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# Microsurgical Epididymal Sperm Aspiration and Assisted Reproductive Techniques

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Infertility can be due to a male factor whenever there are no sperm in the ejaculate (azoospermia), when the sperm present are greatly reduced in number and quality (severe oligoasthenozoospermia), or when the sperm that are present are either dead (necrozoospermia) or present abnormalities that will not allow fertilization.

Traditionally, azoospermia is viewed as irreversible sterility rather than just infertility. Azoospermia results from either lack of production of sperm in the testes (aspermatogenisis, or spermatogenic arrest), or obstruction in the male genital tract. This obstructive cause of azoospermia can be congenital (i.e., congenital absence of the vas deferens) or from inflammatory processes that produce a blockage at the level of the epididymis. Finally, a common type of obstructive azoospermia is postsurgical, such as after vasectomy.

In the United States there are about 4.5 million infertile couples. Statistically, we know that in about 23% of those couples the husband has fewer than 10 million sperm in the ejaculate. Of these men, azoospermia is present in 33%, or 345,000 men. Among these, azoospermia is found in 40% to be caused by an obstruction.[1,2] We believe congenital absence of the vas (CAV) affects about 13-15% of all persons with obstructive azoospermia, and we thus estimate that in the U.S. alone there are about 40,000 men who suffer from this condition. According to series published in the literature by Jequier and Baker, among others, the incidence of congenital absence of the vas deferens in all cases of obstructive azoospermia ranges from 9.5% to 25% and represents about 1-2% of all cases of male factor infertility[1,3] (TABLE 1).

We know that in the United States, 39% of all married couples are surgically sterile by virtue of either vasectomy or a tubal sterilization. There are 400,000 vasectomies performed every year in the United States and we know that about 10 million men have been vasectomized in the United States alone. Some sociological studies have shown that 1-5% of these men are interested in reversal because of remarriage, death of children in the family, or just because they regretted their original decision. Reversal of vasectomy is possible by vasovasostomy or vasoepididymostomy, but if the microsurgeon is inexperienced, 20-70% of these operations will fail to restore viable sperm in the ejaculate. We thus estimate that between 60,000 and 300,000 men in the United States have had an unsuccessful attempt at reversal of vasectomy.

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TABLE 1. Congenital Absence of the Vas Deferens

| | No. of Cases of Male Infertility | Azoospermia (%) | All Cases of Male Infertility (%) | All Cases of Azoospermia (%) | Obstructive Azoospermia (%) |
|---|---|---|---|---|---|
| Jequier (1985, England) | 749 | 13.7 | 1.5 | — | 10.6 |
| Dubin-Anelar (1971, US) | 1294 | 25.3 | 1.8 | 7.3 | 25 |
| Baker (1986, Australia) | 1041 | 18.4 | 0.7 | 3.1 | 9.5 |

About four and a half years ago both of us working in the field of assisted reproduction asked whether there was any way to recover sperm for *in vitro* fertilization from the testes of azoospermic men with irreversible obstruction, men in whom there was otherwise no possibility of performing surgical repair to restore fertility. Dr. Silber felt that until recently people didn't believe that fertilizable sperm could be retrieved directly from the testes. But on the basis of his previous experience with vasoepididymostomy procedures he thought that sperm obtained in sufficient numbers from the proximal epididymis or even the vasa efferentia could fertilize an egg.[4–6] We then decided to examine this subject. When we began, we were surprised at the paucity of information available in the worldwide literature, whether in basic or in clinical science, about sperm from the human testis and epididymis; all the work had been mainly done in the rat, guinea pig, and hamster, but practically none had been done in man.[4,7–9]

## PRELIMINARY AND BASIC STUDIES

We asked ourselves a series of questions to be answered before directly attempting to see whether the human testicular or epididymal sperm could fertilize. As a clinical model, we used men with congenital absence of the vas deferens, men who otherwise under current technology would never have had a chance to have their own genetic child.

We asked first whether men with congenital absence of the vas deferens have normal spermatogenesis.[10] Secondly, if spermatazoa are produced in a normal fashion what happens to them? Where do they go? Next, is the situation of the men with congenital absence of the vas similar to that of men with vasectomy in terms of production of antisperm antibodies? Last, we wanted know whether sperm obtained from the epididymis of men with congenital absence of the vas deferens is able to fertilize a human egg and to produce pregnancy.

