BIOLOGY OF REPRODUCTION 49, 274–280 (1993)

Changes in Movement Characteristics of Human Spermatozoa along the Length of the Epididymis[1]

C.H. YEUNG,[3] T.G. COOPER,[3] F. OBERPENNING,[4] H. SCHULZE,[5] and E. NIESCHLAG[2,3]

Institute of Reproductive Medicine[3] and Urology Clinic,[4] University of Münster Münster, Germany
Urology Department,[5] University of Bochum, Herne, Germany

ABSTRACT

It has been established in laboratory mammals that sperm motility and fertilizing capacity develop during epididymal transit, but sperm maturation along the human epididymis is less well characterized. Spermatozoa were prepared from 5 regions of 8 epididymides from 8 prostatic carcinoma patients undergoing castration and from 8 epididymal spermatocoeles located adjacent to the head of the epididymides and the testes of 5 patients. Sperm movement was characterized by computer-aided sperm analysis (CASA), and percentage motility was estimated by conventional methods. The efferent ducts and spermatocoeles contained the same percentage of motile spermatozoa with similar kinematics. Percentage motility increased from 22.9 ± 4.8 (mean ± SEM) in the efferent ducts to a maximum of 68.3 ± 7.9 in either the mid- or distal corpus epididymidis and declined in the cauda region. Straight line velocity increased from 20.3 ± 3.7 μm/sec to reach a plateau value of 44.0 ± 5.3 μm/sec in the mid-corpus epididymidis; this was more marked than the increase in curvilinear velocity, although the trend was the same. Similar trends in linearity and straightness of the swim paths were not accompanied by any significant changes in the amplitude of lateral head displacement. This objective quantification of sperm movement documents the maturation of sperm motility in the human epididymis, confirming that this maturation pattern is similar to that in other mammals.

INTRODUCTION

It is well recognized that in laboratory mammals, sperm motility improves along the length of the epididymis in parallel with the development of fertilizing capacity (see reviews [1, 2]). Whereas fertilizing capacity is first exhibited by spermatozoa from the distal caput or proximal corpus regions and reaches a maximum in the cauda, the profiles of change in percentage motility in most species studied occur more proximally, such that about one third of the spermatozoa from the distal caput are already motile and maximal motility is attained around the distal corpus (Tables 2 and 4 in ref. [1]). In addition to the percentage of motile sperm, changes in the pattern of sperm movement upon maturation have also been described in various mammalian species. Early findings are confined to qualitative descriptions or quantification of relatively few cells, owing to the tediousness as well as the insensitivity of kinematic measurement methods. With the aid of computers in the analysis of larger samples, more representative and objective data can be obtained. However, documented comparisons have been made mostly between immature and mature cells from only two regions, namely the caput or proximal corpus and the cauda epididymidis, where marked differences have been shown (e.g., ram [3, 4], bull [5], boar [6], chimpanzee [7]). In the rat, where spermatozoa from five regions along the length of the epididymis were examined, not all kinematic parameters showed the same profile of changes and most of these occurred proximal to the distal corpus region [8].

That human spermatozoa also develop the potential for sustained progressive motility in passing through the epididymis has been indicated by comparison of sperm motility from various regions of unblocked human epididymides [9–12]. These studies showed increases in the percentages of motile or progressively motile spermatozoa. Changes in the movement characteristics of spermatozoa along the epididymis have not been investigated as extensively in humans as in non-humans, mainly owing to the difficulty of obtaining material but also because of difficulties in assessing sperm movement objectively. Quantification of such kinematic changes via computer-aided sperm analysis (CASA) should help to clarify the confusion that has been caused by the suggestion that the human epididymis is unnecessary for sperm maturation [13]; this suggestion arises from reports on in vitro and in vivo fertilization with spermatozoa from the caput epididymidis of men suffering from obstructive azoospermia ([14–18], also see [19]). In one of these studies, where movement characteristics were quantified in samples obtained from three epididymal regions in brain-dead subjects, percentage motilities were low (< 25%) [18]. In the present study, we examined the motility of sperm from five different regions of the epididymis and from epididymal spermatocoeles of prostatic carcinoma patients who had received no antiandrogen treatment; we measured the kinematics by CASA to detect and characterize changes with maturation along the length of the organ.

Accepted March 22, 1993.
Received October 26, 1992.
[1]Supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) Ni 130/11.
[2]Correspondence: Prof. Dr. E. Nieschlag, Institute of Reproductive Medicine of the University, Steinfurterstr. 107, D-4400 Münster, Germany. FAX: 049–0251–836093.

