A C T A I C H T H Y O L O G I C A E T P I S C A T O R I A
Vol. VI Fasc. 2 Szczecin 1976

Aleksander WINNICKI, Lucjan TOMASIK

Embryology

„SPERMATOCRIT" AS A METHOD FOR BIOLOGICAL EVALUATION OF FISH SPERM

„SPERMATOKRYT" JAKO METODA BIOLOGICZNEJ OCENY SPERMY RYB

Institute of Ichthyology

A volume of trout (*Salmo trutta* L.) spermatozoa relative a sperm volume unit ("spermatocrit") was studied by means of a microhematocrit centrifuge. It was found out that the method allowed density differences between sperm of various individuals to be assessed quickly and precisely; an amount of water absorbed by spermatozoa during their activation can be measured by this method as well.

INTRODUCTION

During the natural spawning of fish of external fertilization, the eggs are being fertilized in water. Only when in contact with water, spermatozoa become motile (Quatrefages, 1853; Ginzburg, 1968; Tomasik, 1973) displaying progressive and oscillatory movements (Rutter, 1904; Scheuring, 1924; Drabkina, 1961; Gorelov, 1966; Ginzburg, 1968; Hettler jr., 1970; Tomasik, 1973). Thus the water has been assumed to activate spermatozoa (Scheuring, 1924; Stroganov, 1938; Beljaev, 1957). It has been found out, however, that the process of activation in fluids can be accelerated, retarded, restricted, or even completely stopped with the fluid osmotic pressure increase (Scheuring, 1924; Stroganov, 1938; Ellis and Jones, 1939; Tanasijčuk and Vonokov, 1956; Drabkina, 1961; Gorelov, 1966). The above-mentioned fact allows a presumption that water osmotically penetrates an engulfed spermatozoon and thus plays a role of a solvent for both the high-energy substances contained in the spermatozoon and enzymes

releasing the energy necessary to perform any movement. Should this be the case, the unit volume of spermatozoa increases on activation, which is supported by findings of Beljaev (1957) and Dorošev (1967) who measured the spermatozoon head sizes before and after the activation. The first recorded an increase in the *Misgurnus fossilis* spermatozoon head size from 1.5–1.8 to 1.8–2.7 μm, while the other observed a similar situation in *Chalcaburnus chalcoides* with an increase from 2.0 to 5.0–6.0 μm. Moreover, Dorošev also observed an increase in the head size of a spermatozoon activated by NaCl in various concentrations.

The differences quoted are substantial, although the techniques applied are themselves neither precise (no possibility of an exact determination of water absorbed by spermatozoa is offered) nor quick enough (laborious and time-consuming microscoping, staining, etc.); they are not accurate enough either, which, when summed up, renders them insufficiently objective.

The present paper's goal was to develop a quick, reliable, and sufficiently precise method for determining a relative spermatozoa volume in sperm and an average amount of water absorbed by a single spermatozoon; on the other hand, an effect of environmental osmotic pressure on the amount of water absorbed was hoped to be assessed by the method as well.

MATERIAL AND METHODS

Fresh sperm of trout (*Salmo trutta* L.) was used in the investigations run in three variants: a) undiluted sperm, b) 1:10 tap water-diluted sperm, c) sperm diluted with 1, 2, 3, 4, 5, 10, 20, and 30‰ NaCl solutions. Standard tubes of microhematocrit were filled with sperm and its solutions and centrifuged in a microhematocrit centrifuge (5000 r.p.m., 5 min.). The centrifuged residue was found to contain exclusively spermatozoa, as the fish sperm fluid contains no other morphotic elements, which had been stated by Miescher (1896) and Ginzburg (1968) among the others and checked by the present authors.

RESULTS

The studies revealed considerable variations in density between sperm obtained from different mature males. It is illustrated by Fig. 1 showing the microhematocrit tubes with centrifuged sperm. A so-called "spermatocrit", i.e., a spermatozoa volume relative the total sperm volume, expressed in %, ranges – as can be seen in Fig. 1 – in mature males from 20 to 50. Table 1 show a certain correlation to exist between the "spermatocrit" and a number of spermatozoa per volume unit. The correlation found seems to indicate the size of a single spermatozoon (and thus its volume) from different males regardless of their age to be more or less constant.

