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Original Article

Impact of omega-3 fatty acids preconception intake on some fertility parameters and foetuses quality of female rats

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Abstract

Background: Research has demonstrated that certain nutrients can reverse infertility. **Aims:** The present study, "Impact of Omega-3 fatty acids preconception intake on some fertility parameters of female rats and foetuses quality" was conducted to further investigate this subject. **Methods:** Subjects were thirty-four female and twelve male adult Albino rats, weighing 172-181 g. Initially, 12 male rats were assigned to treatment groups 1, 2, 3, and 4, and 24 females were assigned to treatment groups 5, 6, 7, and 8. The treatment protocol was administered orally as follow: groups 1 and 5 (controls), 0.3 ml distilled water (as placebo); groups 2 and 6, 250 mg/kg Omega-3 fatty acids (O3FA); groups 3 and 7, 25 mg/kg cyclophosphamide (CPP, negative control); and groups 4 and 8, 25 mg/kg CPP + 250 mg/kg of O3FA for 28 days. Thereafter, they were cohabitated (1:2 male to female ratio) into mating groups 1 to 7 until mating was confirmed. Ten pregnant rats (5 of which were administered 500 mg/kg O3FA on days 15 and 16 of gestation) were used for abortifacient effect experiment. **Results:** Number of implantation sites (IM), implantation index (IMI), percentage of pregnant females (PPF), life foetal number, foetal weight, foetal crown-rump length (FCRL), corpora luteal number (CLN) and fertility index (FI) in O3FA-treated female rats and their foetuses significantly increased ($P<0.05$) compared with negative control. No noticeable abortifacient effect occurred. **Conclusion:** Preconception intake of O3FA may have impacted female fertility positively.

Key words: Abortifacient, Conception, Early-development, Female rat, Omega-3 fatty acids

Introduction

Despite the fact that allopathic medicines have proved to be the best in treating infertility cases, they have shown some grave side effects involving various vital organs of the body. Therefore, there is a pressing need globally for alternatives with minimal or no side effect. Infertility, is considered to be a decreased ability to produce children (Bonanomi *et al*., 2002), estimated to have affected 186 million people worldwide (Inhorn and Patrizio, 2015). Female infertility occurs as a result of ovulation disorders, uterine or cervical abnormalities, fallopian tube damage or blockage, endometriosis, primary ovarian insufficiency, pelvic adhesions, thyroid problems, cancer and its treatment, and certain medications. Similarly, human exposure to environmental agents may be hazardous to the reproductive function (Bonde, 1996; Spira and Multigner, 1998).

Despite the alarming increase in infertility, studies have demonstrated that certain nutrients can reverse it. Omega-3 fatty acids (O3FA) are a group of polyunsaturated fats found naturally in a wide variety of foods and are unequivocally essential for many physiological processes, including growth, vision, brain development, maintaining health through life, reproduction, and early foetal development (Gurr *et al*., 2002). Previous studies revealed that marine organisms are responsible for almost all-natural production of O3FA (Swanson *et al*., 2012; Kabeya *et al*., 2018). The O3FA can influence reproductive processes through a variety of mechanisms. It provides the precursors for prostaglandin synthesis and can modulate the expression patterns of many key enzymes involved in both prostaglandin and steroid metabolism. It is an essential component of all cell membranes. Spermatozoa require a high amount of polyunsaturated fatty acids (PUFA) to provide the plasma membrane with the fluidity essential at fertilization (Wathes, 2007). The developing foetus requires substantial amounts of O3FA to support rapid cellular growth and activity. Maternal dietary supplementation with O3FA during pregnancy has been shown to enhance foetal growth and reduce the risk of pregnancy complications. Recent studies show that maternal dietary n-3 PUFA supplementation during rat pregnancy can reduce placental oxidative damage (Ball and Peters, 2004).

There is scarcity of literature on the impact of preconception O3FA intake on certain parameters regarding the fertility of female rats and foetus quality. Therefore, the present study was designed to evaluate the aforementioned impact of O3FA intake and potential abortifacient property.

