

## Pregnancy with sperm aspiration from the proximal head of the epididymis: A new treatment for congenital absence of the vas deferens

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It has long been assumed that sperm must pass through a certain length of epididymis to mature, gain progressive motility, and become capable of fertilization. In every animal thus far studied, sperm from the unobstructed proximal head of the epididymis exhibit only weak, circular swimming motions and are incapable of progressive motility or fertilization of the egg. It is thought that only when sperm have traversed through most of the corpus epididymis that they mature sufficiently to become progressively motile and are able to fertilize.<sup>1,2</sup> But our observations suggest that their journey through the epididymis may not be an absolute requirement and that sperm may only require a period of time to mature after leaving the testicle.<sup>3</sup>

Congenital absence of the vas deferens accounts for 11% to 50% of cases of obstructive azoospermia and heretofore has been considered basically untreatable.<sup>4</sup> This is a large and frustrating group of patients who have been shown on countless testicle biopsies to have normal spermatogenesis and are theoretically making sperm quite capable of fertilizing an egg. Yet treatment up until the present time has been very dismal.

We have now developed a treatment protocol involving microsurgical aspiration of sperm from the

proximal region of the epididymis, combined with in vitro fertilization (IVF) and zygote intrafallopian transfer (ZIFT), which may offer good results in this frustrating group of couples.

This paper represents the first documentation of IVF and conception with sperm aspirated from the most proximal region of the head of the epididymis and the first documentation of fertilization and pregnancy utilizing direct microsurgical sperm aspiration from the epididymis for the treatment of congenital absence of the vas deferens.

### MATERIALS AND METHODS

#### Induction of Follicular Development And Oocyte Retrieval

The female partners of two men with azospermia caused by congenital absence of the vas were 25 (patient 1) and 35 (patient 2) years of age, respectively. Patient 1 was gravida 1, para 0 (from a previous marriage), and patient 2 was nulligravida. Both patients had presented with regular menstrual cycles, no history of pelvic inflammatory disease (PID), no use of intrauterine device (IUD), and no occurrence of ectopic pregnancy. Ovulation and normal luteal phases were confirmed by ultrasound, hormone analyses, and/or endometrial biopsies. Normal tubal filling and patency was demonstrated by hysterosalpingograms (HSG) performed within 6 months of the attempted surgical procedures.

Both patients underwent induction of multiple follicular development with the following protocol:

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Leuprolide acetate (Lupron, TAP Pharmaceuticals, North Chicago, IL) 1 mg subcutaneously daily (0800 h) from day 1 of the menstrual cycle until the day of follicular aspiration. Patients received human follicle-stimulating hormone (FSH) (Metrodin, Serono Laboratories, Inc., Randolph, MA) and human menopausal gonadotropins (hMG) (Pergonal, Serono) 150 IU intramuscularly (IM) daily (4:00 pm) from day 2 of the menstrual cycle until days 9 and 8 (patients 1 and 2), respectively. Human chorionic gonadotropin (Profasi, Serono, Randolph, MA) 10,000 IU was administered IM (9:00 pm) on days 9 and 10, respectively. On that day, patient 1 presented with 20 follicles ranging from 12 to 17 mm by vaginal ultrasound, 2978 pg/ml of serum estradiol ( $E_2$ ), and excellent cervical mucus score (abundant, clear, spinnbarkeit 15 cm, ferning 3+). Patient 2 showed, on the day of hCG injection, 13 follicles ranging between 12 and 17 mm, 2270 pg/ml of serum  $E_2$ , and an excellent cervical mucus score (abundant, clear, spinnbarkeit 10 cm, ferning 3+).

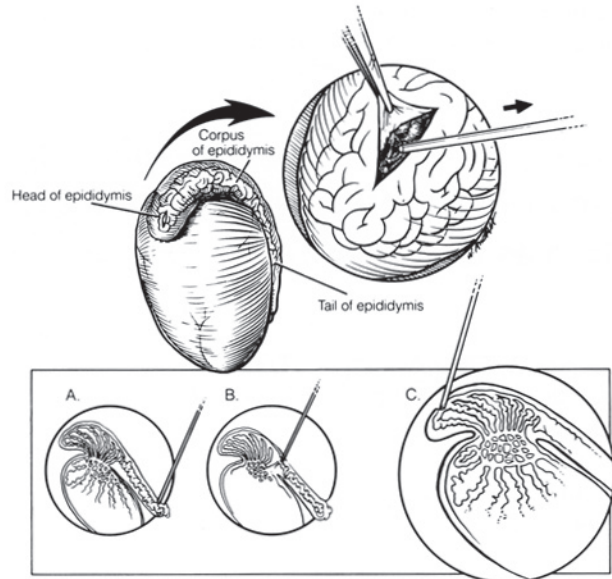
Thirty-six hours after hCG administration, the patients underwent follicular aspiration in the operating room under intravenous sedation with titrating doses of 0.1 to 0.25 mg of fentanyl (Sublimaze, Janssen Pharmaceutical, Inc., Piscataway, NJ) and 5 to 7 mg of midazolam HCl (Versed, Roche Laboratories, Division of Hoffmann-La Roche, Nutley, NJ).

