FERTILITY AND STERILITY
Vol. 62, No. 3, September 1994

Printed on acid-free paper in U. S. A.

Relative force of human epididymal sperm*

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Objective: To assess the relative escape force of human epididymal sperm using a laser generated optical trap and compare it with that of human ejaculated sperm.

Design: Evaluation of the relative force generated by epididymal and ejaculated sperm using an 800-nm laser-generated optical trap system (titanium-sapphire, model 899-01; Coherent Innova, Palo Alto, CA).

Setting: University-based facility at the Beckman Laser Institute and Medical Clinic and Center for Reproductive Health, University of California, Irvine.

Interventions: A total of 2,720 sperm from 28 samples were randomly analyzed. Fifteen were ejaculated samples (1,650 sperm) obtained from men with proven fertilization, and 13 were epididymal samples (1,070 sperm) aspirated microsurgically from patients with obstructive azoospermia. An optical trap equipped with the 100× Neofluar objective was used to analyze an average of 100 sperm per patient.

Main Outcome Measures: Determination of mean relative escape force values in milliwatts for epididymal and ejaculated sperm samples.

Results: The mean relative escape force for epididymal sperm was 32.4 mW, significantly lower than ejaculated sperm, which was 85.1 mW . By correlating epididymal sperm relative force with fertilization in vitro at an arbitrary cutoff value of 30 mW, it was found that no fertilization occurred if a sample had <13% of sperm at that value.

Conclusions: [1] The average relative escape force of the epididymal sperm was found to be 60% weaker than that of ejaculated sperm. [2] It is demonstrated that the noncontact laser optical trap is a sensitive tool that can evaluate single sperm force as a new physiological parameter.

Fertil Steril 1994;62:585–90

Key Words: Sperm force, human epididymal sperm, laser

The development of the microsurgical epididymal sperm aspiration technique associated with IVF offers hope for those cases of male infertility because of obstructive azoospermia secondary to congenital absence of the vas deferens, as demonstrated by live births (1–3). However, a frustrating factor of the microsurgical epididymal sperm aspiration technique and IVF procedure is represented by

Received June 4, 1993; revised and accepted April 13,1994.

* Supported by grant RR01192 from the National Institutes of Health, Bethesda, Maryland; by grant 000–14–91-C-0134 from the Office of Naval Research, Arlington, Virginia; and by grant DE-FG03–91 ER 61227 from the Department of Energy, Washington, D.C.

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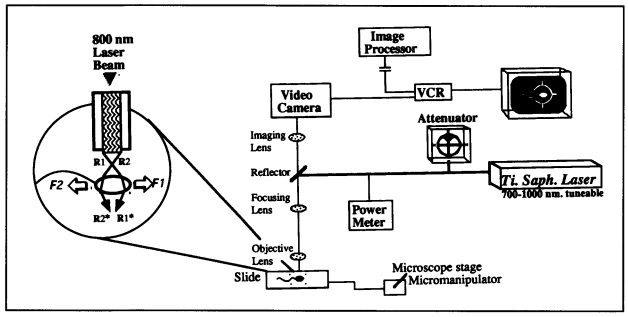

Figure 1 Schematic diagram of the optical trap. The microscope is represented by the objective lens and ray diagram (*left*) depicting the basic principles of the optical trap measurements of sperm force. A near infrared laser beam at a wavelength of 800 nm was selected from the tunable titanium sapphire tube (700 to 1,000 nm). The beam was directed through an objective lens and focused above the sperm. Refraction of the rays R1 and R2 generates a transverse force F2 when the sperm is not centered in the trap, opposite in direction to the force F1 generated by the sperm. The sperm escapes from the trap when F1 > F2.

the low (<20%) and unpredictable fertilization rate of epididymal sperm. Many studies have been performed to understand the reasons for such poor IVF performance (4–7). Unfortunately, none has proved to be conclusive.

In 1989, Tadir et al. (8) first applied a single beam gradient optical trap generated by a neodymium:yttrium aluminum garnet laser to trap sperm. In a second study (9), the relative escape force generated by normal human sperm was measured. The force generated by each individual sperm that results in forward progression is a function of flagellar beat kinematics, sperm morphology, and surface properties. The amount of force generated by each spermatozoa is provided in a very accurate way. At the threshold level where a sperm can swim out of the trap, the force generated by the sperm is equal to the force exerted by the optical trap, which is proportional to the power of the laser beam. This parameter is defined as relative escape force (Fig. 1).

