Microphotokinesigraphic analysis of buffalo spermatozoa
II. Photokinesigraphic characteristics of buffalo vibrating spermatozoa

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ABSTRACT - The study concerns images of vibrating spermatozoa in the ejaculation of buffalo bulls from Bulgarian murrah breed through the method of Tchakarov and Natchev [1962], obtained at 5 sec exposures, optimal for this particular species. The negative registration material has been developed with high-equalising developer by Windisch, which reduces the glow due to overexposure around highaktinic parts of spermatozoa. On the basis of the positive graphic material – kinesigrams, we have established and analysed the main movement patterns of vibrating spermatozoa: with vibrating tail; with vibrating head; with a vibrating tail and head; with whole bilateral vibration; vibrating in circle; chaotically vibrating. We have described the differences in photokinesigraphic images of spermatozoa within given subclass.

Key words: Sex tissue, Vibrating mobile speramatozoa.

INTRODUCTION - By means of the microphotokinesigraphic method [Tchakarov and Natchev, 1962] the following parameters of the studied ejaculated semen could be defined: the number of immobile spermatozoa [%]; the number of vibrating spermatozoa [%]; the number of forward-moving spermatozoa [%]; the speed of the forward-moving spermatozoa [µm/sec.]; the type and the pattern of their movement. Vibrating spermatozoa are defined as kinetic forms, registered in kinesigrams, which are characterised by different moving activity and lack of forward-moving component and rotation around their longitudinal axis. Movement of this type were first registered and described in human spermatozoa, and were reported at the V World Congress on fertility and sterility in Stockholm [Tchakarof and Nachev, 1967]. These kinetic forms alongside with the forward-moving ones comprise the group of the mobile spermatozoa. On the basis of the obtained kinesigrams the aims of the present study are: a) to register and analyse the main types of vibrating movements of the buffalo spermatozoa; b) to find out if each of these subgroups is kinetically homogeneous.

MATERIAL AND METHODS - The study was based on samples of semen of six buffalos of the Bulgarian murrah breed analysed by means of Tchakarov and Natchev’s kinesigraphic method [1962]. The samples were obtained once a week, in a period of four weeks. Ten registrations were conducted from each sample, diluted with glucose-phosphate diluter for buffalo and 2% beef serum albumin. The microscope used was Ergval (Zeiss) with...
a standard phase-contrast condenser. The optic enlargement of the negative kinesigrams was 10x3.2, and the time for the exposition – 5 sec. The negative material (black and white Konika films) was developed with Windisch. The treatment lasted for 12–14 min. at 20°C. Positive kinesigrams’ dimensions were 16x16 cm.

RESULTS AND CONCLUSIONS - Vibrating spermatozoa are differentiated on the basis of their movement: whether the movement concerns just one part of them, or several parts, or the whole cell. Depending on the type of the registered vibrating moment we differentiated between six kinetic subgroups, as follows.

Spermatozoa with vibrating tail (figure 1). The recorded photocinesigraphic images of this subclass of spermatozoa are distinguished for a clearly expressed contour of the head which corresponds to that of the immobile spermatozoa recorded by the same method. Its central non-actinic part is clearly seen there. The image of the vibrating part of the spermatozoa has the shape of an isosceles triangle with an altitude most often bigger than its base.

![Figure 1. Spermatozoa with vibrating tail: a. direction of movement; b. scheme of making the image; c. schematic image; d. kinesigraphic image (fragment of kinesigram).](image)

