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Biochemical composition of the seminal plasma of *Schizothorax niger* in response to different doses of synthetic breeding hormone WOVA-FH

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Abstract
This study investigated the biochemical composition of milt of the snow trout *Schizothorax niger* in response to different doses of synthetic breeding hormone WOVA-FH. Five biochemical parameters which include glucose, triglycerides, cholesterol, total protein and urea were studied. Fishes were divided into 5 treatment groups and a control group and injected with the hormone @ 0.1, 0.2, 0.3, 0.4 and 0.5 ml Kg^{-1} body weight. Control was injected with propylene glycol. The results showed a significant rise in the levels of cholesterol, triglycerides and protein in all hormone treated groups where as a significant decrease in urea concentration was observed with the increasing dose of hormone. Glucose only showed a significant increase in groups treated with higher doses of hormone (T_3, T_4 and T_5).

Keywords: Biochemical composition, seminal plasma, *Schizothorax niger*, WOVA-FH

Introduction
Schizothorax niger Heckel, an important food fish of Kashmir belongs to the family Cyprinidae. Locally known as Alghad snowtrout the fish occurs in good numbers in water bodies of Kashmir like Jhelum, Dal Lake and Manasbal Lake. The fish in natural water bodies is facing immense pressure due to pollution and human interferences in its habitat. So there is a need of developing the sustainable ways to replace declining natural stocks of the fish and also increase the yield of production by producing the high quality fish seed. One of the useful ways to achieve the sustainable and good quality fish seed is through captive breeding or induced breeding, whereby ripe fish brooders are stimulated by natural or synthetic hormones or extracts to breed in captive conditions with environmental manipulation. Good quality sperm is the one which has the higher capacity to successfully fertilize oocytes and further develop into a normal embryo.

Fish semen or milt is a complex biological fluid composed of single type of cells-the spermatozoa, freely suspended in the seminal plasma. Milt production in a male is a result of spermatogenic process going on in testicular lobule. It is a mark of onset of maturation in male brooders. A male brooder in ripe condition is termed as milter. Seminal plasma is an important constituent of fish milt that has a vital role in sperm metabolism, function, survival and sperm motility. Its main role is to create an optimal environment for spermatozoa storage. Seminal plasma also benefits external fertilization by creating a favorable micro-environment for sperm movement. Any quantifiable physical parameter that directly correlates with the fertilizing ability of sperm could be potentially used as a measure of milt quality. There has been a drive towards measuring the parameters or biomarkers of milt quality that directly relates to the fertilizing ability of sperm. Such biomarkers of milt quality so far documented include spermatocrit value, sperm density, osmolarity and pH of seminal plasma, chemical composition of seminal plasma, enzymatic activity, adenosine triphosphate (ATP) concentration, motility, morphology and ultrastructure, fertilizing capacity and several others. The composition of seminal plasma has a great influence on the biological quality of the milt and these factors are directly related to the fertilization success [1]. Biochemical evaluation of seminal plasma is an important criterion for assessment of milt quality [2]. The biochemical composition of seminal plasma might be varied more or less widely within families [3]. Thus the knowledge of quantitative characteristics and chemical composition of sperm is a prerequisite for the successful evaluation of the reproductive ability of different fish species.

So to have a controlled and successful production in fish farming, it is important to have adequate knowledge of sperm characteristics and seminal plasma composition of fish [4].

Materials and methods

The experiment was carried out at the Fisheries Instructional Farm, Shuhama Alusteng of Faculty of Fisheries, SKUAST Kashmir (India) during the breeding season. 36 healthy male brooders were collected from the same farm. They were then transferred to separate ponds for 3 days and then acclimatized in holding tanks for 6-7 days. One week prior to hormone injection, the brooders were randomly divided into five experimental groups (T_1, T_2, T_3, T_4 and T_5) and a control group (T_0) depending upon the hormone dose to be administered. After that they were transferred to the breeding pools and kept under continuous flow of water. The fishes from groups T_1, T_2, T_3, T_4 and T_5 were then administered a single dose of the synthetic hormone (WOVA-FH) in different concentrations @ 0.1, 0.2, 0.3, 0.4 and 0.5 ml Kg^{-1} body weight respectively by trans-muscular injection into the dorsal muscle above the lateral line. Fishes from control group were injected with Propylene Glycol. Milt was collected from 28 brooders having an average weight of 248.37±4.16 grams. Brooders were hand stripped by applying slight pressure on the sides of the fish abdomen as per Richter *et al.* [5] and the semen was collected into graduated plastic tubes and used only if uncontaminated with water, blood, urine and faeces.