We therefore decided to apply the laser-generated optical trap to the epididymal sperm to assess the relative escape force of human epididymal sperm from patients with congenital absence of the vas deferens and compare it with that of human ejaculated sperm. In addition, a possible correlation between the relative escape force threshold and fertilization of human eggs in vitro was studied.

MATERIALS AND METHODS

A total of 2,720 normal appearing and motile sperm from 28 different sperm samples were randomly selected for optical trapping and relative escape force analysis. Fifteen were ejaculated samples (1,650 sperm) obtained from men with normal sperm parameters (as defined in our center as follows: sperm count $>20 \times 10^6$/mL, motility $\geq 40\%$, normal forms $\geq 30\%$ as per methylene-blue staining), and 13 were epididymal sperm samples (1,070 sperm) obtained by microsurgical aspiration of the epididymis of patients with obstructive azoospermia undergoing our microsurgical epididymal sperm aspiration technique and IVF program. The details of the surgical procedure are described in a previous report (3).

All samples were diluted in HEPES-buffered human tubal fluid (HTF; Irvine Scientific, Irvine, CA) supplemented with 0.5% human serum albumin. The samples were centrifuged once at $200 \times g$ for 10 minutes, and the resulting pellets were resuspended in 0.2 to 0.5 mL of culture media (HEPES-HTF). For both types of sperm (ejaculated and epididymal), the aliquots analyzed by the laser-generated optical trap were obtained from the same sample used for the oocyte insemination. All the samples were analyzed within 2 to 3 hours from sperm collection.

The laser beam gradient force optical trap employed in the study was a titanium-sapphire laser (model 899–01; Coherent Innova, Palo Alto, CA), operating continuous wave, at a infrared wavelength of 800 nm and with a power ranging from 0 to 300 mW. The beam was directed into a Zeiss photomicroscope (Zeiss, Thornwood, NY) and focused into the field of view using a 100× Neofluar objective (Fig. 1). A variable attenuator (model 930; Newport Corp., Fountain Valley, CA) was used to control the laser power and was measured to be 1 W at minimum attenuation. A beam polarizer (Melles Griot; Glen Thompson, Irvine, CA) was also used to obtain a linear calibration curve. The spot diameter at the focal plane was in the range of 2 to 3 μm. A motorized X-Y microscope stage was employed to hold the sperm confined in the optical trap while the trapping power was gradually attenuated until the sperm could swim away. Remote real-time viewing of the sperm in the trap was performed using a video camera (Series 68, Dage-MTI; Michigan City, IN) collinear with the path of the trapping beam. A dichroic mirror was used to separate the trapping beam from the image projected onto the video camera and then recorded on a videotape. A photodiode and a calorimeter were used to calibrate the optical trap.

The wives of the 28 men included in the study

Table 1 Sperm Parameters of Each Patient in the Epididymal Sperm Group (Pretreatment)

Patient no.	Sperm count	Motility	Progression (0 to 4)	Normal form	Total motile count
	$\times 10^6$/mL	%		%	
Epididymal sperm					
1	89	30	1 to 2	26	27
2	25	20	1 to 2	12	5
3	34	5	1	24	1.7
4	68	30	1 to 2	14	20.5
5	120	30	1 to 2	24	36
6	100	30	1 to 2	12	30
7	45	20	1	20	8.9
8	11	30	1 to 2	24	3.3
9	52	40	1 to 2	21	21
10	79	30	1 to 2	20	24
11	124	20	1 to 2	36	24.8
12	528	30	1 to 2	32	159
13	57	30	1 to 2	48	17.2
Overall mean ± SEM	102.5 ± 40.1*	26.5 ± 2.4†	1.0 ± 0.1‡	24.4 ± 2.8§	29.1 ± 11.2‖
Ejaculated sperm					
14	94	40	2	30	97
15	76	50	2 to 3	40	110
16	122	40	2 to 3	35	54
17	56	50	2	42	90
18	143	40	2 to 3	34	132
19	134	40	2 to 3	36	134
20	87	40	1 to 2	48	320
21	492	40	2 to 3	42	649
22	366	40	2	29	292
23	170	40	3 to 4	36	68
24	50	40	1 to 2	30	86
25	134	40	2 to 3	31	187
26	95	50	2	30	133
27	30	40	1 to 2	36	47
28	46	40	1 to 2	30	69
Overall mean ± SEM	139.7 ± 32.4*	41.3 ± 1.6†	2.0 ± 0.2‡	35.3 ± 1.5§	171 ± 39.3‖