The length of the light trace is dependent on whether all the parts of the tail - the intermediate and the main part or just the final one take part in the vibratory movement. In the first two cases the peak of the light trace is considerably brighter because the brighter intermediate part of the spermatozoa takes part in the vibration, too. These are the most frequently seen types of vibration from this subclass. More occasionally the vibration is realized only by the final part of the tail when the photocinesigraphic image is fan-shaped with a moderate brightness. The base of the light sector has a low brightness and a small sharpness and it gradually turns into a darkfield ground. For this reason the length of the photocinesigraphic image of the tail is smaller than then the image of the same part of the immobile spermatozoa. The magnitude of the point angle, respectively the ratio altitude - base of the light trace depends on the movement amplitude of the vibrating tail. The intensity of the described triangle (sector expansion) is not one and same in certain cases. This is due to the irregularity of the vibratory movement. Usually the edges of the sector are the brightest parts because under conditions of absolute deviation the angular velocity decreases, and in certain cases it is equal to zero, i.e. there is a moment of detention. During an intermittent type of movement of the tail the light triangle has a striped structure. In buffalos, this subgroup of movement is more rarely observed. Spermatozoa with vibration realized by the intermediate part of the tail are also rarely observed. Kinesthesigraphically their image is characterized by a spindle-shaped expansion of this part which is due to its fixation to the walls of the camera.
Spermatozoa with vibrating head (figure 2) Photokinesigraphically the image of this subgroup of movement is again a triangle (a sector expansion) with an apex coinciding with the distal section of the main or of the distal part. The periphery of the sector is brighter and it corresponds to the image of the bright intermediate part and the spermatozoon tail. When the vibratory movement is realized with a facial position of the head, the peripheral part of the sector is darker than it is in the case when the movement is realized with a profile position of the head and reason for this is the higher actinism of the cellular territory in a profile position. In this subgroup it is of importance, too, whether the movement is smooth or intermittent and as a result of that the light triangle is homogeneous or striped. In the positive registration material obtained by us like the ejaculates of the bull, the vibrating in this way spermatozoa of the buffalo are also rarely observed.

Spermatozoa with a vibrating tail and head (figure 3) In this category of vibrating spermatozoa the photokinesigraphic image presented in two sectors with their points touching or two oval sections, also touching. The oscillatory movements are round the comparatively immobile part of the tail. One of the sectors or the oval images has greater brightness, due to the higher actinism of the head and the intermediate part. This subclass of movement considering the studied animal species is represented rather on a small scale.

Spermatozoa with whole bilateral vibration (figure 4) The light print of this subgroup of spermatozoa is a rectangle with a width corresponding to the amplitude of the vibratory movement and an altitude corresponding to the shortened photokinesigraphic length of the spermatozoon. A characteristic feature of this type of movement is that all the parts move simultaneously bilaterally round a provisionally balanced line. The light print is not homogenous. It is represented by three stripes with a different brightness corresponding to the longitudinal sizes of the head, the intermediate part and main and final part of the tail.
Spermatozoa vibrating in circle (figure 5) The photokinesigraphic images of the vibrating in a circle spermatozoa are circles with a concentric stripiness (wreath-like shape). During a circular vibration with the head being the centre, the central part of the circle is small and non-actinic, surrounded by a clear contour. A wreath with a low actinism is situated peripherally around it and outside there is a wreath with a considerable brightness, a result of the circular movement of the intermediate part. The great linear velocity of the main and mostly of the final part of the tail and the rather low brightness of the latter are reason for the absence of a light image on the negative photograph material. Another category of circular vibrations is realised when a certain point on the tail of the spermatozoon is the center of the vibration. The recorded image has a central low actinic zone, around which there is a brighter wreath consisting of a peripheral wider and brighter zone and a central zone with a lower intensity. In certain cases, when the registration time is less than the time necessary for describing a full circle the photokinesigraphic images have a central part represented by a circle and a periphery of a sector which is usually bigger than 180°. During the intermittent movements the radial stripes are visualised in the image of the vibrating spermatozoon. This category of movement is observed only in some of the buffalo ejaculates studied by us.

Chaotically vibrating spermatozoa (figure 6) The photokinesigraphic images of this subgroup of vibrating spermatozoa integrate in two basic characteristic combinations. The first one is characterized by slower chaotic vibrations and the image formed by a certain spermatozoon most frequently represents an oval picture of the bright rectilinear sections of the actinic intermediate part of the spermatozoon, meeting at angles with different directions. The second one is usually a rounded field with a comparatively low actinism because of the energetic chaotic vibrations and respectively the shorter time needed for detention in the corresponding intermediate position. That is why the images of the intermediate part of the tail are difficult to discern.

In buffalo species the intermittent movements of spermatozoon with vibrating tail and vi-
brating middle part are seldom. Quite seldom are also the intermittent and the smooth movements of spermatozoa with vibrating head.

**REFERENCES** - Tchakarov, E., Natchev, Tch., 1962. Contribution aux méthodes d'étude de la cytophysiologie des spermatozoïdes. Bulg. Acad. Sci. Bulletin Inst. Physiol. 5:147-162. Tchakarov, E., Nachev, Ch., 1967. Spermatokinesigraphic features of the vibrating spermatozoa of human semen, In: Fertility sterililty /B. Westinq N. Wiqvist, eds./ Excepta Medica Fnd. 645-652.