Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 16	Fasc. 2	179-188	2008

QUALITY PARAMETERS AND SELECTED BIOCHEMICAL MARKERS OF ASP, *ASPIUS ASPIUS* (L.), SEMEN OBTAINED AFTER HORMONAL STIMULATION WITH OVAPRIM OR OVOPEL

Beata Irena Cejko, Dariusz Kucharczyk**, Katarzyna Targońska**, Dorota Kubiak*, Beata Sarosiek*, Jan Glogowski *,****

*Departament of Molecular Andrology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, Poland
**Chair of Lake and River Fisheries, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Poland
***Chair of Ichthyology, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. The aim of the study was to determine the basic quality parameters and selected biochemical markers of asp, *Aspius aspius* (L.), semen after spermiation was stimulated with Ovaprim and Ovopel. Sperm motility and concentration, osmotic pressure, total protein content, and activities of acid phosphatase (AcP), lactate dehydrogenase (LDH), and β-N-acetylglucosaminidase (β-NAG) were determined. It was revealed that higher sperm motility and concentration and higher seminal plasma protein content were obtained after stimulation with Ovaprim. The osmotic pressure of the seminal plasma estimated for males following the administration of Ovopel was higher than after they had been treated with Ovaprim. It was determined that enzyme activity in the seminal plasma of the fish stimulated with Ovaprim was higher in comparison with results obtained after they had been treated with Ovopel. Significant, positive dependencies were confirmed between the concentration of sperm and the total protein content in the seminal plasma (r^2 = 0.492) and the activity of β-NAG (r^2 = 0.779); among the total protein content in the seminal plasma and the activities of AcP (r^2 = 0.476), LDH (r^2 = 0.564), and β-NAG (r^2 = 0.738); and between the activities of AcP and LDH (r^2 = 0.483) and between the activities of LDH and β-NAG (r^2 = 0.844).

Key words: ASP, SEMEN, SEMINAL PLASMA, OVAPRIM, OVOPEL

INTRODUCTION

Asp, *Aspius aspius* (L.), is a freshwater, rheophilous cyprinid fish that primarily inhabits running waters (Mamcarz 2000). Formerly this species played an important role in Polish inland fisheries; however, due to river pollution and a lack of fisheries

CORRESPONDING AUTHOR: Beata I. Cejko, Instytut Rozrodu Zwierząt i Badań Żywności PAN, Zakład Andrologii Molekularnej, ul. Tuwima 10, 10-747 Olsztyn. Tel. +48 89 5357429, e-mail: bcejko@poczta.onet.pl

exploitation, it is not now economically significant and its ecological importance is underestimated. Over the past decade there has been increasing interest in rheophilous fish, including the asp, which is the only predator among Poland's indigenous cyprinid species (Babiak et al. 1998, Kujawa et al. 1997, 1999). This is linked to the necessity of taking protective measures regarding, among other things, developing biotechniques for artificial fish reproduction,the production of stocking material or the cryopreservation of semen. While maintaining biodiversity, this renders it easier to introduce species to riverine environments based on technologies of artificial spawning and the rearing of stocking material. This also refers to the aquaculture of asp.

The quality of progeny is shaped by parental characteristics such as the age and weight of spawners or the quality of their gametes, which ensures that the reproductive process and the further growth of juvenile stages both proceed accordingly (Morawska and Żuromska 1989). The quality of milt is determined based on the basic parameters of motility and sperm concentrations (Kruger et al. 1984). Semen is also assessed by determining protein content, the osmotic pressure, or the activities of seminal plasma enzymes. The latter give more detailed information regarding the correct development of the testis, the usefulness of the milt for fertilization, and the effects of long-term storage.

Obtaining stocking material of various species, including rheophilous ones, requires conducting spawning under controlled conditions and the application of natural or artificial substances to stimulate both ovulation and spermiation (Kucharczyk et al. 1999, Szabó et al. 2002). One of these is Ovopel, a substance with two active ingredients: mammalian hypothalamus hormone analogue ((D-Ala^6, Pro^9 NEt)-mGnRH) and metoclopramide, a water-soluble pituitary dopamine receptor antagonist (Horváth et al. 1997). This complex is used in the controlled reproduction of both cyprinids such as carp, *Cyprinus carpio* L. (Brzuska and Białowąs 2002), tench, *Tinca tinca* (L.), and herbivorous fish (Horváth et al. 1997) as well as non-cyprinids like European catfish, *Silurus glanis* L. (Brzuska 2003), or perch, *Perca fluviatilis* L. (Kucharczyk et al. 1998).

