CONGENITAL ABSENCE OF THE VAS DEFERENS

The Fertilizing Capacity of Human Epididymal Sperm

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Abstract *Background.* Congenital absence of the vas deferens has been considered a virtually untreatable cause of male sterility. Furthermore, sperm that have not passed through at least the head of the epididymis have been thought to be incapable of causing pregnancy. We attempted to determine whether human sperm that had never passed through the epididymis could fertilize eggs in vitro and whether the technique could be used for men with congenital absence of the vas deferens.

Methods. Twenty-eight men with congenital absence of the vas deferens underwent microsurgical aspiration of sperm from the epididymis and vasa efferentia for attempted in vitro fertilization of their wives' oocytes, with subsequent transfer of embryos. Thirty-two treatment cycles were begun (four were repeat cycles).

Results. The most motile sperm were found in the proximal epididymis, at or near the vasa efferentia. Embryos were obtained for transfer in 21 cases (66 percent). Ninety-three embryos resulted from 352 mature oocytes (fertilization rate, 26 percent). Clinical pregnancy was achieved in 10 of the 32 treatment cycles (31 percent). Seven women delivered normal infants, and three miscarried. One of the seven live births was of twins. There were six girls and two boys. When fewer than 10 eggs were retrieved, no pregnancy occurred. When 10 or more eggs were retrieved (20 cases), the pregnancy rate was 50 percent.

Conclusions. Sperm from the proximal caput epididymidis and even sperm from the vasa efferentia (which have never passed through the epididymis) can fertilize the human oocyte in vitro and result in pregnancy with live birth. (N Engl J Med 1990; 323:1788-92.)

IT has long been assumed that sperm must pass through a certain length of the epididymis to mature, gain progressive motility, and become capable of fertilization.[1-3] In some animal studies, surgical ligation of the epididymis has allowed sperm to develop motility without traveling the entire length of the epididymis.[4,5] Such sperm, however, have only rarely been shown to fertilize eggs. The transport of sperm through most of the epididymis has still been considered mandatory for fertilization.

In humans, transport through the epididymis has been thought to take approximately 11 days.[6] This concept was recently challenged by Johnson and Varner, who demonstrated that the storage capacity of the human epididymis is extremely limited and that sperm are transported through it in approximately two days.[7] Their finding is consistent with the possibility that human sperm may not require the same degree of epididymal maturation as that of many animals. Fertility data on sperm obtained from the vasa efferentia and proximal epididymis are needed to determine whether epididymal transit is necessary for fertilization.

Men with azoospermia due to congenital absence of the vas deferens represent a good clinical model in which to study the ability of sperm from different regions of the epididymis to fertilize an oocyte and produce pregnancy. We have treated infertility in such men by direct microsurgical aspiration of sperm from the epididymis, combined with in vitro fertilization and embryo transfer. We have studied a large number of such cases to determine whether human testicular sperm require transit through the epididymis to mature enough for fertilization and subsequent pregnancy to occur and to determine whether the procedure is sufficiently reproducible to become an acceptable treatment.

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METHODS

Twenty-eight couples with primary infertility in which the cause was azoospermia due to congenital absence of the vas deferens in the male partner were treated by a combination of microsurgical aspiration of sperm from the epididymis, in vitro fertilization, and embryo transfer. Thirty-two treatment cycles were performed (four of the couples underwent repeat cycles). Each cycle included four phases: controlled ovarian hyperstimulation and transvaginal aspiration of oocytes, microsurgical retrieval of sperm (Fig. 1), laboratory preparation of sperm and in vitro fertilization, and tubal or uterine transfer of embryos.

Controlled Ovarian Hyperstimulation and Transvaginal Oocyte Aspiration

All the wives were between 23 and 38 years of age. They had regular menstrual cycles and normal tubal patency on hysterosalpingography, laparoscopy, or both in the six months preceding ovarian stimulation. In order to synchronize the onset of their menses for participation in a particular cycle, they received 10 mg of

