Nagoya J. Med. Sci. **55.** 71 ~ 75, 1993

MICROASSAY OF SPERM CONCENTRATION IN THE RAT EPIDIDYMIS BY MICROPUNCTURE TECHNIQUE

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ABSTRACT

Micropuncture samples taken from the rete testis, caput, corpus and cauda epididymidis of adult rats were assayed for intraluminal sperm concentrations. The amount of fluid resorbed from the efferent duct and epididymal lumen was calculated based on the sperm concentration. Epididymal sperm concentrations increased from the rete fluid to the cauda fluid through the caput fluid. Eighty-nine percent of the fluid leaving the rete testis is resorbed by the efferent ducts and proximal epididymal tubule, and 96% of the fluid leaving the rete testis is resorbed in the distal cauda epididymidis. Resorption is important for the control of intraluminal fluid by the epididymis.

Key Words: Sperm, Concentration, Microassay, Epididymis

INTRODUCTION

It has been known for many years that spermatozoa transported to the ductuli efferentes from the rete testis can neither swim nor fertilize an ovum. Hammar[1] and Walker[2] noted that spermatozoa from the distal epididymis have greater potential of movement and fertility than those from proximal segments of the duct. Young[3–5] showed that mammalian epididymis played an important role in sperm maturation.

However, the mechanisms of sperm maturation by the epididymis are not fully understood. This process is generally thought to depend on the fluid milieu within the epididymal lumen and on the epithelial cells that produce fluid. The epithelial cells of the epididymis play a role in the electrolyte transport[6,7] and osmolarity of the epididymal tract.[7,8] Epithelial cells also have a role in the reabsorption as well as the secretion of carnitine,[8] inositol[9] and a variety of glycoproteins.[10–12] Thus, epithelial cells produce an everchanging epididymal microenvironment. Unfortunately, characterization of this microenvironment has not yet been completed because many studies must be performed directly by in vivo or in vitro micropuncture techniques available in only a few laboratories. Using these techniques, the intraluminal content of the epididymal duct can be collected, making it possible to study the intraluminal fluid and spermatozoa. In the present study we investigate the removal of intraluminal contents from the lumen of each zone of the rat epididymis by estimation of net resorption of fluid based on sperm concentrations.

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Accepted for Publication in August 13, 1992

MATERIALS AND METHODS

Thirty-nine adult, male, Sprague-Dawley rats (350—540 g) were obtained from a breeder and reared in laboratory at 25°C with a 12 hr: 12 hr light-dark cycle. They were anesthetized with intraperitoneal injections of Inactin (sodium 5-ethyl-5-(1-methylpropyl)-2-thiobarbiturate, Byk Guilden Konstanz, Hamburg, Germany; 100 mg/kg body weight) and subjected to in vivo micropuncture. The micropuncture pipettes were attached to a micromanipulator (Narishige, Tokyo, Japan) for well-controlled movement and connected to a 10-ml syringe with PE-50 tubing to provide positive and negative pressure in the pipette. Sudan-black stained mineral oil was drawn into the micropipette before micropuncture. The micropipette was guided into a selected tubule and the intraluminal position of the pipette tip was confirmed by ejecting a small drop of stained mineral oil. Microsamples were obtained by applying negative pressure the micropipette. The sample with stained mineral oil was always sealed at both ends to prevent exposure to air. The micropipette containing epididymal lumen content (sperm and fluid) was attached to a vertical transfer apparatus (Bunton Instruments, Rockville, MD) and the lumen content was mixed in water-equilibrated mineral oil in a glass mini-beaker (Fig.1). A precalibrated 100-nl microvolumetric pipette (constriction pipette) was used to aspirate duplicate 100-nl aliquots of each micropuncture sample collected (Fig.1). The 100-nl aliquot was transferred to an adjacent minibeaker and inserted into a mineral oil-covered, 25-μl droplet of 1% hyaluronidase in saline. The diluted hyaluronidase solution prevented sperm aggregation and facilitated evenly mixed spermatozoa in the small diluent drops. Twenty-five μl of diluted epididymal fluid was then aspirated into a precalibrated 300-μl pipette. The remainder of the 300-μl pipette was filled with 1% hyaluronidase solution again. Each pipette was vigorously vibrated for 90 sec on an automatic

