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Effect of feeding male mice with palm, fish, and sunflower oils on sperm characteristics and sex ratio of offspring

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Abstract

Several studies have indicated that feeding female animal with fatty acids could result in alteration of offspring sex ratio; however, there is little information available regarding the effect of feeding male animal with fatty acids on sex ratio of offspring. The aim of current study was to investigate the effect of paternal feeding with n-3 and n-6 polyunsaturated fatty acids (PUFAs) on sperm characteristics and sex ratio of offspring in mice. Male mice received 2.50% of palm oil (control), fish oil (n-3 PUFAs) or sunflower oil (n-6 PUFAs) for two months. Sperm kinematics and viability were assessed using computer-assisted sperm analyzer and eosin-Y staining, respectively. Additionally, female mice were randomly introduced to males of three experimental groups to determine reproductive parameters including litter size, conception rate and sex ratio. Fish oil enhanced sperm concentration, motility and viability, whereas sunflower oil decreased sperm concentration. Nevertheless, progressive motility, velocity, linearity and straightness of sperms were not affected by source of fatty acids. Although supplementation with fish oil resulted in male-biased sex ratio, palm and sunflower oils did not impact sex ratio. Besides, source of fatty acids failed to influence conception rate and litter size. In conclusion, the present study provided evidence for the impact of paternal fish oil consumption on sex ratio of offspring for the first time. Moreover, it showed beneficial effect of n-3 PUFAs and adverse effects of n-6 PUFAs on seminal parameters.

Introduction

Polyunsaturated fatty acids (PUFAs) are fatty acids with multiple carbon-carbon double bonds in the chain. Among various types of PUFAs, n-3 and n-6 ones are of more importance as they play essential roles in regulation of different pathways.[1,2] The predominant dietary n-6 PUFA is linoleic acid (C18:2), which is converted to arachidonic acid (C20:4) following entrance into the body. Arachidonic acid is the precursor of various inflammatory mediators such as prostaglandins, prostacyclin, thromboxanes and leukotrienes.[1,2] The main dietary sources of linoleic acid are vegetable oils including corn, soybean and sunflower oils.[1,2] On the other hand, linolenic acid (C18:3), eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) are the predominant dietary n-3 PUFAs,[1,2] which have anti-inflammatory properties by inhibiting the formation of n-6 PUFAs' derivatives.[3,4] The main dietary source of linolenic acid is linseed oil and the main source of EPA and DHA is fish oil.[1,2]

Sex ratio of offspring, which is defined as the proportion of male offspring, could be affected by various environmental and biological factors.[5-9] Among environmental factors, nutrition is considered as a substantial factor contributing to offspring sex ratio alteration.[5] In terms of nutrition, feeding with fatty acids has been indicated to impact sex ratio of offspring in different species.[2,10-12] Initially, Rosenfeld *et al.* have reported that supplementation of maternal feed with fat increases proportion of male pups.[10] Further studies have revealed the influence of PUFAs including n-3 and n-6 PUFAs on sex ratio of offspring in mice, sheep and dogs.[2,11,12] Nevertheless, most of the studies in this field

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have focused on the effect of maternal nutrition on sex ratio of offspring and little information is available on whether enrichment of paternal feed with PUFAs can impact sex ratio of offspring.

In addition, numerous studies have indicated that PUFAs play critical role in regulation of sperm function through modification of testicular tissue, sperm metabolism, membrane integrity and fluidity and hormonal regulatory system in various species.[13-17]

Therefore, the present study was conducted to assess the impact of feeding male mice with n-3 and n-6 fatty acids on sperm characteristics and sex ratio of offspring.

Materials and Methods

Animals and experimental design. All procedures of present study were done under approval of Ethics Committee at AJA University of Medical Sciences (Tehran, Iran, No: 695572). The male mice (n = 45; age = around 12 weeks of age) were maintained in a temperature-controlled environment under 12 L: 12 D photoperiod and had *ad libitum* access to food and water. The male mice were randomly assigned to three experimental groups including: 1) Control group: male mice received palm oil (Table 1) at 2.50% of dry matter intake, 2) n-3 PUFA group: male mice received fish oil (Table 1) at 2.50% of dry matter intake and 3) n-6 PUFA group, male mice received sunflower oil (Table 1) at 2.50% of dry matter intake. The mice were fed with experimental diets for two months prior to the beginning of further assessments. In each experimental group, 10 male mice were subjected to epididymal sperm analysis and five male mice were allocated to mating with female mice for evaluation of conception rate, litter size and sex ratio of offspring.

