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The Correlation Between Semen Fatty Acids with Spermatological Parameters in Iranian Sturgeon (*Acipenser persicus*) Brood Stocks

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Abstract: The fatty acids are one of the important components of fish semen. They are the main source of metabolic energy and improved functionality of reproduction, embryo growth and development. Balanced level of fatty acids for fertility and sperm functionality is important and necessary. This study investigated the fatty acids of *Acipenser persicus* brood stocks semen and their correlation with spermatological parameters. For this purpose, spermatological parameters of 15 *Acipenser persicus* brood stocks were measured and the fatty acids were measured by Gas Chromatograph (GC) set. The results showed that, the highest value of total of Σ-3 fatty acid was observed in N treatment that it was in the maximum value of sperm duration and percentage of sperm motility and the highest value of total of Σ-6 fatty acid was observed in H treatment that it was in the maximum value of spermatocrit and sperm density. The lowest value of fatty acids was observed in B and F treatments that they were found in the minimum value of spermatological parerameters. Therefore it could be concluded that semen fatty acid mixture of *Acipenser persicus* broodstocks are effective in the semen as they maintain spermatological parameters and reproduction process in their best conditions.

Key words: *Acipenser persicus* · Spermatological parameters · Fatty acids

INTRODUCTION

Sturgeons belong to the family of Acipenseridae and exist in the northern hemisphere. They reproduce in freshwater and most of them migrate to the sea, either living in brackish water (Caspian, Azov, Black and Baltic seas) or in the continental shelves of marine environment. They are the most important group among the fishes and 90% of their stocks are found in the Caspian Sea.

Sturgeons are commercially the most important fish species in the world. Sturgeons produce the most valuable caviar in the world [1] and are expensive fish appreciated for its boneless and flavorful flesh. The decline of sturgeon catches from the natural environment (due to overfishing, poaching, water pollution, dredging and gravel mining and blocks in upstream migration) has stimulated the commercial culture of these fishes in farms. However, many conservation programs now include restocking as a mean to rebuild sturgeon population [2]. Sturgeon culture doesn't only supply fish to markets but, it is also a conservation tool to decrease fishing pressure on natural stocks [3]. As for decline of fish stocks at recent years, further attentions conducted to the artificial reproduction of this fish. One of the important effective factors in the success of artificial reproduction, is sperm quality. Seminal plasma contains organicand inorganic compounds and enzymes [4, 5].

Some of the compounds of semen are fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that both of them except polyunsaturated fatty acid (PUFA) have different roles in fish body. DHA and EPA have a structural role in the process of creating organs for example in the cell membrane (muscle cells, brain and retina). They are the precursors of physiologically active molecules such as eicosanoids [6-8].

DHA, which has a competitive relationship with EPA, is particularly important for normal neural development and function, including that of retina and brain [9]. Lipids are a major component of spermatozoa of teleost fish [10]. Lipids are the main energy resources of salmonid

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spermatozoa during immotile storage [11] and therefore they are important to maintain sperm viability. Also in mammals, fatty acids have a positive effect on sperm functionality: in boar spermatozoa, oleic and linoleic acid significantly maintain the motility and viability of spermatozoa, oleic and arachidonic acid enhanced the acrosome reaction [12]. However, in some mammalian species, cryoresistance of spermatozoa could be improved by fatty acids, e.g. in boar semen an increase in semen post-thaw quality was obtained in docosahexaenoic acid-supplemented extenders [13]. Fatty acids are important and necessary for sperm reaction and fertility [14]. Also, in teleost fish, Arachidonic acid is precursor of prostaglandins, that is stimulator of sexual behaviors and cause the synchronization of spawning process and ithas a directly effect on successful reproduction [15].

Fatty acid composition of fish spermatozoa is affected by the diets [16, 17]. The diet significantly modified the fertilizing ability of fresh sperm. The transfer of essential fatty acids from the diet to the semen is effective and this transfer may have biological effects on the fertilizing ability of semen. Relatively little information is available about the fatty acid composition of semen and about the role of fatty acids on sperm reaction. This knowledge can increase our understanding in semen biology and improve the techniques for handling and manipulation of semen. Scarce information is present about the correlation between fatty acids of semen and spermatological parameters, this investigation was carried out to exhibit the correlation between semen fatty acids composition and the reproductive performance of male Iranian sturgeon (*Acipenser persicus*).

