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Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis of Rainbow Trout (*Oncorhynchus mykiss*) Seminal Plasma Proteins and their Correlation with Semen Characteristics

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ABSTRACT

***Background*:** Fish seminal plasma is a complex mixture made of different components. In few studies on some fish species such as rainbow trout, Nile tilapia and jundiá fish (*Rhamdia quelen*) characterization of seminal plasma proteins were shown. Meanwhile there is little information regarding the correlation between specific seminal proteins and semen characteristics in rainbow trout. The objective of the present study was to evaluate the seminal plasma protein profiles and their relation with some semen characteristics in rainbow trout.

***Materials, Methods & Results*:** Nine mature male rainbow trout with total weight 2300 ± 200 g were used. A total of nine male fish were anaesthetized by clove oil bath (50 µl L^{-1}) and semen was collected by abdominal massage. Semen variables (spermatocrit, concentration, duration of activity, percent of motility, total protein) were recorded. Seminal plasma was also separated by centrifugation and subjected to SDS-PAGE analysis. Gel images were analysed to determine molecular weight and relative protein content (pixel density) using the Total Lab TL120 computer program. Spermatocrit (%), sperm concentration (cell mL^{-1}), duration of sperm activity (sec), percentage of sperm motility and total protein (mg dL^{-1}) were 22.67, 12.35×10^9, 36.56, 89.33 and 81.49 respectively. Nine protein bands were identified on the 15% gel ranging from 11.74 to 68.12 kDa which seven were present in all samples. Band 1 (68.12 kDa), 2 (60.20 kDa) and 3 (54.19 kDa) were prominent (56.92% of the bands) in semen samples. Sperm concentration was negatively correlated with band 1 (68.12 kDa), 2 (60.20 kDa), 7 (32.72 k Da) and 8 (20.96). Percent of sperm motility showed positive correlation with band 3 (54.19 kDa) and 9 (11.74). Negative correlation was also revealed between spermatocrit and band 7 (32.72 k Da).

***Discussion*:** In the present study, spermatocrit (%) and duration of sperm activity (sec) were 22.67 and 36.56. In our study values for sperm concentration (cell mL^{-1}) and percentage of spem motility were 12.35×10^9 and 89.33 while in previously study these values were 7.89×10^9 and 88.5. Nine bands with molecular weights ranging from 11.74 to 68.12 kDa were detected in this study. The proteins with molecular weight higher than 54 kDa were prominent (56.92% of the bands). In the current study protein bands 3 (54.19 kDa) and 9 (11.74 kDa) were positively correlated with percent of sperm motility. It can be concluded that protein band 3 (54.19) and 9 (11.74) may modulate sperm function by providing energy and protection for spermatozoa as a complementary substance. Also According to our results, protein bands 1 (68.12), 2 (60.20), 7 (32.72) and 9 (11.74) negatively correlated with sperm concentration. It seems that these protein fractions could relate to other parameters in rainbow trout semen which are detrimental to sperm cells. On the other hand, all protein bands showed no correlation with other spermatological parameters such as total protein and duration of sperm activity. In conclusion, the present study shows that there is a correlation between some of the seminal plasma protein fraction and semen characteristics.

Keywords: *Oncorhynchus mykiss*, rainbow trout, SDS-PAGE, seminal plasma protein, semen characteristics.