Epididymal sperm preparation and evaluation. The mice were initially sacrificed by cervical dislocation. Afterwards, the dissected cauda epididymis was placed in 1.00 mL of pre-warmed T6 culture medium containing 10.00% fetal bovine serum (Sigma, St. Loius, USA). The sperms were released in the medium following dissection of the tissue concomitant with gentle agitation. The resulted semen samples were incubated at 37.00 ˚C for 15 min prior to analysis. The sperm count and motility were analyzed by a computer-assisted sperm analyzer (SCA® CASA, version 5.1; Microptic Co., Barcelona, Spain) which was connected to a phase contrast microscope (Eclipse E-200; Nikon, Tokyo, Japan) equipped with a warm plate. Assessments were implemented using pre-warmed (37.00 ˚C) Leja slides (20.00 µm in depth; Leja, Amsterdam, Netherlands). The loaded chamber was placed on a warm plate of the microscope (37.00 ˚C) for 3 min before the analysis. The CASA outcomes included total motility, progressive motility, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN: VSL/VCL) and straightness (STR: VSL/VAP). Viability was evaluated using eosin-Y staining method, in which 10.00 µL of sperm sample was mixed with 10.00 µL of dye (0.50% w/v; Merck, Darmstadt, Germany). A total of 200 sperm cells were counted for each sample. Evaluation of live (unstained) and dead (red-stained) spermatozoa was carried out using a phase contrast microscopy (Nikon).

Reproductive parameters. Female mice, all of which had received similar standard diet containing no additional source of fatty acids, were subjected to subcutaneous injection of equine chorionic gonadotropin (8.00 IU; Hipra, Girona, Spain) followed by an intraperitoneal injection of human chorionic gonadotropin (8.00 IU; IBSA, Lugano, Switzerland) 47 hr later. Subsequently, female mice were randomly introduced to males of three different experimental groups (two female mice were caged with each male mouse). Afterwards, female mice were followed to determine reproductive parameters including litter size, conception rate and sex ratio. The sex of litters was determined by evaluating anogenital distance two days after birth, which was further confirmed at weaning.[10] Conception rate was defined as the number of female mice conceived divided by the number of those introduced to male mice. Sex ratio was defined as the number of male pups divided by the number of all pups born.

Statistical analysis. Data associated with conception rate and sex ratio were analyzed by logistic regression analysis using GENMOD procedure including function link logit in the model. Logistic regression analyses generated odds ratios as the estimates of strength of difference. Data of total motility, progressive motility, LIN, STR and viability were arcsine-transformed prior to analysis. Afterwards, the respective data in addition to sperm concentration, VCL, VSL and VAP were analyzed using GLM procedure.

Table 1. Fatty acid composition of the palm, fish and sunflower oils fed to male mice in the present study.

Fatty acid	Palm oil (%)	Fish oil (%)	Sunflower oil (%)
Myristic acid (C14:0)	1.10	3.200	0.10
Palmitic acid (C16:0)	39.30	18.80	5.60
Palmitoleic acid (C16:1)	0.20	5.90	0.10
Stearic acid (C18:0)	11.40	3.40	4.20
Oleic acid (C18:1)	36.40	22.70	19.80
Linoleic acid (C18:2)	8.50	5.60	66.90
Linolenic acid (C18:3)	0.30	2.00	0.20
Eicosapentaenoic acid (C20:5)	-	12.40	-
Docosahexaenoic acid (C22:6)	-	17.90	-
Others	2.80	8.10	3.10

Multiple comparisons were performed using LSMEANS statement. All analyses were conducted in SAS (version 9.4; SAS Institute Inc., Carry, USA). Differences at $p < 0.05$ and $0.05 \leq p < 0.10$ levels were considered significant and tended to be significant, respectively. Data are presented as proportions and mean ± SEM.

