# Successful pregnancy in a case of azoospermic infertility by using testicular sperm for intracytoplasmic injection into the oocyte

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**Non-obstructive azoospermia used to be considered an untreatable cause of infertility. By the microinjection technique, however, sperm that has been surgically extracted from the testis can be injected into the oocyte cytoplasm. The injected eggs can be transferred to the uterus or fallopian tubes to initiate a pregnancy. A healthy baby boy conceived by using this method was delivered in November 1997. This micromanipulation technique offers couples in which the man has non-obstructive azoospermia the chance of having their own offspring. The methodology used and a brief discussion of its merits are presented.**

*HKMJ 1999;5:69-71*

*Key words: Fertilization in vitro; Microinjections; Oligospermia; Reproduction techniques*

## Introduction

In 1978, the world's first baby conceived by the in vitro fertilisation (IVF) technique, Louise Brown, was born. Since then, advancements have been made in the IVF technique and its allied technologies. Of all advancements, the most significant are the micromanipulation techniques, which, when used singly or in combination, are instrumental in circumventing male fertility problems that had been considered insurmountable. Intracytoplasmic sperm injection (ICSI) was first described in 1992[1] and has revolutionised the management of severe male infertility; severe asthenospermia, severe oligospermia, failed vasovasostomy, and azoospermia have all been successfully treated by using the ICSI technique.[2,3] This report is on a case of non-obstructive azoospermia that was successfully treated by a combination of IVF technologies in a private IVF programme in Hong Kong.

## Case report

A 32-year-old man and 30-year-old woman who had no significant family or medical problems presented to the IVF Centre at the Hong Kong Sanatorium and Hospital in May 1996. The man was found to be azoospermic from two different semen analyses, and physical examination showed normal secondary sexual characteristics; the testes, epididymides, and vasa were normal. The level of follicle-stimulating hormone was 26.3 IU/L (normal range, 1.0-10.0 IU/L). A testicular biopsy showed mostly Sertoli's cells and in the seminiferous tubules, only approximately 10% showed evidence of spermatogenesis. The couple was counselled about—and subsequently consented to—the ICSI technique and the need to recover sperm by performing a testicular biopsy.

### *The first pregnancy*

In September 1996, controlled ovarian hyperstimulation was achieved by giving the woman intramuscular urofollitrophin (Metrodin; Serono Laboratory, Aubonne, Switzerland) 150 U twice daily, starting on day 3 of the menstrual cycle, for 6 days. Follicular growth was monitored by determining the plasma oestradiol level and by intravaginal ultrasonography. On day 10 of the menstrual cycle, human chorionic gonadotrophin 10000 U was given by intramuscular injection and oocyte collection was scheduled for 36 hours later (day 12).

On the morning of day 12, an open testicular exploration/biopsy under general anaesthesia was performed on the man by a urologist. The biopsy tissue was transferred to a petri dish containing N-[2-hydroxyethyl]piperazine-N-[2-ethanesulphonic

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acid] (HEPES)-buffered Earle's medium (Sigma, St Louis, US) and shredded into small pieces using two 1-mL tuberculin syringes. The suspension was then examined under the microscope and when sperm were identified, the biopsy tissue was removed and the suspension was centrifuged at 300 *g* for 5 minutes. After the sperm pellet was resuspended, approximately 100 live, normal-looking mature sperm were identified.

Oocyte collection was performed by a gynaecological surgeon while the woman was sedated. By using an ultrasound-guided transvaginal needle aspiration technique, 10 oocytes were obtained. They were examined and were all found to be in the metaphase II stage of meiosis and suitable for ICSI. During the ICSI procedure, the woman was given anaesthesia and laparoscopy was commenced. When the ICSI was completed, six injected oocytes—three to each ampulla—were transferred to the fallopian tubes under video guidance.

Pregnancy was confirmed 16 days after the procedure. The pregnancy progressed but spontaneous abortion occurred on the 45th day of gestation.