MATERIALS AND METHODS

Collection of Semen: The experiment was carried out on April 2009 at Central Laboratory of Gorgan University of Agricultural science and Natural Resources, Gorgan, Iran. For this study semen of 15 male breeders (enumerated A – O treatments) were obtained from Shahid Marjani Sturgeon Hatchery Center (Gorgan, Iran) and immediately transferred in glass tubes filled with the hatchery water to the Central Laboratory at the Gorgan University of Agricultural science and Natural Resources, Gorgan, Iran. The males were wiped ventrally with aseptic paper towels and the milt was expelled using gentle pressure on the abdominal region without any pollution [18]. Milts were collected by 5 ml syringe (4 volume of air and 1 volume of sperm).

Spermatological Parameters Measurements: After adding distilled water (50 times of sperm volume, to stimulate sperm mobility) to the collected milts, it was examined under a stereomicroscope device (microscope equipped with a CCD camera attached to computer, Panasonic WV-CP240, Japan) [19] with magnification of 10x to measure the duration of sperm movement and percentage of motile sperms (time started in less than 7 seconds).A digital camera recorded the sperm motility using high resolution. The shelf life of sperm for each sample was measured by stopwatch. The stopwatch started to work once milt was activated by water and stopped when sperm movement stopped [20, 21]. Then Adobe premier software (Version 6) was used to get pictures every 10, 20, 30, 40 seconds after the activation of sperms. These pictures changed to 30 forms (slides) then, four forms were randomly selected (i.e. Form 1, 4, 7 and 10). Positions of 10 spermatozoa in these randomly selected pictures and also the percentage of motile sperms were calculated. All treatments were done thrice and to avoid experimental error, all measurements were observed by the same viewer [19]. For measuring the spermatocrit, tubes containing raw semen were centrifuged at 3000 rpm for 8 minutes (Eppendorf AG 22311 Hamburg, centrifuge 5415D) then, hematocrit reader was used to determine the percentage of sperm to seminal fluid (percentage of white space to the total milt volume) [22]. Sperm concentration was assessed using haemacytometry standard method with diluted sperm in magnification phase contrast black background (count $\times 10^9$ /mL). Furthermore, sperm concentration was measured by haemacytometry standard method with diluted sperm in ratio of 1:2000 by water, using a microscope with 10 magnification phase contrast black background and it was written units per ×10 mL semen.

Fatty Acid Analysis: Fatty acids were analyzed on a GC set (DANI 1000). Total lipids were extracted by the method of Folch *et al*. [23] and measured gravimetrically. The formation of fame was carried out according to the procedure described by Desvilettes *et al*. [24]. The sample was saponified with methanolic sodium hydroxide and the fatty acids were esterified with methanolic sulfuric acid. Fame were analysed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30m, 0.25 mm i.d., 0.25 mm film thickness), a split–splitless injector with Agilent tapered liner (4mm id) and flame ionization detector. The initial column temperature was maintained at 100°C for 1 min and

then raised by 25°C /min to 190°C and held for 10 min and then raised to 220°C and held for 5 min. Nitrogen was used as carrier and makeup gas, at flow rates of 1.0 and 45 mL/min, respectively. The injector and detector temperature were held at 250 and 260°C, respectively. ChemStation software was used for online data collection and processing. Individual fame was identified by comparison with known standards (Sigma, Chemical Co. St. Louis).

Statistical Analysis: Statistical analysis of data was done according to Pearson correlation coefficient test at the level of 95% using SPSS v. 16.0. Statistically significance was set at the level of $p<0.05$ and $p<0.01$ with mean ± standard deviation (SD).

RESULTS

The spermatological parameters are shown in Table 1. The maximum value of spermatocrit and sperm concentration was observed in "H" treatment. Also the maximum value of sperm duration and percentage of sperm motility was observed in "N" treatment. While, the minimum value of spermatocrit and sperm concentration was found in the "B" treatment, also the minimum value of sperm duration and percentage of sperm motility was found in "F" treatment.

The correlation between spermatological parameters and fatty acid are shown in Table 3 and 4.

According to the previous data, there was positively significant correlation ($P<0.01$) between spermatocrit, sperm concentration with the total of saturated fatty acid (ΣSFA), monounsaturated fatty acid (ΣMUFA), polyunsaturated fatty acid (ΣPUFA), omega-3 fatty acid (Σn-3) and omega-6 fatty acid (Σn-6). Also, it was observed a positively significant correlation ($P<0.05$) between sperm duration and percentage of sperm motility with C20:3n-3 (iecosatrinoic acid) and (Σn-3). Although, there was no significant correlation between Sperm motility duration and percentage of sperm motility with the other fatty acids.