Materials and Methods

Drug used

Drugs used were procured from reputable pharmaceutical shops, especially the O3FA which was obtained from Prevention Pharmaceuticals, Lambertville, Michigan, USA. Cyclophosphamide (Cytoxan) Oral Cap: 25 mg, 50 mg used was procured from Baxter Health Care, Corporate address: One Baxter Parkway Deerfield, IL 60015-4625, United States.

Animal care

Thirty-four female and twelve male adult Albino rats weighing 172-181 g (with no history of prior use in any investigation) were obtained from the Genetics and Experimental Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria. The ethical aspects and experimental protocol related to conducting experiments on live animals were strictly observed as approved by the University of Nigeria, Nsukka Senate Committee on Medical and Research ethics. The animals were allowed to acclimatize for two weeks before being used for the experiment. They were housed in stainless wire rat cages supplemented with drinkers and faecal gathering trays, fed with commercial growers' mash (19% protein, Vital Feeds, Grand Cereals Limited, Jos, Nigeria) and portable water *ad libitum*, kept in a 12-hour light/dark cycle.

Experimental design 1

Thirty-six adult Albino rats were used for the first experiment. Twelve male rats were assigned to treatment groups 1, 2, 3, and 4 while 24 females were assigned to treatment groups 5, 6, 7, and 8. The groups were treated as following: groups 1 and 5 (controls) received 0.3 ml distilled water (as placebo), groups 2 and 6 received 250 mg/kg O3FA, groups 3 and 7 received 25 mg/kg cyclophosphamide (CPP) (negative control), and groups 4 and 8 received 25 mg/kg CPP + 250 mg/kg of O3FA. Treatment lasted 28 days, administered using plastic syringes attached to metal oropharyngeal cannula. However, O3FA was administered at two-day intervals, administration of CPP was at 14-days intervals. Cyclophosphamide was used to induce infertility (Fig. 1).

Experimental design 2

The abortifacient effect of O3FA was determined using ten non-treated sexually mature female rats assigned into 2 groups of 5 rats each. The treatment group received intraperitoneal injection of 500 mg/kg of O3FA while the control group received intraperitoneal injection of 0.3 ml of distilled water on days 15 and 16 of gestation (Fig. 2).

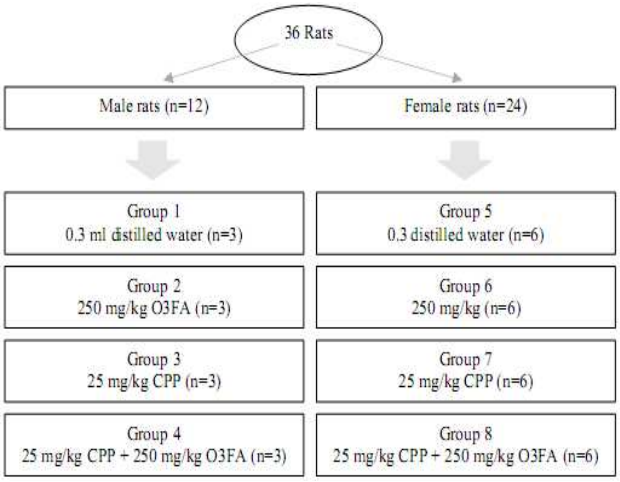

Fig. 1: Experimental design 1. Impact of O3FA preconception intake on fertility index (FI) of female rats. Group 1: Placebo, male; group 2: 250 mg/kg O3FA, male; group 3: 25 mg/kg CPP, male; group 4: 25 mg/kg CPP + 250 mg/kg O3FA, male; group 5: Placebo, female; group 6: 250 mg/kg O3FA, female; group 7: 25 mg/kg CPP, female, and group 8: 25 mg/kg CPP + 250 mg/kg O3FA, female

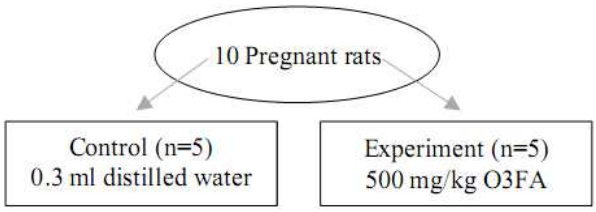

Fig. 2: Experimental design 2. Abortifacient effect of O3FA in female rats

O3FA effect on certain fertility parameters and foetus quality

After 28 days of treatment, the male and female rats from different treatment groups were cohabitated in a ratio of 1:2 into mating groups 1 to 7 (Table 1) until mating was confirmed. Successful mating was confirmed following the method described by Ochiogu *et al.* (2006). This was marked day 1 of gestation.