* P = not significant.
† P = 0.00001.
‡ P = 0.001.
§ P = 0.001.
‖ P = 0.003.

underwent controlled ovarian hyperstimulation (COH) for IVF. It consisted of leuprolide acetate (Lupron; TAP Pharmaceuticals, Chicago, IL), FSH (Metrodin; Serono Laboratories, Randolph, MA), and hMG (Pergonal; Serono Laboratories, Randolph, MA). The details of the COH and oocyte retrieval have been described elsewhere (3).

The epididymal sperm preparation for oocyte insemination was performed by using the mini-Percoll method (10), and the number of sperm used varied between 500,000 and 1×10^6 per pooled oocytes (11). For the ejaculated sperm, however, the regular Percoll technique was used (12). The fertilization rates were then correlated to the results of relative sperm force.

Statistical analysis was performed by using the Student's t-test to compare the overall mean and SEM of the two groups considered in the study. The χ^2 test was used to compare the two groups in terms of number of sperm with >30 mW of relative escape force as well as to compare their fertilization rate.

RESULTS

Both groups of patients (ejaculated and epididymal sperm) were homogeneous for age and number of oocytes collected. Table 1 shows the sperm parameters for each of the sperm samples in both groups. The sperm count was low in only one sperm sample (no.8) of the epididymal sperm group. Four of 13 samples had <30% motility, but in all, the progression was never >2 (scale 0 to 4). Three of the 13 epididymal sperm samples had >30% normal morphology, and the total motile count was $<20 \times 10^6$ in 5 of 13 samples. The mean and SEM of the sperm count was not different between the two groups ($102.5 \pm 40.1 \times 10^6$/mL and $139.7 \pm 32.4 \times 10^6$/mL, respectively). In contrast, the difference

Table 2 Summary of the Relative Escape Force of Epididymal and Ejaculated Sperm Samples

Patient no.	Epididymal	Patient no.	Ejaculated
1	64.8 ± 3.8*	14	106.8 ± 6.9
2	25.8 ± 2.3	15	121.8 ± 6.9
3	25.4 ± 1.7	16	95.5 ± 6.0
4	17.4 ± 1.1	17	108.4 ± 6.6
5	30.2 ± 2.5	18	48.1 ± 2.9
6	37.2 ± 8.5	19	106.7 ± 39.8
7	27.5 ± 3.5	20	38.3 ± 1.9
8	6.1 ± 1.5	21	74.9 ± 3.9
9	35.0 ± 2.5	22	78.6 ± 3.0
10	27.2 ± 2.2	23	61.8 ± 2.6
11	19.0 ± 1.5	24	91.9 ± 2.7
12	15.2 ± 1.2	25	85.7 ± 2.7
13	19.8 ± 2.0	26	103.4 ± 3.3
		27	77.6 ± 3.5
		28	78.7 ± 29.1
All	32.4 ± 1.0†		85.1 ± 1.2†

* Values are means ± SEM of mW.
† $P < 0.0001$.

in motility (26.5% versus 41.3%), progression (1.0 versus 2.0), normal morphology (24.4% versus 35.3%), and total motile count (29.1×10^6/mL versus 171×10^6/mL) between epididymal sperm and ejaculated sperm were statistically significant as shown in Table 1.

Table 2 summarizes the mean relative escape force of each sperm sample expressed by the mean and SEM of the 13 epididymal samples of sperm and 15 of ejaculated sperm. The overall mean relative escape force of the epididymal sperm was 32.4 mW (± 0.98 SEM), significantly lower than that of ejaculated sperm, which was 85.1 ± 1.2 mW ($P < 0.0001$).

