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TEMPORAL CHANGES IN MOTILITY PARAMETERS OF DACE *LEUCISCUS LEUCISCUS* (L.) SPERM OBTAINED FROM SPERMATIC DUCTS AND DIRECTLY FROM TESTICLES*

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Abstract

A Computer Assisted Sperm Analysis system, CASA, enables determination of numerous parameters characterizing sperm motion activity. This system allows for the examination of the effect of various environmental factors on spermatozoa motility parameters. The aim of this work was to compare time-dependent motility changes of sperm obtained from the dace, *Leuciscus leuciscus* (L.), by abdominal massage (sperm from spermatic ducts) and directly from gonads (testicular sperm). The analysis concerned such sperm motility parameters as: percentage of motile sperm (MOT, %), total sperm velocity (VAP, μm s^{-1}), straight line velocity (VSL, μm s^{-1}), curvilinear velocity (VCL, μm s^{-1}), linearity (LIN: VSL/VCL · 100%), straightness (STR: VSL/VAP · 100%), amplitude of the lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz). During 105 seconds of motility, no significant differences were found in the values of MOT between sperm originating from the spermatic ducts and from the testicles. Changes in MOT were only observed during seconds 120–135 of movement, when significantly higher values were found for testicular milt. Significant higher sperm velocities (VAP, VCL, VSL) at the 15s from activation were observed in the sperm originated from spermatic duct. On the other hand 120s after activation of movement, values of sperm velocity (VAP and VCL) of milt originating from spermatic duct significantly decreased in comparison to testicular milt. Our data showed that sperm obtained from spermatic duct have initially higher sperm motility speed compared to that obtained directly from the testis. However testicular sperm are able to swim longer than sperm obtained from spermatic duct.

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ZMIANY W CZASIE PARAMETRÓW RUCHU PLEMNIKÓW JELCA *LEUCISCUS LEUCISCUS* (L.) POZYSKANYCH Z NASIENIOWODÓW ORAZ BEZPOŚREDNIO Z JĄDER

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Słowa kluczowe: jelec, *Leuciscus leuciscus* (L.), CASA, mlecz nasieniowodowy, mlecz jądrowy.

Abstrakt

System komputerowy CASA (*Computer Assisted Sperm Analysis*) umożliwia oznaczanie wielu parametrów charakteryzujących motorykę ruchu plemników. Umożliwia badanie wpływu na ruchliwość plemników różnych czynników środowiskowych, jak również pozwala na szczegółową charakterystykę toru ich ruchu. Celem pracy było porównanie zmian w czasie parametrów ruchu plemników pozyskanych od jelca *Leuciscus leuciscus* (L.) za pomocą masażu powłok brzusznych (nasienie z nasieniowodów) oraz bezpośrednio z gonad (nasienie jądrowe). Analizowano takie parametry ruchu plemników jak: odsetek plemników ruchliwych (MOT, %), całkowita prędkość plemnika (VAP, μm s^{-1}), prędkość ruchu prostoliniowego (VSL, μm s^{-1}), prędkość ruchu krzywoliniowego (VCL, μm s^{-1}), liniowość ruchu (LIN: VSL/VCL · 100%), prostoliniowość ruchu (STR: VSL/VAP · 100%), amplituda odchyleń główki (ALH, μm) oraz częstotliwość uderzeń witki (BCF, Hz). W czasie 105 sekund obserwacji pomiaru parametrów ruchu nie stwierdzono istotnych różnic w wartościach MOT między plemnikami z mlecza pochodzącego z nasieniowodów a plemnikami pochodzącymi z jąder. Zmiany zaobserwowano dopiero w 120–135 sekundzie trwania ruchu, a istotnie wyższe wartości stwierdzono w mleczu jądrowym. W wartościach parametrów VAP, VCL i VSL również zaobserwowano istotne zmiany prędkości plemników w zależności od czasu po aktywacji. W 120 sekundzie trwania ruchu wartości prędkości plemników (VAP i VCL) mlecza nasieniowodowego, w porównaniu z mleczem jądrowym istotnie się obniżyły. W przeprowadzonych badaniach wykazano, że plemniki pozyskane z nasieniowodów charakteryzują się wyższą prędkością w porównaniu z plemnikami pozyskanymi bezpośrednio z jąder. Jednakże ruch plemników jądrowych trwać może dłużej niż plemników nasieniowodowych.

