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COMMENTS ON THE COUNTING AND SIZING OF BULL SPERMATOZOA WITH AN ELECTRONIC PARTICLE COUNTER

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SOMMAIRE

1. L'aptitude du compteur électronique « Coulter », à compter des spermatozoïdes de Taureau, est confirmée. Au moyen de cet appareil on atteint la même précision qu'avec 4 numérations à l'hématimètre.
2. L'aptitude de l'appareil à déterminer le volume des spermatozoïdes du Taureau est discutable, parce que les résultats sont inférieurs au volume calculé à partir des dimensions linéaires trouvées dans la littérature. La distribution des volumes donnée par l'instrument est apparemment trop large.
3. Ce n'est pas la charge négative à la surface des spermatozoïdes qui diminue leur volume apparent mesuré par l'appareil.
4. Toute indication de la valeur de volume absolu doit être accompagnée de la méthode de standardisation utilisée.

INTRODUCTION

Using an electronic particle counter (1) on ejaculates and washed suspensions of bull spermatozoa, we determined the counting error and compared it with the

(1) G. L. Loos en Co's FABRIEKEN N. V., Amsterdam, kindly lent us a counter, made by COULTER ELECTRONICS, Ltd., St. Albans, Herts., England (model A), for three months.
A suspension of small particles in an electrically conductive liquid is forced to flow through a small aperture having an immersed electrode on either side. The flow is caused by the siphonic action of an unbalanced mercury barometer. The passage of a particle through the aperture causes an increase in resistance between the two electrodes and hence produces a voltage pulse of a magnitude proportional to the particle volume. The resultant series of pulses is electronically counted.

error of haemocytometer counts. Since the counter may also be employed for size determination, we used it to define the volume of bull spermatozoa. However, we met with some difficulties in determining volumes with this apparatus which have not been mentioned in earlier publications. It is these difficulties which induced us to write this report.

MATERIALS AND METHODS

Fresh bull ejaculates were diluted 1 : 100 twice. Agglutination of the spermatozoa was practically eliminated by using distilled water for the first dilution instead of physiological saline, saline having an agglutinizing effect on bull spermatozoa (BEDFORD, 1965). Formaldehyde (0.1 per cent w/v) was added to prevent growth of bacteria and algae and to immobilize the spermatozoa.

Counting results obtained with the instrument were compared with fourfold counts of the same ejaculate, performed with 4 Bürker-Türk haemocytometers and 4 erythrocyte dilution pipettes. In order to prevent a systematic error, the 4 chambers and pipettes were used in random combinations, each chamber and pipette being used once. As a diluent, we used 4 vol. per cent of T-pol in distilled water to prevent agglutination.

Latex globules (Dow Chemical) with a diameter of 3.49 μm (± 0.017), prediluted with distilled water to prevent agglutination (WALSTRA, 1965), were used as a standard for volume determinations.

The measurements were performed using a tube with an aperture diameter of 100 μm. The counting volume was 0.5 ml, which corresponds with $500 \cdot 10^{-7}$ ml of the fresh, undiluted semen. (In a haemocytometer the counting volume — about 80 squares of $25 \cdot 10^{-5}$ mm^3 — corresponds with $2 \cdot 10^{-7}$ ml of the undiluted semen only).

To determine the volume of the heads of bull spermatozoa, parts of the fresh ejaculates were homogenized with a homogenizer (BÜHLER, Tübingen, Germany) at maximum speed (40. 000 rpm) for 4 minutes. From smears it appeared that the heads remained unbroken, while only very small remnants of free midpieces and tails were left.

A volume determination was performed on these homogenates and the results compared with the findings on the non-homogenized part of the same ejaculates.

RESULTS

Counting

For 14 ejaculates we found the same very high correlation ($r = 0.99$) between haemocytometer and Coulter counts as did GLOVER and PHIPPS (1962) and IVERSEN (1964 *a*). In contrast to their results, we did not observe a tendency to higher counts using the latter method.

The standard deviation of individual determinations of the spermatozoan concentration within one ejaculate varies from 0.2 to 7.6 per cent of the mean. When determined with a single haemocytometer counting, it ranges from 10.7 to 16.6 per cent. When determined with four haemocytometer countings, using four pipettes at random, the deviation is reduced to 4.9 to 7.6 per cent (KRAMER, 1968). Thus, the counting error range of the Coulter counter is about the same as that of the fourfold haemocytometer counting error range, though in the first about 100 times more spermatozoa are counted than in the latter. This implies that the counting with the electronic counter contains an intrinsic error, increasing the Poisson error by a factor 10.

Sizing

The use of this instrument to determine the volume distribution of spermatozoa in an ejaculate gave rise to the following critical remarks :

1. The volume of bull spermatozoa as determined by GLOVER and PHIPPS (1962), GLOVER (1964), IVERSEN (1965), and us (8 ejaculates) is lower than the volume calculated from the majority of published linear dimensions (table 1). Of these dimensions, the findings of VAN DUIJN are to be preferred because they were obtained on living, immobile (cold) spermatozoa. They show a clearly larger volume than that found with the electronic counter, whether this volume be characterized by its mean or by is mode (table 1).