Ovaprim is a complex, synthetic substance containing salmon hypothalamus hormone analogue ((D-Arg^6, Pro^9 NEt)-sGnRH) and domperidone, a dopamine receptor antagonist. This substance has been applied under controlled conditions in the culture of carp (Brzuska and Adamek 1997), ide, *Leuciscus idus* (L.) (Kucharczyk et al. 2007), European catfish (Brzuska i Adamek 1999), as well as pike, *Esox lucius* L. (Szabó 2003).

The aim of the study was to determine the basic quality parameters of asp semen obtained when spermiation was stimulated with the Ovaprim and Ovopel complexes. The protein content, osmotic pressure, and activities of selected enzymes in the semen were also determined.

MATERIALS AND METHODS

FISH MANIPULATION AND SPERM COLLECTION

The fish were caught in the fall of 2006 in the Pierzchały Dam Reservoir located in northern Poland on the Pasłęka River. Then they were transported to the Czarci Jar Stocking Center (near Olsztyn), where they inhabited earthen ponds until early spring. Fish body weight ranged from 2.1 to 4.7 kg. After they were transported to the hatchery in Olsztyn (Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn), the males were held in basins that permitted controlling thermal conditions. The volume of each basin was $1m^3$ and the water temperature was set to 10°C. After the fish had adapted for two days, the water temperature was gradually increased to 11°C, and after another 24 hours, it was again increased to 12°C.

The fish were divided into two groups from which semen would be obtained. The fish from group I were treated with Ovaprim (Syndel International Inc., Canada), while group II received Ovopel (Unitrade, Hungary) (Table 1). During manipulation (i.e., during spawner examination and when collecting semen) the fish were anesthetized with a solution of 2-phenoxyethanol (Mercx) at a dosage of 0.5 ml l^{-1} water.

TABLE 1

Compounds administered to stimulate spermiation in male asp with dosage, administration method, and the period within which semen was obtained

Drugs stimulating spermiation	Fish group	Dosage	Admin. method	Period within which semen was obtained
Ovaprim ((D-Arg^6, Pro^9 NEt)-sGnRH)	group I (n = 6)	0.25 ml kg^{-1} b.w.	peritoneally	48 h
Ovopel ((D-Ala^6, Pro^9 NEt)-mGnRH)	group II (n = 6)	1/2 granule kg^{-1} b.w.	peritoneally	48 h

ANALYTICAL PROCEDURES

After the semen had been stripped with abdominal massage, sperm motility was determined subjectively under a microscope at a magnification of 400x. A solution of

0.5% NaCl was applied to activate the sperm, and the values determined are reported in percentages. The sperm concentration was determined with spectrophotometry (Ciereszko and Dąbrowski 1993) at a wavelength of 530 nm. In order to determine osmotic pressure, protein, and the selected enzymes of the seminal fluid, the semen was centrifuged for 10 min at 10,000 x g. The plasma collected was stored at a temperature of -70°C until analysis. Protein content was determined (mg ml^{-1}) with the method in Lowry et al. (1951), and seminal plasma osmotic pressure (mOsm kg^{-1}) was measured with a WESCOR® Vapor Pressure 5520 osmometer. The activity of selected enzymes in the seminal plasma was determined with various methods (i.e., acidic phosphatase (AcP) was determined with the method by Bessey et al. (1946), lactate dehydrogenase (LDH) with that of Vassault (1983), and β-N-acetylglucosaminidase (β-NAG) with the method described by Farooqui and Srivastava (1980). The results of the measurements of activity are presented in U l^{-1}. Due to the small volume of semen, the AcP activity in the group stimulated with Ovopel was determined for five specimens, while LDH activity in the group stimulated with Ovaprim was determined for five specimens and with Ovopel for four specimens.

STATISTICAL ANALYSIS

The results were analyzed statistically with the GraphPad Prism 4 Demo program (GraphPad Software, Inc., CA, USA). Pearson's linear correlation was used to identify dependencies among characteristics in samples from both groups combined. The significance of the studied groups for given characteristics was verified with the t-test ($P < 0.05$).