In order to answer the first question—whether spermatogenesis was normal—we performed quantitatively precise testicular biopsies in 22 men with congenital absence of the vas.[11,12] It is now well documented that quantitative evaluation of the number of mature spermatids per tubule on testicular histology is a very accurate reflection of sperm production in the human. An exponential power curve equation, $y = a\ (x^3)$, allows a simple graph to determine sperm production and expected sperm count based upon the number of mature spermatids per tubule. In the present study, we had two objectives in comparing the quantitative production of sperm in patients with congenital

absence of the vas deferens undergoing epididymal sperm aspiration for *in vitro* fertilization: (1) to determine whether or not long-term obstruction results in any diminution in overall spermatogenesis and (2) to see whether the quantitative testicular sperm production correlates with the amount or quality of sperm obtained from these patients for IVF.

TABLE 2 is from a recent study that showed the number of mature spermatids per tube, and the quantity of the sperm production in millions that we expect in these men with congenital absence of the vas deferens. Although some cases are low in terms of numbers of sperm per million, most are within normal limits. It is intriguing that the frequency distribution of sperm production, as determined from testicle biopsy, was considerably lower than would be expected in a normal population of patients. Nonetheless, results in all of the 22 testicular biopsies demonstrated adequate spermatogenesis that would generally be classified within a "normal range" for each individual patient despite up to 40 years of congenital epididymal obstruction.

Next we decided to study what mechanisms of sperm disposal were in operation in the genital tract of men with congenital absence of the vas.[13] Basically we obtained vasa efferential and epididymal fluid at different levels (proximal and distal) and fixed and examined the contents by transmission electron microscopy. That study showed that in men with congenital absence of the vas sperm are trapped in the epididymis over a prolonged period of time and undergo a process of senescent degeneration, followed by phagocytosis by macrophages. This process of phagocytosis is much more evident in distal areas of the epididymis, rather than in the proximal regions. FIGURE 1 shows one macrophage that is obtained from the distal area, very close to the point of blockage; more that 10 spermatazoa are seen to have been phagocytized by just one such cell. FIGURE 2 is an electron micrograph obtained from fluid in the human epididymis at the congenitally blind pouch; it shows a large macrophage containing multiple vacuoles, each from the phagocytosis of an individual sperm. Sperm from more proximal regions of the blocked epididymis are completely normal by electron microscope evaluation and are *not* senescent or degenerative.

We have recently attempted to determine whether the immunologic behavior of sperm antibodies in men with congenital absence of the vas is similar to that of men

TABLE 2. Number of Spermatids and Quantity of Sperm Production

| Mature Spermatids(s) per Tubule | Quantity of Sperm Production ($\times 106$) | Pachytene Spermatocytes (P) per Tubule | P/S Ratio |
|---|---|---|---|
| 17.4 | 7 | 25.4 | 1.46 |
| 20.2 | 11 | 20.3 | 1.00 |
| 22.5 | 16 | 20.3 | 0.90 |
| 21.9 | 16 | 25.3 | 1.10 |
| 23.4 | 17 | 23.9 | 1.02 |
| 23.0 | 18 | 23.4 | 0.97 |
| 23.6 | 19 | 22.8 | 0.97 |
| 23.0 | 20 | 24.7 | 0.93 |
| 26.9 | 23 | 31.4 | 1.17 |
| 22.3 | 25 | 26.1 | 0.85 |
| 28.1 | 26 | 23.0 | 0.82 |
| 27.3 | 28 | 32.0 | 1.17 |
| 27.3 | 28 | 22.4 | 0.82 |
| 27.9 | 29 | 25.2 | 0.90 |