Seminal plasma was collected after centrifugation of the semen at 4000rpm for 10 min at room temperature (20°C) and stored in Eppendorf vials at -20°C for 3 days until the beginning of analysis. Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa. Biochemical parameters like glucose, cholesterol, triglyceride, total protein and urea were determined using spectrophotometer (systronic UV –VIS Spectrophotometer 117) and biochemical Kits procured from Coral clinical system. Glucose was estimated using Glucose Oxidase/Peroxidase method, cholesterol by cholesterol Oxidase/Phenol+Aminophenazone method, triglycerides by glycerophosphate oxidase-peroxidase method, urea by modified berthelot method and total protein by biuret method.

Data obtained was evaluated by one-way ANOVA and Duncan's multiple-range tests using SPSS v20 software. All data was expressed as Mean±SEM and the difference in means was assessed using Duncan's multiple-range tests. Differences were considered to be significant at $P \leq 0.05$.

Results and Discussion

The color of *Schizothorax niger* milt was evaluated visually, immediately after collection. The milt appeared creamy white throughout the experiment in all the treatment groups as well as in control group.

Glucose

The glucose content of seminal plasma is an important biochemical parameter; because it provides membrane protection to spermatozoa [6]. The presence of glucose in seminal plasma has been connected to the high energy demand of the testes during spermatogenesis and thus serves as an energy sources for sperm motility in fish [7, 8]. It has also been linked with the lipid synthesis of spermatozoa [8]. Thus different concentrations of glucose in fish seminal plasma could be related to differences in spermatozoa energy metabolism among fish species. Energy constituents like glucose of the seminal fluid play a very significant role in the activation of sperm [9]. In the current study the glucose was found in the range of 6.1-11.4 mg/dl across different treatment groups (Table 1). It was found varying between 6.1-9.1 mg/dl with a mean value of 7.43±0.88 mg/dl in control group T_0 whereas T_4 recorded a maximum mean glucose level of 9.65±0.41 mg/dl (range=8.4-11.4 mg/dl). Similar range of glucose level (8.2-12.2 mg/dl) in seminal plasma was also found by Khodadadi *et al.* [10] in Shirbot, *Barbus grypus*. This is also in confirmation with the studies carried out by various researchers [11-15] who reported less levels of glucose in the farmed carp. Verma *et al.* [16] also reported the low levels of glucose in cyprinids. Bastami [17] reported the slight higher glucose concentration of seminal plasma in wild common carp as 19.7 mg/dl. Significant increase in the glucose level was recorded in treatment groups T_3, T_4 and T_5 when compared with the control group. Similar increase in the glucose level after the application of carp pituitary gland extract and hCG treatment was reported by Seifi *et al.* [4] in wild carp. The increase of glucose level in seminal plasma can be connected to the high energy demand of the testes during spermatogenesis or for the lipid synthesis of spermatozoa [8]. In contrast Seifi *et al.* [4] reported a decrease in seminal plasma glucose level in farmed carp when fishes were treated with carp pituitary gland extract, ovaprim and hCG.

