

Celebrating ICSI's twentieth anniversary and the birth of more than 2.5 million children—the 'how, why, when and where'

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Why did we start?

The first major breakthrough for the alleviation of tubal infertility occurred in the last quarter of the twentieth century. Louise Brown was born on the 25th July, 1978 in Oldham, England as a result of the pioneering work of Bob Edwards and the late Patrick Steptoe with the help of the late Jean Purdy. Bob Edwards received the 'Nobel Prize for Physiology or Medicine' for this work in 2010. In the early eighties IVF practice was gradually introduced in many countries and became a successful treatment for the alleviation of longstanding female-factor and idiopathic infertility. However, the hypothesis that bringing the male gametes closer to the oocytes would be an effective treatment in couples with reduced numbers of spermatozoa was not supported. As soon as sperm parameters were below a certain threshold, fertilization of oocytes was much reduced or failed completely. It became obvious that conventional IVF could not solve many cases of male-factor infertility. For couples with a very low sperm count or azoospermia it was necessary to resort to the use of artificial insemination with donor sperm (AID). As a consequence, several groups embarked on research aimed at assisting the fertilization process. Micromanipulation procedures were introduced. The two initial assisted fertilization procedures: zona drilling and partial zona dissection were not successful. Zona drilling involved making a hole in the zona pellucida of an oocyte, which was then incubated in a sperm suspension. Zona drilling worked well in mice but not in humans. Partial zona dissection was introduced afterwards. A mechanical slit was made in the oocytes, which were then incubated in a sperm suspension. Fertilization was obtained by this method but when it occurred there was a similar percentage of monospermic and polyspermic fertilization. Although some pregnancies and births occurred, inconsistent results meant that partial zona dissection was not widely applied in the clinic. Around the same time a few case reports were published on the next assisted fertilization procedure: subzonal insemination (SUZI), a micromanipulation technique involving the insertion of a few spermatozoa between the zona pellucida and the membrane of the oocyte. We decided to embark on this approach for assisted fertilization.

What did we do?

We started by investigating the following question, supported by a grant from the Fund for Scientific Research, Flanders—'Does the enhancement of the acrosome of mouse sperm (using chemical means or applying electroporation) result in fertilization and embryo development after a single 'treated' sperm was injected subzonally?'. Our hypothesis proved to be correct: good fertilization and embryo development occurred, many normal pups were born and they were able to reproduce. These experimental results led us to consider introduction of SUZI into the clinic for patients that had failed several cycles of conventional IVF. Ethical approval was asked and obtained from the VUB Hospital Ethical Committee under the condition that all pregnancies and children born, would be part of a thorough follow-up programme. The patients were fully informed on the new procedure and agreed to the follow-up programme including a prenatal diagnosis by either chorionic villous sampling or amniocentesis. Clinical SUZI was started at VUB in 1990 and a number of pregnancies and births occurred after SUZI of a few spermatozoa, which had been prior treated to enhance the acrosome reaction. The technical procedure of SUZI is delicate and occasionally one of the sperm entered into the cytoplasm of the oocyte. In these cases of 'failed SUZI' we observed normal fertilization as well as embryo development. These 'failed' SUZI cases were carefully observed for their development. In the event that only one such embryo was available it was transferred into the patient. We called this procedure intracytoplasmic sperm injection—ICSI. In April 1991 a patient became pregnant after replacing a single ICSI embryo and she delivered on January 14th 1992—almost 20 years ago. After the initial ICSI observations we continued SUZI and also included ICSI on some oocytes in most cycles. It rapidly became very obvious that the results in terms of fertilization were much more consistent after ICSI than after SUZI, and we obtained ethical approval for ICSI under the same strict protocol regarding follow-up of any pregnancy. As of July 1992 the only assisted fertilization procedure practiced at VUB was ICSI. From late 1992 several live-video ICSI workshops were held at the VUB which

helped a lot with the dissemination of ICSI worldwide. The VUB's policy of openness to the world in showing ICSI was similar to the approach taken by the Melbourne groups for the introduction of conventional IVF.

What did we find?

ICSI proved to be a consistent treatment for the alleviation of infertility due to severe semen abnormalities including cryptozoospermia. After the successes with ejaculated spermatozoa, we applied ICSI to epididymal or testicular sperm in cases of azoospermia. Results of ICSI in cases with obstructive azoospermia using epididymal or testicular spermatozoa were similar to the results of ICSI with ejaculated sperm and the results of conventional IVF for female-factor infertility. However spermatozoa can be found in only half of the patients with non-obstructive azoospermia after testicular biopsy, even after extensive and prolonged searching. If no sperm can be found the sole alternative for these couples is the use of AID.

Around the time of the first ICSI successes the first reports appeared of births after preimplantation genetic diagnosis—PGD, a very early form of prenatal diagnosis for couples at high risk of having a child with a genetic disease. PGD involves the removal of one (occasionally two) blastomere(s) from a developing embryo (usually at around the 8-cell stage). The use of ICSI as ART in these couples is indicated for two reasons: to avoid unexpected fertilization failures but also to avoid contamination with sperm DNA or cumulus cells adhering to the zona pellucida at the time of embryo biopsy. At its inception, and even now, questions are asked about the risk for the offspring after ICSI, which is much more invasive than conventional IVF and bypasses a number of steps occurring in natural conception and even conventional IVF. The careful evaluation of pregnancies and children was always a condition of approval by the VUB Ethical Committee. Over the last 20 years, the number of ART children born in the VUB programme is well over 15,000 and it is fair to summarize the outcome results as follows: there is a slight increase in fetal chromosomal abnormalities after ICSI, the congenital malformation rate is also slightly higher than after natural conception but there is no difference between IVF and ICSI. These single-center results are

supported by a number of extensive register-based results on outcome, particularly from Scandinavian countries. In addition, the outcome of well over 1000 PGD children at VUB is no different than the outcome after ICSI.

What does it mean?

Twenty years after the first ICSI birth it is fair to say that the successful introduction of ICSI has meant that the vast majority of cases with severe male-factor infertility, including some cases of azoospermia, can now have their childwish fulfilled. A number of groups have started to use ICSI for all patients with the argument that unexpected fertilization failure can then be avoided. In my view there is no reason to use ICSI when semen parameters are normal or almost normal, except for PGD cases, as mentioned above. The major drawback of this form of ART is no different from other forms of ART: the multiple birth rate is still too high and avoiding this iatrogenic complication is the major challenge for all centres. The solution is available and simple—ie single embryo transfer, perhaps not for all age groups but certainly for patients below 38 years of age in their first treatment cycles.

Clinical research should continue in different areas not the least in the long-term outcome of IVF-ICSI children. In spite of the major advance ICSI provided for the alleviation of male-factor infertility, we still do not understand the basics of infertility; basic research needs full support to entangle the physiopathology of infertility

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