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022

MATERIALS AND METHODS

Subjects

Testes with attached epididymides were obtained with consent from patients undergoing castration because of prostatic carcinoma. Tissues were transported to the laboratory in sterile containers on ice within 20 min after the operation, except for one epididymis that was transported in culture medium (Ham's F-12; Gibco Europe GmbH, Karlsruhe, Germany) on ice and that arrived 80 min after the operation. The epididymides were freed from connective tissue. A small piece cut from the mid-corpus region was minced in a drop of PBS and the exudate was examined to confirm the presence of motile spermatozoa. Epididymides with no motile spermatozoa in the mid-corpus were not processed further for this study. When motile sperm were observed in both epididymides of the same patient, the one containing more motile spermatozoa was used and the other was used for other experimentation. By this procedure, 8 epididymides were obtained from 8 patients aged between 61 and 77 yr (mean, 69 yr). None of the men had been treated with antiandrogen before the operation, and 2 were known to have fathered children.

Additionally, 8 epididymal spermatocoeles, which were located adjacent to the head of the epididymis and the testis and which contained motile sperm cells, were studied. Two of these were found in 2 of the above-mentioned patients and had fluid contents of < 0.5 ml. The other 6 were obtained from 3 patients during surgery in which the spermatocoeles alone had been ligated and excised. These contained 1–7 ml fluid, with one containing more than 100 ml. These patients ranged in age from 53–84 yr (mean, 66 yr) and all of them had children.

Sperm Preparation

Samples from regions of the epididymis were as follows (see Fig. 1): efferent ducts (region 1)—both whitish *coni vasculosi* and dark tubules (epithelia I and III according to Yeung et. al [20]); proximal corpus epididymidis (region 2)—midway between the efferent ducts and the mid-corpus; mid-corpus epididymidis (region 3)—midway between the termination of the caput (defined distally as the junction between the dark tubules and the whitish epididymidis proper [20]) and the origin of the cauda epididymidis characterized by thicker tubules; distal corpus epididymidis (region 4)—midway between the mid-corpus and cauda epididymidis; proximal cauda epididymidis—the initial narrower convoluted tubules of the muscular cauda; distal cauda epididymidis—the more distal, larger-diameter, convoluted muscular tubules. Sperm characteristics from the latter two regions were similar in 3 epididymides examined, and these data were pooled. Only one sample was taken from the cauda (region 5) in the other epididymides.

Loops of epididymal tubules isolated from various regions were minced with iridectomy scissors in 0.5 ml medium containing 123 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 0.4 mM $MgSO_4$, 0.3 mM Na_2HPO_4, 25 mM $NaHCO_3$, 5 mM glucose, 12.5 mM sodium lactate, 0.5 mM pyruvate, 8 μg phenol red/ml, 4 mg BSA/ml, with a pH of 7.4 and osmolality of 310 mOsm/kg (medium H [8]). The minced tubules were transferred with an additional 0.5 ml of medium to capped Eppendorf tubes and were rotated head over head on a rotator at 4 rpm for 5 min at room temperature. After settling for 5 min, the supernatant was transferred to fresh tubes. A drop of this sperm suspension was used to estimate percentage motility by counting flagellating and static spermatozoa, and the rest was centrifuged at $1000 \times g$. The sperm pellet was gently resuspended in a few drops of medium H and used for kinematic analysis within a few min-