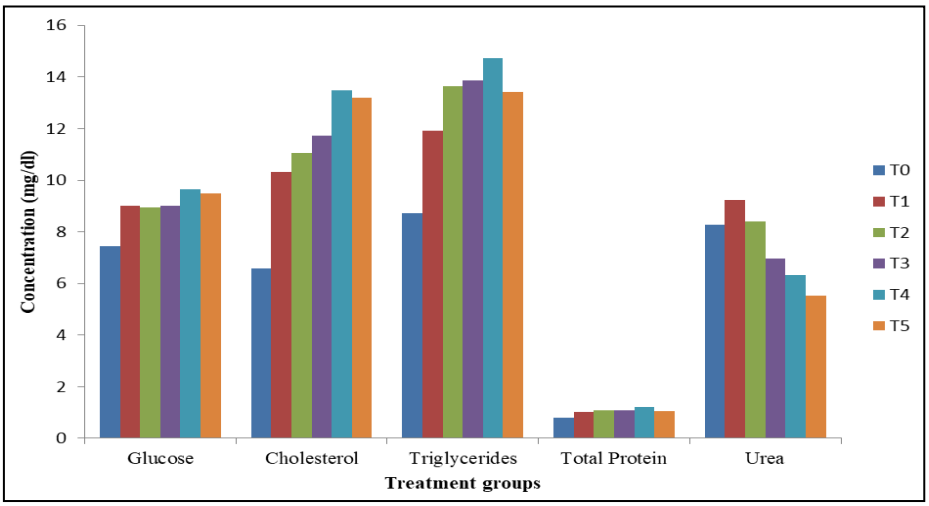

Fig 1: Comparison of different biochemical parameters of seminal plasma in different treatment groups

Cholesterol

There is insufficient information about the role of cholesterol in seminal plasma, inspite of its identification in the seminal plasma of freshwater fishes [2]. Cholesterol might have a protective effect against environmental changes (especially in temperature) that occur when fish semen is released [18]. In some studies the function of cholesterol in seminal plasma has been demonstrated with the aim to improve the sperm deep-freezing technique and composition of semen extenders [19]. In the present study the cholesterol level in seminal plasma varied from 4.68 to 16.34 mg/dl in individual fishes while as mean cholesterol level varied between 6.58±0.63 to 13.50±0.68 mg/dl in control and T_4 groups respectively (Table 1). The results are in close confirmation with Bozkurt *et al.* [20] who found the cholesterol level in the seminal plasma of scale carp to vary from 4-14 mg/dl. Bozkurt *et al*. [18] also recorded the similar mean cholesterol level (12.02±1.18 mg/dl) in seminal plasma of Grass carp, *Ctenopharyngodon idella*. The level of cholesterol in the present investigation are also similar with the findings of Verma *et al.* [16] who recorded the mean cholesterol levels of 14.1±0.8, 17.4±1.7, 22.8±1.3, 15.4±0.6, 14.0±0.7 and 12.7±0.7 mg/dl in *Catla catla* (catla), *Labeo rohita* (Rohu), *Cirrhinus mrigala* (mrigal), *Labeo kalbasu* (Kalbasu), *Hypophthalmichthys molitrix* (Silver carp) and *Ctenopharyngodon idella* (Grass carp) respectively. Borges *et al.* [17] also reported similar cholesterol level of 13.9±0.9 mg/dl in seminal plasma of Jundia (*Rhamdia quelen*). Slightly higher cholesterol levels which varied between 21-29.3 mg/dl with mean levels of 25.71±2.99 mg/dl were observed in seminal Plasma of Shirbot, *Barbus grypus* [10]. In contrast Secer *et al.* [22] recorded very low levels of cholesterol (2.55±2.47 mg/dl) in *Oncorhynchus mykiss* semen while as very high levels (106.85 mg/dl) were recorded by Bastami *et al.* [17] in seminal plasma of wild carp. During present investigation it was found that cholesterol level in treatment groups T_4 and T_5 increased significantly when compared with the non treated group T_0. The results are in confirmation with those of Seifi *et al.* [4] who demonstrated that the level of cholesterol increased in the seminal plasma of wild carp when they were treated with sufficient dose of ovaprim, human chorionic gonadotropin (hCG) and carp pituitary gland (cPG). The higher levels of cholesterol in the T_4 and T_5 can be contributed to the fact that the administration of hormone WOVA-FH helps in the maturation of gonads as also reported by Diwan and Krishnan [23] who stated that the fluctuation of serum cholesterol in males and females of *Etroplus suratensis* is due to maturity.