Table 1: Pairing and treatment of male and female Albino rats

Mated group	Treatment of mated groups	
1	Placebo, male (group 1)	Placebo, female (group 5)
2	Placebo, male (group 1)	250 mg/kg O3FA, female (group 6)
3	250 mg/kg O3FA, male (group 2)	Placebo, female (group 5)
4	250 mg/kg O3FA, male (group 2)	250 mg/kg O3FA, female (group 6)
5	Placebo, male (group 1)	25 mg/kg CPP, female (group 7)
6	25 mg/kg CPP, male (group 3)	Placebo, female (group 5)
7	25 mg/kg CPP + 250 mg/kg O3FA, male (group 4)	25 mg/kg CPP + 250 mg/kg O3FA, female (group 8)

O3FA: Omega-3 fatty acids, and CPP: Cyclophosphamide

The male rats were withdrawn from the females upon successful mating. Laparatomisation of the pregnant female rats under high ether anaesthesia was carried out on day 20 of gestation. The uterine horns were exteriorized and cut at the greater curvature followed by examination and determination of the corpora luteal number (CLN) per pregnant female, live foetuses number (LFN) as well as the foetuses weights (FWs), foetal crown-rump length (FCRL), percentage of pregnant female (PPF), number of implantation sites (IM), and resorbed embryo number (REN). Implantation index (IMI), resorption index (RI), and fertility index (FI) were evaluated following Uchendu *et al*. (2000), Abu and Uchendu, (2011), and Costa-Silva *et al*. (2007).

$$RS = IM - LFN$$

$$RI = \frac{RS}{IM} \times 100$$

$$IMI = \frac{IM}{CLN} \times 100$$

$$FI = \frac{LFN \times FCRL \times PPF}{CLN}$$

Where,
RS: Number of resorption sites
IM: Number of implantation sites
LFN: Live foetal number
RI: Resorption index
IMI: Implantation index
CLN: Corpus luteal number
FI: Fertility index
FCRL: Foetal crown-rump length
PPF: Percentage of pregnant female

Abortifacient effect of O3FA

Ten female rats with confirmed pregnancy (following the method described by Ochiogu *et al*. (2006)) were assigned to two groups, comprising 5 rats each. Group 1 was administered placebo and served as the control, and group 2 received 500 mg/kg O3FA. The two groups were treated at days 15 and 16 of gestation through intraperitoneal injection.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out on the results using the IBM Statistics UK (version 20.0). The means were separated using Duncan's new multiple range tests while differences in the means were considered significant at probability values less than 5% ($P<0.05$). The results were presented as mean±SEM.

Results

O3FA effects on IM, IMI, REN, and RI

Omega-3 fatty acids effects on IM, IMI, REN, and RI are shown in Table 2. Whereas O3FA treated pregnant rats showed significant increase ($P<0.05$) in IM in groups 2, 3, and 4, IMI had a significant increase ($P<0.05$) only in groups 2 and 3 compared to negative control. There was no significant difference ($P>0.05$) in REN and RI among all treatment groups. Additionally, no significant difference ($P>0.05$) was observed comparing O3FA treatment groups with normal control for all parameters (Table 2).

Omega-3 fatty acids effects on PPF, LFN, FW, FCRL, and CLN are shown in Table 3. A significant increase was observed in PPF, life fetal number, fetal weight, FCRL, and CLN of O3FA treatment compared with negative control. One-hundred (100) % PPF was recorded in all O3FA-treated pregnant rats (2, 3, 4, and 7). In Omega-3 fatty acids treatment groups (2, 3, and 4), there was a significantly increased life fetal number and CLN as compared with negative controls (5 and 6). Whereas O3FA treatment group 2 experienced a significant increase in fetal weight, groups 3 and 4 showed an increase in FCRL compared with negative controls (5 and 6). However, there was no significant difference ($P>0.05$) comparing O3FA treatments with normal control for all parameters (Table 3).

