EFFECT OF ORAL FISH OIL SUPPLEMENTATION ON FRESH AND FROZEN RAM SEMEN QUALITY AND SUBSEQUENT FERTILIZATION RATES IN MATURE EWES

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ABSTRACT

Twenty healthy crossbred rams (1/2 Finnish Landrace x 1/2 Rahmani) were used to study if fish oil supplementation would improve fresh and frozen semen characteristics and fertility. Animals were divided into four similar groups of 5 rams each and were fed on the same diet for a preliminary period of two weeks. Treatment groups 1, 2 and 3 received a daily oral dose of 0.5, 1.0 and 1.5 ml of fish oil contained a minimum of 50% docosahexaenoic acid (DHA), respectively, for 12 weeks, however, group 4 served as control. Thereafter, all rams were fed only their basic requirement for subsequent three weeks.

The results showed that the ejaculate volume, sperm cell concentration, sperm motility and live sperm percentage were increased ($P<0.05$) and the percentage of sperm abnormalities was reduced ($P<0.05$) in the semen collected from fish oil treated groups compared to that of control group during treatment period. Seminal plasma total protein, total cholesterol and alkaline phosphatase during treatment tend to be numerically higher than those of control group and pre-treatment period, however, total lipid decreased during treatment and post-treatment compared to that of control diet rams but the differences among groups were not significant. Progressive motility of frozen-thawed semen of rams treated with mid (G1) and high dose (G3) of fish oil was significantly ($P<0.05$) higher than that of control and low dose groups. Conception rate for sixty ewes cervically inseminated at natural estrus with frozen-thawed semen of fish oil treated rams was higher (G1; 46.7 ; G2; 53.3 and G3; 53.3%; $P<0.05$) than that of control group (G4, 33.3%). It was concluded that the addition of 1.0 or 1.5 ml fish oil

daily to the basic diet of ram improved sperm production and quality of fresh and frozen ram semen and enhanced ram fertility.

Keywords: **Ram semen; Semen quality; Cryopreservation; Fertility; Docosahexaenoic acid.**

INTRODUCTION

A great deal of attention has recently been given to the essential roles of polyunsaturated fatty acids of sperm membrane. Semen from all domestic species contains high levels of polyunsaturated fatty acids, in particular, docosahexaenoic acid (DHA) and docosapentaenoic acid (Brinsko *et al.*, 2005). It was claimed that DHA is an essential component of healthy sperm cells, enhancing membrane integrity and tail flexibility, as well as increasing output. Moreover, Ollero and Alvarez (2003) reported that ram spermatozoa are especially rich in DHA, which competes with arachidonic acid for the sn-2 position in membrane phospholipids. There is also evidence that the lipid and fatty acid compositions of chicken sperm play important roles in maintaining semen quality (Cerolini *et al.*, 1997).

The studies of specific requirement for DHA by sperm cells has focused attention on the required physical structure that promotes fertility and their potential association with tissue DHA content e.g. a positive correlation between the state of polyunsaturation and membrane fluidity and function (Maldjian *et al.*, 2003). DHA is the predominant fatty acid in the sperm and was highly correlated with sperm motility (Connor *et al.*, 1998 and Dolatpanah *et al.*, 2008) and other semen characteristics and freezability (Maldjian *et al.*, 2005 and Dolatpanah *et al.*, 2008). As Fish oil is rich in polyunsaturated fatty acids mainly DHA (Cerolini *et al.*, 2006), the aim of this study was to investigate the effect of oral fish oil supplementation on the fresh and frozen semen characteristics and the subsequent fertility rates in mature ewes.

MATERIALS AND METHODS

This work was carried out at Sakha Animal Production Research Station, belonging to Animal Production Research Institute during the period from April 10 to August 25, 2007.