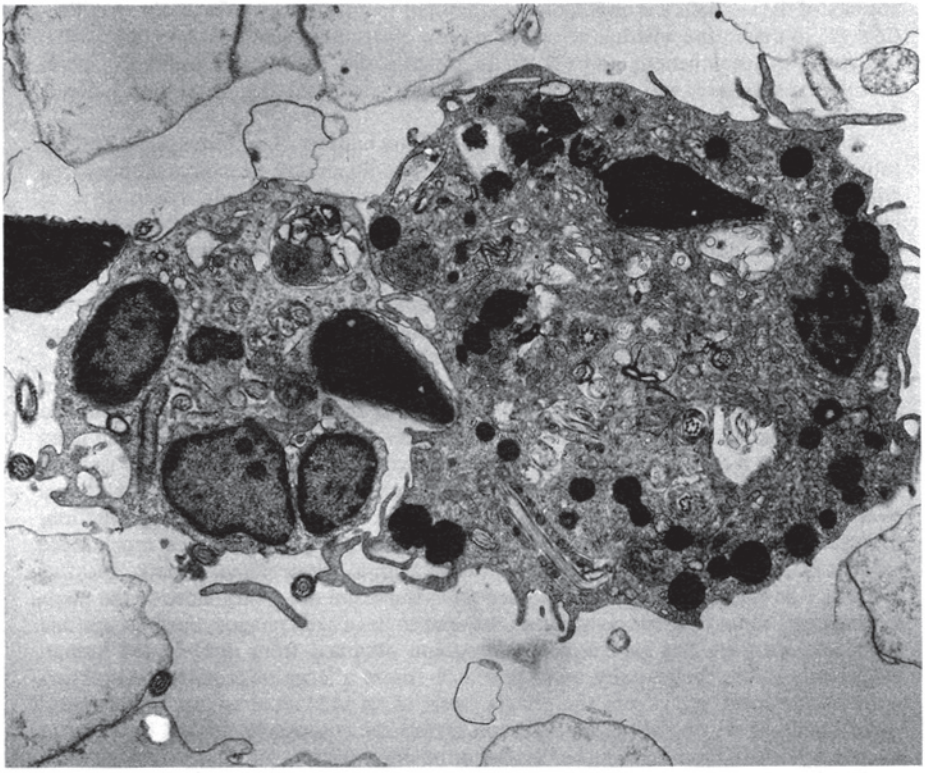

**FIGURE 1.** Microphotograph of a macrophage obtained from the vasa efferentia fluid containing multiple phagocytized sperm.

with chronic obstruction after vasectomy. The levels of antibodies in the men with congenital absence of the vas in this original study was 11% compared to 80% in men with unsuccessful reversal of vasectomy (TABLE 3).

The possible explanations for this low immune reaction are:

1. Epididymal hypertrophy could prevent the leakage of soluble parts of degraded spermatozoa, thus avoiding immunologic exposure.

2. Sperm antigenicity in men with congenital absence of the vas could be different from that of the normal male population.

3. The obstructed epididymis might not be able to properly "coat" the sperm surface with antigens necessary to evoke an immune response.

In any event, we have found that the presence of sperm antibodies do *not* interfere with *in vitro* fertilization anyway. So, for the first question we asked, whether spermatogenesis was normal in men with congenital absence of the vas, the answer was yes. The answer to the second question, which asked about the mechanisms of sperm disposal, was that there was degeneration and phagocytosis by macrophages particularly at distal sites. As to whether the antisperm antibodies develop as a consequence of chronic obstruction, the answer was no: the incidence was very low and, as we will describe later, the presence of antibodies does not appear to affect the chances of fertility of epididymal sperm.

The final and most interesting question was whether these human epididymal sperm were able to fertilize an egg or not. A search of the literature showed almost no data in the human. Data in lower species, such as the fish or the frog, showed that sperm are released from the testes and were very active, and in some cases produced pregnancies if placed in the oviduct. In the rooster it was demonstrated that a small proportion of the sperm coming from the testes are able to fertilize without passing through the epididymis. In the human, the only information available were some biochemical and ultramicroscopic findings during epididymal passage reported by Bedford.[14] These included the following: (1) There was no modification of the acrosomal appearance. (2) Nuclear chromatin was stabilized by S-S cross linkage. (3) Tail organelles (outer dense fibers, mitochondrial membrane, and sheath of principal piece) were also stabilized by S-S bonds. (4) Sperm cell surface changes are determined by response to fixatives, the binding of visible markers, and microelectrophoretic behavior, among other things. (5) Migration and shedding of cytoplasmic droplets occurs.

These data were by no means conclusive regarding the fertilizing capacity of epididymal sperm. Since vasoepididymostomy at our center showed good pregnancy rates despite bypass of large areas of epididymis, we decided to study the fertilizing capacity of human epididymal sperm in men with CAV.

