OPTICAL INVESTIGATIONS ON THE MOVEMENT OF BULL SPERMATOZOA

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1. METHODS

Diluted bull-semen is observed with darkfield illumination with a × 10 objective, N. A. 0.25, combined with a × 15 eye-piece. In the present investigations the stage was kept at about 37°C, though some observations have been made at a lower temparature. The semen was handled as described by Rikmenspoel, van Herpen & Eykhout (1960).

Generally a semen-sample was brought into a microchamber consisting of a perforated plastic foil sandwiched between slides; the depth of the chamber was varied from about 30μ to 100μ or even higher. For obtaining an airliquid interface a micro-chamber of 300μ depth was used. Here a thin drop of diluted semen was placed on the bottom slide; the upper one was covered with a film of T-pol solution to avoid occurrence of condensation droplets.

In order to obtain better information of the orientation in the direction of vision (i. e. vertical) a red and a green filter were placed below the darkfield condenser (fig. 1); this splits the illuminating cone into a red (left) and a green (right) half. The red and green filters were separated by a small dark strip.

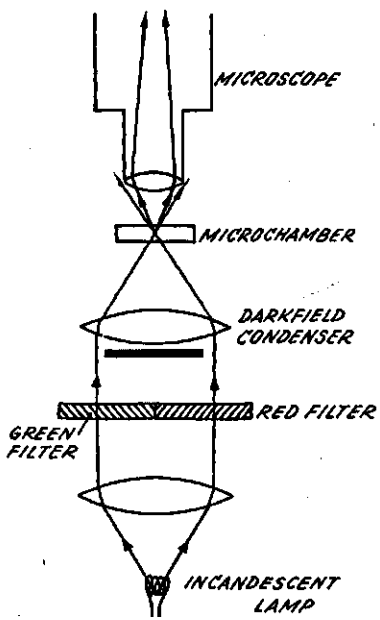

Fig. 1 Optical arrangement

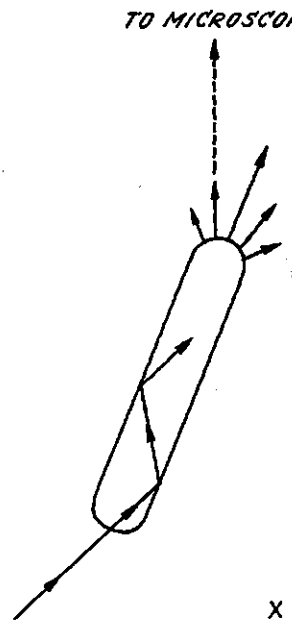

Fig 2. Conduction of light through a sperm head

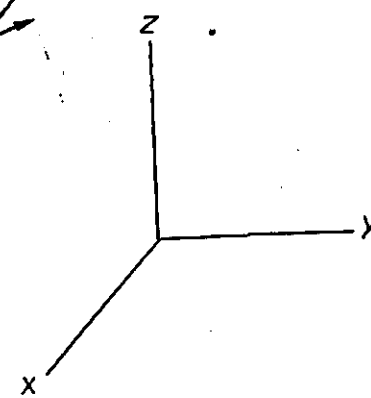

Fig. 3 Directions in sample

When the head of spermatozoon swimming in a horizontal plane is near a vertical position, the upper edge appears bright owing to the light being conducted by total reflection through the head, the latter having a flat shape (fig. 2).

Let the directions in the sample be x, y and z, as denoted in fig. 3. If the longitudinal axis of the spermhead has the x-direction and the vertical cross-section has the position denoted in fig. 2, seen from x (front), it conducts the green light, so that the upper edge appears brightly green (the lower edge appears in the same colour, but less intense). If the deviation from the vertical is to the other side, the head appears red. The same colours remain, when the direction of the longitudinal axis is shifted close to the y-direction. In the exact vertical position the head appears less brilliant.

Information is also obtained of the tail-elements in the yz-plane with the mentioned differential twocolour darkfield (fig. 4). First imagine a very thin tail-element. This element diffracts light in the