Table 3 compares the fertilization of the 13 epididymal sperm samples and that of the 15 ejaculated sperm. The overall epididymal sperm fertilization rate was 5.5% (16/293) as opposed to 87.7% (186/212) for the ejaculated sperm.

In trying to correlate epididymal sperm relative escape force with fertilization in vitro, we were not able to draw any definitive conclusions because of the small number of samples. However, by choosing 30 mW as an arbitrary cutoff of relative escape force, it was observed that in a sample when <13% of the total population of epididymal sperm showed a force ≥ 30 mW, no fertilization occurred.

DISCUSSION

To our knowledge, this is the first study that measured the relative escape force of human epididymal sperm and compared it with that of ejaculated sperm. The laser-generated optical trap allows accurate measurements of this parameter in each individual sperm. From the data presented, it appears that epididymal sperm retrieved from men with obstructive azoospermia are objectively weaker in generating forward progression than normally ejaculated sperm. Their relative escape force was 32.4 ± 0.98 mW compared with 85.1 ± 1.2 mW found in the ejaculated sperm ($P < 0.0001$). It should be mentioned that sperm relative escape force and progression are not the same. Progression represents the speed or velocity of the sperm, whereas relative escape force is the result of the combination of flagellar beat kinematics, sperm morphology, and surface properties. By comparing average sperm parameters, one may find similar count, average motility, progression, and morphology among specimens within the same group; however, the mean relative escape force can be significantly different, as shown in Tables 1 and 2 (see patients 1 versus 4 of the epididymal group).

The epididymal sperm parameters found are consistent with those of previously published data (11). The overall clinical fertilization rate of the epididymal sperm used in this study was very low (5.5%); one possible explanation might be the presence of very weak forward progressive sperm as demonstrated by the laser optical trap. This is particularly evident in three patients (nos. 4, 8, and 12)

Table 3 Comparison of IVF Results Between Epididymal Sperm and Ejaculated Sperm

Patient no.	Epididymal No. embryos/ eggs	Patient no.	Ejaculated No. embryos/ eggs
1	2/32	14	13/15
2	1/27	15	20/20
3	0/14	16	9/10
4	0/8	17	25/28
5	7/39	18	12/13
6	0/19	19	34/36
7	2/19	20	2/2
8	0/27	21	9/9
9	1/7	22	24/27
10	1/17	23	5/5
11	1/29	24	12/24
12	0/12	25	1/2
13	1/43	26	2/2
	—	27	15/15
	—	28	3/4
Total no.	16/293*		186/212*
Overall fertilization rate (%)	5.5		87.7

* $P < 0.001$.

in which the mean relative escape force was extremely low, and none of them achieved fertilization (Table 3).

Several hypotheses can be postulated to explain the low relative escape force of human epididymal sperm. The first is the assumption that the majority of sperm do not acquire the potential to fertilize because of the lack of epididymal transit. This is based on previous animal experiments (13, 14) showing that sperm must pass through a certain length of the epididymis to mature, gain progressive motility, and become capable of fertilization. Contributing to this idea, there are other animal studies (15, 16) showing that sperm from surgically obstructed epididymis have only rarely shown fertilization. However, Johnson and Varner (17) demonstrated that the storage capacity of the human epididymis is extremely limited in time and that, in some cases, sperm are transported through the entire epididymis in approximately 2 days. Their findings demonstrate the possibility that human sperm may not require the same degree of epididymal maturation necessary in many animals.

The second hypothesis is that epididymal sperm from men with congenital absence of the vas deferens or with other obstructive lesions have been retained for a long time in their excurrent pathway and, therefore, there are mixtures of sperm populations in different stage of senescence and maturity.

The third hypothesis is related to the recent discovery that congenital absence of the vas deferens is a congenital abnormality related to cystic fibrosis (CF) disease (18), inherited as an autosomal recessive condition in sons of carriers for different CF mutations. Different CF mutations have been shown to play an important role in the fertilization capacity of epididymal sperm (19). Biochemical alterations of sperm membranes to chloride ions might be negatively reflected in the lower relative escape force value of these sperm. To confirm this hypothesis, however, a comparative study between obstructed azoospermia because of congenital absence of the vas deferens and obstructed azoospermia due to other causes (i.e., vasectomized patients) is necessary. A study is in progress in our laboratories to compare the relative escape force and fertilizability of these sperm with that of normal ejaculated sperm.