Animals and management:

Twenty healthy crossbred rams (1/2 Finnish Landrace x 1/2 Rahmani) 18 months old were used in this study. Rams were fed on 750 gm concentrate fed mixture plus 2.5 kg berseem during April – May and were fed on 750 gm concentrate fed mixture plus 1kg berseem hay during June until the end of experiment. Animal had free access to fresh water and minerals all the day.

Treatments:

Rams were randomly allocated in equal numbers to four groups of 5 rams each. Rams were fed individually on the same basic diet for a preliminary period of last two weeks of April month. This preliminary period served as the control for the subsequent treatment. Starting from May, rams in the first, second and third group received a daily oral supplement of 0.5, 1.0 and 1.5 ml of fish oil (F.O.) contained a minimum of 50% DHA, respectively for 12 weeks, thereafter rams were fed only their basic requirement for the subsequent three weeks, while the fourth group was served as control without fish oil addition.

Experimental procedures:

Semen was collected into graduated tubes with an artificial vagina once weekly from all rams for a period of 17 weeks (2 wks pre-, 12 wks during and 3 wks post-fish oil supplementation) and the ejaculate volume was individually recorded. Each ejaculate was evaluated immediately after collection for mass motility under a microscope at 200x magnification. Sperm viability and morphology were assessed by the eosin-nigrosin method under phase-contrast microscope at 1000x magnification. Spermatozoa considered to be viable (live) and normal when they were not stained and the morphology was normal from head to tail. All other shapes were considered to be abnormal. Three eosin-nigrosin smear were prepared for each ejaculate and 200 spermatozoa were counted on each smear and the proportion of live (unstained) and abnormal spermatozoa were calculated. Sperm cell concentration (x 10^6/ml) was counted using Neubauar Haemocytometer.

Some chemical components of seminal plasma were measured for the first two weeks of pre-, 5 weeks during and 3 weeks post-fish oil supplementation. Total protein, total cholesterol,

total lipids and alkaline phosphatase were estimated using chemical commercial kits according to Varoley (1976).

Only ejaculates with mass motility of 80% or more were diluted for each ram for 3 weeks only during the fish oil administration. Semen was diluted in a rate of 1:4 in heated (37°C) Tris-egg yolk extender. The Tris extender was prepared with Tris (6.05 g), citric acid (3.35 g), glucose (1.5 g), 20 ml egg yolk, Streptomycin (100.000 μg), Penicillin (100.000 IU), and completed with distilled water to 100 ml. Semen samples were placed in a water bath at 37°C, then placed into a refrigerator at 5°C for 4 hrs for equilibration. The cooled semen was frozen in pellet form (0.3 ml semen/pellet) on a special plate with holes engraved in the surface and freezed at -79°C to -196°C by immersion in liquid nitrogen (Evans and Maxwell, 1987) until used for post-thaw motility test. Frozen 2-3 pellets were thawed at 40°C for 10 sec. in a water bath and kept for 2-3 minutes before use for insemination.

Fertility of frozen ram semen:

Sixty mature crossbred ewes divided into four similar groups (fifteen ewes each) were inseminated at the onset of detected estrus (natural estrus) with the frozen-thawed semen of each group via cervical artificial insemination during the breeding season to test the effect of fish oil administration on ram semen fertility.

Statistical analysis:

Data obtained were subjected to statistical analysis using Least square analysis adapted by SPSS program (1997) for User's Guide. Duncan test within program SPSS was done to determine the degree of significant among the means.

RESULTS AND DISCUSSIONS

1. Physical characteristics of fresh ram semen:

Fish oil supplementation with the different doses affected the semen physical parameters (Table 1). The ejaculate volume, sperm cell concentration, sperm motility and live sperm percentages of rams in all fish oil groups were significantly ($P<0.05$) increased during treatment period than that of the control rams. However, the percentage of sperm abnormalities was significantly reduced in fish oil treated groups compared to that for control one.

Table (1): Semen physical characteristics (mean±SE) measured in ejaculates of rams fed a control and fish oil supplemented diet.

