Taiwanese Journal of Obstetrics & Gynecology 55 (2016) 351–356

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Immunohistochemical and ultrastructural analysis of the effect of omega-3 on embryonic implantation in an experimental mouse model

Kemal Sarsmaz [a], Asli Goker [a,*], Serap Cilaker Micili [b], Bekir Ugur Ergur [b], Naci Kemal Kuscu [a]

[a] Department of Obstetrics and Gynecology, Celal Bayar University Faculty of Medicine, Manisa, Turkey
[b] Department of Histology and Embryology, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey

ARTICLE INFO

Article history:
Accepted 11 January 2015

Keywords:
electron microscope
implantation
laminin
leukemia inhibitory factor
omega-3

ABSTRACT

Objective: Implantation is the first step to a healthy pregnancy. Omega-3 supplementation is common to use during pregnancy, for its antioxidant and membrane stabilising effect. In this study we have aimed to study the effect of Omega-3 supplementation on implantation in a mouse model by immunohistochemical methods and electron microscopic evaluation.
Materials and methods: Mice were randomized into three groups to receive standard food, Omega-3 400 mg/kg and Omega-3 1000 mg/kg one menstrual cycle before mating. Mice were sacrificed on third day of estimated implantation and uterine horns were evaluated immunohistochemically for staining of Laminin and Leukemia Inhibitory Factor (LIF) and ultrastructural morphology.
Results: Laminin and LIF immunoreactivity were increased signifcantly in the high dose group when compared to the control and low-dose groups in lumen epithelium basal membrane, gland epithelium basal membrane and endometrial stroma. Electron-microscopic evaluation showed a decrease in epithelial height and microvilli loss in the high dose groups.
Conclusion: Omega-3 supplementation increased implantation markers Laminin and LIF and decreased epithelial height and microvilli thus seems to prepare the endometrium for a favorable environment of implantation.

Introduction

Implantation is the first step of pregnancy, which is a complex sequence of events comprising the blastocyst, endometrium, and regulatory molecules. Steroid hormones, cytokines, integrins, growth factors, adhesion molecules, and pinopodes regulate the implantation process. The implantation window is the period when the blastocyst interacts with the endometrial epithelium and is in the receptive stage [1–4].

Endometrial maturation is associated with the loss of surface microvilli and ciliated cells and the formation of pinopodes, which depends on progesterone [5]. A decidual reaction is the transformation of the endometrium to a receptive state in which connective tissue stores glycogen and fat to grow and form polygonal cells [6]. During decidualization, the following occur: deoxyribonucleic acid, ribonucleic acid, and protein synthesis; reformation of the extracellular matrix; and integrin expression [7]. The apical epithelial surface is nonadhesive; however, during implantation the interaction between trophoectoderm and the luminal epithelium triggers a remodeling in epithelial cell organization. The cells flatten and lose their microvilli and the polarity between apical-basal luminal epithelium decreases [8]. The success of implantation depends on the correct timing of the blastocyst–endometrium encounter.

Fatty acids are classified as saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Saturated fatty acids can be synthesized in the body, whereas some polyunsaturated fatty acids such as linoleic acid and alpha linolenic acid are essential fatty acids [9]. Essential fatty acids are used in the synthesis of prostaglandins, thromboxanes, and leukotrienes [10], are structural components of cell membranes, and are needed for cell functioning [11]. Omega-3 is an essential fatty acid found in

* Corresponding author. Celal Bayar University Faculty of Medicine, Mimar Sinan Bulvarı, Department of Obstetrics and Gynecology, Manisa, Turkey.
E-mail address: asligoker@gmail.com (A. Goker).

http://dx.doi.org/10.1016/j.tjog.2016.04.011

some fish [11]. Insufficient omega-3 fatty acid may lead to increased triglyceride and cholesterol levels, growth retardation, hypertension, impaired wound healing, hair loss, depression of the immune system and postpartum depression [12–15]. Omega-3 integrates into the phospholipids of the cell membrane and is important for mitochondrial-specific functions [16]. This study aimed to investigate the effect of omega-3 fatty acid supplementation on implantation.

Materials and methods

This experimental study was approved by the Ethics Committee of the Research of Laboratory Animals at Dokuz Eylul University Medical School (Izmir, Turkey; approval number, 53/2011). All procedures were performed in accordance with the principles of laboratory animal care.

Twenty-one albino mice [*Mus musculus* (C/C)] weighing 18–22 g were used. The animals were maintained under standardized laboratory conditions in an air-conditioned room at a room temperature of 20–22°C. They had free access to food and water, and underwent light-dark periods of 12 hours. The mice's regular menstrual periods were determined by vaginal smears. They were then divided into three groups. Group I was fed standard animal food pellets; Group II was fed standard animal food pellets and was administered low-dose omega-3 (400 mg/kg omega-3; Marincap 500 mg, Kocak Farma, Istanbul, Turkey) by the oral route; and Group III was fed standard animal food pellets and was administered high-dose omega-3 (1000 mg/kg omega-3, Marincap 500mg; Kocak Farma) by the oral route. Omega-3 supplementation was applied during the estrus phase for one menstrual period to Groups II and III and the mice were allowed to mate. The vaginal plaque was checked for pregnancy the following day and the time of 12:00 was considered embryonic (E) Day 0.5. The mice were sacrificed on the expected day of implantation, namely Day 3.5. Omega-3 supplementation was applied for 8 days. The mice were anesthetized by ether, and 0.1 mL 1% Chicago Blue (Sigma–Aldrich, USA) was applied intravenously. After 10 minutes, a laparotomy was performed. Foci on the uterine horns that were blue were the implantation regions.