Results

Sperm characteristics. Sperm viability was greater in n-3 PUFA group than the control and n-6 PUFA groups ($p < 0.05$). It did not differ between the control and n-6 PUFA groups ($p > 0.05$; Fig. 1). Concentration of sperm was higher in the control and n-3 PUFA groups compared to n-6 PUFA group ($p < 0.05$), but it was not different between the control and n-3 PUFA groups ($p > 0.05$; Table 2). Total motility was higher in n-3 PUFA than the control group ($p < 0.05$) and it tended to be higher in n-3 PUFA group compared to n-6 PUFA group ($0.05 \leq p < 0.01$). However, it was not different between the control and n-6 PUFA groups ($p > 0.05$; Table 2). Progressive motility, VCL, VSL, VAP, LIN and STR did not differ among the control, n-3 PUFA and n-6 PUFA groups ($p > 0.05$; Table 2).

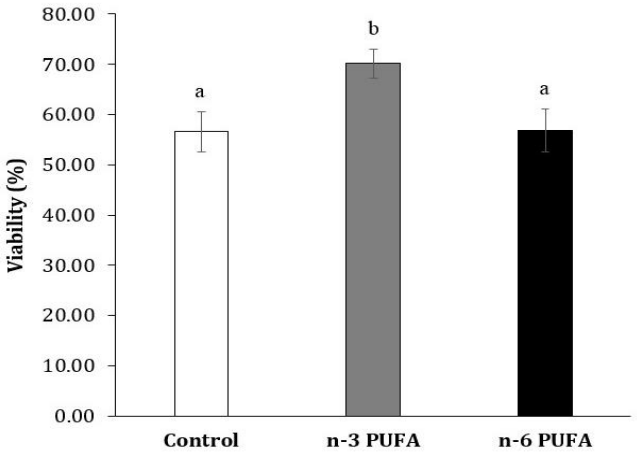

Fig. 1. Sperm viability in male mice received palm oil (control), fish oil (n-3 PUFA) or sunflower oil (n-6 PUFA).
[ab] Different letters indicate significant difference at $p < 0.05$.

Reproductive parameters. Conception rate and litter size were not different among the control, n-3 PUFA and n-6 PUFA groups ($p > 0.05$; Table 3). Sex ratio was higher in n-3 PUFA group compared to the control (odds ratio: 2.48, 95.00% confidence interval: 1.26 - 4.85) and n-6 PUFA (odds ratio: 2.56, 95.00% confidence interval: 1.28 - 5.12) groups ($p < 0.01$). However, it did not differ between the control and n-6 PUFA groups ($p > 0.05$; Table 3).

Table 3. Reproductive parameters of female mice mated by male mice received palm oil (control), fish oil (n-3 PUFA) or sunflower oil (n-6 PUFA). Data are presented as mean ± SEM.

Groups	Conception rate (%)	Litter size	Sex ratio (%)
Control	90.00 (9/10)	8.22 ± 0.38	41.54 (27/65)[a]
n-3 PUFA	100.00 (10/10)	8.00 ± 0.60	63.75 (51/80)[b]
n-6 PUFA	80.00 (8/10)	7.38 ± 0.84	40.68 (24/59)[a]

[ab] Values with different superscripts within columns differ significantly at $p < 0.05$.

Discussion

Feeding male mice with diet enriched with fish oil skewed sex ratio of offspring towards males. Although numerous studies have demonstrated alteration of sex ratio of offspring following maternal consumption of fatty acids,[2,10-12] this is the first report indicating such phenomenon in response to paternal feeding of n-3 PUFAs, to our knowledge. This skewness could be attributed to the greater sperm concentration, viability and motility in mice supplemented with fish oil since evaluating the association between sperm characteristics and gender of offspring in red deer, Gomendio *et al.* have found that male fertility and proportion of morphologically normal sperms were positively correlated with sex ratio of offspring.[18] Although sperm velocity has been suggested as a determining factor for sex ratio bias in human population,[19] we did not find any significant effect of fish oil either on VCL, VSL and VAP or LIN and STR in the present study.