Introduction

In the 1990s, with the development of computer technology, a new method of determining sperm motility emerged, consisting in applying a computer analysis of sperm motility, i.e. CASA (Computer Assisted Sperm Analysis). This system was initially used in clinical laboratories for determining male fertility. Afterwards, it was applied for assessment of mammalian sperm motility (FARREL et al. 1998, MOORE and AKHONDI 1996), and used also in research on the fish semen quality (CHRIST et al. 1996, KIME et al. 1996, 2001, RAVINDER et al. 1997). The CASA system not only allows for the objective

measurement of the percentage of motile spermatozoa, but also for determination of the movement trajectory, head displacement or beat cross frequency (RURANGWA et al. 2004). CASA also offers the possibility of determining sperm velocity, including straight line, curvilinear and total velocity. The ability to determine numerous parameters characterizing spermatozoa movement made it possible to establish the impact of compounds that are toxic for fish, including heavy metals or xenobiotics (KIME et al. 1996, CHYB et al. 2000, 2001, JARMOŁOWICZ et al. 2010), and the effect of hormonal stimulation on sperm motility parameters (CEJKO et al. 2011a, 2012), or changes in the quality of milt after short-term refrigerated storage (RAVINDER et al. 1997, KOWALSKI et al. 2004).

In recent years, the CASA system has been used in the diagnostics of milt originating from species of slightly lower economic significance, i.e. rheophilic fish (CEJKO et al. 2011a, 2012). The decreasing area of their occurrence has forced researchers to seek solutions aimed at supporting their native populations by the reproduction of these fish under controlled conditions, the rearing of juvenile forms and, consequently, carrying out restocking with the material produced. A starting point for the production of the stocking material is optimization of reproductive biotechnology (KREJSZEFF et al. 2008, CEJKO et al. 2011b, TARGOŃSKA et al. 2011) and for particularly endangered species, i.e. the barbel, *Barbus barbus* (L.), nase, *Chondrostoma nasus* (L.), or vimba, *Vimba vimba* (L.), determination of quality markers, including milt quality markers, which are of direct significance for its diagnostics (CEJKO et al. 2012, SAROSIEK et al. 2011).

The latest research indicates that the reproductive success of salmon males is determined by total sperm velocity (GAGE et al. 2004). It is also assumed that ALH (amplitude of the lateral head displacement) can be one of the milt quality markers. It was also found that in barbel males, the value of ALH parameters was reduced in time after hormonal stimulation (CEJKO et al. 2012). Also in the smelt, *Osmerus eperlanus* (L.), significant differences were observed in the values of the ALH parameter between gonad milt (obtained from testicles) and "full milt", i.e. that obtained from spermatic ducts (HLIWA et al. 2009).

As the hormonal stimulation of wild fish not always allows to obtain sperm from spermatic duct, the aim of this study was to compare the changes in time in motility parameters of dace sperm obtained from spermatic ducts and gonads.

Materials and Methods

Fish originating from the Department of River Fishery of the Warmia and Mazury University in Olsztyn were used as material ($n=3$). Semen was collected by gentle abdominal massage. After collecting the semen, the gonads

were removed and milt was collected after gonadal tissue maceration. After collecting the milt, samples were transported on ice (+4°C) to the Department of Gametes and Embryos Biology of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, where further analyses were carried out. In order to activate spermatozoa movement, a 0.5% NaCl solution with an addition of 2 mg ml^{-1} BSA was applied. The semen was diluted 200–400 times (depending on the original concentration), after which samples were placed on a microscopic slide. Sperm motility was analysed with the use of the CASA system, equipped in a black and white CCD camera, a microscope, a video and a computer with Hobson Vision software. The image of moving spermatozoa was recorded through a counter phase lens (10x magnification). The measurements were taken in 15-second intervals up to 135s after activation. The following sperm motility parameters were determined: percentage of motile sperm (MOT, %), total sperm velocity (VAP, μm s^{-1}), straight line velocity (VSL, μm s^{-1}), curvilinear velocity (VCL, μm s^{-1}), linearity (LIN: VSL/VCL · 100%), straightness (STR: VSL/VAP · 100%), amplitude of the lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz).

Results

In the first 15 seconds of movement, no statistically significant differences were observed in the values of MOT (46.3%) of sperm obtained from spermatic ducts in comparison to testicular sperm (49.7%), ($P>0.05$, Figure 1a). At the same time, sperm obtained from spermatic ducts was characterized by significantly higher values of VAP velocity (36.4 μm s^{-1}), VCL (41.7 μm s^{-1}) and VSL (28.1 μm s^{-1}) than sperm obtained from testicles (27.9; 34.3 and 20.5 μm s^{-1} for VAP, VCL and VSL, respectively), ($P<0.05$, Figure 1b–1d). Significant differences were also observed in the value of linearity (LIN) and straightness (STR) of sperm motility. The values of LIN of sperm from spermatic ducts oscillated about 60% while that of testicular sperm was around 50% ($P<0.05$, Figure 1e).