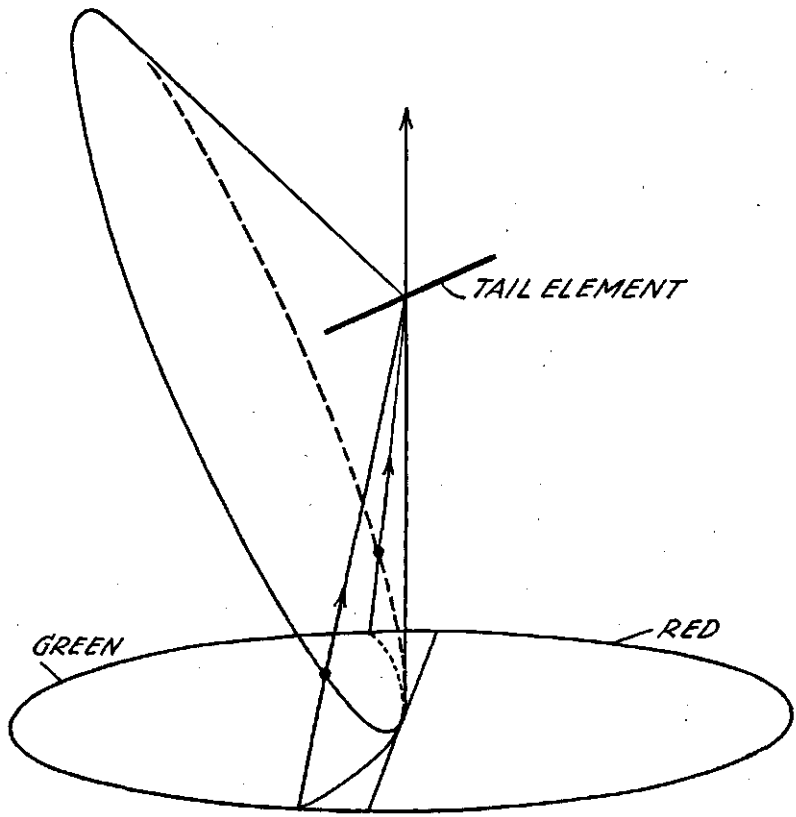

Fig. 4

z-direction if it originates from a direction which makes the same angle with the tail-element as an undiffracted ray in the z-direction, i.e. a cone-mantle as indicated in the figure. In the case shown only two rays, from the green half, obey this diffraction law, and can be diffracted in the direction of the microscope objective. In fact, the tail is not very thin compared with the wavelength of visual light, not completely homogeneous, etc. This does not disturb the rule derived, but there must be a minimum slope for seeing a dominating colour. In motionless cells with sufficient long or steep slopes of the tails this theory may be verified by shifting the focal plane.

2. DATA AND INTERPRETATIONS

Many observations have been made, which ultimately allowed coherent interpretation. Because of the complexity of the data, interpretations are given immediately.

In slowly moving cells swimming in the positive x-direction, the colour always shifts from red to green, when the position of the head passes the vertical, in the mentioned two-colour darkfield. If sperms swim in the opposite direction, the colour shifts from green to red. In normally moving cells which have a high rate of flashing, the same colour shift can be deduced from the colour sequence seen when the stage is moved, rapidly enough for preventing the eye from following the cell.

From these observations it is concluded that the spermatozoa rotate with their heads (and of course with their tails too) in anti-clockwise direction, as seen from behind. No exceptions have been found thus far.

The above statements are in agreement with the observations performed in micro-chambers with such small depth that the sperms are prevented from rotating, but can still deviate with their heads from the horizontal position. Now the sperms *tend* to rotate anti-clockwise. If a sperm is swimming in the positive x-direction (or has a component in this direction) the head appears red, when in the opposite direction it appears green. Owing to absence of rotation, any asymmetry causes them to swim in circular orbits.

Rotation eliminates the overall effect of asymmetry and makes the cells to swim in straight orbits. If viewed under the microscope, however, these cells, too, often have a more or less curved orbit. This is caused by interaction with the interface of the liquid to glass, air or oil, whatever is used as a barrier. The spermatozoa accumulate in the frontier-layer and as a result are affected in great majority even in a thick layer. Looking from liquid to border there may be a weak or strong preponderance of curvature in one direction, either to the right or to the left. Besides there are cells, which swim with their heads close to the glass-surface, not rotating about their long axis, and generally having about circular orbits. Here we may also have a weak or strong preponderance of one direction of curvature. It is said that these cells appear in greater majority in uncarefully handled semen (Rikmenspoel *et al*, 1960).

In aged semen, cells with a screwed tail can often be observed. Waves of small amplitude are travelling over it, giving the cell a slow translatory and rotarory movement. Such a cell, swimming in the y-direction or opposite, shows, in the differential-colour darkfield, a red colour at the minus-x-side and a green colour at the plus-x-side, as denoted in fig. 5. On account of section 1 the screw

Fig. 5

must be negative, in accordance with the anti-clockwise rotation of the head. The twist in the tail is fixed as regard to the head. Generally, the frequency of the travelling oscillations is low enough for clearly seeing the tail. I have the impression that the active oscillations (variations of curvature) of an element of the tail are in the plane through the axis of the screw. Sometimes the frequency is too high to be followed by the eye. Then, in connection with the slow rotation, it may be seen that the oscillation of each element is flat with regard to the head.