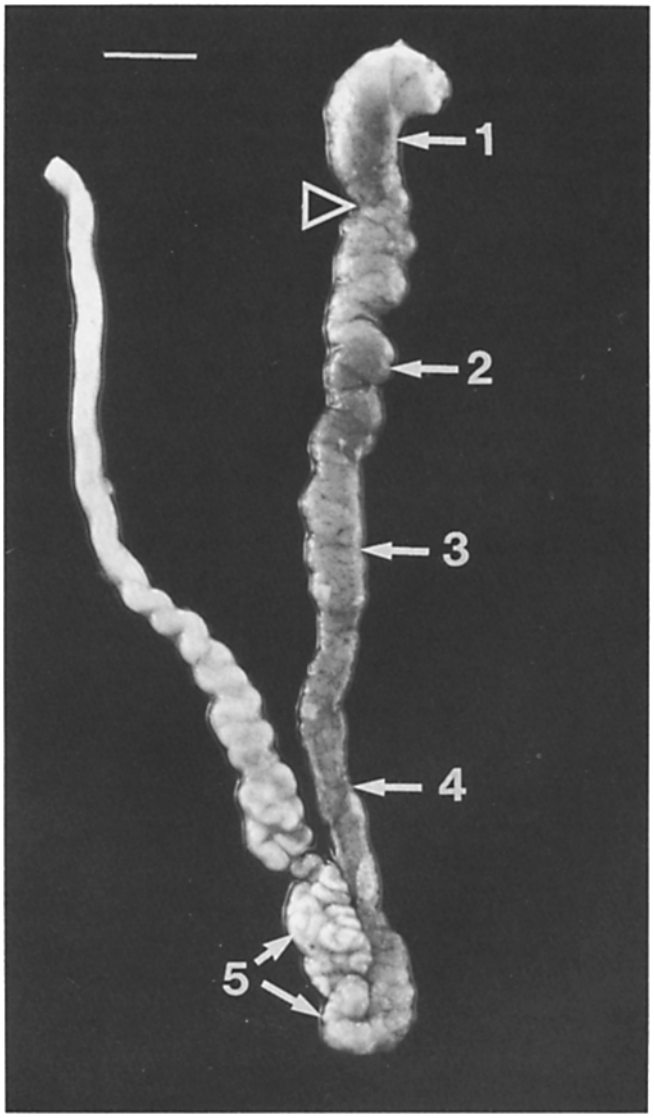

FIG. 1. Photograph of a human epididymis showing the locations of the 5 regions where spermatozoa were obtained for motility measurement. The bar represents 1 cm and the triangle indicates the junction between efferent ducts (1) and the epididymis. See text for detailed description.

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022

utes. The entire sperm preparation procedure was carried out at room temperature and motility measurement was performed at 37°C (see below).

Fluid from the epididymal spermatocoeles was withdrawn by puncture into a syringe through 21-gauge needles. It was centrifuged at 1000 × *g* for 3 min and the sperm pellet was resuspended in medium H as described above.

Effect of Sperm Preparation Techniques on Motility Parameters

In preliminary experiments, luminal contents from cut tubule segments were expressed into medium; sperm motility was examined and videotaped without further processing to check whether the preparation procedure outlined above (which gave a much better yield of spermatozoa, especially in the proximal epididymal regions) would affect sperm motility. Absence of any detrimental effect of sample rotation and centrifugation was also confirmed on ejaculates from 2 healthy donors. Each ejaculate was diluted five times with medium H, and sperm motility before and after rotation, centrifugation, and resuspension as above was compared. Motility of spermatozoa from an epididymal spermatocoele in its native fluid was also compared to that of the pelleted and resuspended sample.

Analysis of Sperm Motility

Percentage motility was estimated as described above. After the sperm suspension was adjusted to a usable concentration, 5 μl was placed in a 10-μm-deep Makler chamber (Sefi Medical Instruments, Haifa, Israel). Sperm motility was observed at 37°C in an Olympus BH-2 positive phase-contrast microscope with an S-Plan 10× objective and 6.7× photo-ocular, then videotaped on a VHS Panasonic NV-870 video recorder via a Panasonic NV-850 video camera and a digital timer (VTG-33; ForA Co. Ltd., Tokyo, Japan). Up to 3 aliquots were analyzed from each sample so that more motile cells could be obtained when sperm concentration or percentage motility was low. All, or up to 200, motile spermatozoa from each sample were analyzed through use of the Hamilton-Thorn CASA system (HTM version 6.4E; Hamilton-Thorn Research Inc., Beverly, MA) with the following settings: tracking duration, 1.07 sec with 20 frames at 19 frames/sec; minimum contrast, 9; minimum size, 12; low/high gate for both size and intensity, 0.5/2.0; threshold for averaged path velocity, 5 μm/sec; magnification, 2.65. The analysis of each field was scrutinized by means of the playback function of the HTM. Occasional tracks arising from non-sperm particles were deleted before the other sperm tracks were saved on disk. The kinematic measurements obtained included curvilinear velocity (VCL), calculated from the path joining consecutive track points; average path velocity (VAP), calculated by smoothing the original path using a 5-point running average; straight line velocity (VSL), calculated from the distance between the first and the last track points; linearity (LIN), calculated as 100 × VSL/VCL; straightness of path (STR), calculated as 100 × VSL/VAP; and amplitude of lateral head displacement (ALH), calculated from the smoothed path. Mean coefficients of variation obtained from duplicate analyses of three samples of spermatozoa from a spermatocoele, the efferent ducts, and cauda epididymidis were 1.9, 2.0, 4.0, 3.7, 4.0, and 2.2% for VCL, VAP, VSL, LIN, STR, and ALH, respectively.