DISCUTION

All the above Figs, shows that there is positive significant correlation between some semen fatty acid and spermatological parameters.

Table 1: Spermatological parameters in *Acipenser persicus* brood stocks.

	Spermatological parameters			
Treatments	Spermatocrit (%)	Sperm concentration ($\times 10^9$)	Sperm duration (s)	Sperm motility (%)
A	0.43±4.21	2.99±32	0.04±135	4.90±80
B	0±1	0±8	0.07±231	8.5±84
C	0.32±1.86	2.21±14	0.03±179	3.56±80
D	0.56±1.60	3.89±13	0.01±143	1.51±76
E	0.15±1.62	1.03±12	0.03±161	4.40±80
F	0.11±1.06	0.8±9.5	0.01±86	1.76±70
G	0.55±4.40	3.83±34	0.02±101	3.36±73
H	0.81±26	5.61±177	0.02±137	4.72±71
I	0.30±3.26	2.1±25	0.02±108	3.01±74
J	0.84±3.17	5.79±27	0.01±119	1.13±72
K	0.07±4.15	0.48±30	0±217	1.10±78
L	1.62±11.86	11.7±81	0.05±173	6.10±73
M	0.29±3.56	1.43±27	0.04±261	5.23±82
N	0.66±13.10	4.56±92	0.01±262	1.76±90
O	0.25±2.42	1.72±19	0.02±217	2.47±81

Table 2: Cocentration of semen fatty acid in *Acipenser persicus* brood stocks.(gr / 100 gr lipid)

Formula	Fatty acid name	Mean±SD
C14:0	Meristic	0.43±1.59
C16:0	Palmitic	0.77±3
C16:ln-7	Palmitoleic	0.74±1.61
C18:0	Stearic	0.79±2.28
C18:ln-9	Oleic	0.93±2.96
C18:ln-7	Vasenic	0.84±1.97
C18:2n-6	Linoleic	0.84±1.93
C18:3n-3	Linolenic	0.72±1.35
C20:0	Arachidic	0.62±0.85
C18:3n-6	Gamma linoleic	0.86±1.15
C20:ln-9	Gadoleic	0.75±1.42
C18:4n-3	Stearidonic	0.85±0.97
C22:0	Behenic	0.96±0.6
C20:3n-6	Dihomo linolenic gamma	0.85±0.97
C20:5n-3	Eicosapentaenoic	0.92±2.38
C20:4n-6	Arachidonic	0.88±1.46
C22:5n-6	Docosapentaenoic	0.94±1.7
C22:5n-3	Docosapentaenoic	0.92±1.77
C22:6n-3	docosahexaenoic	0.87±2.24
C20:3n-3	Eicosatrienoic	1.03±2.64
ΣSFA	Total of saturated fatty acid	0.75±3.11
ΣMUFA	Total of mono unsaturated fatty acid	0.91±3.05
ΣPUFA	Total of poly unsaturated fatty acid	0.86±3.17
DHA/EPA	Ratio of DHA to EPA	0.67±-0.84
Σn-3	Total of omega-3 unsaturated fatty acid	0.92±3.04
Σn-6	Total of omega-6 unsaturated fatty acid	0.78±2.4
Σn-6/Σn-3	Ratio of Total of omega-3 to total omega-6	0.45±0.64

Table 3: Correlations between fatty acid of semen and spermatological parameters

	Spermatological parameters			
Fatty acid	Spermatocrit (%)	Sperm concentration ($\times 10^9$/ml)	Sperm duration (s)	Sperm motility (%)
C14	0.876**	0.859**	0.431	0.415
C16	0.875**	0.847**	0.381	0.370
C16:1n-7	0.801**	0.791**	0.431	0.395
C18	0.907**	0.850**	0.328	0.321
C18:1n-9	0.876**	0.863**	0.411	0.405
C18:1n-7	0.842**	0.858**	0.455	0.455
C18:2n-6	0.909**	0.871**	0.366	0.363
C18:3n-3	0.866**	0.830**	0.343	0.343
C20	0.259	0.221	0.040	0.065
C18:3n-6	0.714**	0.708**	0.361	0.343
C20:1n-9	0.813**	0.729**	0.315	0.255
C18:4n-3	0.777**	0.706**	0.272	0.235
C22	0.222	0.225	0.155	0.164