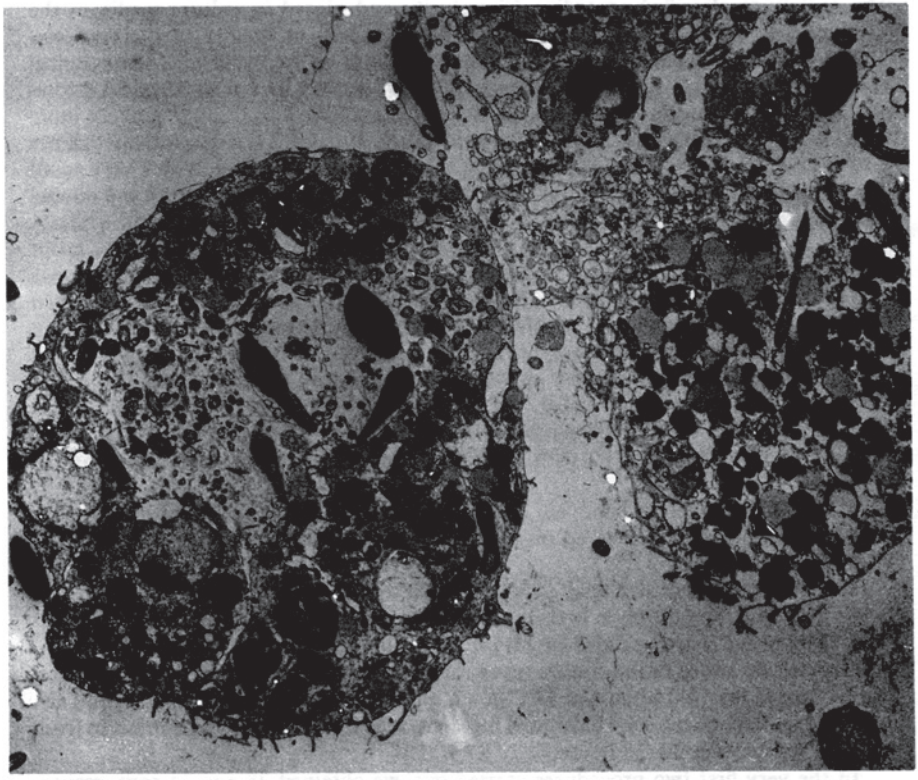

**FIGURE 2.** Microphotograph of a macrophage obtained from the caput epididymis containing multiple vacuoles from phagocytized sperm.

TABLE 3.

| | IBT Serum | | |
|---|---|---|---|
| | Positive | Ig Class | Site Binding |
| Congenital absence of vas ($n = 27$) | 3 (11%) | IgG | Tail, tip of tail |
| Failed reversal of vasectomy ($n = 5$) | 4 (80%) | 3 IgG<br>1 IgG,A,M | Tail, tip of tail<br>Head, tail, tip of tail |

## FERTILITY STUDIES—PATIENT POPULATION

In these studies we treated both the wife and the husband simultaneously. The wives received a combination of medications for controlled ovarian hyperstimulation using leuprolide acetate, human menopausal gonadotropin, and follicle-stimulating hormone, until numerous preovulatory follicles were produced as determined by monitoring with vaginal ultrasound and serum estradiol levels. Human chorionic gonadotropin (hCG) was given, and 36 hours later follicles were aspirated by vaginal ultrasound. The eggs were classified according to maturity and placed in culture media in the incubator. In cases in which the number (less than 5) or quality of the eggs was not adequate, we opted to not perform the procedure in the husband. Microsurgical epididymal sperm collection is relatively simple; it is done by a microsurgical scrotal exploration and identification of the anatomy of the epididymis, beginning in the more distal part of the congenital blind-ended epididymis. FIGURE 3 is a diagram that shows how the tunic is opened, a small cut is made in the epididymis, and, with a very small Medicut pipet, the contents (milky or yellowish in color) that come from the dilated tubule are aspirated. When possible, sperm collection is initiated by incising the epididymis in the most distal part first. The expression of the testicle in order to milk it out has been beneficial in the cases in which sperm are obtained directly from the vasa efferentia. The specimens collected are given to the laboratory, where they are checked for the presence or absence of motile sperm. We continue to advance proximally, up to the vasa efferentia, and we stop only when we are satisfied with the motility of the sperm aspirated. If necessary, the procedure is repeated on the other side. The sperm are processed by a technique called "minipercoll," developed in our laboratory by our biologist, Ms. Teri Ord.[15] This basically involves the following steps:

1. Sperm are diluted 1 : 2 with HTF medium and centrifuged at 200 *g* for 10 minutes.
2. The supernatant is removed, and the pellet resuspended in 0.3 ml of medium.
3. The suspension is layered on discontinuous Percoll gradient (0.3 ml each) at 95%, 70%, and 50%.
4. The sample is centrifuged at 300 *g* for 30 to 40 minutes.
5. The 95% Percoll layer is recovered and washed twice with medium.
6. The pellet is resuspended in 1.0 ml HTF plus 10% serum.