Statistics

Goodness of fit to normal or logarithmic distributions of individual sperm values of each kinematic parameter for each epididymal region were tested for statistical significance with the Kolmogorov-Smirnov test using a sample from that region containing the highest number of analyzed sperm tracks. On the basis of these results, log-transformation was applied to individual sperm data on ALH and angular transformation (arcsin of the square root of the original ratio) to LIN and STR values before the mean values of each sample were calculated. Analysis of variance of sample means using Duncan's test was performed for differences between regions.

RESULTS

Effect of Preparation Procedure on Sperm Motility

The procedures used in the preparation of spermatozoa did not impair their motility regardless of the sites of collection; a slight increase was observed in VCL and VSL of ejaculated spermatozoa from one donor (Table 1).

Changes in Sperm Movement Characteristics along the Post-Testicular Ducts

Percentage motility. The percentage of motile spermatozoa obtained from epididymal spermatocoeles was consistently below 25, with slight if any improvement in sperm from the efferent ducts. Percentage motility from various regions of the epididymis varied greatly between patients, but changes along the length of each organ showed a similar trend (Fig. 2a). The maximum percentage motility achieved by epididymal spermatozoa in all subjects was 68 ± 8% (mean ± SEM), ranging from 58 to 85%, except in one (aged 69, had fathered children earlier in life) where it did not exceed 20%. Increases from the efferent ducts to the proximal corpus epididymidis were observed in 6 of 8 patients, and increases from the proximal to the mid-corpus were found in all except 2. In 3 epididymides the maximum percentage of motile spermatozoa was reached in the mid-corpus and started to decline in the more distal regions, whereas the other 5 showed maximal motility in the distal corpus epididymidis. In all cases sperm motility decreased from the distal corpus to the cauda epididymidis.

Kinematics. In addition to their low percentage motility, spermatozoa from the efferent ducts showed similar

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022

TABLE 1. Motility parameters[a] of spermatozoa from various regions before (A) and after (B) the sperm preparation procedures showing the absence of detrimental effect of the procedures.

Parameter		Spermatocoele	Efferent ducts	Distal corpus epididymidis	Ejaculates	
					Donor 1	Donor 2
% motility	A	13	32	58	62	62
	B	10	32	65	70	72
VCL (μm/sec)	A	34.2–39.0	39.7–55.9	66.6–74.6	72.2–74.8	60.4–63.2
	B	31.0–35.1	43.5–48.4	69.8–74.9	69.9–73.4	66.8–70.2*
VAP (μm/sec)	A	21.1–24.2	28.4–43.1	48.6–54.6	64.1–66.7	48.6–51.4
	B	20.4–23.4	29.3–33.7	48.2–51.7	62.3–65.9	55.7–59.1
VSL (μm/sec)	A	15.5–18.4	17.6–29.9	36.1–43.3	59.3–62.1	44.1–47.1
	B	15.2–18.0	21.3–25.8	36.3–40.3	56.7–60.7	50.4–54.3*
LIN	A	46.3–51.6	36.2–52.5	54.9–65.8	83.4–85.6	83.3–85.6
	B	47.9–52.2	47.8–55.5	54.6–60.6	81.9–84.8	81.9–84.8
ALH (μm)	A	3.8–4.4	4.3–5.3	5.9–7.3	4.8–5.1	4.8–5.1
	B	3.2–4.7	5.1–5.6	6.6–7.3	4.6–5.0	4.6–5.0

[a]Values of each parameter are mean % or ranges (mean − sem) − (mean + sem).
*Value different from that before preparation procedures at $p < 0.01$ (Student's t-test).

kinematics to those from the spermatocoeles and were the most feeble of sperm from all regions examined (Table 2, Fig. 2). The movement was mostly nonprogressive, and beating of the sperm tails was sometimes irregular. In some samples, spermatozoa swam forward slowly with low-amplitude and low-frequency beating; nevertheless, a few fast- and forward-swimming spermatozoa were observed. Whereas regular forward progression was more frequently seen in the proximal corpus epididymidis, spermatozoa from this region occasionally exhibited erratic large-amplitude flagellation resulting in tortuous and nonprogressive motion. Such erratic movement, which appeared like the "thrashing" or "star-spin" motion described for presumed hyperactivated ejaculated sperm [21–23], was also observed in spermatozoa from other regions, although more rarely.