**Significant at P < 0.01

Table 4: Correlations between fatty acid of semen and spermatological parameters

	Spermatological parameters			
Fatty acid	Spermatocrit (%)	Sperm concentration ($\times 10^9$/ml)	Sperm duration (s)	Sperm motility (%)
C20:3n-6	0.628**	0.475	0.006	-0.033
C20:3n-3	0.806**	0.902**	0.593*	0.604*
C20:5n-3	0.804**	0.828**	0.460	0.456
C20:4n-6	0.724**	0.689**	.0324	0.275
C22:5n-6	0.663**	0.735**	0.443	0.460
C22:5n-3	0.790**	0.836**	0.486	0.491
C22:6n-3	0.793**	0.827**	0.483	0.472
ΣSFA	0.882**	0.851**	0.377	0.366
ΣMUFA	0.873**	0.863**	0.422	0.413
ΣPUFA	0.831**	0.869**	0.488	0.489
Σn-3	0.835**	0.886**	0.525*	0.525*
Σn-6	0.797**	0.787**	0.358	0.356
DHA/EPA	-0.048	-0.145	-0.201	-0.233
Σn-3/Σn-6	0.321	0.441	0.449	0.452

**Significant at P < 0.01
*Significant at P < 0.05

According to the above figures, the value of fatty acid such as (C22:6n-3), (C20:5n-3), (C18:2n-6), (C20:4n-6), (Σn-3) and (Σn-6), in the H and N treatment were higher than M, B and F treatment that showed positive effect of semen fatty acid on improvement of spermatological parameters. The result of this study was consistent with several other researches such as Zalata *et al*. [25] in human, Am-In *et al*. [26] in boars, that reported positive significant effect of semen fatty acid on spermatological parameters. These results showed that, the increase in the value of (ΣSFA), (ΣMUFA), (ΣPUFA), (Σn-3) and (Σn-6) in the semen improves spermatological parameters in the *Acipenser persicus* brood stocks.

