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Original Article

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

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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

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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).

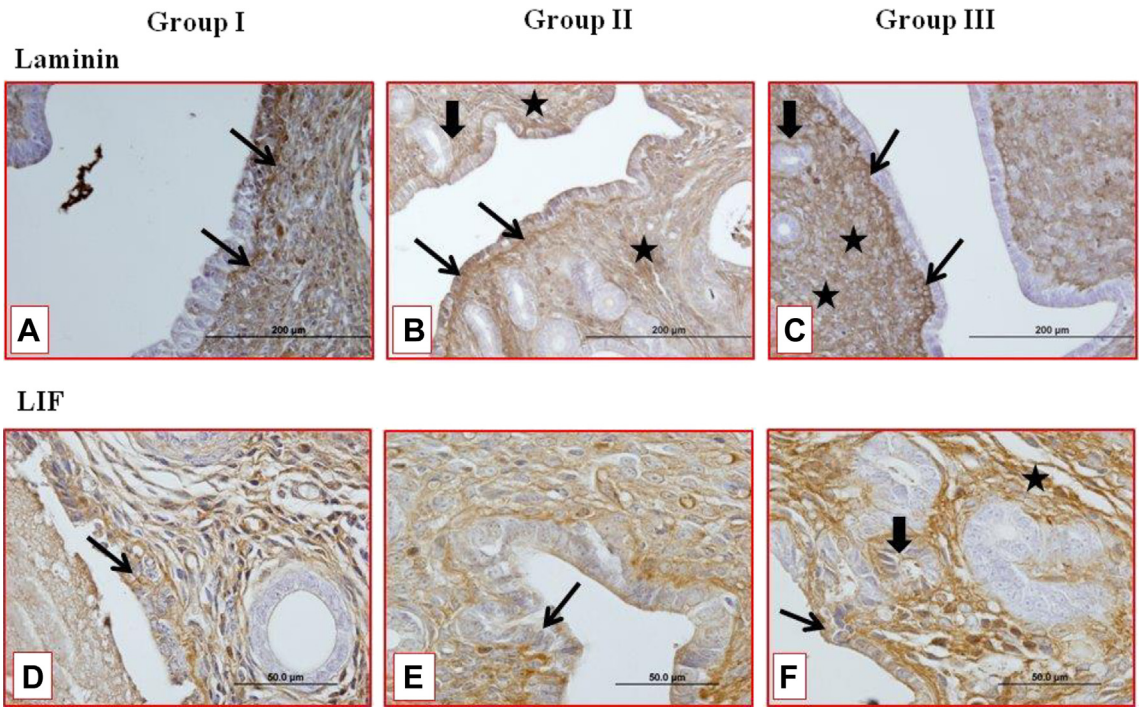

Figure 1. The laminin and leukemia inhibitory factor (LIF) immunoreactivity results. (A) The arrows indicate the surface epithelium basal membrane (B, C) The arrows indicate the surface epithelium basal membrane, the thick arrow indicates the gland epithelium basal membrane, and the star indicates the stroma. (D, E) The arrows indicate the surface epithelium. (F) The arrows indicate the surface epithelium, the thick arrow indicates the gland epithelium, and the star indicate the stroma.

The uterine surface epithelial height was decreased in the high-dose omega-3 group, compared to the low-dose group. Degenerative changes were not observed in the surface epithelium apical faces, organelles of the cytoplasm, or intercellular junctions (Figure 4).

The Kruskal–Wallis test was used to determine whether a significant difference existed between the three groups with regard to epithelial height. A significant difference was detected ($p < 0.001$). The microvilli number per unit area were counted by electron microscopy. The microvillus number was decreased in the low- and high-dose omega-3 groups; the microvillus number in the high-dose group was significantly decreased in comparison to the other groups. These results are shown in Table 1.

There was a significant difference between the control group and high-dose omega-3 group with regard to the implantation ratio. These ratios are shown in Table 2.