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INTRODUCTION

Fish seminal plasma is a complex mixture made of different components. Protein is the main organic part which occur the 200-300 mg dL^{-1} fluid of seminal plasma [9]. Previously, it was found that some seminal plasma proteins are associated with fertility in various species such as bull [7], stallion and boar [3], goat [17], ram [6], and buffalo [1]. Bovine seminal plasma (BSP) contains a family of major proteins designated BSP- Al/A2 and BSP-A3 with molecular masses ranging from 15 to 16.5 kDa and BSP 30 kDa with an approximate molecular mass of 28-30 kDa [13]. BSP proteins, bind to the sperm surface and modulate sperm functions [14]. BSP-like proteins have been extensively investigated in other species. In few studies on some fish species such as rainbow trout [10,11], Nile tilapia [15] and jundiá fish (*Rhamdia quelen*) [4] characterization of seminal plasma proteins were shown. Eight types of proteins with a molecular weight between 14.5 to 78 kDa in rainbow trout semen were identifed [11]. In the study by [9] on rainbow trout semen, 12 proteins bands were detected by SDS – PAGE, ranging from 16 to 135 kDa. Meanwhile there is little information regarding the correlation between specific seminal proteins and semen characteristics in rainbow trout. It was showed that some protein fractions, namely 54, 47 and the 16 kDa positively affected the sperm viability [10]. Therefore, the present study was carried out to asses the protein profile of the rainbow trout seminal plasma by using SDS-PAGE and correlation between seminal plasma proteins and some semen characteristics, namely spermatocrit, sperm concentration, duration of sperm activity, percentage of sperm motility.

MATERIALS AND METHODS

Semen collection

Nine mature male rainbow trout (2-3 years old) with total weight 2300 ± 200 g were used as semen donor in January 2009. They were kept at the fish farm in Tabriz, Iran in concrete pond (1.8 × 1.3 × 1.3 m) with river water at a temperature of 11 ± 1°C and inlet water flow of 2 l s^{-1}. Fish were fed with commercial pelleted diet (Faradaneh, Iran). Fish were feed restricted for two days in order to prevent contamination of semen with faeces during stripping. Total of nine male fish were anaesthetized by clove oil bath (50 µL L^{-1}) and semen was collected by abdominal massage. Only pure samples uncontaminated with faeces, urine or blood were used. Semen samples were transferred to separate sterile microtubes and then taken to the lab on ice for further examinations.

Semen characteristics

- Spermatocrit

Haematocrit capillary tubes were filled with semen and one side of tubes was sealed then they were centrifuged for 6 min at 12,000 *g*. Spermatocrit values were read by haematocrit ruler [5].

- Sperm concentration

For calculating the sperm concentration, artificial seminal plasma solution (ASPS) consisted of 10 mM $NaHCO_3$, 1 mM $MgCl_2$, 1.6 mM $CaCl_2$, 120 mM NaCl, 30 mM KCl, pH 8 was used [5]. After dilution of semen with ASPS at a ratio of 1:20,000, counting was performed using a Neubauer's counting chamber.

- Sperm motility

Ten µL rainbow trout semen which was diluted in artificial seminal plasma solution (ASPS) at a ratio of 1:100 was poured onto the glass slides covered by bovine serum albumin (BSA). Then 20 µL of water was added to activate the sperms. During sperm activation all immotile sperms were counted using light microscopy at 400 x magnification. When activation got stopped completely all sperm cells were counted and motile sperms were calculated by subtracting these two amounts and percentage of motility was estimated. Duration of sperm motility was calculated in seconds from the time of addition of water onto the glass slides until the complete stop of the sperm cells motility [5]. All these steps for estimating the percentage of sperm motility and duration of sperm activity were repeated thrice

Preparation of seminal plasma

The seminal plasma was separated immediately after collection. Fresh semen was centrifuged at 1,500 *g* for 15 min at 5°C. The supernatants were transferred into 1.5 mL eppendorf tubes and centrifuged again at 14,000 *g* for 10 min at 5°C to eliminate the remaining sperm. Total protein concentration was measured by Lowery method [12], based on the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteay phosphomolybdic-

phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. The seminal plasma was then stored at -20°C until used.

SDS polyacrylamide denaturing gel electrophoresis (SDS-PAGE)

The seminal plasma proteins were analysed by SDS–PAGE gel electrophoresis. Seminal plasma samples were suspended in loading buffer (4:1 v/v) containing 50 mM dithiotreitol, 20 mM Tris, 2.5% sodium dodecyl sulphate (SDS), 0.002% bromophenolblue and 5% glycerol, pH 6.8. Electrophoresis was performed in 15% separating and 5% stacking gels according to Laemmli method [8]. Each lane was loaded with 30 µL seminal plasma. Samples were concentrated at 70 V for 10 min, separation was performed at 120 V for 4 h. Gels were stained with Coomassie Brilliant Blue, G-250.

