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# Micro-Spoon Technique in Estimation of Sperm Concentration in Rat Epididymis

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Odabaş, Ö., Timurkan, H., Atilla, M.K., Yilmaz, Y., Şengül, E. and Aydin, S. *Micro-Spoon Technique in Estimation of Sperm Concentration in Rat Epididymis*. Tohoku J. Exp. Med., 2000, **191** (1), 1-5 —— Several technical procedures were described for estimation of epididymal sperm concentration until today. In all of the described techniques, epididymal material was drawn into a micro-pipette before counting procedure. We aimed to proceed this stage without using any pipette because of the difficulty in aspirating very viscous epididymal material into a micro-pipette. For measuring epididymal material we used a 2 $\mu$l reservoir which we named as a micro-spoon. It was filled with the epididymal material and layered in a tube with Ringer's solution of 2 ml containing sodium hyaluronate. Then, it was mixed in order to obtain 1/1000 diluted suspension. Sperm cells in this suspension were counted on a Makler chamber under a light microscope. The procedure was easy and time saving, compared to the other techniques. ———— micro-spoon; cauda epididymis; sperm concentration
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Estimation of epididymal sperm concentration is necessary for experimental studies investigating the effects of some surgical techniques and some medications on epididymal sperm concentrations. The majority of these studies were performed on mice and rats, and variable sperm concentrations were reported. In the published articles, sperm concentrations ranged from $440 \times 10^6$ to $2 \times 10^9$ (Robb et al. 1978; Hibi et al. 1995). Technical variations may contribute these inconsistent results.

A few techniques were described for obtaining epididymal material from the cauda. One of them, described by Turner et al. (1977), the epididymis was externalized though a scrotal incision and placed in a warmer. It was micropunctured, using some sophisticated equipments such as micro-pipettes, a fibre optic light system, a stereo zoom microscope and a micro-manipulator.

In another technique, presented as simple and unsophisticated (Kenipinas

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and Lamano-Carvalho 1988), the sperm was released by cutting the cauda epididymis longitudinally with a pair of fine-pointed scissors and compressing with forceps. The sperm suspension was drawn into a white blood cell pipette and diluted to 1/100.

Material was drawn into a micro-pipette in both of these techniques. Herein we discuss a new technique without using any pipette but a micro-reservoir which we named a "micro-spoon."

## Materials and Methods

Initially, we had some micro-reservoirs made of stainless steel and made in various volumes, and checked them by measuring the filled liquid by micro-pipette. Then we used 2 $\mu$l reservoir to measure the epididymal material (Fig. 1). Before hand, we tested this instrument for determining sperm count of the men who had sperm concentration higher than $100 \times 10^6$ to investigate its reliability. Concentrated sperm samples were selected to obtain reliable result when diluted. Two $\mu$l micro-spoon of ejeculate was diluted with 0.2 ml (1/100) formole saline fixative. The counting procedure was carried on a Makler chamber. The results of the standard technique of Makler chamber and the micro-spoon technique were similar. Afterwards we used this technique in measuring epididymal material which was too viscous to draw into a micro-pipette.

Five male, sexually mature Spraque Dawley albino rats were used for this study. The animals were maintained under a standard light: dark schedule of conditions 14 : 10 hours and were allowed to acclimate for at least one week before experimental use.

Each rat was sacrificed after ether anesthesia and bilateral orchiepididymectomy was performed. Totally ten epididymotesticular units were evaluated with regard to epididymal sperm concentrations. A transection was done at vaso-epididymal junction of the orchiepididymectomy specimen after removing deferential vessels from this area (Fig. 2). The proximal end of the