Discussion

Numerous studies have attempted to enlighten the mysteries of molecular and morphometric changes in the endometrium during the implantation period; however, many triggering mechanisms for these changes need to be identified. Factors that initiate implantation and factors that enhance the process are popular topics of reproduction. Insufficient endometrial receptivity accounts for approximately two-thirds of implantation failure [19]. Omega-3 as a food supplement is widely prescribed by obstetricians to pregnant women and in the preconceptional period. There are numerous studies [20–22] on the effects of omega-3 on pregnancy and the fetus, some of which are favorable and some are not. In particular, the effect of omega-3 on the fetal

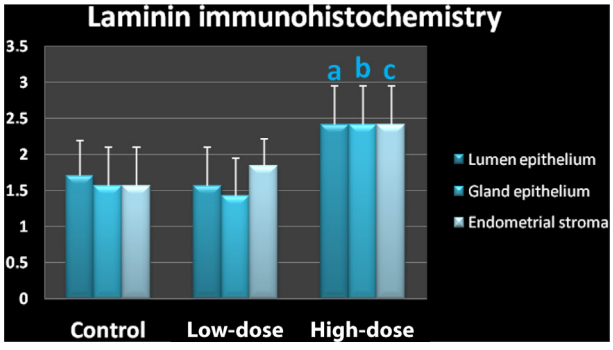

Figure 2. Semiquantitative score of laminin immunohistochemistry. "a" = the high-dose group score is significantly greater than that of the control and low-dose groups ($p = 0.030$ and $p = 0.018$, respectively); "b" = the high-dose group score is significantly greater than that of the control and low-dose groups ($p = 0.018$ and $p = 0.010$, respectively); "c" = the high-dose group score is significantly greater than that of the control and low-dose groups ($p = 0.018$ and $p = 0.044$, respectively).

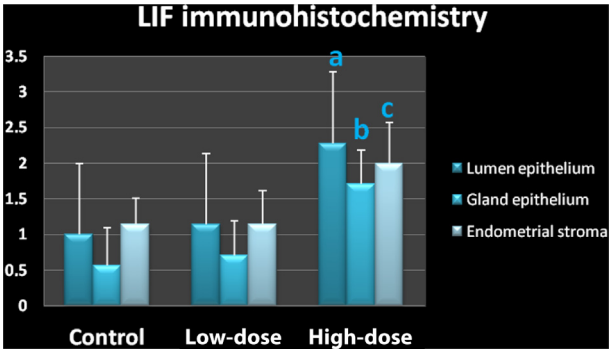

Figure 3. Semiquantitative scores of LIF immunohistochemistry. "a" = the high-dose group score is significantly higher than that of the control and low-dose groups ($p = 0.003$ and $p = 0.002$, respectively); "b" = the high-dose group score is significantly higher than that of the control and low-dose groups ($p = 0.005$ and $p = 0.006$, respectively); "c" = the high-dose group score is significantly higher than that of the control and low-dose groups ($p = 0.010$ and $p = 0.010$, respectively); LIF = leukemia immunohistochemistry factor.