Groups (doses)	Pre-treatment	During treatment	Post-treatment
Ejaculate volume (ml)			
G1 (0.5ml F.O.)	0.72 ± 0.08^{c}	0.98 ± 0.04^{aA}	0.93 ± 0.03^{ab}
G2 (1.0ml F.O.)	0.73 ± 0.07^{c}	0.95 ± 0.03^{aA}	0.93 ± 0.04^{ab}
G3 (1.5ml F.O.)	0.74 ± 0.08^{b}	1.03 ± 0.03^{aA}	0.96 ± 0.03^{a}
G4 (control)	0.74±0.07	0.75 ± 0.02^{B}	0.74±0.04
Sperm concentration (x 10^6/ml)			
G1 (0.5ml F.O.)	293 ± 8.6^{b}	302.9 ± 2.3^{aA}	295 ± 3.6^{b}
G2 (1.0ml F.O.)	282 ± 10.2^{b}	311.8 ± 3.5^{aA}	295.5 ± 4.2^{ab}
G3 (1.5ml F.O.)	290 ± 3.7^{b}	312.4 ± 3.1^{aA}	297.6 ± 4.5^{ab}
G4 (control)	287.6±11.0	290.9 ± 1.8^{B}	288.0±4.1
Sperm motility (%)			
G1 (0.5ml F.O.)	81.5 ± 1.07^{b}	83.54 ± 0.2^{aA}	79.70 ± 0.6^{b}
G2 (1.0ml F.O.)	80.9 ± 0.70^{b}	84.51 ± 0.3^{aA}	81.67 ± 0.8^{b}
G3 (1.5ml F.O.)	81.22 ± 0.90^{b}	84.08 ± 0.2^{aA}	82.0 ± 0.9^{b}
G4 (control)	80.1 ± 0.9^{b}	80.33 ± 0.3^{bB}	79.0 ± 0.76^{b}
Live sperm (%)			
G1 (0.5ml F.O.)	76.9 ± 1.12^{b}	79.1 ± 0.33^{aA}	76.93 ± 0.91^{b}
G2 (1.0ml F.O.)	77.3 ± 1.12^{b}	79.1 ± 0.31^{aA}	77.66 ± 1.07^{b}
G3 (1.5ml F.O.)	77.6±1.17	78.93 ± 0.31^{A}	77.93±0.81
G4 (control)	77.0±0.94	76.80 ± 0.31^{B}	77.0±0.86
Abnormal spermatozoa (%)			
G1 (0.5ml F.O.)	8.8±0.61	7.64 ± 0.32^{B}	8.26 ± 0.56^{B}
G2 (1.0ml F.O.)	8.9±1.00	7.52 ± 0.31^{B}	7.9 ± 0.47^{B}
G3 (1.5ml F.O.)	9.55 ± 0.62^{a}	7.16 ± 0.32^{bB}	7.83 ± 0.55^{Bab}
G4 (control)	9.50±0.62	8.95 ± 0.27^{A}	9.73 ± 0.47^{A}

^{a,b,c} Means within a row with different superscripts are significantly different ($P<0.05$).
^{A,B} Means within the same column with different superscripts are significantly different ($P<0.05$).

These results agree with those of Penny *et al.* (2000) and Dolatpanah *et al.* (2008) who reported that feeding fish oil to boars or to bucks resulted in an increase in total sperm number, sperm concentration, motility score, percentage of normal sperm, and percentage of viable cells. Moreover, Maldjian *et al.* (2003) and Speak *et al.* (2003) found that DHA in boars is positively correlated with ejaculate concentration and total sperm. Thus, other studies

