. However, no strong predictors for successful testicular sperm recovery were determined except for testicular histopathology.

Optimal sperm recovery from a testicular biopsy can be obtained by finely mincing the tissue. Very often, vital spermatozoa can only be successfully recovered for ICSI after processing the biopsy specimens with red blood cell lysis buffer and/or an enzymatic collagen digestion medium. Cryopreservation of supernumerary spermatozoa recovered from the epididymis or the testis is an important issue because microinjection of cryo-thawed sperm cells can avoid repeated surgery in future ICSI cycles. Successful ICSI using cryo-thawed epididymal sperm has been described. Although pregnancies resulting from ICSI with cryopreserved testicular sperm have been reported, cryopreservation of testicular sperm is less frequently used and more difficult because only few spermatozoa are present.

A successful ICSI programme depends on ovarian stimulation, which is essentially similar to conventional IVF. The combination of gonadotrophin-releasing hormone (GnRH) agonists, human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (HCG) allows the retrieval of a high number of cumulus-oocyte complexes and ovulation is induced using HCG. In conventional IVF, oocytes are inseminated while they are lodged within the cumulus complexes. Prior to fertilization by means of micromanipulation, oocytes need to be denuded from the surrounding cumulus and corona cells, allowing not only precise injection of the oocytes, but also the assessment of their maturity, which is of critical importance for ICSI. Cumulus and corona cells are removed using a combination of enzymatic (hyaluronidase) and mechanical procedures. Denuded oocytes can be observed under the inverted microscope ( $\times 200$  magnification) in order to assess nuclear maturity. Observations include assessment of the zona pellucida and the oocyte, and the presence or absence of a germinal vesicle or a first polar body. As only metaphase II oocytes can be fertilized normally, ICSI is carried out on such oocytes.

During ICSI special attention should be given to the following points: (i) the selection and immobilization of a viable sperm cell; (ii) the correct positioning of the oocyte prior to injection and (iii) the rupture of the oolemma prior to the release of the sperm cell into the oocyte. Figure 7.1 illustrates the whole injection procedure and further explanation is written in the legend. Recent experiments using Hoechst-stained oocytes for microinjection clearly showed no interference with the spindle if oocytes are injected with the polar body at the 6 o'clock position (Figure 7.2).

The injected oocytes can be examined for intactness and fertilization about 16–18 h after ICSI. An average damage rate of ~10% of the injected oocytes can be expected, irrespective of the origin of the spermatozoa used (Figure 7.3). The number and aspect of polar bodies and pronuclei are recorded. Oocytes are considered to be normally fertilized when two individual or fragmented polar bodies are present together with two

clearly visible pronuclei (2PN) that contain nucleoli (Figure 7.4). The expected fertilization rate after ICSI using ejaculated spermatozoa is 60–70% when expressed per number of injected oocytes. The normal fertilization rate for ICSI using the three other types of spermatozoa (fresh and frozen-thawed epididymal spermatozoa and testicular spermatozoa) is somewhat lower (55–60%), compared with ejaculated spermatozoa. Abnormal fertilization may occur as one pronuclear (1PN) oocytes (~3% of the injected oocytes). If such abnormally fertilized 1PN oocytes cleave, they are not transferred, because they are likely to be parthenogenetically activated as a result of mechanical or chemical factors. The occasional finding of three-pronuclear (3PN) oocytes (~4%) after injection of a single spermatozoon into the ooplasm is probably caused by non-extrusion of the second polar body at the time of fertilization. Neither type of embryo resulting from 3PN oocytes is transferred to patients. Since 1PN and 3PN oocytes have a similar potential to result in apparently good quality embryos as 2PN oocytes, it is of major importance to observe the presence of two pronuclei to ascertain normal fertilization.

After a further 24 h *in vitro*, the cleavage characteristics of the fertilized oocytes are evaluated: numbers and sizes of blastomeres and the presence of anucleate cytoplasmic fragments are recorded. About two thirds of the 2PN oocytes after ICSI develop into cleaved embryos which are suitable for transfer or freezing. Embryo replacement of at least one embryo is possible in ~90% of the treatment cycles and is not influenced by the source of spermatozoa used. Within an ICSI programme, the embryo transfer policy is similar than for conventional IVF.

Follow-up of almost 2000 children born after ICSI did not show a higher incidence of malformations in comparison with conventional IVF and the general population (Bonduelle *et al.*, 1999).