Although there might be a correlation between mean relative escape force and the fertilizing capacity of epididymal sperm in vitro, current trends in IVF suggest that such sperm may offer better fertilization rates if injected into the egg cytoplasm, and as such, relative escape force may not be clinically useful as a predictive parameter. In conclusion, the data gathered from this study offer relevant basic information on sperm physiology at the level of the human epididymis.

REFERENCES

1. Silber SJ, Balmaceda JP, Borrero C, Ord T, Asch RH. Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. Fertil Steril 1988;50:525–8.
2. Patrizio P, Silber SJ, Ord T, Balmaceda JP, Asch RH. Two births after microsurgical epididymal sperm aspiration in congenital absence of the vas deferens [letter]. Lancet 1988;2:1364.
3. Silber SJ, Ord T, Balmaceda JP, Patrizio P, Asch RH. Congenital absence of the vas deferens. N Engl J Med 1990;323:1788–92.
4. Davis RO, Overstreet JW, Asch RH, Ord T, Silber SJ. Movement characteristics of human epididymal sperm used for fertilization of human oocytes in vitro. Fertil Steril 1991;56:1128–35.
5. Asch RH, Patrizio P, Silber SJ. Ultrastructure of human sperm in men with congenital absence of the vas deferens: clinical implications. Fertil Steril 1992;58:190–3.
6. Rojas FJ, La A-T, Ord T, Patrizio P, Balmaceda JP, Silber SJ, et al. Penetration of zona-free hamster oocytes using human sperm aspirated from the epididymis of men with congenital absence of the vas deferens: comparison with human in vitro fertilization. Fertil Steril 1992;58:1000–5.
7. Patrizio P, Silber SJ, Ord T, Moretti-Rojas I, Asch RH. Relationship of epididymal sperm antibodies to their in vitro fertilization capacity in men with congenital absence of the vas deferens. Fertil Steril 1992;58:1006–10.
8. Tadir Y, Wright WH, Vafa O, Ord T, Asch RH, Berns MW. Micromanipulation of sperm by a laser generated optical trap. Fertil Steril 1989;52:870–3.
9. Tadir Y, Wright WH, Vafa O, Ord T, Asch RH, Berns MW. Force generated by human sperm correlated to velocity and determined using a laser generated optical trap. Fertil Steril 1990;53:944–7.
10. Ord T, Patrizio P, Marello E, Balmaceda JP, Asch RH. Mini-Percoll: a new method of semen preparation for IVF in severe male factor infertility. Hum Reprod 1990;58:987–9.
11. Ord T, Marello E, Patrizio P, Balmaceda JP, Silber SJ, Asch RH. The role of the laboratory in the handling of epididymal sperm for assisted reproductive technologies. Fertil Steril 1992;57:1103–6.
12. Berger T, Marrs RP, Moyer DL. Comparison of techniques for selection of motile spermatozoa. Fertil Steril 1984; 43:268–73.
13. Orgebin-Christ MC. Studies on the function of the epididymis. Biol Reprod 1969;1(Suppl):155–75.
14. Bedford JM. Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit. J Exp Zool 1966;163:319–29.
15. Glover TD. Some aspects of function in the epididymis: experimental occlusion of the epididymis in the rabbit. Int J Fertil 1969;14:216–21.

16. Geddum PJ. Sperm maturation in the male reproductive tract: development of motility. Anat Rec 1968;161:471–82.
17. Johnson L, Varner DD. Effect of daily spermatozoan production but not age on the transit time of spermatozoa through the human epididymis. Biol Reprod 1988;39:812–7.
18. Patrizio P, Asch RH, Handelin B, Silber SJ. Aetiology of congenital absence of vas deferens: genetic study of three generations. Hum Reprod 1993;8:215–20.
19. Patrizio P, Ord T, Silber SJ, Asch RH. Cystic fibrosis mutations impair the fertilization rate of epididymal sperm from men with congenital absence of the vas deferens. Hum Reprod 1993;8:1259–63.