Follicular aspiration was performed using a transvaginal probe (GE H4222 TV) adapted to an ultrasound system (GE RT3,000, General Electric Company, Milwaukee, WI) with a needle set for ovum aspiration and follicle flushing (Labotect, Bovenden-Gotingen, FRG) (#4060-2, length 300 mm, 1.4 mm outside diameter, 1.1 mm inside diameter) connected to a Craft Suction Unit (Rocket USA, Branford, CT) (#33-100) at a maximum vacuum pressure of 120 mmHg.

Each case of follicular aspiration was performed without complications in less than 30 minutes and patients were discharged two hours after the outpatient procedure. The follicular fluids and follicular washings (with TALP-HEPES medium) were given immediately to the embryology laboratory adjacent to the operating room.

### Epididymal Sperm Aspiration

At the same time, the husband underwent scrotal exploration with the intention of aspirating sufficient numbers of motile spermatozoa to utilize



**Figure 1** Technique for epididymal sperm aspiration that begins in the distal corpus region of the epididymis and moves proximally until motile sperm are recovered. In most cases, motility is observed only in the most proximal region of the epididymis.

for IVF of the aspirated eggs, with subsequent transfer into the wife's fallopian tube.

The surgical technique (Fig. 1) in the male was as follows: scrotal contents were extruded through a small incision, the tunica vaginalis was opened, and the epididymis was exposed. Under 10 to 40 $\times$  magnification with an operating microscope, a tiny incision was made with microscissors into the epididymal tunic to expose the tubules in the most distal portion of the congenitally blind ending epididymis. Sperm were aspirated with a #22 medicut on a tuberculin syringe directly from the opening in the epididymal tubule. Great care was taken not to contaminate the specimen with blood, and careful hemostasis was achieved with microbipolar forceps. The epididymal fluid was immediately diluted in HEPES buffered media, and a tiny portion examined for motility and quality of progression. If there was no motility or poor motility, another aspiration was made 0.5 cm more proximally. We thus obtained sperm from successively more and more proximal regions until progressive motility was found. In both of these cases, motile sperm were not obtained until we reached the most proximal portion of the caput epididymis (Fig. 1).

Once we reached the area of motile sperm, epididymal fluid was aspirated in sufficient quantity over a period of 10 to 15 minutes, to obtain a total sperm count of 20,000,000 in patient 1 and of



10,000,000 spermatozoa in patient 2. The scrotum was then closed with 000 Vicryl interrupted sutures, the patients were kept in the hospital overnight, and had minimal postoperative pain.

#### IVF Techniques

Microsurgically aspirated sperm were immediately diluted into 3 ml of a HEPES-buffered modified Tyrodes's solution—TALP-HEPES supplemented with 0.5% human serum albumin (Sigma, Holmdel, NJ, fraction V powder). An aliquot was examined under a microscope for motility. When motile sperm were recovered, the samples were further diluted with 3 ml of TALP-HEPES, centrifuged at  $300 \times g$  for 10 minutes and the supernatant removed. The sperm pellets were resuspended in 100  $\mu$ l TALP-HEPES and incubated at 37°C for 1 hour in patient 2, layered with 100  $\mu$ l medium, and incubated for 1 hour by a swim-up technique in patient 1. After 1 hour of incubation, 10 to 25  $\mu$ l of either the resuspension or resulting swim-up was added to the culture tubes containing the oocytes and 1 ml of Menezo's B<sub>2</sub> medium (IMV International, Minneapolis, MN) supplemented with 15% heat-activated human fetal cord serum. Between five and seven oocytes were cultured together in each tube at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> and air. Before insemination, excess cumulus cells were removed from the oocytes by means of fine-gauge needles. Oocytes were incubated with the sperm 12 to 15 hours, then transferred to fresh culture medium and examined for signs of fertilization (presence of two pronuclei).

#### Tubal Embryo Transfer

Fifty-five hours after insemination, five embryos were transferred to the fallopian tubes of each patient, via minilaparotomy with a technique similar to the one for gamete intrafallopian transfer (GIFT), via a Tomcat catheter (Monoject, St. Louis, MO) 2.5 cm inside the fimbrial ostium. The entire surgical procedure lasted approximately 30 minutes, and the patients were discharged the next day and underwent an uneventful postoperative recovery. Both patients received progesterone in oil, 25 mg IM/day since the day of embryo transfer.