Oocytes are added to the culture tubes containing sperm at the time of insemination and incubated for 12-15 hours at 37°C in a humidified atmosphere of 5% $CO_2$ and air. After incubation, oocytes are examined for signs of fertilization and transferred to fresh medium for another 30-36 hours.

In the very first two procedures carried out, we obtained 28 and 24 eggs, respectively. After insemination in the first case we obtained 15 embryos, and in the second

6. In both patients, we performed tubal embryo transfer (TET), transferring 5 embryos to their fallopian tubes 54 hours after insemination.[16] The extra embryos were frozen and cryopreserved.[16] The first two patients conceived, one of whom had a miscarriage. The first baby to be born, conceived by the procedure of microsurgical epididymal aspiration and IVF-TET, was a healthy girl in May 1988.[17,18] Since then we have carried out the procedure in 54 couples in which the cause of infertility was congenital obstructive azoospermia due to absence of the vas deferens.[19] Of the 54 couples, in only two (4%) were we not able to recover motile sperm; In the remaining 96% we obtained motile sperm. In 33 cases, those sperm were able to fertilize at least one egg (61%). In 19 cases we did not obtain any fertilization at all. Fertilization rate per oocyte was 31%, which is very low when compared to the rate of 65-80% obtained with ejaculated sperm in our laboratories. Of these 33 cases in which we did obtain at least one embryo, we performed TET in 24 cases and conventional intrauterine transfer in 8 cases. Of the 24 cases of TET, we obtained 12 pregnancies (50% pregnancy rate), very similar to our data on TET for other male cases of infertility. In the 8 cases with IVF, we achieved only one pregnancy. Overall, pregnancy rate per patient is 24%, per transfer is 41%, per TET is 50%, and per IVF is 12%.

From our experience to date we have identified some variables that we believe are important for the outcome of the epididymal sperm aspiration.

(1) In TABLE 4, we show the relationship of the site of aspiration to the fertilization and pregnancy rates. Note that in 10 cases we obtained sperm directly from the vasa

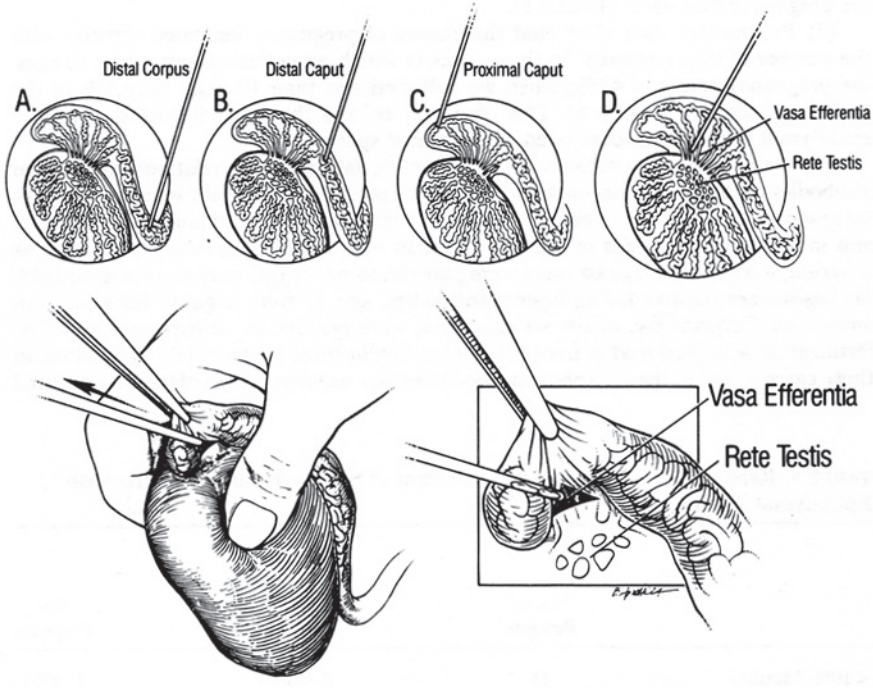


**FIGURE 3.** Artistic diagram of microsurgical sperm aspiration from the epididymis and vasa efferentia.

TABLE 4. Lack of Relationship of Pregnancy Rate to Distal-most Site Where Sperm with Progressive Motility Were Recovered