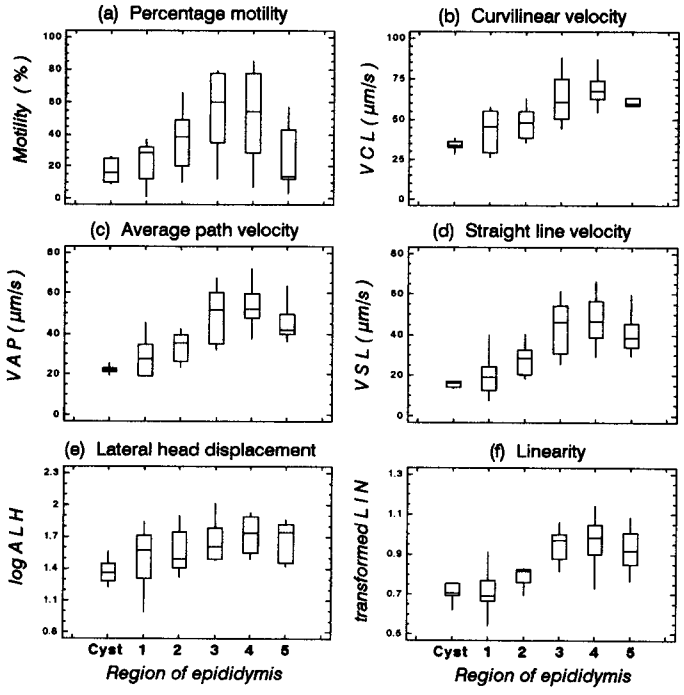

FIG. 2. Motility characteristics (ordinates) of spermatozoa from 5 regions of 8 human epididymides (region 1: efferent ducts; 2: proximal corpus; 3: mid-corpus; 4: distal corpus, 5: cauda epididymidis) and 8 spermatocoeles (cyst). The line within each box indicates the median; the box includes the upper and lower quartiles and the whiskers the ranges (see Table 2 for mean values of retransformed data and statistical differences).

The trend of increase in sperm velocities along the epididymis was obvious in all patients; this trend attained plateau levels in the mid-corpus (Fig. 2, b-d). Spermatozoa from the distal corpus epididymidis usually exhibited the most consistent flagellation in terms of symmetry, beat frequency, and amplitude, as reflected in regular and straightforward progression in addition to high velocities. In the cauda, both VCL and VSL were either maintained or decreased (by about 25 and 40% in two patients). The highest mean velocities reached in any one region from each epididymis were 71 ± 4 and 50 ± 8 μm/sec (mean ± SEM of all patients) for VCL and VSL, respectively.

There were gradual increases in the linearity of sperm paths from the efferent ducts toward the mid-corpus in all but one epididymis (Fig. 2f). However, no obvious changes could be found in the lateral head displacement of spermatozoa from various regions (Table 2; Fig. 2e).

Homogeneity of Sperm Kinematics in Various Regions of the Epididymis

When all the kinematic parameters, especially velocities, are considered, variations within samples were generally large in all regions; the largest was in sperm from the efferent ducts (Table 3). Such variations decreased significantly, although slightly, in the mid- and distal corpus epididymidis, reflecting an increase in the homogeneity of movement characteristics of the sperm population within the region. However there was a tendency toward increasing variability again in the cauda epididymidis.

DISCUSSION

In view of the high mean values of the maximal percentage motility and velocities achieved by the epididymal

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022

TABLE 2. Mean values of percentage motility (MOT, %) and kinematic parameters* of spermatozoa along the length of the human epididymis.

Region	n	MOT†	VCL	VAP	VSL	ALH	LIN	STR
Spermatocoele	8	17.6[a]	33.9[a]	22.1[a]	16.5[a]	3.8[a]	71.6[a]	87.6[a]
Efferent ducts	7	22.9[a]	41.6[ab]	28.2[ab]	20.3[a]	4.5[ab]	68.7[a]	83.8[ab]
Proximal corpus	8	36.5[ab]	47.6[b]	33.6[b]	27.7[a]	4.8[ab]	75.4[ab]	90.6[bc]
Mid-corpus	7	54.5[b]	63.1[c]	49.6[c]	44.0[b]	5.3[b]	83.6[c]	94.2[c]
Distal corpus	8	51.6[b]	68.7[c]	53.6[c]	47.4[b]	5.9[b]	75.3[c]	91.4[c]
Cauda	7	24.7[a]	61.1[c]	45.8[c]	41.0[b]	5.7[b]	71.1[bc]	90.7[c]