### RESULTS

Patient 1 epididymal aspirate yielded a total count of 20,000,000 sperm with only 5% motility, and forward progression of 1 to 2 as noted on a scale

of 1 to 4. As described above, a wash and swim-up was performed on this specimen, and the resulting fraction of sperm recovered had a motility of 30%, with a progression of 3 to 4. However, the normal forms both before and after the swim-up were low—approximately 20%. Ten  $\mu$ l of the sample, about 1,000,000 sperm, were used for insemination.

The specimen from patient 2 had a total sperm count of 10,000,000 with less than 5% motility, progression of 1 to 2, and 20% normal forms. This sample was washed, centrifuged, and resuspended in 100  $\mu$ l medium as above, but the sperm resuspension was incubated at 37°C for 1 hour. After incubation, the motility improved from less than 5% to 20%, but the progression remained at 2. Twenty-five  $\mu$ l of the resuspension, about 2,000,000 sperm, were added to the oocytes. Sperm from both patients remained motile in culture for 72 hours after the time of aspiration.

Twenty-eight oocytes were recovered from the first patient, 8 of which were classified as grade 4 or 5, 19 as postmature, and 1 as degenerate. All oocytes were inseminated, resulting in 16 embryos (8 from the preovulatory and 8 from the postmature oocytes). Five were transferred to the fallopian tubes, all at the 4-cell stage of development. The remaining embryos were frozen and are stored in liquid nitrogen. In patient 2, 24 oocytes were recovered at aspiration and inseminated. Ten of these were classified as grade 4 or 5, 13 as postmature and 1 as immature. Six embryos were generated from the insemination (all of them from the preovulatory oocytes), five of which were transferred to the fallopian tubes. One embryo was at the 2-cell stage and four were at the 4-cell stage of development at the time of transfer.

Both patients conceived after tubal embryo transfer according to increasing concentrations of serum  $\beta$ -hCG.

Patient 1 on day 14 after embryo transfer had 180 m IU/ml of serum  $\beta$ -hCG, 410 m IU/ml on day 18, and 11,239 m IU/ml on day 25. On days 28 and 35, a transvaginal ultrasound revealed the presence of three intrauterine gestational sacs and multiple ovarian corpora lutea cysts. The patient continues at home with an uneventful third trimester pregnancy.

On day 12 after embryo transfer, patient 2 had 47 m IU/ml of serum  $\beta$ -hCG and 450 m IU/ml on day 18. On day 20 post-transfer, the patient began to exhibit vaginal spotting and a  $\beta$ -hCG revealed 155 m IU/ml. Progesterone administration was then discontinued, and the patient exhibited a



withdrawal bleeding. A transvaginal ultrasound revealed absence of a gestational sac.

## DISCUSSION

Both of the first two patients on whom we attempted this combined technique achieved fertilization and pregnancy. One has a continuing healthy ongoing pregnancy in her ninth month. This is the first instance of pregnancy with epididymal sperm aspiration for congenital absence of the vas deferens.

A pregnancy has been achieved in Australia, with sperm aspiration from the distal corpus epididymis. That case involved a failed vasectomy reversal rather than epididymal occlusion or congenital absence of vas.<sup>5</sup> This approach has not previously been reported to work for congenital absence of the vas deferens and has never been attempted with sperm from the head of the epididymis.

Pregnancies that have occurred readily after vasoepididymostomy to the caput epididymis (and even in some cases to the vasa efferentia) suggest that immature sperm that have not had a chance to transit the epididymis might mature on their own during storage in the vas deferens.<sup>3</sup> If this theory were true, it might explain why we have been able to achieve success by aspirating more proximally, not being limited (because of theoretical considerations) to distal regions of the epididymis where the

sperm are generally senescent and nonmotile in the obstructed state.

Other factors in the success of this technique that may be equally important are (1) obtaining large numbers of oocytes in order to increase the odds for fertilization, (2) incubation of sperm outside of the milieu of the obstructed epididymis, and (3) transfer of the embryos into the fallopian tube (ZIFT) rather than into the uterus.

Although these results will have to be considered preliminary until greater numbers are obtained, for the moment it is safe to conclude (1) sperm from the most proximal caput epididymis are capable of fertilization of the human egg in vitro; (2) passage of time after emergence from the testicle may be adequate for sperm maturation without the absolute need for transit through the rest of the epididymis; and (3) we now have an approach for achieving pregnancy in couples with a heretofore dismal condition, congenital absence of the vas deferens.

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