## References

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## Figure Legends

### Figure 7.1

The ICSI procedure on a human metaphase II oocyte. (7.1.1) In the injection pipette, which is filled with polyvinylpyrrolidone (PVP), a single living morphologically normal looking spermatozoon is aspirated. Viability is shown by the motility of the sperm cell, even if it is only a slight twitching of the tail. Prior to injection, the sperm cell is immobilized. Immobilization of a sperm cell involves rubbing the tail with the pipette against the bottom of the dish, which results in a breakage at one point, preferably below the midpiece. Immobilization of spermatozoa has been proven to be important for oocyte activation which is achieved by release of sperm cytosolic factors via the ruptured membrane. After immobilization, the sperm cell is again aspirated tail-first, which will allow the injection of a minimal volume of medium together with the sperm cell. The oocyte is held in position by means of minimal suction by the holding pipette. The polar body is located at the 6 o'clock position to avoid damage to the spindle. If both the holding pipette and the oocyte are in perfect focus, the injection needle, containing the immobilized sperm cell near the tip, can be introduced in the equatorial plane of the oocyte at the 3 o'clock position. It is important to keep the tip of the injection pipette in permanent focus to ensure that it remains in the equatorial plane of the oocyte. (7.1.2) Passing through the zona pellucida is fairly easy and achieved by simply advancing the injection pipette. On the contrary, the oolemma is not always immediately pierced by simple injection of the needle and often slight suction needs to be applied. The ooplasm then enters the injection pipette and sudden acceleration of the flow indicates membrane rupture. The aspiration is immediately stopped and the sperm cell is then slowly released into the oocyte with a minimal volume of medium. (7.1.3) The injection pipette can then be withdrawn carefully. The injected spermatozoon can easily be appreciated in the centre of the oocyte (courtesy of A.DeVos, Brussels, Belgium. Published with permission from Van Steirteghem, A.C., Joris, H., Liu, J. *et al. Hum. Reprod. Update* (1995b) 1, CD ROM).

### Figure 7.2

Hoechst 33258 staining of human oocytes before, during and after ICSI. Recent experiments using Hoechst stained human oocytes for

microinjection clearly showed no interference with the spindle if oocytes are injected with the polar body at the 6 o'clock position. The staining procedure allowed visualization of the different events occurring within 6 h of ICSI. (7.2.1) A mature metaphase-II oocyte, showing the first polar body and the metaphase of the oocyte ; (7.2.2) aspiration of the oolemma during ICSI, the metaphase plate of the oocyte is not disrupted during the procedure ; (7.2.3) condensed sperm head, 1 h after ICSI ; (7.2.4) decondensed sperm head, 90 min after ICSI ; (7.2.5) extrusion of the second polar body, 90 min after ICSI ; (7.2.6) the second polar body is completely extruded 2 h after ICSI ; (7.2.7) decondensed sperm head and female pronucleus, 4 h after ICSI ; the same 2PN zygote, 6 h after ICSI, under fluorescent (7.2.8) and light microscopy (7.2.9). Original magnification  $\times 600$  in all cases (courtesy of A.Van de Velde, Brussels, Belgium).

### Figure 7.3

A human metaphase II oocyte lysed during ICSI. The injected oocytes can be examined for intactness and fertilization ~16–18 h after ICSI. An average damage rate of ~10% of the injected oocytes can be expected, irrespective of the origin of the spermatozoa. Immediate rupture of the oolemma upon insertion of the microneedle without any aspiration has been associated with lower oocyte survival rates (courtesy of L.Gianaroli, Bologna, Italy).

### Figure 7.4

A normal fertilized human oocyte containing two pronuclei. The number and appearance of polar bodies and pronuclei are recorded ~16–18 h after injection. Oocytes are considered to be normally fertilized when two individualized or fragmented polar bodies are present together with two clearly visible pronuclei (2PN) that contain nucleoli. This oocyte shows 2PN which are closely in apposition with polarized nucleoli and the axis of the 2PN almost in line with a polar body (at the 07:30 position) (courtesy of J.Liebermann, Würzburg, Germany).