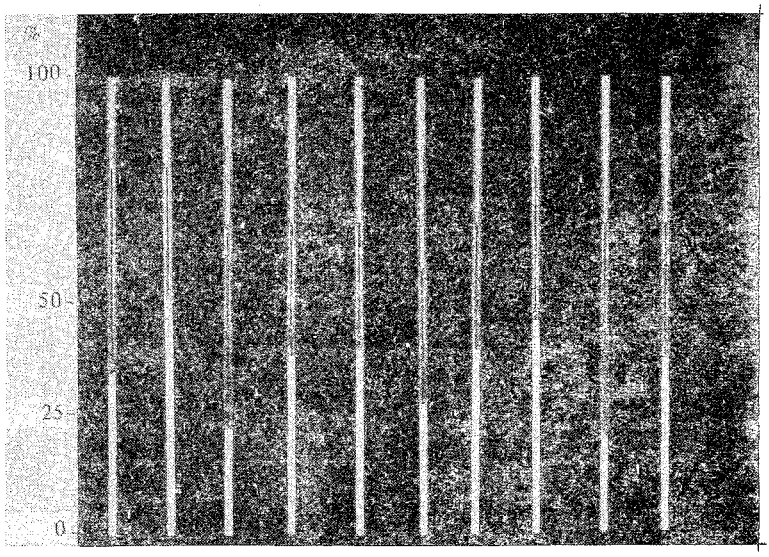

Fig. 1. Spermatocrits of various trout individuals

Table 1

"Spermatocrit" and density of spermatozoa in different males

No	"Spermatocrit" %	Number of spermatozoa per 1 mm^3
1	20	8800000
2	24	10140000
3	24	12140000
4	36	12960000
5	36	13760000
6	41	16080000
7	44	18080000
8	46	20160000
9	48	19740000
10	49	19730000
11	53	19340000

The graph on Fig. 2 presents a comparison between sperm 1:10 diluted with tap water and various NaCl solutions and the control (undiluted sperm). The comparison points out that during the water-activation of spermatozoa, their total and individual volumes significantly increase (almost four-fold in some instances). The volume increase can be regarded as a measure of the amount of water absorbed. This amount reaches its maximum when tap water and $1^{\circ}/_{oo}$ NaCl solution are used to dilute sperm, and decreases with a rise in salinity to settle on an even level (no volume increase) in solutions of salinities from $5^{\circ}/_{oo}$ on.