Triglycerides

Seminal plasma lipids are generally associated with metabolism in spermatozoa [24]. Triglycerides serve as energy resources for spermatozoa during immotile storage and during the regeneration phase after motility [25]. In the present study the triglyceride level varied between 6.72-18.13 mg/dl in individual samples where as in different groups, mean triglyceride level was observed to vary from 8.72-13.42 mg/dl in control and treatment group T_5 respectively (Table 1). This is in confirmation with the earlier studies like the one by Verma *et al.* [16] who found the mean triglyceride level in *Labeo rohita*, *Cirrhinus mrigala* and *Labeo calbasu* to be 11.7±1.1, 19.4±0.1 and 15.0±1.22 mg/dl respectively. Bozkurt *et al.* [18, 20] also reported the similar triglyceride level in scale carp (14.58 mg/dl) and mirror carp (5-12 mg/dl) respectively. Borges *et al.* [21] also reported the mean triglyceride level to be 10.9±0.8 mg/dl in the seminal plasma of *Rhamdia quelen*. Aramli *et al.* [26] reported the triglycerides level as 15.2 mg/dl for Persian sturgeon, *Acipenser persicus*. The mean triglyceride level in the present study was slightly higher (8 mg/dl) than that recorded in *Onchorhynchus mykiss* [22]. Low levels of triglycerides were also found in the seminal plasma of cyprinids [27]. Low triglyceride levels have been reported to be an indicative of inadequate energy resources, reduced motility rate and fertilization capacity [27]. This variation in the level of triglycerides can be related to hormonal stimulation of spermiation along with other factors like environmental condition, frequency of stripping, sampling period and sampling methods [2, 28, 29] and also to contamination of sperm by urine [30]. In the current study a significant rise in the triglyceride levels was observed in all the groups treated with WOVA-FH when compared with the control.

Total Protein

Fish seminal plasma, in contrast to that of higher vertebrates, is characterized by a low total protein concentration, substantial mineral compounds [sodium (Na), potassium (K), calcium (Ca), magnesium (Mg)] and low concentrations of organic substances [18]. The specific role of protein in fish semen is unknown. It is believed to protect the spermatozoa against the oxidative damage (i.e transferring, superoxide dismutase), microbial attack (i.e, transferrin, anti protease), and premature activation [31]. White and Macleod [32] indicated that protein had a protective role in fish semen and a high protein concentration is considered as positive characteristic of fish semen. Some proteins are believed to have a key role in the motility of fish spermatozoa through buffering the seminal plasma which prolong the viability of spermatozoa [33]. Fishes have no accessory glands which contribute to the production of most seminal plasma proteins as in mammals [34], thus the low concentrations of seminal plasma proteins can be related to the absence of accessory glands in fish. In the present study seminal protein ranged from 0.69-1.34 mg/dl in the studied individuals. The least mean seminal protein level was found in the control group where it recorded a mean value of 0.80±0.03 mg/dl and the maximum was recorded in treatment group T_4 with a mean value of 1.22±0.05 mg/dl (Table 1). Present findings are in confirmation with the studies carried out by Verma *et al.* [16] who recorded a seminal protein concentration of non treated males as 0.60 and 0.80 mg/dl in silver carp and grass carp respectively. Khodadadi *et al.* [10] recorded a mean seminal protein concentration of 1.27±0.29 mg/dl in Shirbot, *Barbus grypus*. Lower protein concentration of 0.34 mg/dl was recorded by Bhatt *et al.* [35] in *Schizothorax plagiostomus.* In the current study a significant increase in the seminal protein concentration was observed in all the treatment groups administered with hormone WOVA-FH as compared to control. These findings are in agreement with the earlier findings [36] who also recorded higher protein contents in the seminal plasma of *Aspius aspius* obtained after hormonal stimulation with ovaprim and ovapel. They observed a two fold increase in seminal protein of the specimens treated with ovaprim. Increase in the seminal protein by the application of hormones like carp pituitary gland extract and hCG was also observed by Seifi *et al.* [4] in wild carp.