REUSLTS

Greater motility, higher sperm concentrations, and higher protein contents in the seminal plasma were determined in the semen samples obtained following hormonal stimulation with Ovaprim. After the males were administered Ovopel, the osmotic pressure of the seminal plasma was confirmed to be higher at 291 ± 19.1 mOsm kg^{-1} (Table 2). No significant differences were confirmed between the fish groups or the characteristics studied (t-test, $P > 0.05$). A positive dependency was identified between sperm concentration and total protein content in the seminal plasma ($r^2 = 0.492$; Fig. 1a).

Higher enzyme activity parameters were noted in the semen of fish stimulated with Ovaprim (Table 2); however, no statistically significant differences were noted among

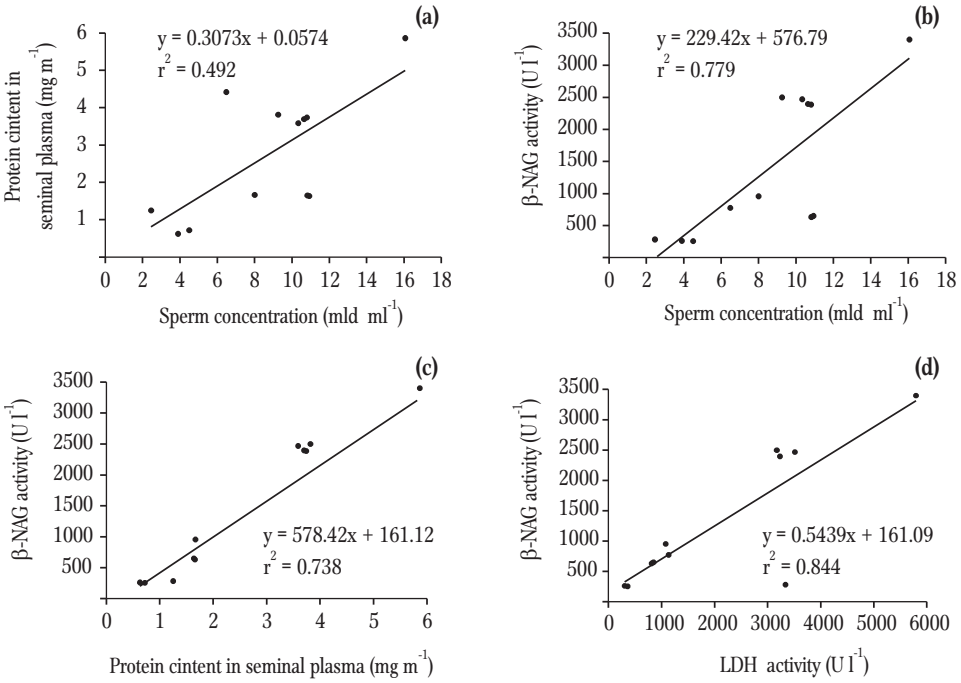

Fig. 1. Relation between sperm concentration and protein content in seminal plasma (a) and β-NAG activity (b) as well as between β-NAG activity and protein content in seminal plasma (c) and LDH activity (d).

the studied groups of fish. This was likely due to the size of the sample. If a greater number of fish had been analyzed, such average differences between the studied groups would be significant.

TABLE 2

Quality parameters of asp semen and the activity of selected enzymes in the seminal plasma obtained from fish stimulated with Ovaprim or Ovopel

Compounds stimulating spermiation	Statistical measure	Sperm motility (%)	Sperm concentration (million ml^{-1})	Total protein in seminal plasma (mg ml^{-1})	Seminal plasma osmotic pressure (mOsm kg^{-1})	AcP activity (U l^{-1})	LDH activity (U l^{-1})	β-NAG activity (U l^{-1})
Ovaprim	$\bar{x}$	61.67	10.65	3.50	279.0	9.41	2545.56	1739.55
	SD	19.41	3.13	1.64	11.8	9.19	1988.24	1200.82
	N	6	6	6	6	6	5	6
Ovopel	$\bar{x}$	40.00	6.72	1.95	291.1	3.74	1662.35	1091.57
	SD	25.30	3.60	1.42	19.1	4.24	1511.68	1043.20
	N	6	6	6	6	5	4	6

$\bar{x}$ - *mean value, SD – standard deviation, N – number of samples*

A significant and positive dependency was noted between sperm concentration and β-NAG activity (r^2 = 0.779; Fig. 1b) as well as among β-NAG activity and the protein content of the seminal plasma (r^2 = 0.738; Fig. 1c) and LDH activity (r^2 = 0.844; Fig. 1d).