showed that the proportion of motile spermatozoa in human, boars and checken semen is positively correlated with DHA (Cerolini *et al.*, 1997; Zalata *et al.*, 1998; and Rook *et al.*, 2001). Also, Brinsko *et al.* (2005) found that mean sperm concentration in ejaculates of stallions fed fish oil was 1.8 times higher than that of stallions fed the control diet. Therefore, feed supplementation with DHA increased its bioavailability and this could allow spermatogenesis to operate to its maximum extent. On the other hand, Zaniboni *et al.* (2006) reported that sperm concentration was not changed between control diet and fish oil diet fed to Turkey breeders. Moreover, other studies reported no effect of fish oil supplementation on quality parameters of fresh semen in different animal species (Paulenz *et al.*, 1999; Conquer *et al.*, 2000 and Maldjian *et al.*, 2005). Additionally, the present study indicated that fish oil supplementation improved the physical characteristics of ram semen during the 12 weeks of treatment and enhanced ejaculate volume, sperm concentration and sperm abnormalities after stopping treatment compared to pre-treatment period. This improvement in physical characteristics of rams semen may be due to the specific enrichment of ram semen with polyunsaturated fatty acids mainly DHA after fish oil supplementation.

2. Chemical characteristics of fresh ram semen:

The effect of different treatments and experimental period on chemical parameters of ram semen are presented in Table (2). The overall cocentrations of total protein, total cholesterol and activity of alkaline phosphatase in the seminal plasma were slightly higher and concentration of total lipids was slightly lower in rams treated with fish oil as compared to the control rams, but differences were not significant. Increased intake of DHA of fish oil may ameliorate the abnormalities of lipid metabolism such as hyperlipidemia and imbalances of (n-6) and (n-3) fatty acids (Song *et al.*, 2000).

Also, the oral fish oil supplementation in this study increased seminal plasma total protein, total cholesterol and alkaline phosphatase during treatment period (Table 2). However, total lipids decreased during treatment period and after stopping treatment compared to that of control rams. These lipids had an improvement role in membrane integrity, fluidity, stability and

permeability (Kuiper *et al.*, 1971; Engelhard *et al.*, 1978 ; Clandinin *et al.*, 1985 and Yeagle, 1985). The above mentioned results are in agreement with that of Fontdevila and Obregon (1993) who found that male rabbits fed on fish oil increased their cholesterol and LDL-cholesterol serum levels after 15 days of diet. Moreover, high concentration of cholesterol could improve membrane fluidity (Connor *et al.*, 1998). In addition both DHA and cholesterol are polyunsaturated and may contribute to membrane fluidity necessary for bending and flexing of tails required for motility.

Table (2): Some chemical characteristics of fresh semen as affected by fish oil (F.O.) supplementation.

Groups (doses)	Pre-treatment	During treatment	Post-treatment	Overall mean ±SE
Total protein (gm%)				
G1 (0.5ml F.O.)	5.45±0.01	8.36±0.93	9.7±1.8	9.35±1.53
G2 (1.0ml F.O.)	7.73±0.46	9.45±1.3	10.3±2.2	9.16±0.88
G3 (1.5ml F.O.)	7.27±3.63	10.36±1.4	10.7±0.8	9.12±0.97
G4 (control)	7.27±1.82	7.36±1.7	7.1±1.1	7.24±0.98
Total lipids (mg/100 ml)				
G1 (0.5ml F.O.)	552.5±22.5	520.5±21.3	428±27.2	500.3±30.9
G2 (1.0ml F.O.)	566±48.8	537±28.5	400.8±39.6	501.3±41.6
G3 (1.5ml F.O.)	592.5±47.5	507±27.9	494.2±15.6	531.2±18.4
G4 (control)	585±45.0	546±12.1	567.5±36.1	566±44
Total cholesterol (mg/dl)				
G1 (0.5ml F.O.)	96.6±30.0	97.3 ± 16.0^{B}	111.1 ± 21.9^{AB}	101.7±26
G2 (1.0ml F.O.)	90.0±10.1	115.0 ± 32.3^{A}	95.6 ± 19.0^{BC}	100.2±30
G3 (1.5ml F.O.)	100.0±16.01	132.0 ± 33.4^{A}	128.9 ± 31.9^{A}	120.4±36
G4 (control)	93.3±26.7	94.0 ± 14.4^{B}	93.5 ± 14.7^{C}	93.6±22
Alkaline phosphatase (IU/l)				
G1 (0.5ml F.O.)	33.4±11.7	48.4±4.3	57.5±5.1	46.4±5.3
G2 (1.0ml F.O.)	30.9±2.8	49.3±2.9	42.9±4.7	41.0±4.0
G3 (1.5ml F.O.)	34.6±0.6	39.9±4.9	39.3±5.1	38.6±2.8
G4 (control)	33.2±2.4	35.8±2.1	33.2±5.4	34.0±2.5