Urea

Urea contamination of semen usually results in reducing the sperm motility and fertilizing ability [37] which in turn causes the change in other semen parameters [38]. It is believed to have a relationship with protein metabolism and total proteins which contains N_2 [18, 20, 22]. In the present study, urea (mg/dl) was calculated immediately after milt collection and varied between 5.53±0.44 to 9.22±1.15 in different groups (Table 1). It was recorded lowest in treatment group T_5 and highest in T_1. This is in confirmation with the findings of Verma *et al.* [16] who recorded the urea levels as 5.0 mg/dl in seminal plasma of grass carp, *Ctenopharyngodon idella*. This was slightly higher (3.16 mg/dl) than the levels recorded in rainbow trout by Secer *et al.* [22] but very less than the findings of Bozkurt *et al.* [20] who reported the urea content in seminal plasma of scale carp as 24.45± 7.96 mg/dl. The urea content in the mirror carp was also reported to be in the higher range of 38-97 mg/dl by Bozkurt *et al.* [20]. Only in treatment group T_5, the urea level was significantly higher than the control group. It is understandable that the content of ammonia-N tends to be higher in the intensively cultured systems [39], which was not the case in the present study. This might be the reason for the decreased level of urea in the current study.

Conclusion

In summary, the results of the present study on biochemical composition of the seminal plasma of Schizothorax niger in response to different doses of Synthetic breeding hormone WOVA-FH provides us a knowledge of changes in the important biochemical components of milt which could be useful to determine the optical quality of milt to be used for fertilization purposes. This will also lead to more efficient gamete management and increased fry yields, and aid suitability of semen for cryopreservation.

Table 1: Mean±SEM of different biochemical parameters of the seminal plasma of *Schizothorax niger* in different treatment groups

	Glucose			Cholesterol			Triglycerides			Total Protein			Urea		
Treatment Group	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Min	Max	Mean±SEM	Min	Max	Mean±SEM
T0	6.10	9.10	7.43±0.88	4.68	8.49	6.58±0.63	6.72	11.54	8.72±0.70	0.69	0.92	0.80±0.03	6.48	13.65	8.28±1.09
T1	8.20	9.70	9.00±0.44	5.38	14.26	10.32±1.22	7.93	13.76	11.93±0.87	0.85	1.17	1.02±0.05	5.17	13.52	9.22±1.15
T2	8.10	9.60	8.95±0.34	6.18	14.25	11.06±1.16	12.53	14.52	13.66±0.29	0.92	1.21	1.08±0.05	7.13	9.82	8.40±0.45
T3	7.90	10.20	9.02±0.35	8.31	16.25	11.73±1.16	11.23	15.80	13.86±0.63	0.99	1.19	1.08±0.03	4.61	9.19	6.98±0.66
T4	8.40	11.40	9.65±0.41	11.41	16.34	13.50±0.68	11.38	18.13	14.73±0.88	1.02	1.34	1.22±0.05	3.09	7.82	6.33±0.69
T5	8.20	11.20	9.48±0.43	9.17	15.31	13.19±0.91	9.41	15.17	13.42±0.88	0.88	1.21	1.04±0.05	4.18	7.29	5.53±0.44
Total	6.10	11.40	9.07±0.21	4.68	16.34	11.06±0.54	6.72	18.13	12.72±0.43	0.69	1.34	1.04±0.03	3.09	13.65	7.46±0.37