The tissues were fixed by 10% buffered formalin for 48 hours, and then embedded in paraffin. The paraffin blocks were placed in a rotary microtome (RM 2255; Leica Microsystems, Wetzlar, Germany) and 5-mm thick sections were obtained [17]. After deparaffinization and rehydration, all sections were stained with hematoxylin and eosin. The images were analyzed by using a computer-assisted image analyzer system consisting of a microscope (BX51; Olympus, Tokyo, Japan), and the images were transferred into the computer using a digital video camera (DP71; Olympus).

For immunohistochemistry, antibodies to LIF (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and laminin (Santa Cruz Biotechnology, Inc.) were applied. After deparaffinization and rehydration, the sections were treated with trypsin (Cat No: 00-3008 Digest All 2A; Zymed, San Francisco, CA, USA) at 37°C for 15 minutes. To inhibit endogenous peroxidase activity, the sections were incubated in a solution of 3% hydrogen peroxide for 15 minutes, and then with normal serum blocking solution. The sections were again incubated in a humid chamber for 18 hours at +4°C with anti-LIF antibody (1/100 dilution) and anti-laminin antibody (1/100 dilution). They were thereafter incubated with biotinylated immunoglobulin G (IgG), followed by streptavidin conjugated to horseradish peroxidase for 15 minutes each. The sections were prepared in accordance with the kit instructions (85-9043; Invitrogen Corporation, Camarillo, UK). The sections were finally stained with diaminobenzidine (1718096; Roche, Mannheim, Germany), counterstained with Mayer hematoxylin, and analyzed by using a light microscope [18]. Immunohistochemical staining was evaluated by a semiquantitative method. Staining was classified as strong (+++, 3), moderate (++, 2), weak (+, 1), and ambiguous (−, 0). Two histologists inspected the slides.

Uterine tissues (~1 mm^3) were fixed with 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.2) for 48 hours at 4°C. The tissues were washed in the same buffer overnight after the primary fixation. The tissues were postfixed with 1% osmium tetroxide in sodium phosphate buffer for 1 hour at 4°C. The postfixed tissues were then washed in the same buffer and dehydrated by a graded series of ethanol starting at 50% for each step for 10 minutes, and finally with propylene oxide. The tissue specimens were embedded in araldite. Ultrathin sections were cut from the blocks on an ultramicrotome (Leica, Deerfield, IL, USA) and mounted on copper grids, and double-stained with uranyl acetate and lead citrate before they were examined with a transmission electron microscope (Libra 120; Carl Zeiss, Germany) and digitally photographed [17].

The data were statistically evaluated using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were analyzed using the Kruskal–Wallis test and further analysis was performed by the Mann–Whitney *U* test. Values of $p < 0.005$ were considered significant.

Results

Light microscopic evaluations of the specimens revealed that the endometrium consisted of the lamina propia, which was characterized by endometrial lumen epithelium and endometrial glands in the most inner part, the myometrium in the middle part, and the perimetrium covering the outer part. The lumen epithelium consisted of a single layer of prismatic epithelial cells. Stromal cells and uterine connective tissue were visible. Cells of the uterine lumen were short. The muscle cells of the myometrium had a normal structure. The primary and secondary decidual regions were identified as implantation markers.

Laminin immunoreactivity calculated for the control group (lumen epithelium basal membrane, 1.71 ± 0.48; gland epithelium basal membrane, 1.57 ± 053; endometrial stroma, 1.57 ± 053) and the low-dose group (lumen epithelium basal membrane, 1.57 ± 0.53; gland epithelium basal membrane, 1.42 ± 0.53; endometrial stroma, 1.85 ± 0.37) was not significantly different, but it was significantly higher in the high-dose group (lumen epithelium basal membrane, 2.42 ± 0.53; gland epithelium basal membrane, 2.42 ± 0.53; endometrial stroma, 2.42 ± 0.53 ($p < 0.05$; Figures 1 and 2).

Leukemia inhibitory factor immunoreactivity calculated for the control group (lumen epithelium basal membrane, 1.00 ± 0.57; gland epithelium basal membrane, 0.57 ± 0.53; endometrial stroma, 1.14 ± 0.37) and the low-dose group (lumen epithelium basal membrane, 1.14 ± 0.37; gland epithelium basal membrane, 0.71 ± 0.48; endometrial stroma, 1.14 ± 0.37) was not significantly different, but it was significantly higher in the high-dose group (lumen epithelium basal membrane, 2.28 ± 0.48; gland epithelium basal membrane, 1.71 ± 0.48; endometrial stroma, 2.00 ± 0.57; $p < 0.05$; Figures 1 and 3).

Ultrastructural findings showed a prismatic surface epithelium of the uterus, euchromatic nuclei parallel to the long axis, and morphologically normal organelles. Junctions between the microvilli on the apical cell surface and between cells were normal. Glandular tissue and the stroma had a normal morphology (Figure 4). Morphometric calculations showed decreased epithelial height in the low-dose omega-3 group than in the control group. There were no degenerative changes in the surface epithelium apical faces, organelles of the cytoplasm, or intercellular junctions (Figure 4).