Irrespective of the effect of fish oil on sex ratio of offspring, the present study showed that diet enriched with fish oil as a source of n-3 PUFAs improved seminal parameters including sperm concentration, viability and motility, whereas diet fortified with sunflower oil as a source of n-6 PUFAs diminished concentration of sperm. Likewise, Yan *et al.* have observed enhancement of sperm density and motility in rats as the ratio of n-3 relative to n-6 PUFAs increased.[14] Furthermore, Ferramosca *et al.* have reported that male rats fed diet containing n-3 PUFAs had higher concentration of spermatozoa with greater motility compared to those fed diet composed of saturated fatty acids.[17]

Table 2. Sperm characteristics in male mice received palm oil (control), fish oil (n-3 PUFA) or sunflower oil (n-6 PUFA). Data are presented as mean ± SEM.

Groups	Concentration ($\times 10^6$ sperm mL^{-1})	Total motility (%)	Progressive motility (%)	VCL (μm sec^{-1})	VSL (μm sec^{-1})	VAP (μm sec^{-1})	LIN (%)	STR (%)
Control	6.80 ± 0.31[a]	49.60 ± 4.28[a]	32.54 ± 3.67	84.92 ± 9.04	25.86 ± 3.30	39.23 ± 3.84	30.43 ± 2.13	64.58 ± 2.33
n-3 PUFA	7.89 ± 0.27[a]	64.30 ± 3.06[b]	41.36 ± 2.54	85.15 ± 6.93	27.45 ± 5.20	41.02 ± 4.76	32.71 ± 5.55	63.52 ± 4.51
n-6 PUFA	5.17 ± 0.55[b]	52.10 ± 3.79[ab]	33.98 ± 4.59	94.56 ± 6.27	26.33 ± 1.50	40.40 ± 2.38	28.46 ± 1.80	65.28 ± 2.81

VCL: curvilinear velocity, VSL: straight line velocity, VAP: average path velocity, LIN: linearity, and STR: straightness.
[ab] Values with different superscripts within columns differ significantly at $p < 0.05$.

Greater testicular morphology including enhanced development of spermatogonia and spermatocytes as progenitors of spermatozoa has been observed in response to feeding proper ratio of n-3/n-6 PUFAs to male rats and roosters.[14,16] Furthermore, appropriate ratio of n-3 PUFAs augmented the concentration of GnRH, LH, FSH and testosterone (hormones which are responsible for regulation of spermatogenesis in the male animals) in male rats and roosters.[14,16,20] In addition, Feng *et al.* have revealed that the increment in ratio of n-3 PUFAs in diet of roosters leads to up-regulation of GnRH, LH and FSH receptors[16] as well as steroidogenic acute regulatory protein playing a pivotal role in internalization of cholesterol into mitochondria and testosterone synthesis in Leydig cells.[21] Feeding n-3 PUFAs has also indicated to expand testosterone positive area and metabolism in mice testes.[22] Collectively, the positive impact of fish oil on sperm concentration in the present study could have originated form enhancement of testicular germinal layer and/or hormonal secretion in the male mice.

Fish oils containing EPA and DHA are well-known to attenuate oxidative stress, thereby inhibiting corresponding apoptotic processes in various types of cells.[23,24] Additionally, fish oil has been demonstrated to alleviate cisplatin-induced toxicity in testicular tissue via prevention of oxidative stress in rats.[25] Supplementation with n-3 PUFAs has also been reported to reduce lipid peroxidation in sperm of dogs[26] and mice.[17] Given that oxidative stress and resultant lipid peroxidation can adversely affect sperm function,[27,28] the enhanced sperm viability and motility in male mice fed diet supplemented with fish oil might have resulted from antioxidant effects of EPA and DHA as prominent components of fish oil.

Moreover, Ferramosca *et al*. have found that supplementation with n-3 PUFAs enhances mitochondrial respiratory efficiency, thus increasing ATP generation in rats.[17] Considering that mitochondrial functionality could substantially impact sperm quality,[29] enhanced mitochondrial functions could also explain the greater sperm viability and motility in the fish oil group in the present study.

In conclusion, the present study revealed that paternal supplementation with fish oil could not only enhance seminal characteristics but also skews the sex ratio of resultant offspring towards males. Conversely, consumption of sunflower oil as a source of n-6 PUFAs reduced semen concentration.

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Conflict of interest

The authors do not have any potential conflict of interest to declare.

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