65. Separation of Human X- and Y-Bearing Sperm using Free-Flow Electrophoresis^{†)}

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Various techniques¹⁾⁻³⁾ including electrophoresis⁴⁾⁻⁶⁾ have been applied for separating X- and Y-bearing sperm of mammals. The observation by Schroeder⁴⁾ that Y-bearing sperm moved towards the cathode and X-bearing sperm to the anode suggested the possibility of separating the two types of cells by galvanization. Bhattacharya *et al.*⁵⁾ and Daniell *et al.*⁶⁾ also reported similar findings with the convection counter streaming galvanization.

We have attempted to observe the electropotential differences between human X- and Y-bearing sperm using free-flow electrophoresis, and succeeded in the separation of the two types of cells.

The ejaculates of various semen qualities were obtained after 4 days sexual abstinence. Sperm density was determined with a hemocytometer and the percentage of motile sperm was estimated. Y-bearing sperm were determined by the fluorescence staining with quinacrine mustard (Sigma, St. Louis, Mo., USA) according to Barlow and Vosa.⁷⁾ Sperm carrying a fluorescent body in the center of head was identified as Y-bearing, and those without the body as X-bearing.

The combined semen from 11 donors (sperm density, 1 to 6×10^7 /ml; motility, 20 to 70%) was diluted 5-fold with a sperm culture medium (20 mM HEPES, 138 mM NaCl, 2 mM Na_2HPO_4 , 2 mM CaCl_2 , 2 mM MgCl_2 , 10 mM Glucose, N-NaOH 7.5 ml, N-KOH 2.5 ml, pH 7.4, 290–300 m osmole).⁸⁾ After filtration through a nylon mesh (40 μm pore size), sperm were washed by centrifugation at $80 \times g$ for 20 min at room temperature, and the resultant loose pellet of sperm was resuspended in the sperm culture medium to make up the final concentration of 3×10^7 sperm/ml. The percentage of Y-bearing

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sperm in the combined semen was $47 \pm 2.6\%$, whereas that of washed sperm was slightly reduced, $42 \pm 3.5\%$, as judged from the quinacrine mustard staining, probably due to a lower recovery of Y-bearing sperm in the sediment.

The separation of X- and Y-bearing sperm was performed in a free-flow electrophoresis apparatus (Elphor VaP-5, Bender and Hobein, Munich, F.R.G.). Immediately before placing the washed sperm to the electrophoretic chamber, they were diluted 2-fold with the chamber buffer (5 mM triethanolamine acetate, 1 mM N,N'-ethylenediamine tetraacetic acid (EDTA), 0.25 M sucrose, pH 7.4).⁹⁾ The electrophoresis was carried out at 20 to 23°C and 115 V/cm electric field. A 6.0 ml sample was injected continuously into the chamber, and the effluents from the bottom of the chamber were collected into 90 fractions for 30 min.

Fig. 1. Free-flow electrophoresis of washed human sperm.

As shown in Fig. 1, the electrophoretic profile of sperm gave two separated main peaks. Both X- and Y-bearing sperm moved towards the anode in contrast to the results reported by the previous authors.⁴⁾⁻⁶⁾ The first peak nearer the anode (fraction nos. 2-11) contained only X-bearing sperm, whereas the second peak was mainly

composed of Y-bearing sperm. The tailing of X-bearing sperm reduced the purity of the second main peak and the highest percentage of Y-bearing sperm was found to be 83% in the fraction no. 22. The fractions behind the second main peak contained immature sperm with cytoplasmic droplets, resulting in a drop in the percentage of Y-bearing sperm. The results were reproducible and similar distribution profiles of X- and Y-bearing sperm were obtained with 5 other sperm samples. In some other cases, agglutinated sperm were found after resuspension and disturbed the separation to some extent, the agglutinates being recovered behind the second main peak (fraction nos. 30–50). Both careful preparation of washed sperm and carrying out of electrophoresis as soon as possible were thus essential for successful separation.

The recovered sperm were immobilized probably due to the presence of EDTA in the chamber buffer. To avoid the loss of motility during electrophoresis, another buffer without EDTA (2 mM HEPES-NaOH, 3 mM NaCl, 0.25 M sucrose, pH 7.4) was examined under the same conditions. Similar electrophoretic profiles of sperm were obtained in several runs regardless of the modification of the chamber buffer. Motility, however, was still reduced, although a few sperm moving forward were observed after electrophoresis. Further improvement of the buffer composition will enable us to separate X- and Y-bearing sperm without loss of motility.

In conclusion, the electrophoretic mobility of X-bearing sperm was faster than that of Y-bearing ones, showing that the zeta potential on the cell surface of X-bearing sperm was higher than that of Y-bearing ones. The present study achieved the isolation of X-bearing sperm. Re-electrophoresis of Y-bearing sperm rich fractions will provide the better isolation of Y-bearing sperm. Physical, chemical and morphological differences between X- and Y-bearing sperm will further be examined.

Summary. The separation of human X- and Y-bearing sperm was achieved using free-flow electrophoresis. The mobility of X-bearing sperm was faster than that of Y-bearing sperm and the electrophoretic pattern showed the separated two main peaks. One nearer the anode contained only X-bearing sperm, whereas the other was mainly composed of Y-bearing sperm. This result indicates that the zeta potential on the cell surface of X-bearing sperm was higher than that of Y-bearing sperm.

References

- 1) A. M. Roberts: *Nature*, **238**, 223 (1972).
- 2) R. J. Ericsson, C. N. Langevin, and M. Nishino: *ibid.*, **246**, 421 (1973).
- 3) W. Rhode, T. Porstmann, S. Prehn, and G. Dörner: *J. Reprod. Fert.*, **42**, 587 (1975).
- 4) V. Schroeder: *Biol. Zhurnal*, **3**, 465 (1934).
- 5) B. C. Bhattacharya, P. Shome, and A. H. Gunther: *Int. J. Fert.*, **22**, 30 (1975).
- 6) J. F. Daniell, C. M. Hervert, J. Repp, C. A. Torbit, and A. C. Wentz: *Fertil. Steril.*, **38**, 233 (1982).
- 7) P. Barlow and C. G. Vosa: *Nature*, **226**, 961 (1970).
- 8) S. Kaneko and C. Moriwaki: *J. Pharm. Dyn.*, **4**, 443 (1981).
- 9) K. Hannig and H. G. Heidrich: *Methods in Enzymology*. vol. 31, Academic Press, New York, pp. 746-761 (1974).