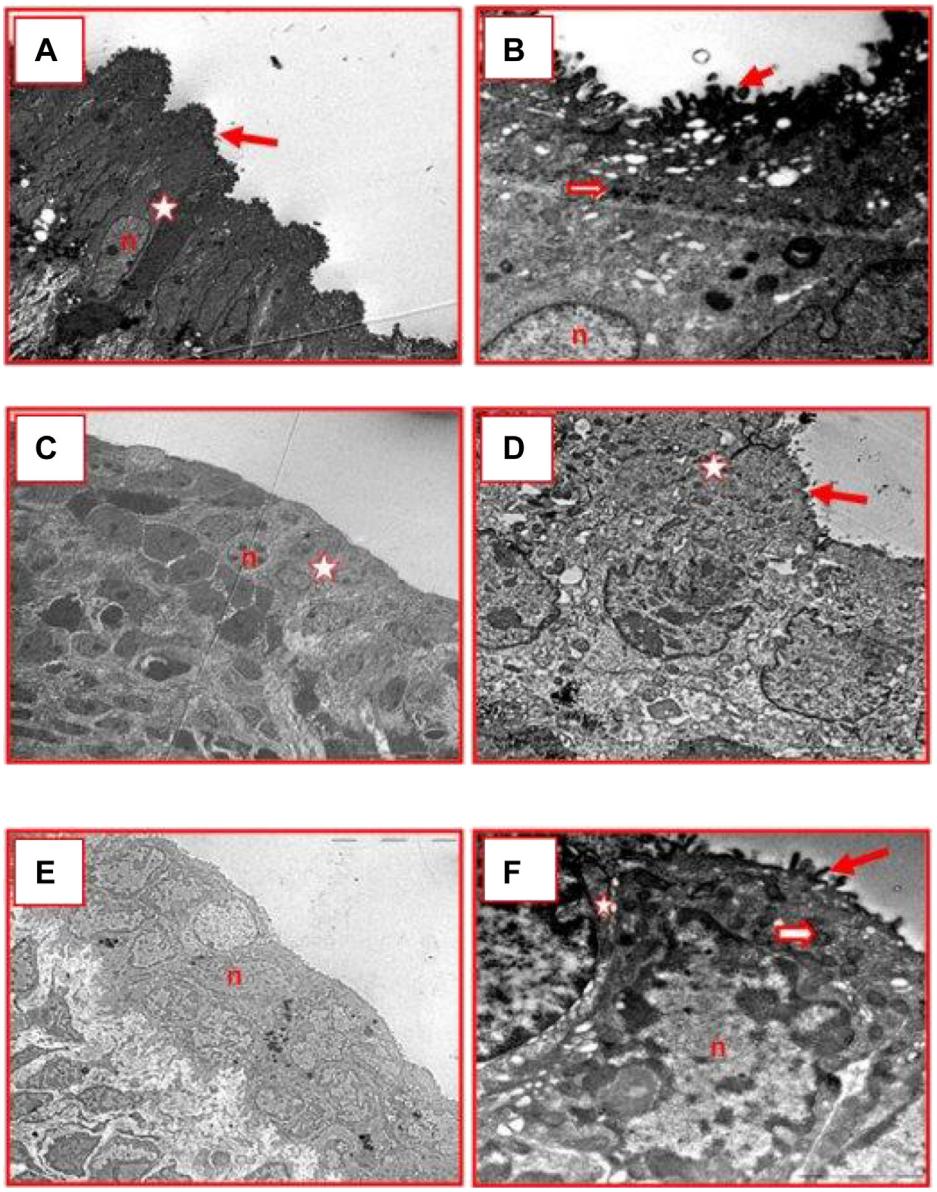

Figure 4. Ultrastructural findings. (A, B) The control group. (C, D) The low-dose omega-3 group. (E, F) The high-dose omega-3 group. (A–F) The letter "n" indicaes the nucleus, the arrows indicate apical alteration, the stars indicate the intercellular junctions, and the white arrows indicate the mitochondria.

Table 1
The number of microvilli per unit area and lumen epithelium height (i.e., ultrastructural feature).

	Microvilli number/1000 nm	Mann–Whitney *U* test (*p*)	Epithelium average height (nm) ± standard deviation	*p*
Control group	3.80	0.599	18175.94 ± 2979.3	0.008
Low-dose group	2.67	0.000 *	17844.71 ± 719.9 *	0.004
High-dose group	2.16	0.000 p*	7051.08 ± 682.3 *	0.004

* Statistically significant.

Table 2
Implantation ratio.*

	Control group	Low-dose group	High-dose group
Implantation ratio	8.5 ± 0.75	9.2 ± 0.46	10.0 ± 0.77

* For the ratio between the control and low-dose groups, $p = 0.03$; between the control and high-dose groups, $p = 0.002$; and between the low-dose and high-dose groups, $p = 0.03$.

neural system has been substantially studied and findings show it has positive effects on neuronal development, differentiation, and synaptic network formation in the cerebellum [20]. High omega-3 diets appear to cause growth retardation [21] or to increase birth weight [22]. Omega-3 has favorable effects in the cardiovascular system and thus may increase endometrial perfusion and enhance pregnancy rates. To date, there has been no study to our knowledge that has investigated the effect of omega-3 on implantation

by using immunohistochemical markers and ultrastructural analysis.