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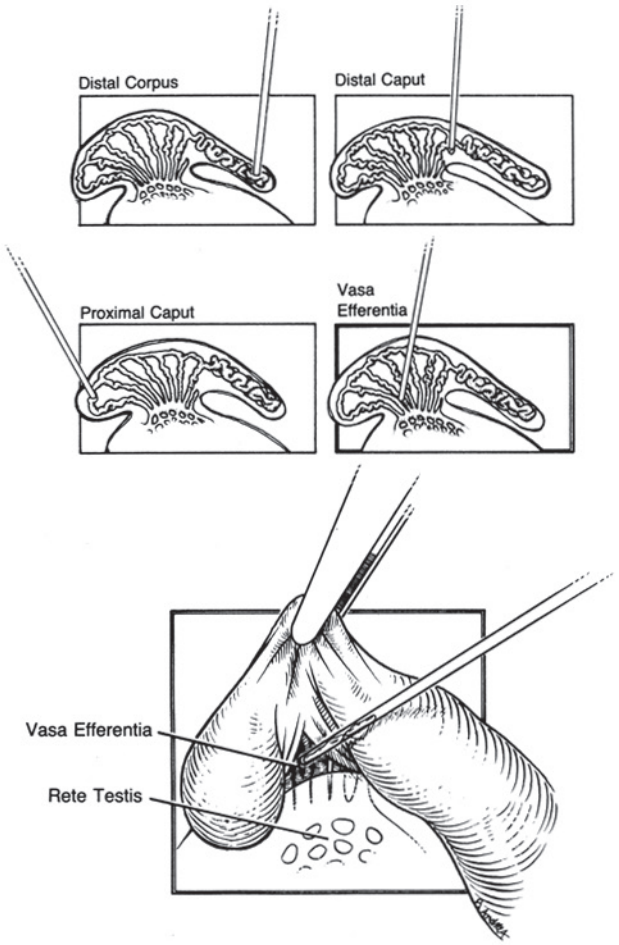

Figure 1. Aspiration of Epididymal and Rete Testis Fluid from the Vasa Efferentia and Other Sites.

If sperm were absent or their motility was poor in the most distal portion of the epididymal tubule, aspirations were performed at successively more proximal points until progressive motility was observed.

norethindrone acetate for 9 to 37 days. The onset of menses occurred in all the women between two and four days after the discontinuation of norethindrone acetate.[8]

The women then underwent controlled ovarian hyperstimulation with a combination of leuprolide acetate (1 mg every 24 hours, given by subcutaneous injection; Lupron, TAP Pharmaceuticals, Chicago), urofollitropin (150 IU per day; Metrodin, Serono Laboratories, Randolph, Mass.), and menotropins (150 IU per day; Pergonal, Serono).

Follicular development was monitored by daily vaginal ultrasound examinations (Model RT 3600, General Electric Medical Systems, Milwaukee) and serum estradiol measurements, and the length of administration of urofollitropin and menotropins was determined accordingly. A dose of 10,000 IU of chorionic gonadotropin (Profasi, Serono) was administered by intramuscular injection when two of the largest follicles had reached a diameter of 18 to 20 mm and the serum estradiol level had reached at least 734 pmol per liter per mature follicle.

The retrieval of oocytes was performed transvaginally with ultrasound guidance, 36 hours after the injection of chorionic gonadotropin.

Microsurgical Retrieval of Sperm

After the oocytes had been aspirated from the wife, the husband underwent scrotal exploration. The contents of the scrotum were exposed through an incision of approximately 2 cm, the tunica vaginalis was opened, and the epididymis and testicle were exposed. At a magnification of 10 to 40 under an operating microscope, the epididymal tunic was incised to expose the epididymal tubule in the most distal portion (Fig. 1). Interstitial tissue was then removed from the epididymal tubule, and a 1-to-2-mm longitudinal incision was made with microscissors. A number 22 Medicut needle (Sherwood Medical, St. Louis) on a tuberculin syringe was used to aspirate the fluid flowing from the opening in the epididymal tubule directly. Careful hemostasis was achieved with microbipolar forceps, and care was taken to avoid contaminating the specimen with blood. The total volume of aspirate varied from about 50 to 500 μl.

Aspirated epididymal fluid was immediately taken to the adjacent laboratory, diluted in HEPES-buffered medium, and an aliquot examined for the presence of sperm and the percent and quality of motility of the sperm. If sperm were absent or their motility was poor, aspirations were performed at successively more proximal regions (up to the straight portion of the vasa efferentia) until progressively motile sperm were seen. The quantity and quality of motility in the various regions of the epididymis were noted. In cases in which a vasa efferentia tubule was opened microsurgically, direct squeezing of the testicle resulted in a brisk flow of rete testis fluid. The fluid sampled from the vasa efferentia thus appeared to be not epididymal fluid but rete testis fluid (Fig. 1).