*Data are mean values of n patients, retransformed when appropriate. For each kinematic parameter, values with different superscripts are different from one another at the 95% significance level (Duncan's test). VCL, VAP, and VSL are in μm/s; ALH in μm.
†% Motility was measured manually whereas other parameters were obtained by CASA.

sperm samples, the epididymides studied were most probably normal healthy organs despite the age of the subjects. These values are similar to those obtained from ejaculated spermatozoa, treated under the same experimental conditions, from healthy donors. It has been shown by comparison between fertile young men and sexually active grandfathers that old age alone does not influence the quality of semen [24]. In the present study epididymal spermatozoa were not examined in their native fluid and it is possible that dilution in medium affects motility, by, for example, stimulation by bicarbonate and Ca^{2+} via raised intracellular cAMP [25, 26]. The current study did, however, reveal the change along the epididymis in the ability of spermatozoa to swim in a physiological solution.

Although the application of CASA in the measurement of sperm movement has increased tremendously in recent years, analysis by CASA has its limitations [27, 28]. Errors in the identification of both motile and immotile spermatozoa have been found in our previous studies [28–30]. In the present study where contamination of sperm samples by tissue debris was unavoidable, fully automated analysis would be even more error prone. To ensure quality of the present data, each CASA analysis was scrutinized to eliminate erroneous tracks, and percentage motility was measured manually. As long as motile spermatozoa are correctly identified, velocity measurements by CASA are accurate, as has been demonstrated by validation with a manually operated digitizer in both humans [31] and rats [8].

Motility of spermatozoa from the efferent ducts, which in the human comprise the caput epididymidis [20], are of particular interest in view of reports that spermatozoa recovered from the caput epididymidis of patients with blocked epididymal tubules or congenital absence of the vas deferens can fertilize ova in vitro [14–18]. Spermatozoa from such an origin have similar percentage motility but higher VCL [18, 32] than those obtained in the present study from the efferent ducts. One study [18] also showed that such cells swam better than the few nonprogressive motile caput spermatozoa obtained from brain-dead subjects (epididymides assumed to be non-occluded), suggesting that the better (or abnormal) quality of the former was a result of blockage. An increase in the percentage motility of caput sperm in blocked compared to nonblocked epididymides has been reported for men [9, 33] as well as non-human species (see Cooper [1]). These observations support the notion that in blocked epididymides there may be diffusion proximally into the patent caput region of more distally originating epididymal secretions that are necessary for sperm maturation [19].

The spermatocoeles from which spermatozoa were sampled in the current study were located adjacent to the head of the epididymis and the testis. All motility parameters measured from spermatozoa residing there were similar to those of spermatozoa from the efferent ducts. This suggests that these spermatocoeles were derived from efferent ducts, possibly from their blind-ending tubule branches [20], thus accounting for the fact that the maturational state was the same for both groups of spermatozoa. On the other hand, similar spermatocoeles have been shown to contain purely testicular secretions [34]. If the spermatozoa from these spermatocoeles were truly in rete testis fluid, the present findings would suggest that the luminal environment of the

TABLE 3. Mean variations[a] of kinematic parameters within each sperm population sampled from each of the different regions of 8 human epididymides.

Region	VCL	VAP	VSL	ALH	LIN	STR	Rank[b]
Spermatocoele	51.5	52.3	68.4	44.0	27.0	24.2	4.5
Efferent ducts	48.7	53.7	69.3	37.6	31.7	27.6	5.3
Proximal corpus	46.9	56.2	72.9	29.2	29.5	23.4	4.7
Mid-corpus	37.7	42.6	52.8	28.7	26.6	20.3	1.8*
Distal corpus	37.1	40.7	49.9	28.2	27.5	20.7	1.7**
Cauda	42.1	48.7	58.6	29.6	27.7	19.7	3.0

[a]The variation of each sperm sample is the standard deviation expressed as the percentage of the sample mean.
[b]Rank = average rank using Friedman's rank test on all kinematic parameters; significantly different from efferent duct spermatozoa at $*p < 0.05$ and $**p < 0.01$.

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022

efferent ducts, whose spermatozoa were as poorly motile as the spermatozoa from spermatocoeles, does not cause any maturational changes in sperm motility.

