

SUCCESSFUL *IN VITRO* FERTILIZATION AND PREGNANCY BY THE MICROMANIPULATION WITH SPERM FROM EFFERENT DUCT RETRIEVED BY MICROPUNCTURE TECHNIQUE IN A PATIENT WITH CONGENITAL ABSENCE OF THE VAS DEFERENS

Masanori Yamamoto, Satoshi Katsuno, Keisuke Yokoi,
Hatsuki Hibi and Koji Miyake

From the Department of Urology, Nagoya University School of Medicine

A case of successful *in vitro* microfertilization and pregnancy of 30-year-old woman with sperm from efferent duct retrieved by a micropuncture technique combined with perivascular nerve stimulation from a patient with congenital absence of the vas deferens is reported. Treatment of couples involving men with surgically unreconstructible vasal obstruction is now possible. Our experience indicates that sperm from the efferent duct retrieved by this new technique, when combined with assisted reproductive technologies, permit fertilization and subsequent pregnancy for the men with surgically irreparable vasal obstruction.

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Key words: Microfertilization, Sperm, Efferent duct, Micropuncture

INTRODUCTION

Until recently, the treatment option for men with irreparable obstruction of the reproductive tract involved creation of alloplastic spermatoceles for subsequent percutaneous aspiration of sperm. These sperm were used for intrauterine injection, which has been reported to result in successful pregnancy in a maximum of 4% of cases¹⁾. However, since the report by Temple-Smith et al.²⁾ on the successful use of microsurgically aspirated epididymal sperm *in vitro* fertilization-embryo transfer (IVF-ET), an effective fertility treatment for patients with congenital bilateral absence of the vas deferens has become possible. The combination of microsurgical epididymal sperm aspiration and IVF-ET has been shown to offer couples who are suffering from infertility because of congenital bilateral absence of the vas deferens a chance to have their own genetic children³⁻⁵⁾. However, the success of this combined procedure depends on both the number of motile sperm aspirated and the

number of oocytes retrieved at ovum pickup⁵⁾

Schlegel et al. reported that the epididymal micropuncture technique to minimize blood contamination in the collection of sperm was useful if oocyte micromanipulation was available⁶⁾. Recently, we developed a new technique for epididymal sperm retrieval by combination of micropuncture and nerve stimulation of the spermatic nerve to obtain a large volume of epididymal fluid. Here we demonstrate the first successful pregnancy after microfertilization with sperm from the efferent duct retrieved by this new technique.

CASE REPORT

A 34-year-old man who had been married for two years, was seen for evaluation of azoospermia found on a semen analysis ordered by his wife's gynecologist. His history was normal. On physical examination his testicular size was bilaterally normal, but neither vas deferens were palpable. Seminal volume was only 0.5 ml and seminal pH was 6 (very acidic). The

semen analysis showed fructose negative. The endocrine levels including serum luteinizing hormone, follicle stimulating hormone, testosterone and prolactin were within the normal limit. Right testicular biopsy specimen revealed normal spermatogenesis. The test for antisperm antibody was negative. The antisperm antibody was assayed according to Isojima's method⁷⁾. Transrectal ultrasound revealed absence of seminal vesicles. Renal ultrasound disclosed normal bilateral kidneys. Based on these clinical findings, he was diagnosed with congenital absence of the vas deferens (CAV). Epididymal sperm retrieval by a micropuncture procedure for microfertilization was performed as follows.

Pipette preparation

Small bore (75 μm) micropuncture pipettes were prepared as described by Yamamoto et al⁸⁾. The pipettes were briefly washed in soap and then rinsed several times with distilled water and dried in acetone. The pipettes were then polished on a fine grinding wheel, and the tip sharpened to assist puncture of the epididymal tunics, washed again, coated with silicone and allowed to dry completely. The pipettes were sterilized by ethylene oxide gas just prior to use. The pipettes were placed on the micromanipulator and attached via short lengths of medical grade silastic tubing to a 20 gauge needle, a three way plastic stopcock and a very clean 10 cc glass syringe.

Surgical technique

Under general anesthesia, a right scrotal incision was made and the testis and epididymis were retrieved and stabilized in the testicular holder with the aid of 2% agar (Fig. 1). The micropipettes were placed in a micromanipulator (Narishige, type MM-133) fixed on the metal plate and connected to a 10 cc glass syringe with silastic tubing. A pair of Ag-AgCl ring electrodes were placed around the spermatic cord for perivascular nerve stimulation with D.C. (intensity, 136 V; frequency, 20 Hz) for 30sec (Fig. 1). Electrical stimulation was performed simultaneously with epididymal fluid retrieval every minute

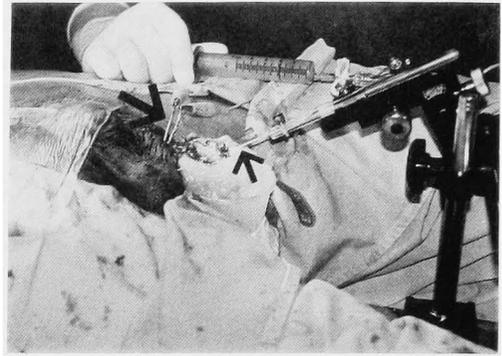


Fig. 1. *In vivo* human epididymal micropuncture. Micropipette is held on the micromanipulator fixed on the metal plate and intraluminal fluid from the efferent duct is aspirated. Large arrow indicates a pair of Ag-AgCl ring electrodes. Small arrow indicates micropipette.