Image acquisition

Gel images were analysed to determine molecular weight and relative protein content (pixel density) using the Total Lab TL120 computer program[1].

Statistical analysis

Data analysis was performed using SPSS software program (version 15.0 for Windows). All values were expressed as mean ± standard error of mean (S.E.M.). The Spearman's correlation coefficient test was applied to examine the correlation between seminal plasma protein fractions with all the parameters of the semen. Correlations with $P < 0.05$ were considered significant.

RESULTS

The results of the semen quality parameters of nine rainbow trout are summarized in Table 1, and shown as mean ± S.E. In these two year-old broodstock, spermatocrit (%), sperm concentration (cell mL-1), duration of sperm activity (sec), percentage of sperm motility and total protein (mg dL^{-1}) were 22.67, 12.35×10^9, 36.56, 89.33 and 81.49 respectively.

As is shown in Figure 1, a total of nine protein bands with a molecular weight of 68.12, 60.20, 54.19, 48.51, 45, 40.49, 32.72, 20.96, 11.74 kDa were found. Of the nine proteins found, band 5 (45) was present in 4 samples and band 9 (11.74) was just found in sample 9. Three proteins were more abundant in seminal plasma samples collected from fish broodstock: the bands 1 (68.12, 21.70%); 3 (54.19, 17.65%); 2 (60.20, 17.57%) [Table 2].

Of all protein fractions, 68.12 (r = -0.870, $P = 0.002$), 60.20 (r = -0.803, $P = 0.009$), 32.72 (r = -0.812, $P = 0.008$) and 20.96 kDa (r = -0.711, $P = 0.03$) were negatively correlated with sperm concentration in fresh semen. Protein bands 3 (r = +0.733, $P = 0.02$) and 9 (r = +0.683, $P = 0.04$) were positively correlated with percent of sperm motility. Meanwhile, Band 7 (r = -0.760, $P = 0.01$) was negatively correlated with Citation herespermatocrit. Conversely, no correlation was found between protein bands and total protein and duration of sperm activity.

Table 1. Semen characteristics in fresh ejaculates of rainbow trout.

Parameter	N	Minimum	Maximum	Mean	Standard error of Mean
Spermatocrit (%)	9	20.00	26.00	22.67	0.62
Sperm concentration (cell mL^{-1})	9	11.13×10^9	14.35×10^9	12.35×10^9	0.34×10^9
Duration of sperm activity (sec)	9	31.00	40.00	36.56	0.89
Percentage of sperm motility	9	87.94	90.56	89.33	0.35
Total protein (mg dL^{-1})	8	68.18	115.55	81.49	5.57

Table 2. Correlation between protein fractions of different molecular weight (MW) and semen quality parameters.

Band number	MW (kDa)	Percentage of the presence of band	Spermatocrit (%)	Sperm concentration (cell mL^{-1})	Duration of sperm activity (Sec)	Percent of sperm motility	Total protein (mg dL^{-1})
1	68.12	21.70	-	-0.870	-	-	-
2	60.20	17.57	-	-0.803	-	-	-
3	54.19	17.65	-	-	-	0.733	-
4	48.51	8.18	-	-	-	-	-
5	45.00	6.14	-	-	-	-	-
6	40.49	7.03	-	-	-	-	-
7	32.72	8.77	-0.760	-0.812	-	-	-
8	20.96	13.04	-	-0.711	-	-	-
9	11.74	9.72	-	-	-	0.683	-

-: no correlations.