Sperm motility was graded with a 0-through-4 system. Grade 0 sperm had no motility. Grade 1 sperm vibrated in place, with no forward progression. Grade 2 sperm had slow forward progression with poor linearity. Grade 3 sperm had forward progression with good linearity but low velocity. Grade 4 sperm had good linearity as well as rapid velocity.

The average operating time was two to three hours but decreased with experience. Once all sperm were aspirated, the opening in the proximal epididymal tubule or vasa efferentia tubule was closed with 10-0 nylon interrupted sutures to avoid complicating subsequent sperm aspirations by scar reaction. The tunica vaginalis of the testis was closed with 4-0 Vicryl interrupted sutures after filling with heparin-treated saline. The scrotum was closed with 3-0 Vicryl interrupted intracuticular sutures. Drains left in each scrotal sac were removed the next day. All the men had minimal pain after the operation and were discharged the following day.

Sperm Preparation and in Vitro Fertilization

Microsurgically aspirated sperm were immediately diluted with HEPES-buffered human tubal fluid (Irvine Scientific, Santa Ana, Calif.), examined for motility and morphologic features, and centrifuged at 200×g for 10 minutes. Pellets were suspended in 0.3 ml of medium and layered on a discontinuous Percoll gradient ("mini-Percoll") consisting of 0.3 ml each of 50, 70, and 95 percent isotonic Percoll (Pharmacia, Uppsala, Sweden).[9] The gradient was centrifuged at 400×g for 45 minutes. After centrifugation the 95 percent Percoll layer was removed, washed twice, and resuspended in culture tubes with 1 ml of human tubal fluid and 10 percent fetal-calf serum. No other special procedures were performed on the sperm. Oocytes were added to the culture tubes containing sperm and incubated for 13 to 15 hours at 37°C in a humidified atmosphere of 5 percent carbon dioxide and air. After incubation, the oocytes were examined for signs of fertilization, indicated by the presence of two pronuclei, and were transferred to fresh growth medium (human tubal fluid) for another 30 to 36 hours. Fertilization was considered confirmed only if cleavage occurred. Embryos were transferred at the two-to-four-cell stage of development. No micromanipulation of the eggs was used. Although donor sperm was available as backup in one case, none of the in vitro procedures in this study involved donor sperm.

Embryo Transfer

Embryos were transferred to the fallopian tubes or uterus according to the quantity and quality of embryos developed in vitro.[10,11] Two embryos from the first insemination was the minimum necessary to perform a tubal transfer. Embryos were transferred to the fallopian tubes through a 3-cm Pfannenstiel minilaparotomy in 15 cases. In a Tomcat catheter (Sherwood Medical), one to five embryos (total volume, 20 μl) were transferred 2.5 cm inside each fallopian tube through the fimbriated end. The entire sur-

gical procedure lasted less than 30 minutes. The women were discharged the next day, and recovery was uneventful in all cases. In five women, embryos were placed in the uterus in the conventional fashion, with a Frydman catheter. All the women received progesterone in oil (25 mg per day, given intramuscularly) from the day of the transfer until the day of the pregnancy test (14 days after the transfer), or for 56 days if the pregnancy test was positive.

Results

Rates of Fertilization and Pregnancy

Table 1 is a detailed summary of the results of each cycle. Four of the 32 cycles were repeats after an initial failure. Table 2 shows the overall rates of fertilization and pregnancy. The overall pregnancy rate was 31 percent (10 of 32 cycles). Seven of the women delivered healthy babies. One of the seven births was of twins. There were six girls and two boys. Three of the four repeat cycles resulted in pregnancy and live birth. Twenty-one of the 32 cycles (66 percent) produced at least one cleaving embryo.

On the basis of routine sperm indexes, it was not possible to determine the cases in which fertilization would occur, except that more than 10 percent motility was clearly required. No further analysis of the sperm data we obtained predicted fertilization. Most predictive of success was the number of eggs retrieved from the wife. When fewer than 10 were retrieved, there were no pregnancies. When 10 or more eggs were retrieved, 10 of 20 women (50 percent) became pregnant. In cycles in which more than two embryos were produced, the pregnancy rate per tubal transfer was 75 percent (9 of 12).

Table 2. Rates of Fertilization and Pregnancy.