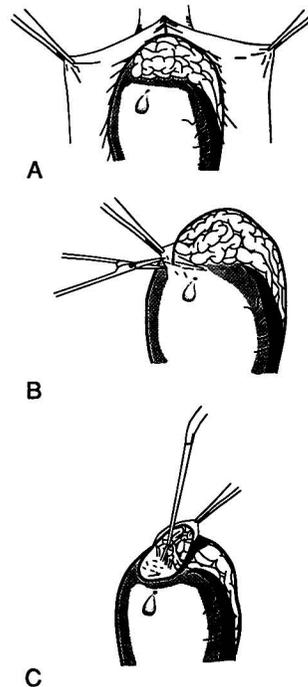


Fig. 2. Surgical technique for accessing the efferent duct. (A) The tunica vaginalis has been opened, exposing the caput epididymis. (B) The epididymal tunic is incised at its junction with the testis over the caput epididymis. (C) After bluntly peeling back the caput to expose the efferent ducts, micropuncture retrieval of sperm from the efferent duct is shown schematically.

until no further fluid was obtained.

Only caput epididymis was recognized. The epididymal tunic was incised and the loop of epididymal tubules was exposed under an operating microscope with a 10- to 25-power magnification. The epididymal tubule was punctured in an area devoid of blood vessels. We performed the first micropuncture on the testicular (proximal) side of the grossly dilated, white tubules that were located in the caput epididymis. However, no motile sperm was found in any area of the caput epididymis after several punctures. The epididymal tunic was incised at its junction with the testis over the caput epididymis (Fig. 2). After bluntly peeling back the caput, micropuncture retrieval of sperm from the efferent duct was successfully performed. When no further fluid could be collected, the pipette was removed from the tubule and the collected fluid was transferred immediately into Ham's F-10 fluid (GIBCO, Grand Island, NY). The aspirated volume of epididymal fluid was 980 μ l. It took approximately 15 minutes to complete the collection of epididymal fluid. Sperm parameters at retrieval were sperm count of 43 million/ml with 25% motility. The percentage of normal morphology was 32%. The total operative time was 2 hours.

Sperm Preparation

The retrieved spermatozoa were centrifuged with 80% percoll and were then collected in a human tubal fluid layer containing pentoxifylline (1 mg/ml). The motile sperm were collected by the swim up technique. The final specimen was diluted to 0.8 ml with the wife's follicular fluid. Microinsemination by use of subzonal insertion of spermatozoa was carried out. Consequently, two embryos were obtained and transferred. Successful pregnancy was confirmed by identification of a fetal heart at ultrasound scan.

DISCUSSION

As recently as a few years ago, CAV was untreatable until it was shown that sperm aspirated from the epididymis could be used for IVF⁵. The introduction of the microsurgical epididymal sperm aspi-

ration technique offered new hope for couples suffering from this form of infertility. However, treatment of couples using sperm obtained from the epididymis is a technically difficult process. Coordination between an experienced IVF-ET team and urologic microsurgeons is important to achieve optimal results. Sperm should be collected from the epididymis with elimination of contaminating blood cells from outside the epididymal lumen because exposure of sperm to these blood cells impairs their fertilizing ability⁹.

Since Silber's optimal results in 1987³, several investigators reported clinical pregnancies achieved by IVF using sperm aspirated from the epididymis in the cases of CAV^{6,8}. Schlegel et al. reported that they applied the epididymal micropuncture technique to 48 couples with surgically unconstructible vasal obstruction and obtained a final pregnancy rate of 27.5%⁶. They demonstrated that optimal sperm quality within a short epididymis was found in the efferent ducts⁶. However, they did not mention about successful pregnancy using sperm retrieved from the efferent duct. The concentration of sperm obtained from the efferent duct was sufficient for IVF, but sperm motility was poor in the present case. Because the fertilization level obtained with epididymal sperm is often less than optimal, the microinsemination technique is a useful adjunct to the epididymal sperm retrieval. In our case we used subzonal insertion of spermatozoa as a microfertilization technique. In the near future a more advanced technique namely intracytoplasmic sperm injection will be available in our reproduction center. For this technique neither motility nor morphology is a real matter for concern^{10,11}. Therefore, this technique may be useful to achieve better fertilization rates with epididymal sperm.

The advantages of our new method are as follows: (1) a large volume of uncontaminated epididymal fluid can be facilitated (2) repeated micropuncture is possible. The present successful pregnancy indicates that sperm retrieved by our new technique when combined with

micromanipulation of the oocyte allows fertilization and subsequent pregnancy for a significant number of patients who have surgically unreconstructible vasal obstruction.

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和文抄録

先天性精管欠損症患者の精巣輸出管から微小穿刺法により 取り出した精子を用いた顕微授精の1成功例

名古屋大学医学部泌尿器科学教室 (主任: 三宅弘治教授)

山本 雅憲, 勝野 暁, 横井 圭介

日比 初紀, 三宅 弘治

先天性精管欠損症患者より微小穿刺法と精索血管周囲神経刺激の組み合わせにより精巣輸出管から精子を取り出し、ついで30歳の妻の卵に顕微授精を行い妊娠に成功した1例を報告する。外科的に修復不可能な精管閉塞症の夫婦の治療は可能である。今回の成功によ

り、われわれの開発した新しい技術と生殖操作の技術を組み合わせることにより、精巣輸出管より取り出した精子を用いて上記のような不妊夫婦が拳児をえることが可能であることが示された。

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