*Journal of Chromatography*, 358 (1986) 279–283
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 519

## Note

# Possible separation of ram and bull spermatozoa by a novel method: industrial autofocusing

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(Received January 31st, 1986)

The idea of breeding-quality control by selection of spermatozoa is very old. Its great importance lies not only in the scientific field but also in its economical effect on animal production by increasing breeding quality. The first reports on this appeared in 1933[1], but a real chance to solve this problem came only recentles, with the fast development of molecular genetics, embryogenetics and cytogenetics. Many experimental works[2–5] offer possibilities of breeding preselection, mainly according to sex ratio. The endeavour to solve this problem is shown by the fact that from January 1982 until December 1983, in various scientific journals, there were more than 400 papers[6] published on it. In a field in which so much is descriptive and so little is integrative, we must pause occasionally and ask whether we are getting any closer to the solution of the problem of spermatozoa separation or whether we are in danger of drowning in a sea of unrelated facts. In this problem, the situation is akin to trying to read a novel in which there are too many characters, and we feel that each character shows a different aspect of his personality each time he appears on the pages of the book.

At present, there are many new separation approaches and methods, *e.g.* the flow technique in an improved form used by Sarkar and co-workers[7–9] or Hagele *et al.*[10]. However, most of the experimental works described here have not had any significant effect. It is therefore to be expected that the problem is very complicated and, for further solution, it is necessary to elaborate on new methods and techniques.

The above definitions were the starting point for the study of spermatozoa separation by a novel, recently published method of industrial autofocusing[11,12]. The principles of this method have been reported elsewhere[13].

## MATERIALS AND METHODS

Bull and ram spermatozoa obtained from the ejaculation of five healthy animals of each species are incubated with an equal volume of isotonic solution containing 24.055 g of glycerol and 5 g of fructose in 1000 ml of distilled water. The osmotic presure of this solution is 0.635 MPa, the pH is 8.51 and the conductivity is 57 $\mu$S/cm. The mixture is centrifuged for 10 min at 1000 rpm, at 4°C, for elimination

0021-9673/86/$03.50 

of the proteins and peptide compounds. The sediment composed of the spermatozoa is then resuspended in 70 ml of the same isotonic solution and loaded into an autofocuser[13] of 70-ml volume. Autofocusing is then carried out at 20°C with a d.c. power of 3 W and electric field strength varying from 200 to 1000 V until the current decreases to zero (in *ca.* 36 h)[12,13]. Then, the current is disconnected and the vessel opened. The autofocused solution is divided into twenty equal fractions and measured for pH and spermatozoa concentration, calculated according to observation in a Bürker chamber.

## RESULTS

During autofocusing, the pH gradient in each treatment was automatically formed as usual, without carrier ampholytes[14,15]. Fig. 1 shows the autofocusing of the isotonic solution used in experiments without spermatozoa. The pH gradient of the autofocused solution appears smooth in the physiological pH range of 5.5–8.5, provinding a good medium for the separation of spermatozoa. The conductivity of the single fractions shows that most of the free ions that would otherwise form unstable surface charges, were focused to both negative and positive electrodes, thus provididing the great advantage of this method.

Figs. 2 and 3 present the average values of the five experiments of the ram and bull spermatozoa autofocusing. The ram spermatozoa (Fig. 2) were separated into three expressive peaks with p*I* 4,62, 5,97 and 6,35. It was found that minor peaks were created at pH 8.69 and 10.36. In the presented peaks, the spermatozoa concen-

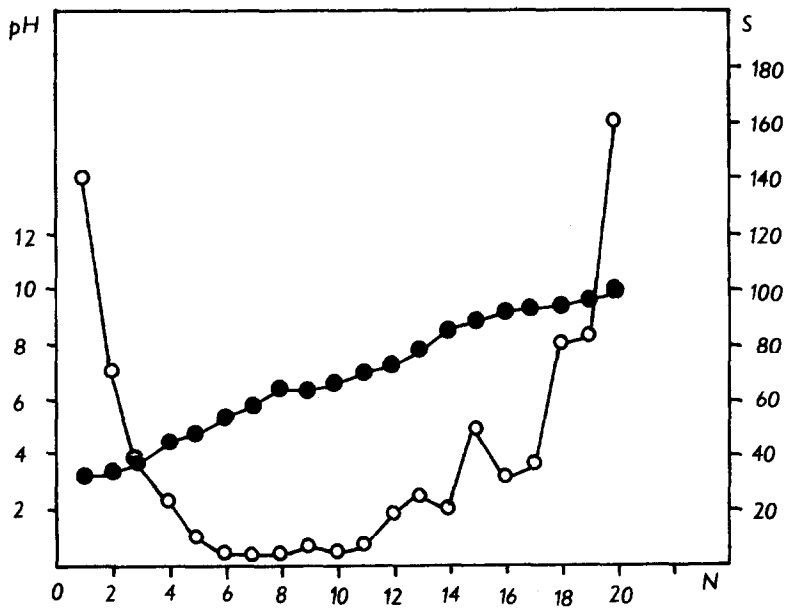


Fig. 1. Autofocusing of the isotonic solution without spermatozoa: (●) pH gradient, (○) conductivity S (values are given in μS/cm); *N* = fraction number.