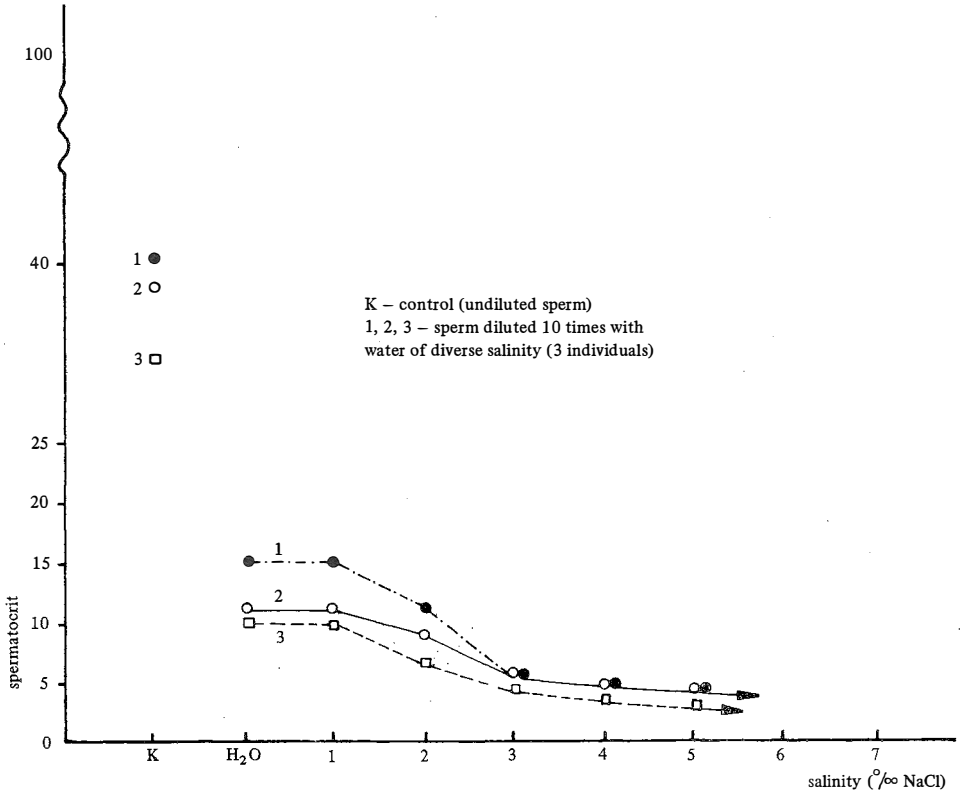

Fig. 2. Relative volume of spermatozoa in solutions of different salinity

DISCUSSION OF RESULTS AND CONCLUSIONS

The results obtained in the course of studies described not only confirm the findings of other authors on a spermatozoon head volume increase (Beljaev, 1957; Dorošev, 1967), but also allow a more or less precise determination of water amount absorbed by spermatozoa during their activation to be carried out. A regular feature emerges from the observations, that is a mature and ready to be activated spermatozoon absorbs, under a normal salinity regime, the amount of water exceeding its original volume 3–4 times. The behaviour of spermatozoa in water of changing salinity, assuming that the osmotic penetration of water ceases after an osmotic equilibrium has been settled on both sides of the case, allows to determine the spermatozoon head osmotic pressure as ca 130 mosm/l. A lower osmotic pressure in the swollen spermatozoon head than that given above cannot be exluded and hydrophilic colloids can play some part in water absorption as it is the case in the egg periviteral fluid, according to the theory advanced by Bogucki (1930).

The method used in the studies presented meets, as it has been said in the introductory section, the necessary requirements. It offers a possibility to assess qualitatively the applicability of a given sperm to fertilization, since the spermatozoa unactivated in water (non-motile) have not been observed to increase their size.

In view of the fact that sperm obtained from various males differs in its quality (differences in, or lack of, motility), a procedure, worked out empirically by fish farmers, of fertilizing the eggs with sperm supplied by a number of males, can be considered absolutely justified. Failures of incubation of the so-called "pure samples" (eggs from one female and sperm from one male), frequently observed in laboratories, may have resulted from a lack of expertise in the biological quality of sperm used in fertilization.

The method described is possible to be employed in fish farmer's practice for a current biological evaluation of sperm since it is reasonably simple, precise, and relying on a small amount of material.

REFERENCES

Beljaev E.V., 1957: Nekotorye osobennosti fiziologii spermy i jaic ryb.-Tr. Mosk. Techn. In-ta Rybn. Promysl. i Choz-va, **8**: 271–277.

Bogucki M., 1930: Recherches sur la perméabilite des membranes et sur la pression osmotique des oeufs des salmoides.– Protoplasma, **9**: 345–369.

Dorošev S. I., 1967: O vlijanii jonov natrija i kalcija na spermii presnovodnych ryb. – Dokl. Ak. N. SSSR, **172**: 1238–1240.

Drabkina B.M., 1961: Vlijanie vody rozličnoj solenosti na vyźivaemost'spermy, ikry i ličinok osetra.– Dokl. Ak. N. SSSR, **138**: 492–495

Ellis W., Jones J., 1939: The activity of the spermatozoa of Salmo salar in relation to osmotic pressure.– J. exp. Biol.: **16**, 530–534.

Ginzburg A.S., 1968: Oplodotvorenie u ryb i problema polispermii. Moskva, Izd. „Nauka".