^{A,B,C} Means within the same column with different superscripts are significantly different ($P<0.05$).

Treatment with fish oil led to increase seminal plasma proteins (Table 2) which adsorbed into the cold-shocked ram sperm surface and that this adsorption is able to reverse the membrane alterations induced by cold-shock and maintain high percentage of frozen-thawed ram sperm motility (Perez-Pe *et al.*, 2001).

3. Freezability of ram semen:

Data presented in the Table 3 indicated that post-thaw progressive sperm motility of ram semen in G2 and G3 supplemented with 1.0 and 1.5 ml fish oil was higher (P<0.05) than that in G1 supplemented with 0.5ml fish oil and G4 control rams during treatment period (50.9 and 51.6% vs. 44.7 and 41.9%). This trend was continued to maintain a high level after stopping the treatment (56.5 and 56.5 vs. 52.5 and 43%, respectively). The present results were confirmed by the finding of Brinsko *et al.* (2005) who found that more dramatic improvements in the quality of fresh, cooled and frozen-thawed stallion semen fed a DHA-enriched nutriceutical. In addition, Maldjian *et al.* (2005) reported that an increased amount of DHA should increase membrane fluidity and it can therefore be hypothesized that it would decrease the damage to the plasma membranes during freezing. In contrast, these results disagreed with that of Paulenz *et al.*(1999) and agrees with that of Brinsko *et al.*(2005) who observed a significant improvement in motion characteristics of sperm of cooled semen. Therefore, enhancing DHA content of the plasma membrane via changing of lipid content of the feed could improve membrane fluidity and would increase the resistance of spermatozoa to damage caused by cooling and freezing/ thawing.

Table (3): Effect of fish oil supplementation on post-thaw progressive sperm motility of ram semen collected during and post.

Groups	Motility during treatment			Motility post-stopping treatment			Overall mean of post-thaw motility±SE
	Initial Progressive motility	Pre-freezing motility	Post-thaw motility	Initial Progressive motility	Pre-freezing motility	Post-thaw motility	
G1	81.5±1.1	71.6±0.40	44.7±1.2^{B}	80.0±0.98	70.0±0.01	52.5±0.01^{B}	48.6±0.9^{B}
G2	81.0±0.7	71.6±0.50	50.9±1.3^{A}	80.0±0.9	71.3±0.04	56.5±0.03^{A}	53.7±0.4^{A}
G3	82.0±0.9	73.1±0.30	51.6±1.8^{A}	81.0±0.7	72.5±0.03	56.5±0.04^{A}	54.1±0.6^{A}
G4	80.1±0.9	70.0±0.01	40.9±0.8^{C}	79.0±1.2	70.0±0.01	43.0±0.01^{C}	42.4±0.3^{C}

^{A,B,C} Means within the same column with different superscripts are significantly different (P<0.05).

4. Fertility of frozen ram semen:

The fertility rate is considered to be the best parameter to assess the quality of frozen thawed semen (vale, 1997). The results of present study (Table 4) showed that rams supplemented with fish oil diet (G1, G2 and G3) yielded a comparatively higher conception rates (46.7, 53.3 and 53.3%, respectively) as compared to that for

rams fed control diet (G4, 33.3%). These results may be attributed to the effect of oil DHA which is an essential component of healthy sperm cells, enhancing membrane integrity from damage and tail flexibility and fertility ability (Bezard *et al.*, 1994; Lenzi et al, 1996; Penny *et al.*, 2000).