A sharp increase in the percentage of motile spermatozoa from the distal caput to the corpus epididymidis has also been reported in brain-dead subjects [11]. A similar increase in percentage progressive motility has been reported for men undergoing vasectomy or epididymovasostomy [12]. The present data document that concurrent with the increase in percentage motility, in all subjects—despite large variations between subjects in the absolute values—there were marked changes in the movement characteristics of the motile spermatozoa from the efferent ducts along the epididymis to the mid-corpus, more marked in straight line than in curvilinear velocities. There was a significant improvement in the straightness of swim paths although no changes were detected in the lateral displacement of the sperm head. These increases in the vigor of flagellation and efficiency in forward progression exhibited by spermatozoa from more distal sites in the human epididymis are similar to those reported for rats [8], although the rat did not show a significant increase in percentage motility from the mid-caput through to the distal cauda. Plateau values of kinematic parameters were reached in the mid-corpus epididymidis in men, but more distally (distal corpus) in the rat [8]. Whereas in rats the decline in percentage motility of spermatozoa from the proximal to the distal cauda epididymidis (a mean of 25 percentage points) was not statistically significant, in men the decline from the distal corpus to the cauda was (a mean of 27 percentage points). This decline may reflect long-term storage of matured spermatozoa in these aged patients. Indeed, long abstinence by older man has been suggested as a cause for the decreased motility of ejaculated sperm [24] and the change in the ability of these spermatozoa to fuse with zona-free hamster eggs [35]. An inferiority in the competitive fertilizing capacity of spermatozoa from the vas deferens compared to the proximal cauda in rabbits has been reported [36], although percentage motility of sperm from these two regions was not mentioned.

Variations in kinematic data of individual spermatozoa within each sample were generally large, indicating the heterogeneity of human spermatozoa. As these variations were largest in the efferent ducts and smallest in the mid- and distal corpus epididymidis, they probably reflect heterogeneity in the acquisition of motility maturation among individual sperm cells.

In conclusion, the present study has provided, both objectively and quantitatively, evidence for the changes of movement characteristics of human spermatozoa along the epididymis. These findings strengthen the evidence that in the human, as in other mammals, the epididymis plays a role in sperm maturation.

ACKNOWLEDGMENTS

The authors thank Eva Möllmann and Külli Nurmik for the transportation of tissues and Sonja Raczek for the isolation of the organs.

REFERENCES

1. Cooper TG. The epididymis, sperm maturation and fertilisation. Berlin: Springer-Verlag; 1986.
2. Amann RP. Function of the epididymis in bulls and rams. J Reprod Fertil Suppl 1987; 34:115–131.
3. Amann RP, Hay SR, Hammerstedt RH. Yield, characteristics, motility and cAMP content of sperm isolated from seven regions of the ram epididymis. Biol Reprod 1982; 27:723–733.
4. Pariset CC, Feinberg JMF, Dacheux JL, Weinman SJ. Changes in calmodulin level and cAMP-dependent protein kinase activity during epididymal maturation of ram spermatozoa. J Reprod Fertil 1985; 74:105–112.
5. Acott TS, Katz DF, Hoskins D. Movement characteristics of bovine epididymal spermatozoa: effects of forward motility protein and epididymal maturation. Biol Reprod 1983; 29:389–399.
6. Bork K, Chevrier C, Paquignon M, Jouannet P, Dacheux JL. Analys de la motilité et du mouvement flagellaire des spermatozoides de verrat au cours du transit épididymaire. Reprod Nutr Dev 1988; 28:1307–1315.
7. Gould KG, Young LA, Hinton BT. Alterations in primate sperm motility with maturation and during exposure to theophylline. Am J Primatol 1988; 15:325–336.
8. Yeung CH, Oberländer G, Cooper TG. Characterization of the motility of rat spermatozoa by computer-aided objective measurement. J Reprod Fertil 1992: 96:427–441.
9. Mooney JK, Horan AH, Lattimer JK. Motility of spermatozoa in the human epididymis. J Urol 1972; 108:443–445.
10. Bedford JM, Calvin H, Cooper GW. The maturation of spermatozoa in the human epididymis. J Reprod Fertil Suppl 1973; 18:199–213.
11. Dacheux JL, Chevrier C, Lanson Y. Motility and surface transformations of human spermatozoa during epididymal transit. Proc Natl Acad Sci USA 1987; 513:560–563.
12. Sutherland PD, Matson PL, Moore HDM, Goswamy R, Parsons JH, Vaid P, Pryor JP. Clinical evaluation of the heterologous oocyte penetration (HOP) test. Br J Urol 1985; 57:233–236.
13. Silber SJ. Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. Hum Reprod 1989; 4:298–303.
14. Silber SJ, Ord T, Balmaceda J, Asch R, Borrero C. Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. Fertil Steril 1988; 50:525–528.
15. Silber SJ, Ord T, Balmaceda J, Patrizio P, Asch RH. Congenital absence of the vas deferens. The fertilizing capacity of human epididymal sperm. New Engl J Med 1990; 323:1788–1792.
16. Jequier AM, Cummins JM, Gearon C, Apted SL, Yovich LM, Yovich JL. A pregnancy achieved using sperm from the epididymal caput in idiopathic obstructive azoospermia. Fertil Steril 1990; 53:1104–1105.
17. Bladou F, Grillo JM, Rossi D, Noizet A, Gamerre M, Erny R, Luciani JM, Serment G. Epididymal sperm aspiration in conjunction with in-vitro fertilization and embryo transfer in cases of obstructive azoospermia. Hum Reprod 1991; 6:1284–1287.
18. Mathieu C, Guérin J-F, Cognat M, Lejeune H, Pinatel M-C, Lornage J. Motility and fertilizing capacity of epididymal human spermatozoa in normal and pathological cases. Fertil Steril 1992; 57:871–876.
19. Cooper TG. In defense of a function for the human epididymis. Fertil Steril 1990; 90:965–975.
20. Yeung CH, Cooper TG, Bergmann M, Schulze H. Organization of tubules in the human caput epididymidis and ultrastructure of their epithelia. Am J Anat 1991; 191:261–279.
21. Burkman LJ. Characterization of hyperactivated motility by human spermatozoa during capacitation: comparison of fertile and oligospermic sperm populations. Arch Androl 1984; 13:153–165.
22. Burkman LJ. Discrimination between nonhyperactivated and classical hyperactivated motility patterns in human spermatozoa using computerized analysis. Fertil Steril 1991; 55:363–371.
23. Grunert JH, DeGeyter C, Nieschlag E. Objective identification of hyperactivated human spermatozoa by computerized sperm motion analysis with the Hamilton-Thorn sperm motility analyser. Hum Reprod 1990; 5:593–599.
24. Nieschlag E, Lammers U, Freischem CW, Langer K, Wickings EJ. Reproductive functions in young fathers and grandfathers. J Clin Endocrinol & Metab 1982; 55:676–681.