Effect of omaega-3 fatty acids on FI

Effect of O3FA on FI of mated groups are shown in Fig. 3.

Omeg-3 fatty acids treatment groups (2, 3, and 4) showed higher FI than the negative controls (5 and 6).

Abortifacient effect of O3FA

The high dose of O3FA (500 mg/kg) administered to pregnant female rats on days 15 and 16 of gestation did not produce any noticeable abortifacient effects.

Table 2: O3FA effects on IM, IMI, REN, and RI

Mated groups	IM	REN	RI	IMI
1	8.67 ± 0.33^{b}	0.50 ± 0.34^{a}	6.01 ± 4.21^{a}	80.00 ± 4.38^{abc}
2	9.00 ± 0.26^{b}	1.00 ± 0.37^{a}	11.39 ± 4.34^{a}	90.46 ± 3.50^{c}
3	7.83 ± 0.48^{b}	0.50 ± 0.34^{a}	7.41 ± 5.49^{a}	94.17 ± 4.17^{c}
4	8.00 ± 0.45^{b}	1.00 ± 0.37^{a}	13.66 ± 5.47^{a}	88.98 ± 4.16^{bc}
5	2.83 ± 0.91^{a}	1.00 ± 0.52^{a}	22.50 ± 11.09^{a}	51.53 ± 17.15^{ab}
6	3.17 ± 1.56^{a}	0.33 ± 0.21^{a}	4.63 ± 3.01^{a}	45.95 ± 20.64^{a}
7	5.17 ± 0.65^{a}	1.17 ± 0.54^{a}	29.17 ± 13.57^{a}	81.75 ± 8.29^{abc}

Values with different alphabet superscript in a column were significantly different ($P<0.05$). Data are presented as mean±SE. O3FA: Omega-3 fatty acids, IM: Number of implantation sites, IMI: Implantation index, REN: Resorbed embryo number, and RI: Resorption index. Mated groups: 1 = Placebo (control) female mated with placebo (control) male, 2 = O3FA female mated with placebo male, 3 = Placebo female mated with O3FA male, 4 = O3FA female mated with O3FA male, 5 = CPP female mated with placebo male, 6 = Placebo female mated with CPP male, and 7 = CPP + O3FA female mated with CPP + O3FA male

Table 3: O3FA effects on PPF, LFN, FW, FCRL, and CLN

Mated groups	PPF (%)	LFN	FW (g)	FCRL (cm)	CLN
1	100	8.67 ± 0.33^{b}	4.97 ± 0.25^{abc}	4.41 ± 0.15^{c}	9.17 ± 0.40^{d}
2	100	9.00 ± 0.26^{b}	6.05 ± 0.21^{c}	4.21 ± 0.09^{bc}	10.00 ± 0.37^{d}
3	100	7.83 ± 0.48^{b}	5.09 ± 0.18^{abc}	4.34 ± 0.34^{c}	8.33 ± 0.42^{cd}
4	100	8.00 ± 0.45^{b}	5.75 ± 0.21^{bc}	4.68 ± 0.03^{c}	9.00 ± 0.37^{cd}
5	67	2.83 ± 0.90^{a}	3.76 ± 1.19^{ab}	2.76 ± 0.87^{ab}	3.83 ± 1.32^{ab}
6	50	3.17 ± 1.56^{a}	2.96 ± 1.32^{a}	2.04 ± 0.91^{a}	3.50 ± 1.75^{a}
7	100	5.17 ± 0.65^{a}	5.68 ± 0.13^{bc}	4.08 ± 004^{bc}	6.33 ± 0.42^{bc}

Values with different alphabet superscript in a column were significantly different (P<0.05). Data for LFN, FW, FCRL, and CLN are presented as mean±SE. O3FA: Omega-3 fatty acids, PPF: Percentage of pregnant females, LFN: Life foetal number, FW: Foetal weight, FCRL: Foetal crown-rump length, and CLN: Corpora luteal number. Mated groups: 1 = Placebo (control) female mated with placebo (control) male, 2 = O3FA female mated with placebo male, 3 = Placebo female mated with O3FA male, 4 = O3FA female mated with O3FA male, 5 = CPP female mated with placebo male, 6 = Placebo female mated with CPP male, and 7 = CPP + O3FA female mated with CPP + O3FA male