On the other hand, the values of STR were above 75% for sperm obtained from spermatic duct milt and 50% for sperm of testicular milt ($P<0.05$, Figure 1f). Within 30 seconds of movement, the values of ALH and BCF of sperm originating from testicles were significantly higher (2.3 μm s^{-1} for ALH and 1.47 Hz for BCF) in comparison to that from spermatic ducts (1.1 μm s^{-1} for ALH and 1.0 Hz for BCF) – Figure 1g, 1h). However, spermatozoa originating from testicles were characterized by significantly higher values of MOT in seconds 120–135 of movement as compared to spermatozoa originating from spermatic ducts ($P<0.05$; Figure 1b). The veloc-

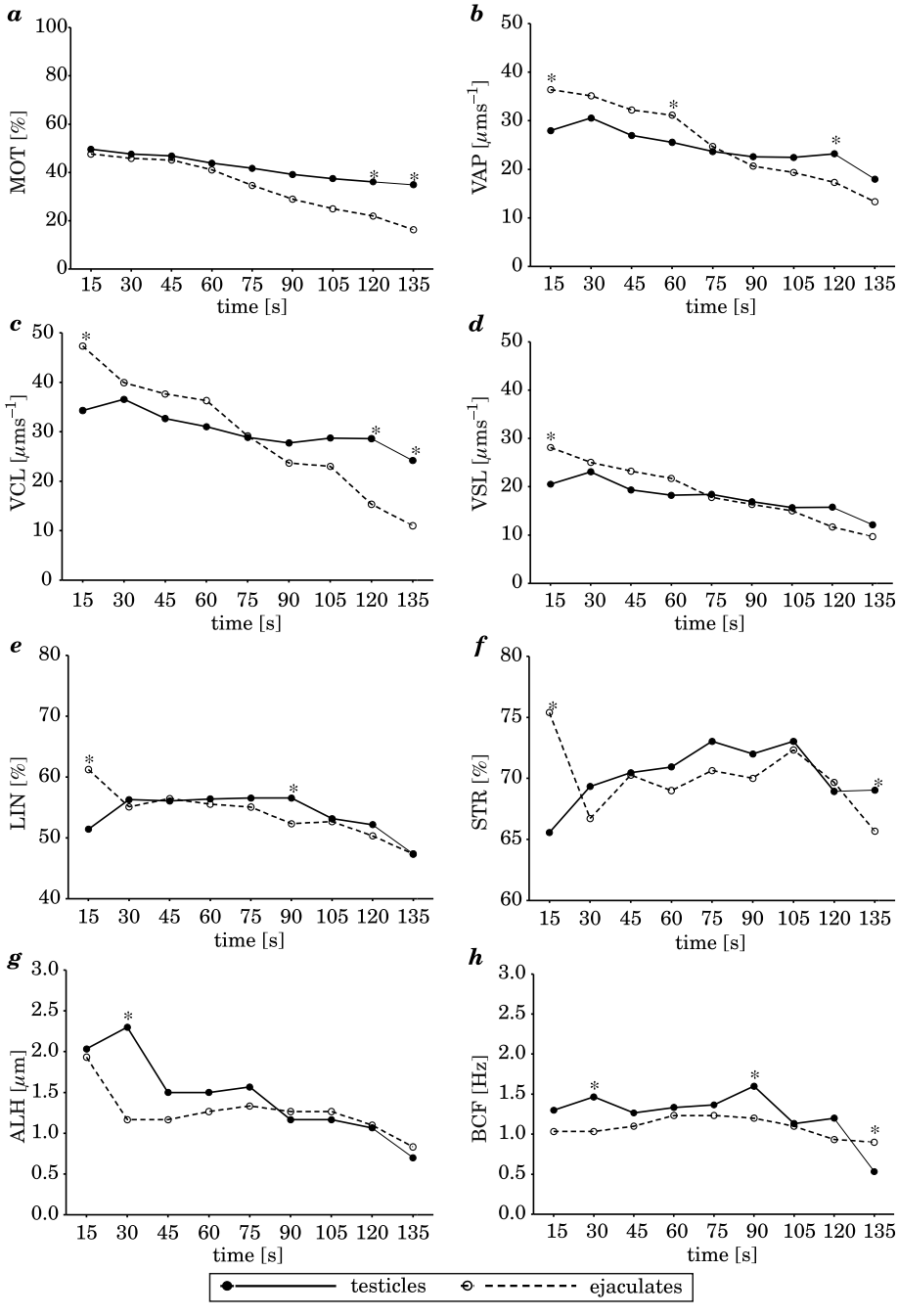

Fig. 1. Changes in the values of MOT (a), VAP (b), VCL (c), VSL (d), LIN (e), STR (f), ALH (g) and BCF (h) during the movement of dace *Leuciscus leuciscus* (L.) spermatozoa. Statistically significant differences in mean values of the analysed parameters are marked with an asterisk (n=3)

ity of spermatozoa from the milt obtained from spermatic ducts rapidly decreased at second 90 of movement (22.6; 27.7 and 15.4 μm s^{-1} for VAP, VCL and VSL, respectively), to finally, i.e. at second 135, evolve into the stage of vibration (17.9; 24.2 and 12.1 μm s^{-1} for VAP, VCL and VSL, respectively) – Figure 1b–1d.