Variable	No. (%)
Patient cycles	32 —
Pregnancies	10 (31)
Miscarriages	3 (30)
Women with live births	7 (22)*
Mature oocytes	352 —
Embryos	93 (26)
Cycles producing at least 1 embryo	21 (66)
Cycles producing 2 embryos	12 (38)
Pregnancy rate per transfer	10/21 (48)
Pregnancy rate per tubal transfer of 2 embryos	9/12 (75)
Pregnancy rate when <10 eggs retrieved	0/12
Pregnancy rate when at least 10 eggs retrieved	10/20 (50)

*One delivery was of twin girls; there were thus eight babies altogether. The percentage was calculated in terms of the total number of treatment cycles.

Relation of Epididymal Level to Sperm Motility and Fertilization

Progressively motile sperm were found in 20 of the 32 procedures (62 percent) in the 28 men. However, the percent motility and the number of rapidly motile

Table 1. Summary of Fertilization and Pregnancy Results.*

Patient Cycle No.	Age of Wife	No. of Mature Oocytes	No. of Embryos	Total Sperm Retrieved		Motility		Progression		Embryo Transfer	Outcome
	yr			$\times 10^{-6}$		%					
1	37	28	6	—		—		1–2		Tubal	Pregnant, miscarried
2	26	24	15	—		—		1–2		Tubal	Pregnant, girl born
3	34	7	2	—		5		1		Uterine	—
4	33	3	2	Very low		10		1		Tubal	—
5	22	20	0	—		0		0		—	—
6	29	6	1	1.68		10		1		Uterine	—
7	24	8	0	26.0		10		1–2		—	—
8	31	3	0	9.4		20		1		—	—
9	36	4	1	—		—		—		Uterine	—
10	35	13	2	16.4		20		1		Tubal	—
11	30	9	2	—		—		—		Tubal	—
12	26	11	0	—		20		1		—	—
13†	37	20	9	—		2		1–2		Tubal	Pregnant, girl born
14	38	13	0	Very low		5		1		—	—
15	32	11	0	—		10		1–2		—	—
16	26	2	1	—		1		1		Uterine	—
17	35	2	0	—		5		1		—	—
				PRE	POST	PRE	POST	PRE	POST		
18	26	14	4	—	10.2	10	10	1–2	3–4	Tubal	—
19	25	8	0	59.0	—	1	1	1	1	—	—
20	28	12	5	24.8	5.3	15	20	1	2	Tubal	Pregnant, miscarried
21	33	11	2	37.2	3.4	10	10	1–2	1–2	Tubal	Pregnant, girl born
22	35	10	3	—	0.48	30	60	1–2	3	Tubal	—
23‡	22	14	0	13.7	6.9	1	1	1	1	—	—
24	26	6	0	—	13.0	5	20	1	2	—	—
25	31	10	7	47.4	9.7	30	60	1–2	3–4	Tubal	Pregnant, boy born
26§	35	25	3	37.8	10.9	10	30	1–2	2–3	Tubal	Pregnant, boy born
27¶	26	10	8	119.0	34.0	20	30	1–2	1–2	Tubal	Pregnant, twin girls born
28	37	10	5	101.2	10.2	10	20	2	2	Tubal	Pregnant, girl born
29	40	11	9	34.8	3.8	30	60	2–3	3–4	Tubal	Pregnant, miscarried
30	34	11	5	—	47.0	1	10	1	1–2	Tubal	—
31	24	12	0	31.0	11.5	10	30	1	1–2	—	—
32	40	4	1	—	1.1	1	10	1	1–2	Uterine	—

*Pre and post denote number of sperm, percent motility, and progression before and after preparation with Percoll. Before cycle 18, such data were not available.
†Repeat of cycle 1. ‡Repeat of cycle 5. §Repeat of cycle 10. ¶Repeat of cycle 12.

Table 3. Relation between Pregnancy Rate and Site from Which Sperm with Progressive Motility Were Recovered.

Site of Aspiration for IVF*	Cycles	Cycles with Sperm with Progressive Motility	Cycles with at Least 1 Egg Fertilized	Cycles with Pregnancy
	no. (%)	*no.*	*no. (%)*	
Vasa efferentia	10 (31)	6	5 (50)	4 (40)
Proximal caput	14 (44)	9	9 (64)	3 (21)
Distal caput	5 (16)	2	2 (40)	2 (40)
Corpus	3 (9)	3	1 (33)	1 (33)

*Most distal site in which motile sperm were present. IVF denotes in vitro fertilization.

sperm were always low. Table 3 shows that the sperm with the greatest motility were found most proximally. The most distal site from which progressively motile sperm were recovered was the corpus epididymidis (3 of 32 procedures [9 percent]). In all the other cases, progressively motile sperm were not recovered from the distal epididymis; it was in the caput or in rete testis fluid from the vasa efferentia that the motility was greatest.