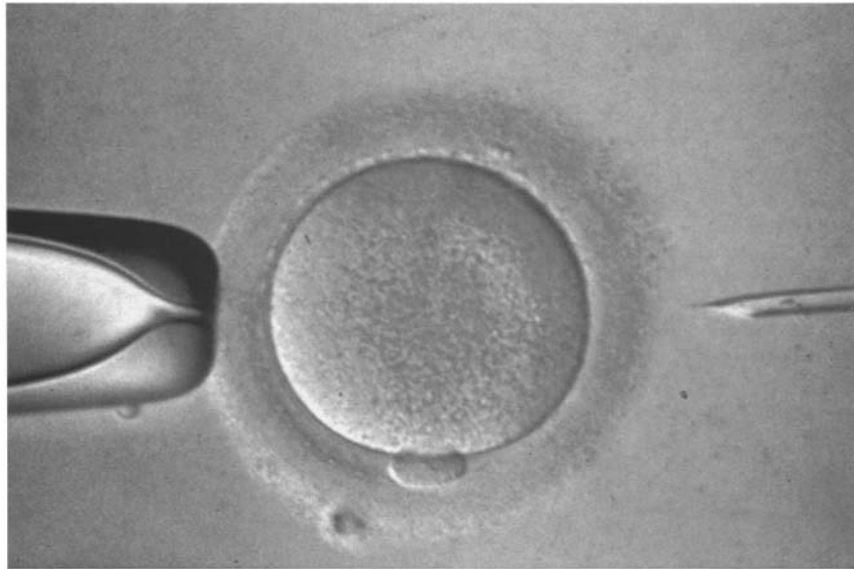


Figure 7.1.1



Figure 7.1.2

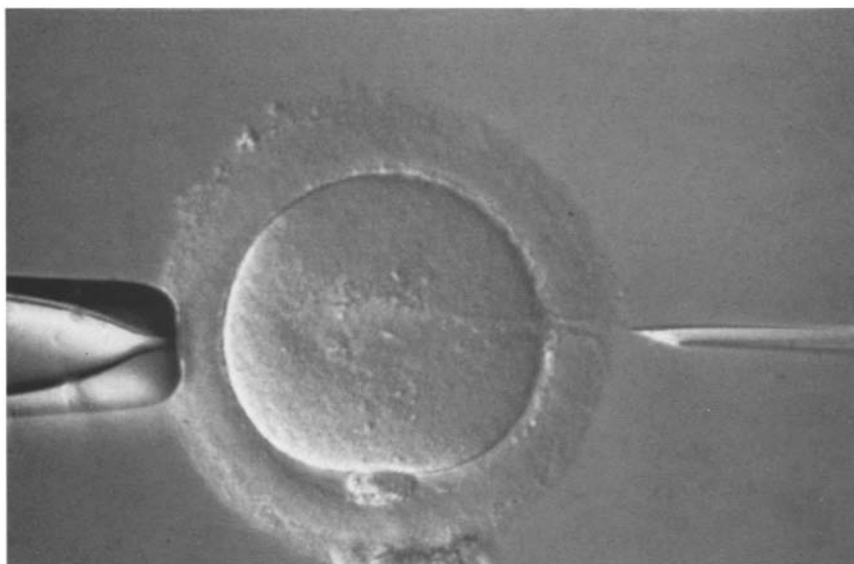


Figure 7.1.3

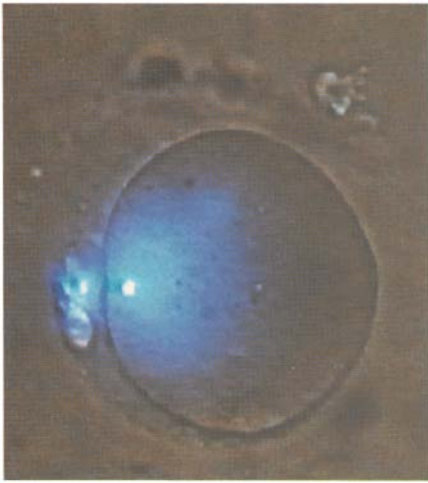


Figure 7.2.1



Figure 7.2.2

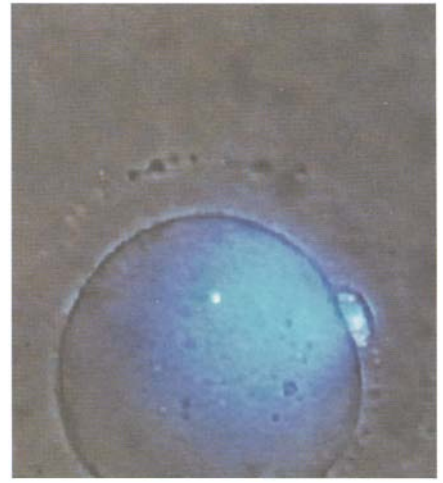


Figure 7.2.3



Figure 7.2.4

