A SIMPLE METHOD FOR AUTOMATIC COUNTING OF RABBIT SPERMATOZOA

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Automatic counting of spermatozoa in ejaculates from rabbits by means of electronic methods is complicated by the occurrence of numerous small particles in the seminal fluid, many of them being of the same size as the spermatozoa. A synthetic washing material has been used to eliminate these particles (Kihllström & Fjellström, 1967). Recently, however, this washing material was observed partly to destroy the cell structures, especially after treatment for some hours, thereby considerably disturbing the counts recorded. An alternative method of eliminating these particles with a minimum of damage to the spermatozoa is reported below.

Semen was collected from eighteen sexually mature rabbits, most of them of the Bourgoigne Breed, by means of an artificial vagina. Semen (0.1 ml) was thoroughly mixed with 4.9 ml physiological sodium chloride solution containing 2% chloramine T. Of the suspension thus obtained, 0.2 ml was diluted to 100 ml with physiological saline. Electronic counting was then performed by means of Celloscope 202 (Lars Ljungberg & Co., Stockholm, Sweden), retaining the homogeneity of the suspension by magnetic stirring. The sample tube of the counter had a capillary aperture of 75 μm , and each reading lasted about 55 sec. During this time, 1 ml of the suspension passed through the aperture and a maximum of 30,000 particles was counted. Measurements were performed with the attenuator set at 1/1, the current intensity control at 7.50, and the two discriminators at 18 and 80, respectively. With this adjustment, the two recorders registered all particles with a volume corresponding to that of spherical non-conductive particles having diameters between 1.9 and 3.1 μm . Precautions were taken to keep the solutions dust-free and they were filtered when necessary. Before use, all glassware was washed in acid and rinsed with physiological sodium chloride solution. After use, washing with distilled water was sufficient to remove the spermatozoa completely, provided that the glassware was prevented from drying before washing. Before using it again, the glassware was rinsed with dust-free physiological saline.

Manual microscopic duplicate counts were performed using a Buerker haemocytometer. In all ejaculates tested, the total number of particles automatically counted within the size range given above coincided closely with the number of sperm cells counted in the haemocytometer. In Text-fig. 1, the Celloscope counts (y) were plotted against the corresponding haemocytometer counts (x). The equation of the regression line was $y = 0.982x + 0.150$ and the

TEXT-FIG. 1. Celloscope counts obtained from twenty-four rabbit ejaculates plotted against the corresponding haemocytometer counts.

correlation coefficient was 0.948. The mean value of the Celloscope counts was 4.116×10^8 , and that of the haemocytometer counts was 4.038×10^8 .

The results show that electronic methods are suitable for rapid counting of rabbit spermatozoa. The lack of discrimination between spermatozoa and irrelevant particulate matter is unimportant after treatment with chloramine T, as shown by the minute difference between the haemocytometer and Celloscope counts.

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REFERENCE

- KIHLSTRÖM, J. E. & FJELLSTRÖM, D. (1967) Automatic counting of spermatozoa in rabbit semen. *J. Reprod. Fert.* **14**, 155–157.