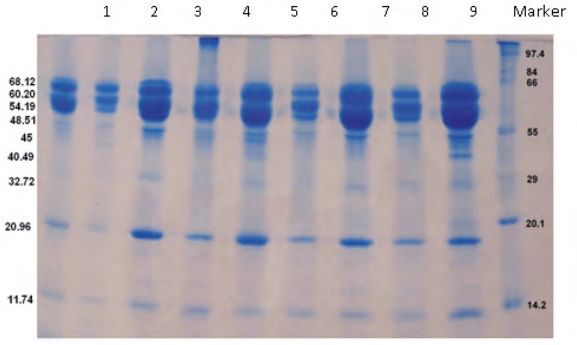

Figure 1. Molecular weight (kDa) of proteins in the seminal plasma as revealed by SDS–PAGE gel electrophoresis. Gels were stained with Coomassie Brilliant Blue. Numbers indicate the molecular weight of the standard (right) and sample (left) proteins in kDa.

DISCUSSION

In the current study, the seminal plasma protein profile and correlation between specific seminal plasma protein and semen characteristics were investigated by one-dimensional SDS-PAGE. In the current study, semen characteristics were nearly in the range of previous studies [5,16]. In the present study, spermatocrit (%) and duration of sperm activity (sec) were 22.67 and 36.56 while in study [5] these values were 20.1 and 35.4 respectively. Meanwhile, in our study values for sperm concentration (cell mL^{-1}) and percentage of spem motility were 12.35 × 109 and 89.33 while in previously study [16] these values were 7.89×10^9 and 88.5. It seems that difference in broodstock age; season and rearing condition are contributed to such variations [2]. Nine bands with molecular weights ranging from 11.74 to 68.12 kDa were detected in this study. The proteins with molecular weight higher than 54 kDa were prominent (56.92% of the bands). In another study, eight proteins with molecular weights between 14.5 and 78kDa were characterized [11]. Eight types of proteins with a molecular weight from 13.6 to 91.7 kDa were found previously [9]. SDS-PAGE analysis of seminal plasma proteins indicated the presence of 12 proteins with molecular weights from 16 to 135 kDa; a group of proteins with molecular weights of 65 and 54 kDa were found in highest quantities (34-45% of the total quantified protein content)[10]. According to various studies, we can conclude that protein bands with molecular mass from 54 to 68 kDa were more prominent. Differences of molecular weights shown in studies could be explained by different approaches of sampling, preparation of seminal plasma, different

strategies of culture and feeding, selection of different ages or strains of broodstocks and season of semen sampling [18].

Correlation between seminal plasma proteins and fertility of the male has been reported in some species of domestic animals such as bull [7], boar [3], goat [17], ram [6] and buffalo [1]. Physiological effects of seminal plasma proteins in prolongation and stabilization of sperm viability in rainbow trout was shown previously [9]. In the current study protein bands 3 (54.19 kDa) and 9 (11.74 kDa) were positively correlated with percent of sperm motility. In recent study it was observed that sperm motility could be activated by protein fractions which shared 54, 47 and 16 kDa protein[10]. It can be concluded that protein band 3 (54.19) and 9 (11.74) may modulate sperm function by providing energy and protection for spermatozoa as a complementary substance. Conversely, in Nile tilapia (*Oreochromis niloticus*) inhibition of sperm motility by seminal plasma protein fractions was shown [15]. According to our results, protein bands 1 (68.12), 2 (60.20), 7 (32.72) and 9 (11.74) negatively correlated with sperm concentration. It seems that these protein fractions could relate to other parameters in rainbow trout semen which are detrimental to sperm cells. For example some acidic proteins in ram semen are negatively correlated to sperm motility and concentration [18]. On the other hand, all protein bands showed no correlation with other spermatological parameters such as total protein and duration of sperm activity.

CONCLUSION

The present study shows that there is a correlation between some of the seminal plasma protein fraction and semen characteristics. However additional studies are necessary to define the protein fractions that affect different parameters in semen.

SOURCE AND MANUFACTURER
[1]Total Lab TL120 computer program (v2009), USA.

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