In somewhat less aged semen often a rather great number of cells with an other type of movement may be seen. At first sight, observing the tail, one gets the impression of a slowly screwing translatory movement, the view of the screwing tail being blurred by a three dimensional tailwave of high frequency. However, the head appears to rotate at a high speed anti-clockwise, whereas the colour of the blurred tail is opposite to the first mentioned type, the screw being positive.

This apparently paradoxal type of movement may be described in the following way. Let flat

sinusoidal waves travel over the tail, it being straight and not screwed in equilibrium, but the plane of vibration twisted anti-clockwise. Further, let the plane of vibration at the proximal end be the plane of the flat head. Then, as in the first mentioned type, the translation is accompanied by an anti-clockwise rotation. Thus, each tail-element has, regarded from outside, a combined vibratory and rotatory movement (regardless of the translation). Let one period of vibration, $\alpha\beta\gamma\delta\varepsilon$, (fig. 6a),

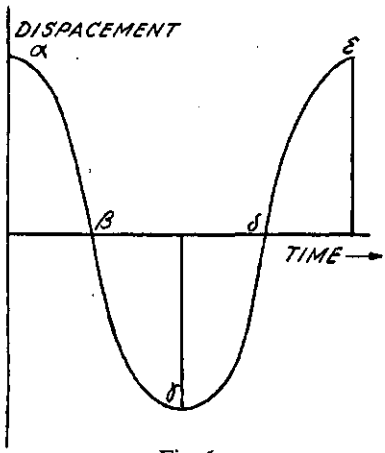

Fig. 6a

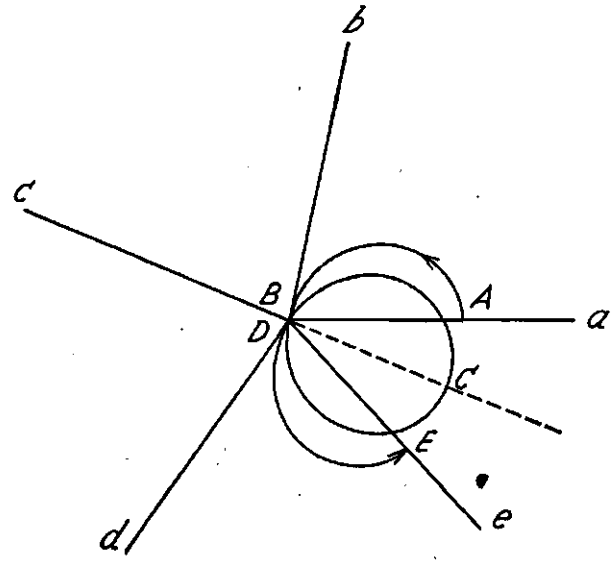

Fig. 6b

be connected with somewhat less than one rotation abcde (fig. 6b). Then the combined movement is the looping curve ABCDE. This kind of movement, however, is affected by the head, which can move much more easily in its own plane than perpendicular to it and consequently will

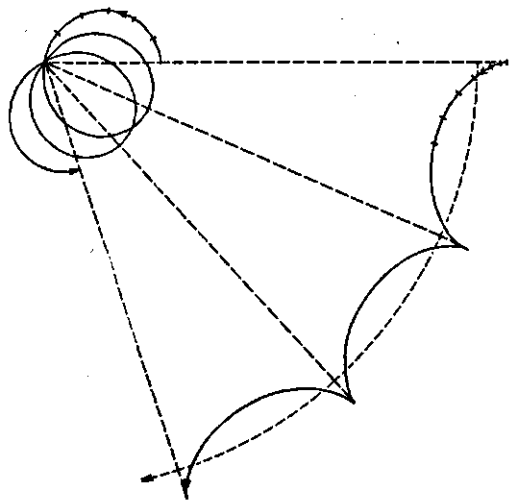

Fig. 7

have rather a peaked orbit than a looping one (fig. 7). This result may be also, roughly, considered as the original looping movement, superimposed by a slow clockwise circular movement. This 'precession' will be followed, and may even be enhanced, by the tail .This explains the slow, positively screwing, movement observed, combined with a high rate of rotation of the sperm as a whole, and vibration of each tail-element, which the eye is not able to follow. The single peaks or loops may be much closer to each other than as denoted in the figures nos. 6b and 7, and the precession may be as slow as one period per second with 10 to 20 periods of tail vibration.

These cells move slowly and may sometimes be seen in an inclined or perpendicular position with their heads against the glass wall. Near the end of their lives they may change into the former type, with fixed screw.