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022

25. Garbers DL, Tubb DJ, Hyne RV. A requirement of bicarbonate for Ca^{2+}-induced elevations of cyclic AMP in guinea pig spermatozoa. J Biol Chem 1982; 257:8980–8984.
26. Okamura N, Tajima Y, Soejima A, Masuda H, Sugita Y. Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. J Biol Chem 1985; 260:9699–9705.
27. Boyers SP, Davis RO, Katz DF. Automated semen analysis. Curr Probl Obstet Gynecol Fertil 1989; 12:168–200.
28. Knuth UA, Neuwinger J, Nieschlag E. Computer based techniques for semen analysis and their application in andrology. In: Guardabasso V, Rodbard D, Fort G (eds.), Computers in Endocrinology: Recent Review. New York: Raven Press; 1990: 149–158.
29. Knuth UA, Nieschlag E. Comparison of computerized semen analysis and sperm motion characteristics with the conventional procedure in 322 patients. Fertil Steril 1988; 49:881–885.
30. Yeung CH, Weinbauer GF, Nieschlag E. Movement characteristics of ejaculated sperm from cynomolgus monkeys (*Macaca fascicularis*) analyzed by manual and automated computerized image analysis. J Med Primatol 1989; 18:55–68.
31. Olds-Clarke P, Baer HM, Gerber WL. Human sperm motion analysis by automatic (Hamilton-Thorn motility analyzer) and manual (Image 80) digitization systems. J Androl 1990; 11:52–58.
32. 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; 56:1128–1135.
33. Lindholmer C. The importance of seminal plasma for human sperm motility. Biol Reprod 1974; 10:533–542.
34. Cooper TG, Raczek S, Yeung CH, Schwab E, Schulze H, Hertle L. Composition of fluids obtained from human epididymal spermatocoeles. Urol Res 1992; 20:275–280.
35. Zenzes MT, Reed TE, Nieschlag E. Non-Poisson distribution of sperm from grandfathers in zona-free hamster ova. J Androl 1991; 12:71–75.
36. Bedford JM. Sperm dynamics in the epididymis. In: Asch RA, Balmaceda JP, Johnson I (eds.), Gamete Physiology. Norwell: Serono Symposia; 1990: 53–67.

Downloaded from https://academic.oup.com/biolreprod/article/49/2/274/2762347 by U.S. Department of Justice user on 16 August 2022