Discussion

Sperm motility is one of the basic indicators of the quality of milt used in its diagnostics. Sperm movement corresponds with the reproductive strategy of a given species, and can last a few hours, e.g. in the Acipenseridae, several minutes in the Cyprinidae species, or a few seconds in the Salmonidae species (RURANGWA et al. 2004). Differences in values of specific sperm motility parameters have also been observed between closely-related species, i.e. rheophilic cyprinids of the genus Leuciscus (KOWALSKI et al. 2006). Consequently, the milt diagnostics on the basis of sperm motility was carried out for each species individually.

The mean initial velocity VCL of dace sperm (43 μm s^{-1}) was lower than the velocity of chub, *Squalius cephalus* (L.), sperm in which this parameter reached 70 μm s^{-1} (LAHNSTEINER et al. 2004) and carp, *Cyprinus carpio* (L.) sperm, with VCL between 80–90 μm s^{-1} (CHYB et al. 2001). The values of VCL were also lower than the values determined for the crucian carp, *Carassius carassius* (L.), in which the VCL velocity exceeded 50 μm s^{-1} (DIETRICH et al. 2003). Even lower values of the VCL parameter were found in gonadal milt (34 μm s^{-1}), which can indicate immaturity of the sperm in the testicles. It is interesting that our observations reveal that the values of dace sperm velocity can be definitely higher than the currently presented data. In the research on the effect of hormonal stimulation of the dace, we found that depending on the applied hormonal preparation, the velocity of dace sperm significantly differed. After stimulation with Ovopel [(D-Ala^6Pro^9 NEt)-mGnRH] + metaclopramide, the values of velocity were significantly lower (VCL: 96 μm s^{-1} and 76.5 μm s^{-1}) than the values after stimulation with LHRHa (VCL: 133 μm s^{-1} and VSL 107 μm s^{-1}), (CEJKO et al. 2011b). The lower values of the velocity could result from the individual variability and a different degree of sperm maturity.

The amplitude of lateral head displacement, i.e. the ALH parameter, can constitute an important quality indicator of milt. While analysing the motility of smelt sperm significantly lower values of ALH were found in gonadal milt as compared to milt from the spermatic duct (HLIWA et al. 2009). It was also found that 72 h after hormonal stimulation, the value of the ALH parameter decreased, which can be explained by sperm aging and loss of its fertilizing abilities (HLIWA et al. 2009). Similarly, in the case of barbel, *Barbus barbus* (L.),

a reduction in motility was observed with time after hormonal stimulation, including the ALH parameter, while a significant decrease was noted 36 h after stimulation (CEJKO et al. 2012). These results slightly differ from those previously presented, where ALH values amounted to: 0.9–1.1 μm (CEJKO et al. 2011a) and they indicate that dace sperm is rather similar to the crucian carp sperm (ALH about 3 μm) as regards the ALH parameter. Semen of the *Acipenseridae* (GLOGOWSKI et al. 2004; 10 μm), the *Salmonidae* (DIETRICH et al. 2005; 9–12 μm) and the *Percidae* (SAROSIEK et al. 2004, KOWALSKI et al. 2004, 6–15 μm) is characterized by significantly higher values of ALH. It should be emphasized that during the movement, the values of ALH decrease at a faster rate during the first 30 seconds (milt from spermatic ducts) and 45 seconds (milt from testicles). A slower decrease in the ALH parameter of testicular sperm, as compared to sperm from the spermatic ducts, can be related to their lower initial velocity, which makes them lose their energy reserves at a slower rate. Despite the sperm obtained directly from the testis is able to maintain their motility for longer time, it is not necessarily beneficial for the reproduction. Eggs of dace are able to be fertilized till 90s after contact with water (KUCHARCZYK, unpublished data). Therefore longer motility observed in sperm obtained from testis might not bring benefits in term of fertilization success.

The results presented indicated certain differences in the motility activity of dace sperm originating from spermatic ducts and from gonads. Spermatozoa originating from spermatic ducts are characterized by faster initial movement and a quite high rate of losing the ability to move (a sprinter type), while spermatozoa originating from gonads are characterized by lower initial velocities and a slower decrease of those parameters during the movement (a marathoner type). Those differences could result from immaturity of testicular spermatozoa. In view of the fact that for fish, a parameter determining reproductive success is sperm velocity (GAGE et al. 2004), the fertilizing capacity of testicular spermatozoa might be lower, although they move for longer time.

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