References

1. Rurangwa E, Kime DE, Ollevier F, Nash JP. The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture. 2004; 234:1-28.
2. Billard R, Cosson J, Crim LW, Suquet M. Sperm physiology and quality. In: Brood Stock Management and Egg and Larval Quality (ed. by N.R. Bromage & R.J. Roberts), Blackwell Science, Oxford, UK, 1995, 25-52.
3. Baccetti B, Burrini AG, Callaini G, Gilbertini G, Mazzini M, Zerunian S. Fish germinal cells. I. Comparative spermatology of seven cyprinid species. Gamete Research. 1984; 10:373-396.
4. Seifi T, Imanpoor MR, Golpour A. The Effect of Different Hormonal Treatments on Semen Quality Parameters in Cultured and Wild Carp. Turkish Journal of Fisheries and Aquatic Sciences. 2011; 11:595-602.
5. Richter CJJ, Eding EH, Goos HJT, De-Leeuw R, Scott AP, Van-Oordt PGWJ. The effect of pimozide/LHRH-a and 17α-hydroxyprogesterone on plasma steroid levels and ovulation in the African catfish, *Clarias gariepinus*. Aquaculture. 1987; 63:157-168.
6. Maisse G. Cryopreservation of fish semen: a review. Proceedings of the Refrigeration Science and Technology Conference, Refrigeration and Aquaculture. Institute International du Froid, Paris, France. 1996, 443-467.
7. Stoss J. Fish gamete preservation and spermatozoan physiology. In: Fish physiology (Eds. Hoar, W.S., Randall, D.J. and Donaldson, E.M.), NewYork: Academic Press, 1983, 305-350.
8. Soengas JL, Sanmartin B, Barciela P, Aldegunde M, Rozas G. Changes in carbohydrate metabolism in domesticated rainbow trout *Oncorhynchus mykiss* related to spermatogenesis. Comparative Biochemistry and Physiology. 1993; 105:665-671.
9. Goodall JA, Blackshaw AW, Capra MF. Factors affecting the activation and deviation of motility of the spermatozoa of the summer whiting (*Sillago ciliata*). Aquaculture. 1989; 77:243-250.
10. Khodadadi M, Arab A, Jaferian A. A Preliminary Study on Sperm Morphology, Motility and Composition of Seminal Plasma of Shirbot, *Barbus grypus*. Turkish Journal of Fisheries and Aquatic Sciences. 2016; 16:947-951.
11. Hardy D, Audet C. Evaluation of plasma glucose as an indicator of mild chronic stress in brook charr (*Salvelinus fontinalis*). Bull Aquaculture Association Canada. 1990; 90:54-56.
12. Torres P, Tort L, DePauw N, Joyce J. Effects of stress and metal exposure on blood parameters and liver metabolism in rainbow trout. Special Publication. European Aquaculture Society. 1991; 14:312-313.
13. Cech JJ, Bartholow SD, Young PS, Hopkins TE. Striped bass exercise and handling stress in freshwater: physiological responses to recovery environment. Transactions of the American Fisheries Society. 1996; 125:308-320.
14. Santos MA, Pacheco M. Stress biomarkers recovery in clean water and secondary treated pulp mill effluent. Ecotoxicology and Environmental Safety. 1996; 35:96-100.
15. Svoboda M, Kouril J, Hama C, Kova J, Kala BP, Savina L, *et al.* Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre- and post-spawning period. Acta Veterinaria Brno. 2011; 70:259-268.
16. Verma DK, Routray P, Dash C, Dasgupta S, Jena JK. Physical and biochemical characteristics of semen and ultrastructure of spermatozoa in six carp species. Turkish Journal of Fisheries and Aquatic Sciences. 2009; 9:67-76.
17. Bastami K, Rezaei M, Haghparast S, Eghtesadi AP, Najafi HE, Khansari A. *et al.* A comparative study on the biochemical parameters of seminal and blood plasma and