TABLE I

The volume of bull spermatozoa, as calculated from linear data published in literature, compared with the volume, as determined withe the electronic counter

Le volume des spermatozoïdes du taureau soit calculé partant des dimensions linéaires trouvées dans la littérature, soit déterminé au moyen du compteur électronique

Author		Instrument used (1)	Volume of spermatozoa (μm^3)	Volume of head (μm^3)	Volume of head as % of total volume
CLARKE-ROTHSCHILD	1957	LM	58.8		
BRETHERTON-ROTHSCHILD	1961	—	34.8	24.1	69.3
BLOM-BIRCH ANDERSEN	1960, 1961	EM	18.3	11.5	62.8
BAHR-ZEITLER	1964	—	30.4	15.7	51.6
VAN DUIJN	1960, 1965	LM (2) and IM (2)	48.8	37.9	76.1
VAN ROSMALEN-VAN DUIJN	1963				
IVERSEN	1964 *b*	IM		12.0	
GLOVER-PHIPPS	1962	Coulter	17.3 (mode)		
GLOVER	1964	—	22.0 —		
IVERSEN	1965	—	21.1-25.4 —		
KRAMER		—	12.9-18.1 —		
			17.7-21.4 (mean)		50.7-52.6

(1) LM = light microscope. EM = electron microscope. IM = interference microscope. Coulter = Coulter counter.

(2) The spermatozoa are measured in a living, immobile state (cold).

2. The absolute value of the spermatozoan volume obtained depends on the method of standardizing the apparatus. In the equation $\overline{V} = \overline{q i'}$ (1) the value of q depends on the definition of i' as, viz : either the arithmetic mean, the mode, or the median of the i' distribution. These values differ as the i' distribution is skew. We

(1) $\overline{V}$ = mean volume of e. g. latex globules, the mean diameter of which is known.
q = calibration factor, constant for given aperture size, electrolyte resistivity, and amplification.
i' = threshold settings, indicating volume classes of the particles (particles which produce a voltage pulse above a given threshold setting are counted, while smaller ones are not).

used the mean, but the mode yields an 11 per cent larger value of q. This demonstrates the necessity to mention the method for determining q when absolute values are published. Perhaps much of the difference between our results and those of GLOVER (1964) and IVERSEN (1965) may be explained in this way.

3. The volume distribution of the spermatozoa, as shown by the instrument, is positively skew and falls within the measuring error range with a logarithmic distribution. This distribution is also very broad, the standard deviations of the distribution varying from 25.7 to 38.6 per cent of the means. Probably the particular apparatus used added considerably to the actual variation of spermatozoan volume. This is borne out by our experience while standardizing the apparatus with latex globules. The standard deviation of their volume distribution, as given by the manufacturer, is 1.5 per cent. With the Coulter counter we found 19.0 per cent.

4. The repetition percentage of volume determination is not high. We found 10 per cent differences between repeatedly determined mean volumes within one ejaculate, and 14 per cent differences between modal values.

At least par of the inconstancy of the measuring method may be imputed to the way the apparatus was used in this study. In reproducing routine measurement conditions as closely as possible, we disregarded some other very precise measures such as : constancy of temperature, regular cleaning of the aperture (spermatozoa have a tendency to stick to the aperture wall and its proximity), frequent renewal of the electrolyte in the aperture tube (to prevent the settling of a cloud of particles in it), and the use of counting vessels of one and the same model and volume (10 ml vessels yielded a shift to the left of the distribution curve as compared with 50 or 100 ml vessels).

5. The heads of the spermatozoa have half the apparent volume of the whole, intact cells. Linear dimension calculations show that head volume measurement has a higher percentage of systematic error than whole, intact spermatozoa measurement (table 1).

Surface charge of spermatozoa.

The following causes may be advanced for the difference between the volume of bull spermatozoa as determined by the instrument and the volume as calculated from the most reliable linear dimensions :

1. The properties of the spermatozoa differ from those of latex globules, erythrocytes, and cells, which can be considered to be di-electric particles with negligible capacitive effects (GREGG and STEIDLEY, 1965).

2. The theoretical relation between pulse height and particle volume is not valid in the case of such a peculiar shape as that of the bull spermatozoon.

3. The negative electric charge on the surface of bull spermatozoa influences the electric field in the aperture, and consequently, the pulse height.

To inquire further into the third above mentioned possibility, we determined the volume distribution at pH 7.4 and 3.4, the iso-electric point of the spermatozoa (NEVO, MICHAELI, and SCHINDLER, 1961). Two buffer solutions were made, one of

pH 3.4. and another of pH 7.4, both having the same conductivity and osmotic value (1).

The modal values of volume distributions of the spermatozoa of 3 ejaculates of different bulls suspended at equal concentration in the two buffer systems, were the same in 3 repeated countings. This indicates that the negative electric surface charge at neutral pH does not interfere with the change in resistivity in the aperture caused by the spermatozoan volume.

As expected, the differences between the first and second countings at one thresold setting (the electrode polarity being reversed) were considerably decreased at pH 3.4, and in many cases were reversed in direction. Obviously, these remaining differences are caused by the variability in spermatozoan number of subsequent counting volumes (sampling error).

CONCLUSIONS AND SUMMARY

1. The suitability of the Coulter electronic particle counter for counting bull spermatozoa is confirmed. This method is as accurate as fourfold haemocytometer counting, and is preferable when large numbers of ejaculates are to be counted daily.

2. Its suitability for estimating the volume of bull spermatozoa is questionable because the results are too low as compared with the volume calculated from data on linear dimensions in the literature.

3. The deviation in volume determination with the instrument is not caused by the negative surface charge of the spermatozoa.

4. The volume distribution given by the apparatus is apparently too broad.

5. The method of standardizing should be mentioned when absolute volume values are published.

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(1) The buffer solution with pH 7.4 consisted of : 213 ml 0.16 Mol. Na_2HPO_4, 37 ml 0.16 Mol. NaH_2PO_4, and 1 000 ml 0.9 p. 100 (w/v) NaCl. The buffer solution with pH 3.4 consisted of : 75 ml. 0.20 Mol. Na_2HPO_4, and 175 ml 0.10 Mol. citric acid ; this being adjusted to pH 3.4 with citric acid, 1 000 ml 1.1 p. 100 (w/v) NaCl was added. The conductivity was checked with the Coulter counter itself.

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