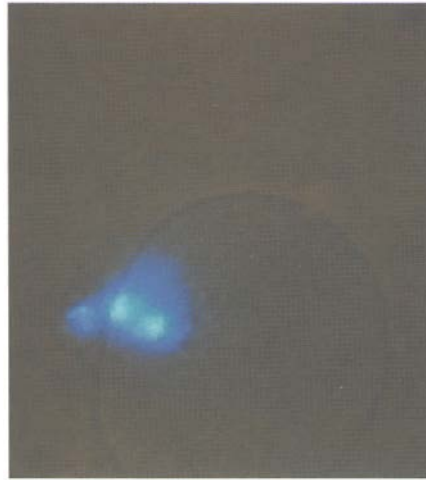


Figure 7.2.5

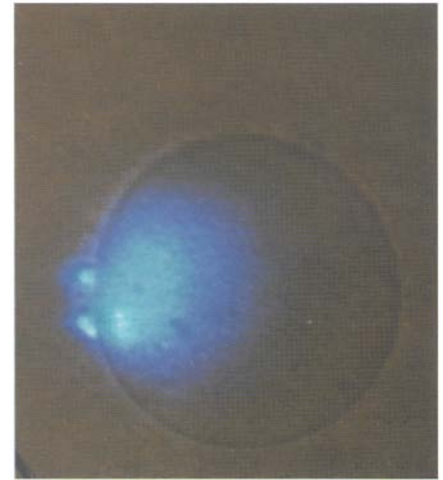


Figure 7.2.6

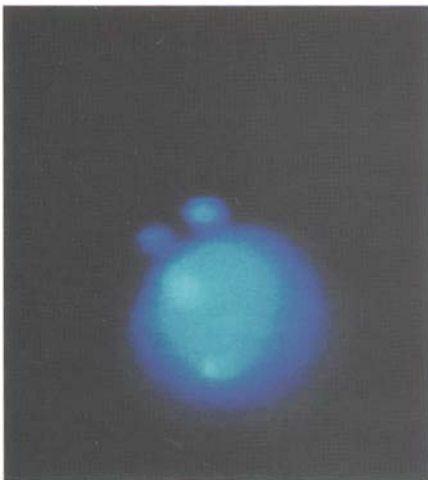


Figure 7.2.7

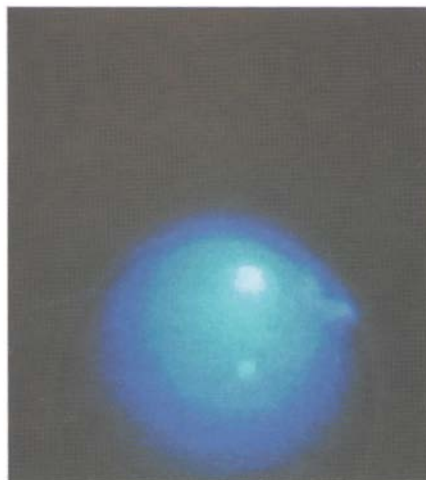


Figure 7.2.8



Figure 7.2.9

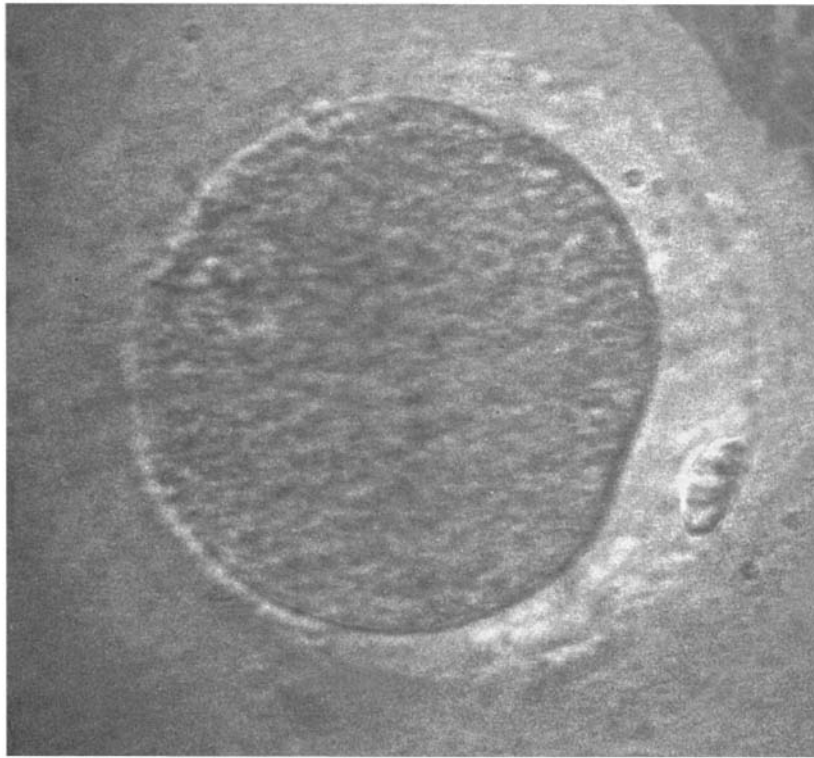


Figure 7.3



Figure 7.4