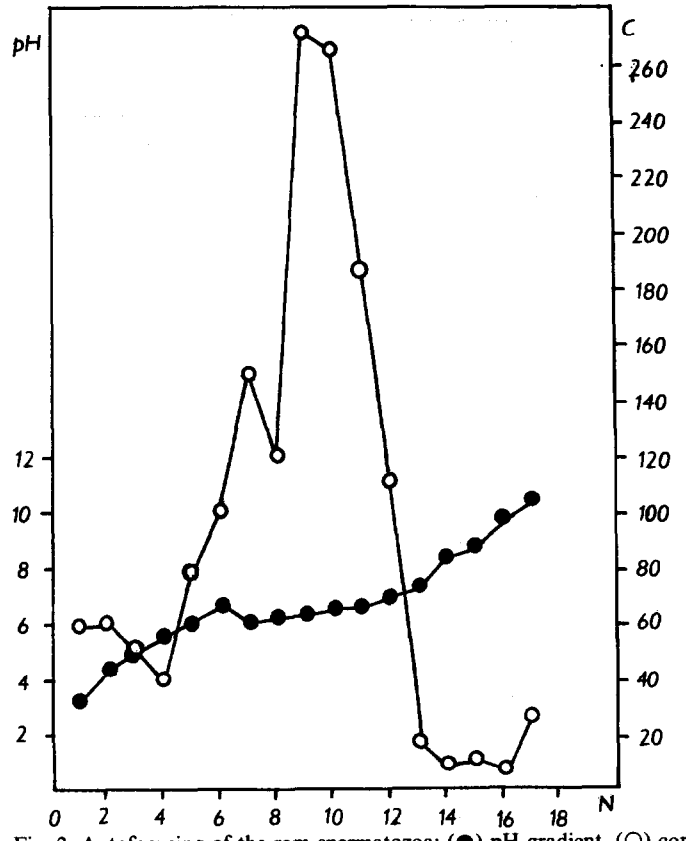


Fig. 2. Autofocusing of the ram spermatozoa: (●) pH gradient, (○) concentration of the spermatozoa given in $10^4$ spermatozoa/mm$^3$; $N$ = fraction number.

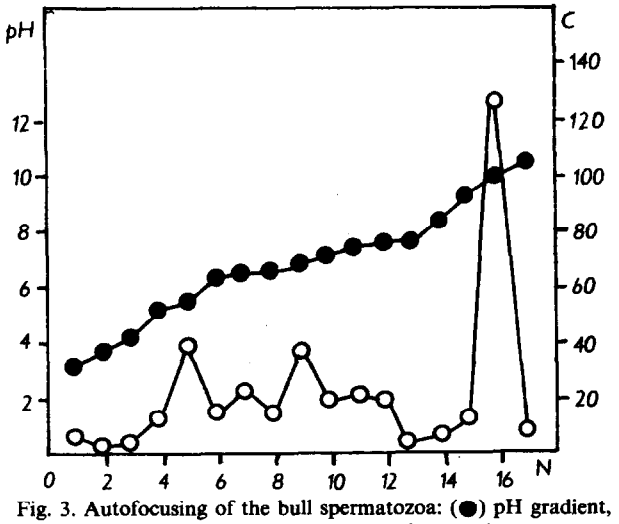


Fig. 3. Autofocusing of the bull spermatozoa: (●) pH gradient, (○) concentration of the spermatozoa given in $10^4$ spermatozoa/mm$^3$; $N$ = fraction number.

tration increased in the following way: in the first peak, the concentration is as much as $60 \cdot 10^4$, in the second, $148 \cdot 10^4$ and in the third, $271 \cdot 10^4$ spermatozoa/mm$^3$.

The autofocusing of the bull spermatozoa also indicated changes in the concentration on increasing pH. In Fig. 3, there are six peaks, at pH 3.15, 5.52, 6.47, 6.84, 7.40 and 9.81. The spermatozoa concentration in the peaks fluctuated between $21 \cdot 10^4$ and $39 \cdot 10^4$ spermatozoa/mm$^3$, except the last peak where a high cumulation of spermatozoa ($126 \cdot 10^4$/mm$^3$) was observed.

## DISCUSSION

The separation of spermatozoa by autofocusing has not been reported prior to this study. The fact that spermatozoa react to the autofocusing system as single particles having their own isoelectric points could be elucidated by the existence of other examples where different whole cells or viruses were separated[14–16]. This fact, in relation to spermatozoa, was not known until now.

The results achieved show that the spermatozoa, like the whole-cell system, dispose of a surface electric charge and in the proces of autofocusing are separated according to their isoelectric points, forming well-defined peaks. At the same time, the differences between the separation of the ram and bull spermatozoa were well observed because the ram spermatozoa were concentrated mainly at pH 6.35 and the bull spermatozoa at pH 9.81. This means that the isoelectric points of the majority of spermatozoa in both species are different. According to analysis of the isotonic solution used in these experiments, it does not contain polar molecules and its conductivity and buffering capacity are minimal so that the pH gradient in the course of the separation and the concentration in the individual peaks are produced by the spermatozoa themselves. This fact cannot influence either of the materials contained in the seminal plasma because they are centrifuged and the sediment is diluted in a ratio of 1:70. This operation completely eliminates these possible influences.

In the present work, the principal mode of spermatozoa separation in an apolar isotonic solution by autofocusing was studied. However, the vitality of the spermatozoa or other factors have not been investigated and require further study.

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