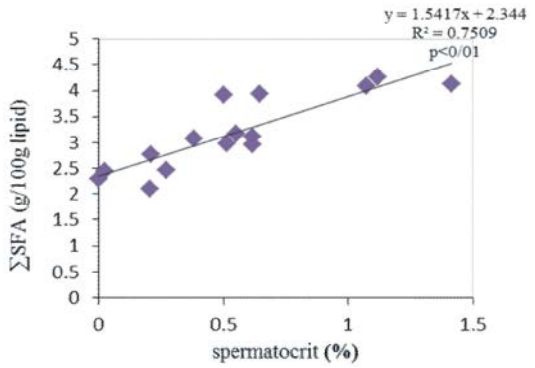

Fig 1: The correlation between spermatocrit and ΣSFA P<0.01

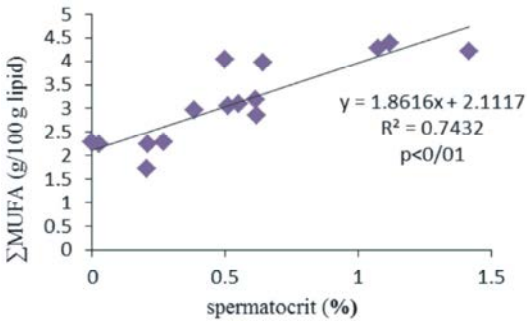

Fig 2: The correlation between spermatocrit and (ΣMUFA) P<0.01

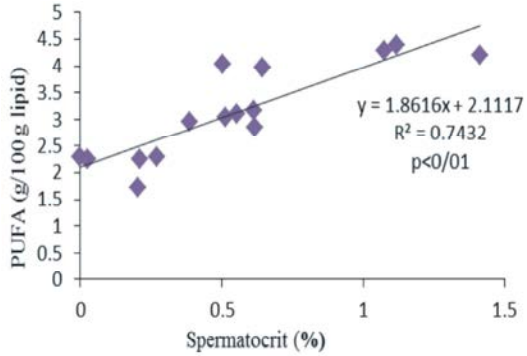

Fig 3: Correlation between spermatocrit and ΣPUFA P<0.01

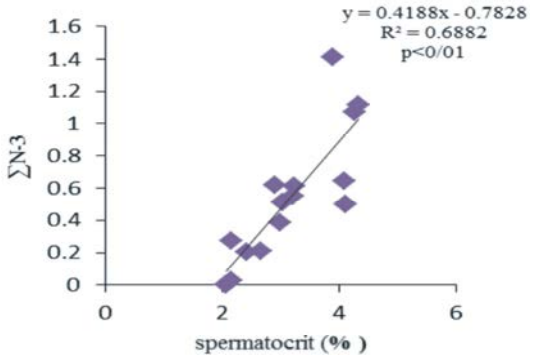

Fig 4: Correlation between spermatocrit and ΣN-3

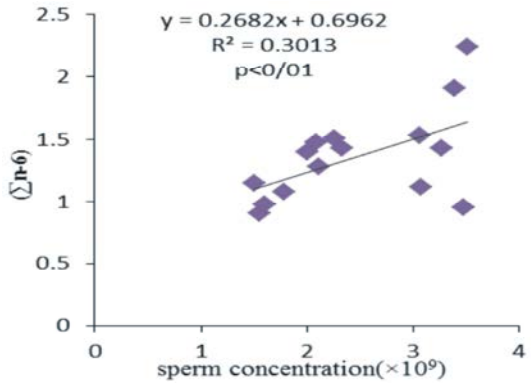

Fig 5: The correlation between sperm concentration and (Σn-6) P<0.01

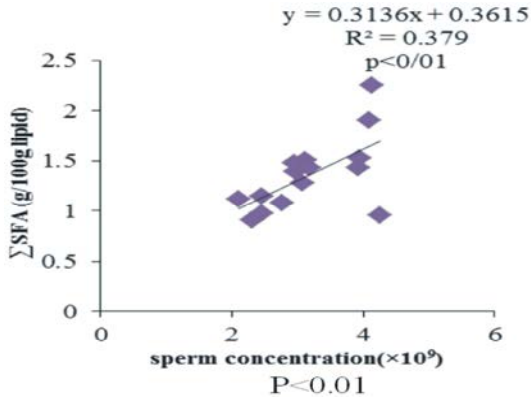

Fig 6: The correlation between sperm concentration and ΣSFA P<0.01

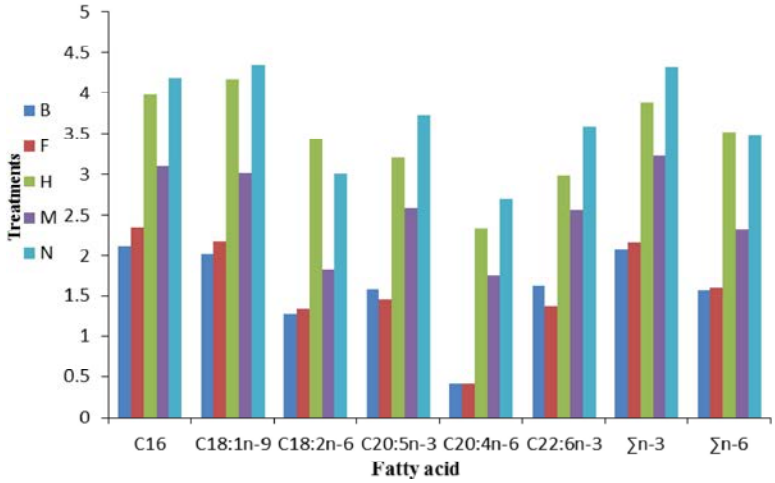

Fig 7: Comparison value of fatty acids in some treatments

Sperm requires fatty acids for their survival and movement. Fatty acids oxidation in sperm cells membrane by mitochondria produces ATP [27, 28] that is save in mid zone of sperm and tail of sperm to be used for their movement. So, the increase of fatty acids in non sexual cell of testicle rise the ability of testicles to produce sperm, therefore increase spermatocrit, sperm concentration, sperm duration and percentage of sperm motility. Understanding the importance of fatty acid and their effect on spermatological parameters, diet formulationfor brood stocks is very effective on semen contents of fatty acids, so addition of fatty acids and their balance in the diet of brood stocks can improve semen quality and finally cause successful reproduction. In conclusion, results of this study indicated that semen fatty acids can be effective in the semen of of *Acipenser persicus* brood stocks as they maintain spermatological parameters and reproduction process in their best conditions.

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