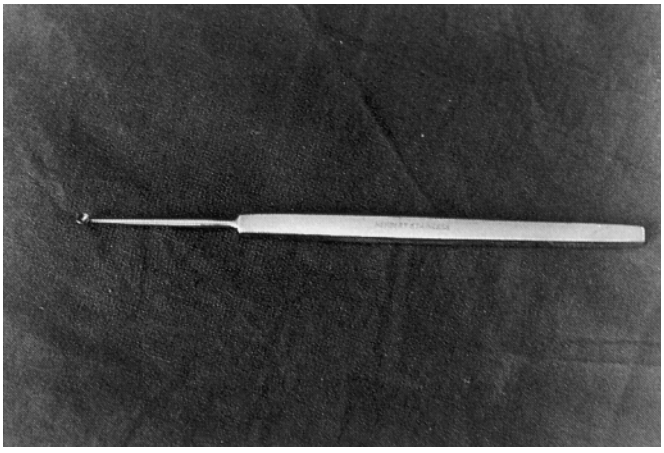


Fig. 1. The micro-spoon made of stainless steel.

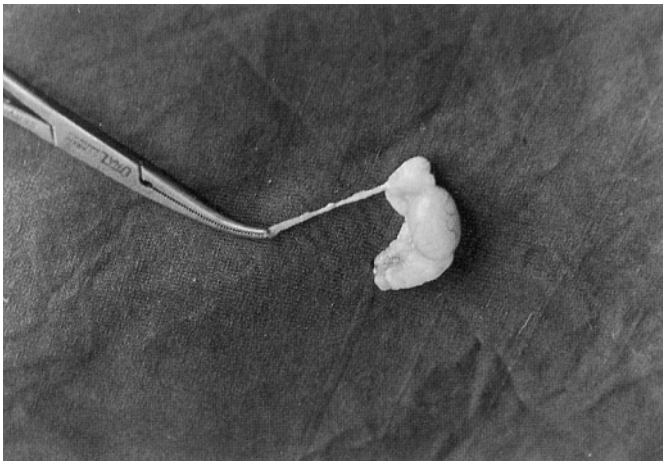


Fig. 2. Orchiepididymectomy specimen shows vaso-epididymal junction and vas deference with serosal layer and vessels.

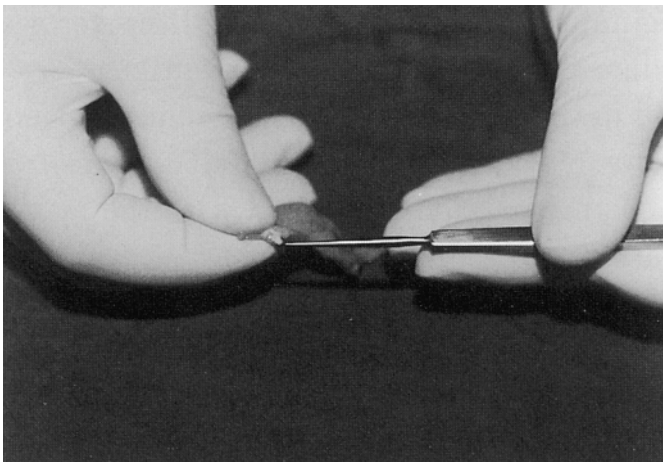


Fig. 3. Epididymal material is discharged in micro-spoon by pressing cauda epididymis between thumb and forefinger.

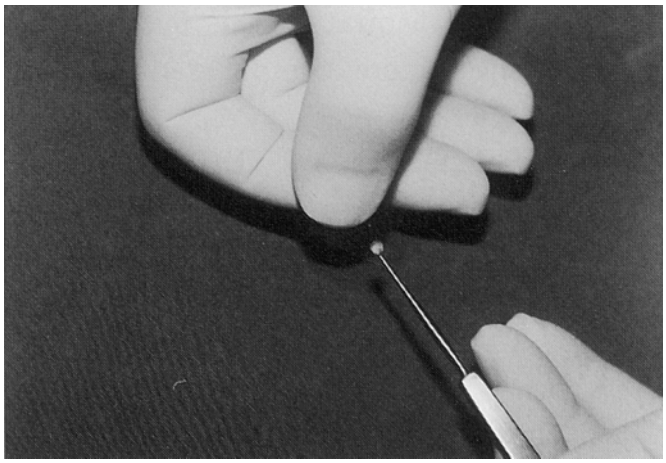


Fig. 4. A thin slide is put on micro-spoon for removing excess material.