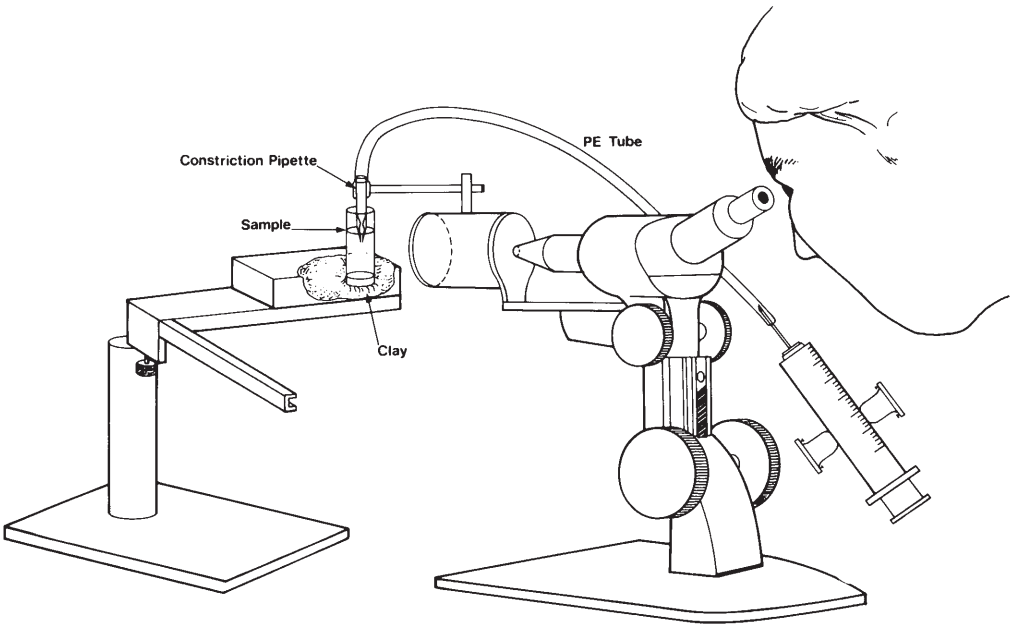

Fig. 1. Schema of vertical transfer apparatus. The sample in the original collection micropipette is placed into this apparatus. This apparatus is designed for transferring known volumes of samples through a volumetric constriction micropipette.

pipette shaker (Clay-Adams, NY) to completely mix the sample. Drops of the pipette contents were applied to a hemocytometer to determine the sperm density of the fluid in the 300-μl pipette. Each counting was performed in quadruplicate, and sperm density in the original micropuncture samples was calculated by the appropriate dilution figures.

The formulae used for the several calculations involved are as follows:

$$\frac{SC_D - SC_P}{SC_P} \times 100 = \%\Delta_{sc}$$

where SC_D=mean sperm concentration in any distal zone; SC_P=mean sperm concentration in any proximal zone, and $\%\Delta_{sc}$=percentage of the change of intraluminal sperm concentrations.

$$\frac{SC_D - SC_P}{SC_D} \times 100 = FR$$

where % FR=percentage of fluid resorption between the proximal and distal zone.

$$Conc_p \times \frac{SC_D}{SC_P} \times Conc_D$$

where $Conc_p$=mean concentration of any substance in a proximal zone and $Conc_D$=predicted mean concentration of a substance in a distal zone which is calculated from a known concentration in a proximal zone.

RESULTS

The rat sperm concentrations in the fluid ($\times 10^9$/ml) of rete testis, caput, corpus and cauda epididymidis were 0.06 ± 0.01, 0.72 ± 0.07, 1.56 ± 0.11, and 1.85 ± 0.045, respectively (Table 1) showing sequentially increased concentrations from rete to cauda fluid. This demonstrates that 92% of the fluid leaving the rete testis is resorbed by the efferent ducts and proximal epididymal tubule. Totally, 97% of the fluid leaving the rete testis is resorbed by the time the end of the distal cauda epididymidis is reached (Table 1). This fluid resorption resutls in a 12-fold increase in sperm cell concentrations between the rete testis and the caput epididymidis and a 31-fold increase between the rete testis and the distal cauda epididymidis. The percentage of intraluminal

Table 1. Sperm Concentrations (mean ± s.e.) and Fluid Resorption from the Rat Epididymal Duct.