Gorelov V.K., 1966: Reakcija spremiev nekotorych presnovodnych ryb na izmenenie osmotičeskogo davlenija vnešnej sredy.– in: Biologičeskie osnovy rybnogo choz-va na vodoemach srednej Azii i Kazachstana. Alma-Ata.

Hettler V.jr., 1970: Effect of paternal death on sperm viability in the orangethroat darter.– **32**: 209–211.

Miescher F., 1896: Physiologisch chemische Untersuchungen über die Lachsmilch.– Arch. exp. Pathol. Pharmak., **37**: 100–155.

Quatrefages A.de, 1853: Recherches sur la vitalité de spermatozoiden de quelques poissons d'eau douce.– Ann. Sci. nat., **19**: 341–369.

Rutter C., 1904: Natural history of the quinnat salmon. A report of investigations in the Sacramento river, 1896–1901.– Bull. U.S. Fish Commission for 1902, **22**: 65–141.

Scheuring L., 1924: Biologische und physiologische Untersuchungen an Salmonidensperma.– Arch. Hydrobiol., **Suppl.–B.** 4, L.2: 181–318.

Stroganov N.S., 1938: Vyźivaemost' spermy volžskoj seldi (Caspialosa volgensis) pri raznych vnešnych uslovijach.– Zool. źurn., **17**: 316–336.

Tanasijčuk V.S., Vonokov I.K., 1956: Vlijanie vody raznoj solenosti na spermu i ikru vobly i lešča severnogo Kaspija.– Tr. VNIRO, **32**: 284–292.

Tomasik L., 1973: Specific and indyvidual differences in motility between Salmonid spermatozoa.– Acta Ichthyol. Pisc., **3**, 1: 11–17.

„SPERMATOKRYT" JAKO METODA BIOLOGICZNEJ OCENY SPERMY RYB

Streszczenie

Przy użyciu wirówki mikrohematokrytowej badano względną objętość plemników troci (*Salmo trutta* L.) w jednostce objętości spermy („spermatokryt").

Zastosowana metoda pozwala na szybkie i precyzyjne oznaczanie gęstości plemników w jednostce objętości spermy, oraz daje możliwość ustalenia różnic osobniczych. Ustalono, że „spermatokryt" u ciekających samców waha się od 20 do 50%. Z drugiej strony metoda ta umożliwia oznaczenie wchłanianej przez plemniki wody w trakcie ich aktywacji. Zwiększenie objętości plemników jest bowiem miarą ilości wchłanianej przez nie wody. Ilość wchłanianej wody jest największa w przypadku rozcieńczenia spermy wodą wodociągową, taka sama w przypadku rozcieńczenia spermy wodą o zasoleniu 1‰ i maleje w miarę wzrostu zasolenia, aby ustalić się na jednakowym poziomie w roztworach o zasoleniu około 5‰ i większym.

„СПЕРМАТОКРЫТ" - МЕТОД БИОЛОГИЧЕСКОЙ ОЦЕНКИ СПЕРМЫ РЫБ

Резюме

При помощи микрогематокритовой центрофуги исследовали относительный объём сперматозоидов кумжи (Salmo trutta L.) в единице объёма спермы („сперматокрыт").

Применяемый метод позволяет быстро и точно определить густоту сперматозоидов в единице объёма спермы и даёт возможность установить индивидуальные различия. Установлено, что „сперматокрыт" у текучих самцов колеблется от 20 до 50. С другой стороны этот метод позволяет определить количество поглощаемой сперматозоидами воды в процессе их активации так как увеличение объёма сперматозоидов является показателем количества поглощаемой ими воды.

Количество поглощаемой воды является найбольшим при разведении спермы водопроводной водой; та же самая картина наблюдается при разведении спермы водой солёностью 1‰. Количество поглощаемой воды уменьшается по мере увеличения солёности и стабилизируется в растворах солёностью ок. 5‰ и большей.

Address:

Recived: 25 VI 1976 r.

Prof. dr hab. Aleksander Winnicki, dr Lucjan Tomasik
Instytut Ichtiologii AR
71–550 Szczecin, ul. Kazimierza Królewicza 4
Polska – Poland