DISCUSSION

In cyprinids, the mean concentration of sperm fluctuates around 11 million ml^{-1} for bream, *Abramis brama* (L.), (Glogowski et al. 1999), 15-17 million ml^{-1} for vimba, *Vimba vimba* (L.) (Hliwa et al. 2003) to 18 million ml^{-1} for carp (Sikra and Linhart 1987). In the current study, the value of this parameter was approximately 7 million ml^{-1} for the fish stimulated with Ovopel and approximately 11 million ml^{-1} for the group stimulated with Ovaprim. In a study by Babiak et al. (1998), in which the fish were hormonally stimulated with natural compounds (pituitary homogenate + human chorion thyrotropin), the asp sperm concentration in the semen obtained was 6.2 million ml^{-1}. The results of a study by Kowalski et al. (2003) of the proteolytic activity of semen plasma of ten fish species indicated that the sperm concentration of rheophilous fish ranged from 5.67 million ml^{-1} in ide to 15.26 million ml^{-1} in European chub, *Leuciscus cephalus* (L.).

The period of sperm motility depends primarily on the reproductive strategy of the given species. The injection of synthetic gonadoliberin stimulates the secretion of gonadotropin and spermiation (Donaldson 1996). Sperm motility is also dependent on the dosage and number of times the substance stimulating milt release is administered. In the current study, stimulation with the chosen complex was administered once (Table 1), and better motility was noted in the asp semen samples following the administration of Ovaprim.

Among the parameters that characterize semen quality, seminal plasma osmotic pressure is the most significant factor in the activation of sperm motility in cyprinid fish (Morissa et al. 1983). The osmotic pressure of the asp seminal plasma exceeded 270 mOsm kg^{-1} (Table 2), and this value of this parameter was higher in the fish that had been stimulated with Ovopel (291 mOsm kg^{-1}). It is worth adding that the variability in seminal plasma osmotic pressure might be caused by hormonal stimulation (Alavi and Cosson 2006), and this could have been reflected in the results of the current study. However, there is a lack of more complete information regarding the impact of

hormonal stimulation on this parameter of semen quality. Nevertheless, it should be emphasized that in other rheophilous fish such as vimba bream, ide, or European chub, the seminal plasma osmotic pressure was decisively lower than that noted in asp (Hliwa et al. 2003, Kowalski et al. 2003).

The principle organic component of seminal plasma is protein, but in comparison to that of mammals, fish semen is protein poor. The protein content noted in the fish stimulated with Ovaprim (3.50 mg ml^{-1}) was nearly two-fold of that in specimens that had been stimulated with Ovopel (1.95 mg ml^{-1}). A significant, positive correlation was also noted between the protein content of seminal plasma and sperm concentration ($r^2 = 0.492$, $P < 0.05$); this could be evidence that the origin of the protein from dead or damaged sperm.

The enzymatic activity of the seminal plasma characterizes the quality of the milt, the fertilization ability of sperm, and the usefulness of it for cryopreservation (Glogowski and Strzeżek 1980, Ciereszko et al. 1992). Higher activities of the studied enzymes were detected in the samples from fish that had been stimulated with the salmon analogue (Ovaprim). AcP activity in the fish stimulated with Ovaprim was 9.41 U l^{-1}, while it was 3.74 U l^{-1} in those administered Ovopel. AcP activity was noted to be highly variable in both groups of fish (Table 2).

The role of β-NAG is related to the structure of the acrosome in mammalian and ascipenserid sperm. Along with acrosin, this organelle plays a role in the sperm penetration of the oocyte while also counteracting polyspermia (Lambert et al. 1997). High activity levels of this enzyme in asp seminal plasma, in which sperm are devoid of this structure, was surprising and prompts undertaking further study with the aim of clarifying its role in fish sperm that do not have acrosomes. The small sample size in which this enzyme was determined did not permit drawing unequivocal conclusions regarding the role of this enzyme in the fish species studied. The highest values of the correlation coefficient were noted between the activities of β-NAG and LDH as well as between those of β-NAG and the seminal plasma protein content.

The variability of the parameters determining semen quality prompt undertaking more probing study of each one in order determine the dependencies between them and to identify which is of key importance in shaping semen quality. Since there is little information in the literature regarding the biochemical characteristics of the semen of rheophilous fish, the analysis of the results presented herein are primarily of a cognitive character.