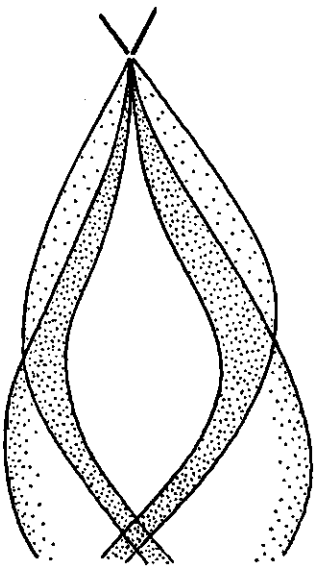

Fig. 8a

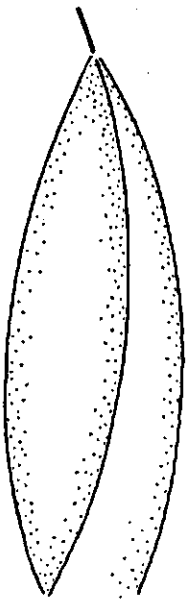

Fig. 8b

Normal cells often have (apart from their translation) a stationary pattern of interference between vibration and rotation at a high rate. The appearance may be symmetrical, as fig. 8a, or asymmetrical, as fig. 8b. They are identical with the last described 'precession'-type, except that the proportion of their rotation and vibration frequency is not almost 1 : 1, but 1 : 2 and 1 : 3, respectively. Their 'cross-sections' have 4 and 3 loops or peaks, respectively. Exact synchronisation is brought about by interaction with the wall.

The distal part of the tail is often moving in a more complicated manner. A further complication is the asymmetry of the tail wave, which can have a great magnitude. Research is being continued.

Summary

Bull spermatozoa have been observed with differential two-colour darkfield (fig. 1) in order to analyse their movements. Near the vertical position the flat head of a sperm conducts the light (fig. 2). From the colour-changes observed it is concluded that the cells rotate anti-clockwise, as seen from behind. The tail may also show colours, if it is not in a horizontal plane. Observations and interpretations are given of the movements of two types of cells with low velocity, and of normally moving cells. In the latter probably flat waves travel across the tail, the plain of the tailwaves being twisted, which results in rotation. Influence of the bottom or top boundary can lead to synchroni-

sation of rotation and vibration, producing a stationary interference pattern as seen by an observer following the translation movement (fig. 8). The orbit of each element of the tail and the head shows peaks or loops (regardless of the translation of the cell).

Résumé

Pour en analyser le mouvement on a observé des spermatozoïdes de taureaux à éclairage oblique (sur champ noir) et à deux moitiés différemment colorées (figure 1). Le plan de la tête plate étant en position verticale, la tête conduit la lumière ce qui en fait s'éclaircir vivement le bord supérieur (figure 2). Le virement de couleur, qui en résulte, donne lieu à constater une rotation négative des spermatozoïdes (vue par derrière). Quand il ne s'étend pas dans un plan horizontal, le flagelle peut aussi être vue en couleur.

Sont communiquées des observations et leurs interprétations sur trois types de mouvement, deux où le déplacement est lent, l'autre cellules normales.

Chez ces dernières il paraît que les ondes planes s'avancent sur le flagelle, le plan en question étant tourné de la tête jusqu'au bout du flagelle.

Il en résulte que la propulsion est accompagnée d'une rotation autour de l'axe longitudinal. L'influence des faces supérieures et inférieures peut donner lieu à la synchronisation de la rotation et de la vibration où l'observateur suivant de l'oeil un spermatozoïde remarque une frange d'interférence stationnaire (figure 8).

Abstraction faite de l'avancement, chaque élément de la tête ou de la queue suit une trajectoire rotatoire avec des pics ou des boucles.

Zusammenfassung

Beim Studium der Bewegung eines einzelnen Spermiums des Bullen durch mikroskopische Beobachtung, sieht (oder photographiert) man im Wesentlichen nur zwei Dimensionen der meistens räumlichen Bewegung. Gezeigt wird, dass Teilung der Dunkelfeldbeleuchtung in zwei Farben nähere Information gibt über die dreidimensionale Bewegung von Kopf und Schwanz der Samenzellen. Aus den Wahrnehmungen geht hervor, dass frei schwimmende Samenzellen immer negativ rotieren, von Hinten gesehen; jedenfalls wurden keine Ausnahmen beobachtet. Das Bewegungsbild des Schwanzes ist kompliziert, konnte aber in allgemeinen geklärt werden mittels Analyse der visuellen Beobachtungen. Die Untersuchungen werden fortgesetzt.

Reference

Rikmenspoel, R., van Herpen, G. & Eijkhout, P. (1960).
Cinematographic observations of the movements of bull sperm cells. *Physics in Med. and Biol.*, **5**, 167.

PREPRINT.

PROCEEDINGS IV th INTERNATIONAL CONGRESS ON ANIMAL REPRODUCTION - The Hague (Holland), June 5-9, 1961.