cutting was laid on the micro-spoon and epididymal content was discharged in it by pressing cauda epididymis between thumb and forefinger (Fig. 3). After filling the micro-spoon, a thin slide was put on it for removing excess material (Fig. 4). The material in the micro-spoon was, this adjusted exactly to 2 $\mu$l. Thereafter, the micro-spoon was layered in a tube with Ringer's solution of 2 ml containing sodium hyaluronate in order to obtain 1/1000 diluted suspension. The suspension was placed to an incubator in 37°C for 15 minutes after mixing properly. Sperm cells in this suspension were counted on a Makler chamber under light microscope.

## Results and Discussion

The procedure to prepare sperm suspension was easy and not time consuming in this technique. The time for preparing cauda epididymis, obtaining epididymal material and preparing sperm suspension (in 1/1000 dilution) took 5 minutes at the beginning of the experiment. However we could manage to complete the procedure in only 3 minutes afterwards.

Instrument used in this technique was only a micro-spoon excepted mosquito clamp and penscet for removing vessels and fatty tissue from vaso-epididymal junction. Viscous sperm was easily collected with the lack of disadvantages of micro-pipette aspiration.

The mean number of spermatozoa in cauda epididymis was $4.16 \times 10^9 \pm 7.38 \times 10^8$/ml.

Measuring the epididymal material is necessary not only for the estimation of sperm concentration, but also for determining some substances (such as proteins and ions) in it (Robaire and Henno 1988; Turner 1991). The techniques described earlier required a white blood cell pipette or some other micro-pipettes for this purpose. Not many investigators reported difficulty in aspirating very viscous (sometimes almost solid) epididymal material by a micro-pipette, but our earlier experiences indicated that aspirating this material for experimental purpose was difficult and required more complicated instruments, especially in micropuncture technique.

The technique, described by Turner et al. (1977), the epididymis was externalized through a scrotal incision and placed in a warmer specially prepared for this purpose. Then the epididymis was moistened with water equlibrated mineral oil. Afterwards, it was micropunctured, using some sophisticated equipments such as micro-pipettes, a fibre optic light system, a stereo zoom microscope and a micro-manipulator.

Another technique, presented as simple and unsophisticated (Kenipinas and Lamano-Carvalho 1988) was as follow: After fat, blood vessels and connective tissue were cut away, cauda epididymis was placed in a hollow plate. Than the sperm was released by cutting the cauda epididymis longitudinally with a pair of fine-pointed scissors and compressing with forceps, and were therefore deposited

free of epididymal tissue into the cavity. The sperm suspension was drawn into a white blood cell pipette and diluted to 1/100 with proteolytic enzyme solution. This technique required a meticulous dissection of cauda epididymis for the removal of other tissues. Otherwise the result might be affected from any little fluid except from epididymal ducts.

We used only a micro-spoon for obtaining and measuring the epididymal material. The only work was to remove the vessels on the vas deferens in order not to mingle any blood with epididymal material. The procedure including the discharging epididymal material into a micro-spoon and diluting it with Ringer's solution took only three minutes for the last specimen.

Kempinas (Kempinas and Lamano-Carvalho 1988) suggested collagenase and trypsin for obtaining homogeneous sperm suspension. We prefered dilution solution with sodium hyaluronate for this purpose. Our results were satisfactory, too.

The spermatozoa count ($4.16\times10^9\pm7.38\times10^8$) in our study was higher than those in the recent literature. This might be the result of techniqual differences. Additionally, it is known that sperm concentration in cauda epididymis increases from proximal to distal direction. In our method we obtained the epididymal material directly from the epididymal channell at vaso-epididymal junction. Therefore, it was not surprising to find out the material obtained from the most distal part of cauda epididymis was more concentrated than that obtained from whole caudal tissue. Kempinas (Kempinas and Lamano-Carvalho 1988), who used whole cauda epididymal tissue for obtaining sperm, reported sperm cocentrations from $1.52\times10^9$ to $2.3\times10^9$.

We concluded that the micro-spoon technique is more easy, reliable and time saving procedure than the others.

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