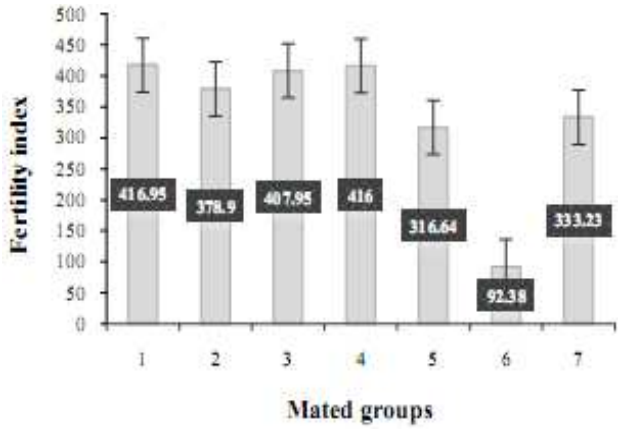

Fig. 3: O3FA effect on FI of various mated groups. Mated groups: 1 = Placebo (control) female mated with placebo (control) male, 2 = O3FA female mated with placebo male, 3 = Placebo female mated with O3FA male, 4 = O3FA female mated with O3FA male, 5 = CPP female mated with placebo male, 6 = Placebo female mated with CPP male, and 7 = CPP + O3FA female mated with CPP + O3FA male. The error bars show mean+SE

Discussion

Implantation involves a sequence of processes controlled by several types of signaling pathways between the embryo and the uterus during the early period of gestation. It mainly entails positioning, attachment, and invasion of the embryo (blastocyst) in the uterus (Wang and Dey, 2005). The significant increase in the IM and IMI caused by the activities of O3FA as demonstrated by this study suggests that O3FA positively impacted fertilized eggs and uterine wall relationship. This effect may have been mediated through the O3FA influence on the micro-uterine environment making it conduce for proper positioning, attachment, and invasion of the embryo. Conversely, the activities of O3FA caused no significant difference in REN and RI in all treatment groups. Webel *et al*. (2004) reported that supplementing O3FA from fish oil in the diet of sows improved early embryo survival.

Maternal preconception nutrition is believed to affect fertility outcomes (Mmbaga and Luk, 2012). Similar research have demonstrated that feeding unsaturated fatty acids tended to increase the fertilization rate, significantly increased the proportion of excellent and good quality embryos, and decreased degenerated embryos (Cerri *et al*., 2009). Generally, a significant increase was observed in PPF, life fetal number, fetal weight, FCRL and CLN of subjects in the O3FA treatment groups compared with negative control. The 100% PPF recorded in O3FA-treated animals was similar to the findings of Burke *et al*. (1996), but our findings on LFN and FW was incongruent with Muhlhausler *et al*. (2011). Similarly, clinical data regarding the effect of maternal O3FA supplementation on the outcomes of human pregnancy also showed no significant difference in the number of offspring, but in contrast there was a significant increase in birth weight (Makrides *et al*., 2006, 2010). Fetal crown-rump length is a parameter for fetal growth (Abu and Uchendu, 2011). Our findings on FCRL agreed with Makrides *et al*. (2006) and Szajewska *et al*. (2006). The CLN result was similar to Perez *et al*. (1995) but disagreed with Kojima *et al*. (1997).

This present study also demonstrated that O3FA may possess FI boosting property. Importantly, there was no observed significant difference between the normal control and O3FA treatment group for all parameters.

Any agent/substance that induces abortion is said to be abortifacient (Rajeswari and Rani, 2014). The 500 mg/kg of O3FA administered to the pregnant rats did not cause any noticeable abortifacient effect. This may simply indicate that O3FA lacks the capacity/property of inducing abortion. This observation may imply that consuming O3FA is safe for pregnant women in the third trimester.

The preconception intake of O3FA may have positive impact on female fertility. There was no observed abortifacient activity, reprotoxicity effect, or teratogenicity effect caused by intake of O3FA.

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Conflict of interest

There is no conflict of interest among the authors.

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