In the 10 procedures in which sperm were obtained exclusively from rete testis fluid from the vasa efferentia, fertilization was achieved in 5 (50 percent) and pregnancy in 4 (40 percent). All but one pregnancy occurred with sperm from either the caput epididymidis or vasa efferentia rete testis fluid. Rates of fertilization and pregnancy were similar whatever the most distal site from which motile sperm were obtained.

Discussion

Congenital absence of the vas deferens accounts for 10 percent or more of the cases of noniatrogenic obstructive azoospermia and has generally been considered an untreatable cause of male infertility.[12-14] Men with this condition have been shown on testicular biopsy to have adequate spermatogenesis and are theoretically producing sperm capable of fertilizing an oocyte. Yet treatment has been very disappointing.[15-18]

We turned our attention to the possibility of using direct microsurgical aspiration of sperm from the vasa efferentia or epididymis combined with in vitro fertilization. Such an approach had previously met with disappointing results. No pregnancy had been reported with this approach to congenital absence of the vas deferens, although a single pregnancy was reported from Australia in a case involving a failed reversal of a vasectomy.[19,20] The fertilizations and pregnancies that occurred in the wives of the men in our series, with sperm aspirated from the proximal epididymis and vasa efferentia, thus represent a strong departure from previous experience. The reasons for this difference may include the following: the early Australian work retrieved a small number of eggs as compared with the number that can be obtained with modern stimulation protocols involving gonadotropin-releasing–hormone agonists; we used "fresher" sperm from the most proximal regions of the epididymis; we transferred embryos to the fallopian tubes rather than the uterus, which produces a higher rate of implantation than standard in vitro fertilization; and we used the "mini-Percoll" method of sperm preparation rather than standard "swim-up" or simple wash techniques.[10,11]

The most striking finding in the retrieval of sperm from a chronically obstructed epididymis was the inversion of the pattern of motility one would expect in a nonobstructed epididymis. Sperm in the distal regions of an obstructed epididymis had the weakest motility, and sperm in the proximal regions had the strongest. One may speculate that the distal sperm are aging, and the more recently produced proximal sperm have had time to mature on their own. In fact, the chief problem in fertilization with these sperm is aging rather than immaturity.

This finding should not be as surprising as it may seem. In a large series of men undergoing vasoepididymostomy for noncongenital obstructive azoospermia, sampling of sperm from various levels of the epididymis has consistently demonstrated that highly progressive motile sperm can usually be found in the most proximal region of the epididymis, even though only poorly motile or nonmotile sperm are found in the distal epididymis.[21] Krylov and Borovikov have made similar observations in the Soviet Union.[22] The phenomenon has prompted us to change our approach in deciding the level at which to perform a vasoepididymal anastomosis.

There is strong experimental support for this concept, dating as far back as 1930 with Young's studies in guinea pigs.[23] Young ligated the epididymis at various levels and examined the proximal sperm that had been trapped for various periods of time. Contrary to expectation, the more distal sperm had the poorest motility and the more proximal sperm the best. He concluded that in the obstructed epididymis the more distal sperm are senescent, whereas the more proximal sperm have had time to mature despite not having passed through the epididymis. Our study in humans confirms Young's original studies in animals — some elements of sperm maturation are intrinsic in nature.

Clinical studies with vasoepididymostomy in humans have demonstrated equivalent rates of pregnancy whether the sperm pass through a long or short length of the corpus epididymidis.[21,24] Even when sperm have only passed through a portion of the caput, there have been reasonable rates of pregnancy. In fact, two pregnancies have been documented (with proved paternity) after end-to-end anastomosis of the vas deferens to the vasa efferentia, with normal motile sperm found in the postoperative ejaculate. It was therefore suggested that sperm do not require transit through the epididymis in order to fertilize an oocyte.[25] The present series offers direct proof of this concept.

The cost per cycle of the procedure is about twice that of standard in vitro fertilization. Because of the higher implantation rate, transfer of the embryos to the fallopian tube is preferable to transfer to the uterus in cases in which fertilization is so difficult to achieve, but this surgery is more expensive.[10,11] Also, the aspi-

ration procedure in the man involves microsurgery and general anesthesia. Although the procedure is clearly repeatable (probably many times), cost may therefore become an important restriction.