***The second pregnancy***

In March 1997, a second round of ovarian hyperstimulation was done. A second open testicular biopsy yielded only six live, mature, normal-looking spermatozoa. Transvaginal aspiration obtained six oocytes, five of which were in the metaphase II stage of meiosis. Following ICSI, two and three sperm-injected oocytes were transferred into the left and right fallopian tube, respectively.

Pregnancy was confirmed 16 days after the procedure and progressed normally. At 17 weeks of gestation, an amniocentesis was conducted and showed the baby to be a normal male. Pregnancy progressed without complication, and a healthy baby boy, in footling breech presentation, was delivered by caesarean section in November 1997.

## Discussion

Azoospermia used to be regarded as absolute sterility, and the available options were to adopt children or to artificially inseminate oocytes with donor sperm (donor insemination). The liberalisation of abortion laws, however, has made adoption more difficult. In addition, donor insemination has associated problems of a low availability of donor sperm, poor semen quality, and a possibility of transferring human immunodeficiency virus. In Hong Kong, semen donors are scarce, and the long waiting lists and age limit imposed on recipients in the local donor insemination programme effectively rule out many couples from the programme.

Experience around the world has shown that in most men who present with non-obstructive azoospermia for testicular sperm extraction, the testes have islets of normal spermatogenesis in the seminiferous tubules and thus contain normal sperm.[2-4] Unfortunately, the number of normal sperm is usually insufficient for IVF, which requires 50 000 motile sperm. Allied procedures are thus needed. Intracytoplasmic sperm injection has brought hope to a situation where "the wife is producing more eggs than the husband's spermatozoa—situations never even contemplated in our wildest dreams 2 to 3 years ago".[5] Such situations occurred on this couple's second attempt at pregnancy, when only six usable sperm were identified.

Micromanipulation followed by immediate fallopian tube transfer (MIFT) was first reported in 1994 by McLachlan et al,[6] who replaced oocytes after performing subzonal sperm injection. We believe that ICSI followed by immediate fallopian tube transfer is a better procedure, because there is a higher fertilisation rate. Treating infertility by using assisted reproduction techniques such as gamete (sperm and egg) intrafallopian transfer (GIFT) may be better than using IVF, because of the more physiological tubal environment for fertilisation and embryo development.[6] For the same reason, MIFT has a higher pregnancy rate than does ICSI-IVF.[6,7] Because of the lower fertilisation rate we have achieved with ICSI (IVF fertilisation rate: 75% of oocytes; ICSI fertilisation rate: 55% of oocytes; unpublished data), we compensate by placing slightly more oocytes than is usual in cases of unexplained infertility.

Sperm retrieval can be achieved by open testicular biopsy or by epididymal aspiration.[2,4] A technique that immediately cryopreserves sperm-bearing testicular biopsies has recently been reported[8] and consequently, ICSI-IVF or MIFT can be carried out at a later, more convenient date. This method also obviates the logistic difficulty of conducting concurrent operative procedures on the couple.

## Conclusion

We report on two pregnancies in a couple where the husband has non-obstructive azoospermia. In both attempts, using testicular sperm extraction and a direct

ICSI technique, coupled with immediate fallopian tube transfer, the woman became pregnant. The first pregnancy resulted in spontaneous abortion, but the second resulted in the delivery of a healthy, normal baby boy in November 1997. Testicular sperm retrieval and assisted reproduction techniques may now enable such couples to conceive.

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## Editorial comment

Multiple pregnancy is an unwanted outcome of assisted reproductive technology and is associated with a relatively high perinatal morbidity and mortality. Measures are now being taken in some countries to reduce the numbers of multiple pregnancies resulting from these procedures. In the United Kingdom, the maximum number of embryos that can be transferred to patients, by law, is three. The American Society for Reproductive Medicine suggests that no more than three good embryos be transferred to women aged under 35 years, no more than four good embryos to women aged 35 to 40 years, and no more than five good embryos to women older than 40 years or who have had multiple failed attempts.[1] There is currently no legislation in Hong Kong regarding the number of embryos that can be transferred.

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