LITERATURE

Alavi S.M.H., Cosson J. 2006 – Sperm motility in fishes: (II) Effects of ions and osmolality: A review – Cell Biol. Int. 30: 1-14.

Babiak I., Glogowski J., Kujawa R., Kucharczyk D., Mamcarz A. 1998 – Cryopreservation of sperm from Asp *Aspius aspius* – Prog. Fish-Cult. 60: 146-148.

Bessey O.L.A., Lowry O.H., Brock M.J.Z. 1946 – A method of the rapid determination of alkaline phosphatase with five cubic millimeters of serum – J. Biol. Chem. 164: 321-325.

Brzuska E. 2003 – Artificial propagation of European catfish (*Silurus glanis*): application of a single dose of pellets containing D-Ala^6, Pro^9 NEt-mGnRH and dopamine inhibitor metoclopramide to stimulate ovulation in females of different body weight – Czech J. Anim. Sci. 48: 152-163.

Brzuska E., Adamek J. 1997 – Attempts to use Ovaprim to stimulate ovulation in female carp – Komun. Ryb. 1: 19-21 (in Polish).

Brzuska E., Adamek J. 1999 – Artificial spawning of European catfish, *Silurus glanis* L: stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary extract – Aquacult. Res. 30: 59-64.

Brzuska E., Białowąs H. 2002 – Artificial spawning of carp, *Cyprinus carpio* (L.) – Aquacult. Res. 33: 753-766.

Ciereszko A., Glogowski J., Strzeżek J. Demianowicz W. 1992 – Low stability of aspartate aminotransferase activity in boar semen – Theriogenology 37: 1269-1281.

Ciereszko A., Dąbrowski K. 1993 – Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using spectrophotometric technique – Aquaculture 109: 367-373.

Donaldson E.M. 1996 – Manipulation of reproduction in farmed fish – Anim. Reprod. Sci. 42: 381-392.

Farooqui A.A., Srivastava P.N. 1980 – Isolation of β-N- acetylhexoaminidase from rabbit semen and its role in fertilization – Biochem. J. 191: 827-834.

Glogowski J., Strzeżek J. 1980 – Molecular forms of alkaline phosphatase of ram seminal plasma – some properties and changes in pathological processes of reproductive organs – Anim. Reprod. Sci. 3: 307-323.

Glogowski J., Babiak I., Kucharczyk D., Łuczyński M., Piros B. 1999 – Some properties of bream *Abramis brama* L. sperm and its cryopreservation – Aquacult. Res. 30: 765-772.

Hliwa P., Król J., Kowalski R., Glogowski J. 2003 – Quality and biochemical markers in the semen of vimba bream, *Vimba vimba* (L.) – In: Predatory fish. Reproduction, rearing, prophylactics (Eds.) Z. Zakęś., K. Demska-Zakęś., T. Krzywosz., J. Wolnicki, Wyd. IRS, Olsztyn: 193-199 (in Polish).

Horváth L., Szabó T., Burke J. 1997 – Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species – Pol. Arch. Hydrobiol. 44: 221-226.

Kowalski R., Glogowski J., Kucharczyk D., Goryczko K., Dobosz S., Ciereszko A. 2003 – Proteolytic activity and electrophoretic profiles of proteases from seminal plasma of teleosts – J. Fish Biol. 63: 1008-1019.

Kruger J. C., De W., Smit G.L., Van Vuren J.H.J., Ferreira J.T. 1984 – Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreochromis mossambicus* (Peters) – J. Fish Biol. 24: 263-272.

Kucharczyk D., Szczerbowski A., Łuczyński M.J., Kujawa R., Mamcarz A., Skrzypczak A., Wyszomirska E., Szabó T. 1998 – Reproduction of perch (*Perca fluviatilis* L.) with Ovopel stimulation – In: Hatcheries 1997-1998 (Ed.) J. Waluga, Wyd. IRS, Olsztyn: 101-104 (in Polish).

Kucharczyk D., Kujawa R., Mamcarz A., Wyszomirska E., Ulikowski D. 1999 – Artificial spawning of ide (*Leuciscus idus*) under controlled conditions – EJPAU 2: 1-7.

Kucharczyk D., Borejko A., Targońska K., Rożek W., Chwalczyk R., Kowalski R., Glogowski J. 2007 – Impact of Ovaprim on ide (*Leuciscus idus*) reproduction – In: Reproduction, rearing, prophylactics of lacustrine fish and other species (Eds.) J. Wolnicki., Z. Zakęś., R. Kamieński, Wyd. IRS, Olsztyn: 31-35 (in Polish).