Fluid from:		Sperm conc ($\times 10^9$/ml)	%FR		FI		$\%\Delta_{sc}$	
Rete testis	(12)	0.06 ± 0.01	92		12		1100	
Caput	(7)	0.72 ± 0.07	54	97	2.2	30.8	117	2983
Corpus	(9)	1.56 ± 0.11	16		1.2		18.6	
Cauda	(11)	1.85 ± 0.05						

FR : Fluid resorption from zone to zone.

FI : Fold-increase in intraluminal sperm concentrations from zone to zone.

$\%\Delta_{sc}$: Increase in sperm concentrations from zone to zone.

Numbers in parentheses indicate the number of animals.

sperm concentration from the rete testis to the caput epididymidis and to the cauda epididymidis were also increased at 1100 and 2983, respectively (Table 1).

DISCUSSION

Transport of spermatozoa through the lumen of the epididymis is due in large part to spontaneous, peristaltic contractions of the smooth muscles surrounding the epididymal duct, but is assisted by the hydrostatic pressure since the mean pressure in the caput is significantly greater than the mean pressure in the proximal cauda epididymidis.[13] Sperm concentrations in the rete testis are low and increase dramatically along the ductuli efferentes and epididymis. This occurs because of the absorption of fluid from the ductal lumen, particularly in the ductuli efferentes and caput epididymidis. Spermatozoa in the lumen of the epididymal duct system are not appreciably metabolized in situ, nor diffused from the lumen, nor taken up by the epithelial cells. Intraluminal sperm concentrations can therefore be an indicator of fluid resorption or secretion. If sperm concentrations in the epididymis increased or decreased from one zone to another, we would expect the concentrations of any other intraluminal substances not diffused, metabolized, or taken up by cells to increase or decrease by the same amount. Using the values of sperm concentration and the concentrations of the other intraluminal substances, we can estimate the net secretion or absorption of substances throughout the excurrent duct system.[14] Net loss or gain of a substance from the efferent ducts or epididymal lumen has received little or no attention in the previous literature.[9,10] The present calculations provide a new emphasis on resorption and secretion in the epididymal physiology. The key of this study was the use of intraluminal sperm concentration as a direct marker for intraluminal fluid.

The fact that 97% of the fluid leaving the rete testis is resorbed by the time co-migrating spermatozoa reach the cauda epididymidis indicates the possibility of a 3000% increase in any other intraluminal substances not diffused, metabolized or taken up by cells. Total protein concentrations in the rete testis and caput epididymidis are approximately 1.3 mg/ml and 25 mg/ml, respectively.[13] Predicted protein concentration in the caput fluid based on sperm concentration is 15.6 mg/ml, calculated as follows: $1.3\times(0.72\div0.06)=15.6$ mg/ml. Therefore, a net secretion of protein can occur between the rete testis and the caput epididymidis. Protein concentration in the cauda fluid is 29 mg/ml.[13] Predicted protein concentration in the cauda fluid is 64.2 mg/ml, calculated as follows: $25\times(1.85\div0.72)=64.2$ mg/ml. A net resorption of intraluminal proteins can occur between the caput and the cauda epididymidis. Potassium concentrations increase throughout the male excurrent duct system.[6] Potassium concentrations in the rete testis, caput epididymidis and cauda epididymidis are approximately 16.1, 27.6 and 52.4 mEq/l, respectively.[6] Predicted potassium concentrations in the caput and cauda epididymidis based on sperm concentration are 193.2 and 70.9 mEq/l, respectively. These data demonstrate that there is actually a net resorption, not secretion, of potassium between the rete testis and the caput epididymidis, and also between the caput and the cauda epididymidis. Thus, there is a resorption of proteins and potassium ions from the epididymal lumen despite increases in intraluminal concentrations of these substances.

In conclusion, the present results demonstrate that the epididymal tubule has active resorptive function, which corroborates other results that some organic compounds are removed from the lumen fluid in large amounts. Knowledge of the absorptive and secretory functions of the epididymis is important in understanding the epididymal physiology more completely.

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