| Site of Aspirations | *n* | Sperm with Progressive Motility Recovered | Fertilization | Pregnancy |
|---|---|---|---|---|
| Vasa efferentia | 10 | 6 | 5 (50%) | 4 (40%) |
| Proximal caput | 14 | 9 | 9 (64%) | 3 (21%) |
| Distal caput | 5 | 2 | 2 (40%) | 2 (40%) |
| Corpus | 3 | 3 | 1 (33%) | 1 (33%) |

efferentia, before they entered the epididymis. Fertilization was achieved in five of the ten (50%), and in four of those five (40% of the total) pregnancy occurred. Sperm that were never exposed to the epididymal milieu are different from any other sperm that are obtained from the proximal or the distal part of the corpus. So apparently it is not important where the aspiration is performed in the epididymis for the chances of fertilization and pregnancy to occur.

(2) According to the motility at the moment of aspiration of the sperm, one can predict the likelihood of fertilization and pregnancy. In the cases in which sperm presented less than 10% motility, the fertilization rate was 45% and pregnancy rate only 9%. If sperm motility was greater than 10%, the fertilization rate was 76% and the pregnancy rate 43% (TABLE 5).

(3) Preliminary data show that the chance of pregnancy increased directly with the number of eggs retrieved. In those cases in which we collected more than 10 eggs, the pregnancy rate was 41%; when we collected less than 10 eggs, only 5% of the patients conceived (TABLE 6). This obviously reflects the lower fertilization rate of epididymal sperm when compared to ejaculated sperm.

(4) We have recently obtained very interesting data about the relationship of sperm antibodies to the epididymal sperm to pregnancy in couples where the man has congenital absence of the vas. Of 18 cases in which we measured antisperm antibodies by direct and indirect immunobeads in the sperm and in the fluid of the epididymis as well as in serum, it was found that six cases were positive, some for IgG only and some for IgM. Six cases were positive for antisperm antibodies, and 12 were negative for antisperm antibodies. Surprisingly, of the six cases that were positive for antisperm antibodies, fertilization was produced in five (83%), resulting in three pregnancies. In contrast, in those cases in which the antisperm antibody test was negative, seven of twelve produced

TABLE 5. Relationship of Pregnancy to Percent of Sperm Motility in "Pre-wash" Epididymal Aspirate[a]

| | No. of Patients | No with Fertilization and Embryo Transfer | No. Pregnant |
|---|---|---|---|
| < 10% Motility | 11 | 5 (45%) | 1 (9%) |
| > 10% Motility | 21 | 16 (76%) | 9 (43%) |

[a] First 32 cases.

fertilization (58%) and only two produced a pregnancy. In this small population of CAV patients, it does not seem that antisperm antibodies are detrimental to these sperm obtained in the epididymis despite high titers in some cases.

(5) Because of the still low pregnancy rate after this procedure, the possibility of repeated aspirations is a very crucial point. Four of the first 32 cycles we performed were second attempts by the same patient after an initial failure. In three of those four with an initial failure a pregnancy occurred with a second attempt. We have patients who underwent microsurgical epididymal aspiration up to three times on the same side on the same epididymis or vasa efferentia who still produce a good number of sperm for fertilization *in vitro*. In our initial counseling with couples we stress the possibility of repeating the procedure because this type of aspirated sperm may not be successfully cryopreserved.

## CONCLUSIONS

As recently as 4 years ago, men with congenital absence of the vas deferens could not have their own genetic children. We believe that now we are able to offer an effective

TABLE 6. Relation to Number of Mature Eggs Retrieved from Wife

| No. of Eggs | Pregnancy |
|---|---|
| > 10 Eggs | 14 of 34 (41%) |
| < 10 Eggs | 1 of 19 (5%) |

treatment for infertility due to this congenital absence. In developing this treatment, which has both social and scientific value, we have learned that in the obstructed epididymis, the "worst" sperm are the ones that are more distal, the ones that have been in the epididymis for the longer period of time. We have also learned that sperm directly from the vasa efferentia can fertilize and produce successful pregnancies, which casts serious doubts on whether the epididymis in the human, in contrast to some smaller species, is so critical for sperm function. Men with congenital absence of the vas deferens or those with failed reversals of vasectomy or other nonoperable inflammatory obstructive azoospermia are clearly good candidates for the technique of microsurgical epididymal sperm aspiration. We also believe that in cases of very severe oligoasthenospermia this procedure might be used in combination with epididymal sperm storage to provide greater numbers of sperm for IVF.

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