Finally, the interpretation of these findings must be limited in certain respects. The fertilizing capacity of sperm in various regions of an obstructed epididymis is not likely to be the same as that in a nonobstructed epididymis. The pattern of motility of sperm under obstructive conditions is clearly the inverse of that previously described for nonobstructed conditions.[26,27] Furthermore, although transit of the sperm through the epididymis is not necessary for fertilization, our results do not rule out the possibility that retrograde epididymal secretions may still support or be necessary for sperm maturation.

References

1. Orgebin-Christ MC. Sperm maturation in rabbit epididymis. Nature 1967; 216:816-8.
2. *Idem*. Studies on the function of the epididymis. Biol Reprod 1969; 1:Suppl 1:155-75.
3. Bedford JM. Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit. J Exp Zool 1966; 162:319.
4. Glover TD. Some aspects of function in the epididymis: experimental occlusion of the epididymis in the rabbit. Int J Fertil 1969; 14:216-21.
5. Gaddum PJ. Sperm maturation in the male reproductive tract: development of motility. Anat Rec 1968; 161:471-82.
6. Rowley MJ, Teshima F, Heller CG. Duration of transit of spermatozoa through the human male ductular system. Fertil Steril 1970; 21:390-6.
7. Johnson L, Varner DD. Effect of daily spermatozoan production but not age on transit time of spermatozoa through the human epididymis. Biol Reprod 1988; 39:812-7.
8. Zorn JR, Boyer P, Guichard A. Never on a Sunday: programming for IVF-ET and GIFT. Lancet 1987; 1:385-6.
9. Ord T, Marello E, Balmaceda JP, Asch RH. The use of modified Percoll technique in IVF for cases of severe oligoasthenospermia. Presented at International Symposia of Gamete Physiology, Newport Beach, Calif., November 6–10, 1988.
10. Yovich JL, Yovich JM, Edirisinghe WR. The relative chance of pregnancy following tubal or uterine transfer procedures. Fertil Steril 1988; 49:858-64.
11. Balmaceda JP, Gastaldi C, Remohi J, Borrero C, Ord T, Asch RH. Tubal embryo transfer as a treatment for infertility due to male factor. Fertil Steril 1988; 50:476-9.
12. El-Itreby AA, Girgis SM. Congenital absence of the vas deferens in male sterility. Int J Fertil 1961; 6:409-16.
13. Girgis SM, Etriby AA, Ibrahim AA, Kahil SA. Testicular biopsy in azoospermia: a review of the last ten years' experiences of over 800 cases. Fertil Steril 1969; 20:467-77.
14. Sakatoku J, Yoshida O, Komatsu Y, Takayama H, Harada T. Congenital aplasia of vas deferens. Hinyokika Kiyo 1967; 13:769-84.
15. Schoysman R, Drouart JM. Recent progress in the surgery of male and female sterility. Acta Chir Belg 1972; 71:261-80.
16. Kelami A. Alloplastische Spermatocele: vorlanfige Mitteilung. Acta Urol Belg 1976; 7:167-76.
17. Vickers MA Jr. Creation and use of a scrotal sperm bank in aplasia of the vas deferens. J Urol 1975; 114:242-5.
18. Jimenez-Cruz JF. Artificial spermatocele. J Urol 1980; 123:885-6.
19. Pryor J, Parsons J, Goswamy R, et al. In-vitro fertilization for men with obstructive azoospermia. Lancet 1984; 2:762.
20. Temple-Smith PD, Southwick GJ, Yates CA, Trounson AD, de Kretser DM. Human pregnancy by in vitro fertilization (IVF) using sperm aspirated from the epididymis. J In Vitro Fert Embryo Transf 1985; 2:119-22.
21. Silber SJ. Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. Hum Reprod 1989; 4:298-303.
22. Krylov VS, Borovikov AM. Microsurgical method of reuniting ductus epididymis. Fertil Steril 1984; 41:418-23.
23. Young WC. The study of the function of the epididymis: functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea pig. J Exp Biol 1931; 8:151-62.
24. Silber SJ. Apparent fertility of human spermatozoa from the caput epididymidis. J Androl 1989; 10:263-9.
25. *Idem*. Pregnancy caused by sperm from vasa efferentia. Fertil Steril 1988; 49:373-5.
26. Bedford JM, Calvin H, Cooper GW. The maturation of spermatozoa in the human epididymis. J Reprod Fertil Suppl 1973; 18:199-213.
27. Mooney JK Jr, Horan AH, Lattimer JK. Motility of spermatozoa in the human epididymis. J Urol 1972; 108:443-5.