Kujawa R., Mamcarz A., Kucharczyk D. 1997 – Effect of temperature on embryonic development of asp (*Aspius aspius* L.) – Pol. Arch. Hydrobiol. 44: 139-143.

Kujawa R., Kucharczyk D., Mamcarz A. 1999 – A model system for keeping spawners of wild and domestic fish before artificial spawning – Aquacult. Eng. 20: 85-89.

Lambert C., Goudeau H., Franchet C., Lambert G., Goudeau M. 1997 – Ascidia polyspermy by two independent mechanism: one at the egg plasma membrane volving the follicle cells – Mol. Reprod. Dev. 48: 137-143.

Lowry O.H., Rosenbrough N.J., Farr A.R., Randall K.J. 1951 – Protein measurement with the Folin phenol-reagent – J. Biol. Chem. 193: 265-275.

Mamcarz A. 2000 – Asp – In: Freshwater Fish of Poland (Ed.) M. Brylińska, PWN, Warszawa: 294-300 (in Polish).

Morawska B., Żuromska H. 1989 – Quality of carp spawners and progeny abundance and quality. Impact of maternal and paternal factors on the quantity and quality of hatch – Gosp. Ryb. 2: 3-5 (in Polish).

Morisawa M., Suzuki K., Shimizu H., Morisawa S., Yasuda K. 1983 – Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes – J. Exp. Biol. 107: 95-103.

Szabó T., Medgyasszay C., Horváth L. 2002 – Ovulation induction in nase (*Chondrostoma nasus*, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone – Aquaculture 203: 389-395.

Szabó T. 2003 – Ovulation induction in northern pike *Esox lucius* L. using different GnRH analogues, Ovaprim, Dagin and carp pituitary – Aquacult. Res. 34: 479-486.

Sikra O., Linhart O. 1987 – The spermatocrit value as a parameter of sperm concentration in some fish – Bull. VÙRH Vodńany 2: 12-18.

Vassault A. 1983 – Lactate dehydrogenase. UV-method with pyruvate and NADH – In: Method in Enzymatic Analysis (Eds.) H.U. Bergmeyer – Verlag Chemie, Weinheim 13: 118-126.

Received – 11 July 2007

Accepted – 20 November 2007

STRESZCZENIE

WYZNACZNIKI JAKOŚCIOWE I WYBRANE PARAMETRY BIOCHEMICZNE NASIENIA BOLENIA *ASPIUS ASPIUS* (L.) POZYSKANEGO PO STYMULACJI HORMONALNEJ PREPARATAMI OVAPRIM LUB OVOPEL

Celem badań było określenie podstawowych wyznaczników jakościowych i wybranych parametrów biochemicznych nasienia bolenia, *Aspius aspius* (L.) po stymulowaniu spermacji preparatami Ovaprim i Ovopel (tab. 1). Określono ruchliwość i koncentrację plemników, ciśnienie osmotyczne, zawartość białka

ogólnego oraz aktywność fosfatazy kwaśnej (AcP), dehydrogenazy mleczanowej (LDH) i β-N-acetylogluk-ozoaminidazy (β-NAG). Wykazano że, wyższą ruchliwością, koncentracją plemników oraz zawartością białka w plazmie nasienia charakteryzowały się próby mlecza, które pozyskano po stymulacji hormonalnej Ovaprimem (tab. 2). Ciśnienie osmotyczne plazmy nasienia, które oznaczono dla samców po podaniu Ovopelu, była wyższa niż po podaniu rybom Ovaprimu. Stwierdzono, że aktywność enzymów w plazmie nasienia ryb stymulowanych za pomocą Ovaprimu była wyższa w porównaniu z rezultatami uzyskanymi po podaniu Ovopelu. Istotne i dodatnie zależności stwierdzono pomiędzy koncentracją plemników a zawartością białka ogólnego w plazmie nasienia (r^2 = 0,492) oraz aktywnością β-NAG (r^2 = 0,779); zawartością białka w plazmie nasienia a aktywnością AcP (r^2 = 0,476), LDH (r^2 = 0,564) i β-NAG (r^2 = 0,738) oraz pomiędzy aktywnością AcP a aktywnością LDH (r^2 = 0,483) i pomiędzy aktywnością LDH a aktywnością β-NAG (r^2 = 0,844) (rys. 1).