The present results indicated that conception rates obtained after cervical artificial insemination with ram frozen semen fed fish oil ranged between 46.7 to 53.5%. These percentages were lower than that reported by Sallam (1999) who found that conception rate was 66.7% and slightly higher than 41.7% that reported by El-Sharawy (2005). This may explained by the findings of Maxwell (1984) and Dettena *et al.* (1992) who reported that cervical artificial insemination with frozen semen resulted in low fertility due to the slow transport of frozen- thawed spermatozoa and their short survival in the female reproductive tract.

Table (4): Conception rate of ewes conceived with frozen-thawed semen collected from the rams supplemented with fish oil and control one.

Groups	No. of inseminated ewes	No. of conceived ewes	Conception rate
G1	15	7	46.7^{B}
G2	15	8	53.3^{A}
G3	15	8	53.5^{A}
G4	15	5	33.3^{C}

^{A,B,C} Means within a column with different superscripts differ ($P < 0.05$).

It was concluded that, the addition of graded oral doses of fish oil to the daily ration significantly improved the quality parameters of fresh ram spermatozoa (physical and chemical) and sperm production. In addition, fish oil supplements led to improve freezability of ram semen and enhanced their conception rates in ewes after cervical artificial insemination with frozen-thawed semen at natural estrus.

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تأثير التجريع بزيت السمك على جودة السائل المنوي الطازج والمجمد للكباش ومعدلات الخصوبة في النعاج

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استخدم لهذه الدراسة ٢٠ كبش خليط (٢/١ فنلندي × ٢/١ رحماني) لدراسة تأثير تجريع زيت السمك للحيوانات على صفات السائل المنوى والخصوبة فى الكباش ، قسمت الحيوانات إلى أربعة مجاميع متساوية كل منها ٥ كباش تم تغذيتها منفردة على نفس العلائق الأساسية لها لمدة أسبوعين كفترة تمهيدية ثم أعطي عن طريق الفم جرعات يومية ٠,٥ ، ١ ، ١,٥ مل زيت سمك يحتوى على ٥٠% DHA على الأقل للمجموعات ١ ، ٢ و ٣ على الترتيب لمدة ١٢ أسبوع بينما استخدمت المجموعة الرابعة كمجموعة ضابطة ، بعدها غذيت كل المجموعات على العليقة الأساسية فقط لمدة ٣ أسابيع تالية.

أظهرت النتائج زيادة معنوية ($P<0.05$) في حجم القذفة والحيوية وكذلك نسبة ألحى من الحيوانات المنوية بالنسبة للحيوانات المعاملة بزيت السمك مقارنة بتلك الغير معاملة، على العكس أدت المعاملة إلي انخفاض معنوي ($P<0.05$) في نسبة الشواذ في الحيوانات المنوية مقارنة بالمجموعة الغير معاملة (المجموعة الضابطة أو الكنترول). كما أظهرت المعاملتان ٢ ، ٣ زيادة في الحركة التقدمية للحيوانات المنوية مقارنة بالمجموعة المعاملة بجرعة منخفضة وكذا المجموعة الضابطة.

كانت نسبة الحمل المتحصل عليها بعد تلقيح ستون من النعاج التي أظهرت شبق طبيعي عن طريق عنق الرحم بالسائل المنوي للكباش المعاملة بزيت السمك نسبة عالية معنويا ($P<0.05$) وكانت ٤٦,٧ ، ٥٣,٣ ، ٥٣,٣% لكل من المجموعات ١ ، ٢ ، ٣ على الترتيب مقارنة بالنعاج التي لقحت بكباش المجموعة الضابطة (٣٣,٣%).

والخلاصة أن إضافة ١ أو ١,٥ مل من زيت السمك يوميا إلى العليقة الأساسية للكباش أدت إلى تحسين إنتاج وجودة السائل المنوي الطازج والمجمد للكباش وكذا تحسين نسبة الخصوبة.