their correlation in the wild common carp (*Cyprinus carpio*). Comparative Clinical Pathology. 2012; 21:781-784.
18. Bozkurt Y, Ogretmen F, Ercin U, Yildiz U. Seminal plasma composition and its relationship with physical spermatological parameters of grass carp (*Ctenopharyngodon idella*) semen with emphasis on sperm motility. Aquaculture Research. 2008; 39:1666-1672.
19. Beer-Ljubic B, Aladrovic J, Marenjak TS, Laskaj R, Majic-Balic I, Milinkovic-Tur S. Cholesterol concentration in seminal plasma as a predictive tool for quality semen evaluation. Theriogenlogy. 2009; 72:1132-1140.
20. Bozkurt Y, Ogretmen F, Secer FS. Effect of different extenders and storage periods on motility and fertilization success of Grass Carp (*Ctenopharyngoden idella*) sperm during spawning season. Journal of Agricultural Sciences. 2009; 15:277-284.
21. Borges A, Scotti LV, Siqueira DR, Jurinitz DF, Wasswemann GF. Biochemical composition of seminal plasma and annual variations in semen characteristics of jundia *Rhamdia quelen* (Quoy and Gaimard, Pimelodidae). Comparative Biochemistry and Physiology. 2007; 31:45-53.
22. Secer S, Tekin N, Bozkurt Y, Bukan N, Akçay E. Correlation between biochemical and spermatological parameters in rainbow trout (*Oncorhynchus mykiss*) semen. Israeli Journal of Aquaculture-Bamidgeh. 2004; 56:274-280.
23. Diwan AD, Krishnan L. Levels of cholesterol in blood serum and gonads in relation to maturation in *Etroplus suratensis.* Indian Journal of Fisheries. 1986; 33:241-245.
24. Piironen J. Composition and cryopreservation of sperm from some Finnish freshwater teleost fish. Finnish Fish Research. 1994; 15:27-48.
25. Lahnsteiner F, Patzner RA, Weismann T. Energy resources of spermatozoa of the rainbow trout, *Oncorhynchus mykiss.* (Pisces, Teleostei). Reproduction Nutrition Development. 1993; 33:349-360.
26. Aramli MS, Kalbassi MR, Nazari RM. Comparative analysis of blood and seminal plasma composition of Persian sturgeon, *Acipenser persicus*. Journal of Applied Biological Sciences. 2013; 7:65-67.
27. Lahnsteiner F, Patzner R, Weismann T. The testicular main duct and the spermatic duct in some cyprinid fishes: II. Composition of the seminal fluid. Journal of Fish Biology. 1994; 44:459-467.
28. Linhart O, Mims SD, Boris-Gomelsky B, Hiott AE, Shelton WL, Cosson J. Ionic composition and osmolality of paddlefish (*Polyodon spathula,* Acipenseriformes) seminal fluid. Aquaculture International. 2003; 11:357-368.
29. Ciereszko A. Chemical composition of seminal plasma and its physiological relationship with sperm motility, fertilizing capacity and cryopreservation success in fish. In: Fish spermatology (Eds. S. M. H. Alavi, J. Cosson, K. Coward and G. R. Rafiee) Alpha Science, Oxford, 2008, 215-240.
30. Perchec G, Cosson J. Andre F and Billard R. Degradation of the quality of carp sperm by urine contamination during stripping. Aquaculture. 1995; 12:133-137.
31. Dietrich GJ, Dietrich M, Kowalski RK, Dobosz S, Karol H, Demianowicz W *et al*. Exposure of rainbow trout milt to mercury and cadmium alters sperm motility parameters and reproductive success. Aquatic Toxicology. 2010; 97:277-284.
32. White I, Macleod J. Composition and physiology of semen. In: Mechanisms Concerned with Conception (Ed. Hartman, C.G.), Pergamon Press, London, 1963, 135-172.
33. Lahnsteiner F, Mansour N, Berger B. Seminal plasma proteins prolong the viability of rainbow trout (*Onchorhynchus mykiss*) spermatozoa. Theriogenology. 2004; 62:801-808.
34. Billard R. Spermatogenesis and spermatology of some teleost fish species. Reproduction Nutrition Development. 1986; 2:877-920.
35. Bhatt G, Singh S, Bahuguna V, Chowdhary AK, Bhardwaj S, Bahuguna SN. Semen quality and sperm ultrastructure of Himalayan snowtrout *Schizothorax plagiostomus* Heckel, 1838. Indian Journal of Fisheries. 2017; 64:49-55.
36. Cejko BI, Kucharczyk D, Targonska K, Kubiak D, Saroseik B, Glogowski J. Quality parameters and selected biochemical markers of asp, *Aspius aspius* (L.), semen obtained after Hormonal stimulation with ovaprim or ovopel. Archives of Polish Fisheries. 2008; 16:179-188.
37. Dreanno C, Suquet M, Desbruyeres E, Cosson J, Delliou H, Billard R. Effect of urine on semen quality in turbot (*Psetta maxima*). Aquaculture. 1998; 169:247-262.
38. Glogowski J, Kwasnik M, Piros B, Dabrowski K, Goryczko K, Dobosz S *et al*. Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success. Aquaculture Research. 2000; 31:289-296.
39. Shen XM, Zhang HY, Hua R. Effect of environmental factors on haematological characters of blunt-snout bream (*Megalobrama amblycephala*). Acta Ecologica Sinica. 1991; 11:92-94.