The implantation window is characterized by differentiation in cellular morphology and by molecular changes [23,24]. There is a noticeable increase in pinopodes, LIF, and LIF receptors during the blastocyst implantation phase [25,26]. Leukemia inhibitory factor has an important role in implantation, as well as in stem cell differentiation [27,28]. The uterus glandular epithelium of mice on the 4th day of implantation contain LIF messenger ribonucleic acid [29]. Human endometrium contains LIF and LIF receptors during blastocyst implantation [25]. Leukemia inhibitory factor also contributes to trophoblast adhesion and differentiation [30]. Women with high LIF immunoreactivity during the implantation period have high pregnancy rates [31], whereas infertile women with endometriosis do not express LIF in their endometrium [32]. Mice with insufficient LIF have implantation failure, and aspirin increases LIF immunoreactivity [33–35]. The present study showed that mice that received omega-3 supplementation had an increased secretion of LIF during the implantation window. This finding led us to conclude that omega-3 has a positive effect on implantation.

Extracellular matrix proteins have important roles in proliferation, differentiation, migration, and adhesion [36,37]. Laminin is an extracellular matrix protein that increases in the basal membrane after implantation [38]. It contributes to embryogenesis, cell migration, differentiation, and cell growth [38]. Laminin is a major glycoprotein of the basal membrane and extracellular matrix, and has a role in cell growth, differentiation, migration, and function—especially during the embryonic period [39–42]. Laminin activity gradually increases during pregnancy in the basal membrane and subepithelial tissue [43], and favors trophoblastic invasion into the extracellular matrix [1,44]. Laminin exists in all membranes of a blastocyst [36,38]. The present study showed an increase in laminin immunoreactivity in the lumen epithelium of the basal membrane, glandular epithelium basal membrane, and endometrial stroma of mice treated with omega-3, which suggests a positive effect on embryonic implantation.

Important structural changes in the endometrium of rodents during the receptive period are decreased in microvilli in apical membranes of secretory cells and in the formation of pinopodes [45,46]. Sarani et al [47] reported that the basal membrane of the human luminal endometrium reaches its narrowest height on the sixth day of the luteinizing hormone (LH) peak, and this feature is the morphological clue for an implantation window with the most favorable environment for blastocyst invasion. In this study, we found that omega-3 supplementation enhances these changes. Epithelial height was significantly shorter and the loss of microvilli was significantly more in the omega-3 groups; thus, the endometrium was better prepared for implantation and trophoblastic invasion.

This study showed that mice treated with omega-3 have a more favorable endometrium for implantation. This conclusion is drawn from the fact that LIF and laminin were increased in these mice, the epithelium height was decreased, and the microvilli were decreased, especially in the high-dose group. We demonstrated by electron microscope evaluation that mice treated with omega-3 had a significant decrease in the endometrium epithelium height, which increases the success of implantation.

Our results led us to conclude that mice treated with omega-3 supplementation in the preconceptional period have high levels of LIF and laminin in their endometrial basal membrane, shorter epithelial height, and decreased microvilli, all of which are positive markers for successful implantation. Omega-3 supplementation seems to have good effects on implantation and reproduction.

Conflicts of interest

The authors have no conflict of interest to declare relevant to this article.

References

[1] Kimber SJ. Molecular interactions at the maternal-embryonic interface during the early phase of implantation. Sem Reprod Med 2000;18:237–53.
[2] Paria BC, Lim H, Wang XN, Liehr J, Das SK, Dey SK. Coordination of different effects of primary estrogen and catecholestrogen on two distinct targets mediates embryo implantation in the mouse. Endocrinology 1998;139:5235–46.
[3] Sunder S, Lenton E. Endocrinology of the peri-implantation period. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:789–800.
[4] Diedrich K, Fauser BC, Devroey P, Griesinger G, Evian Annual Reproduction (EVAR) Workshop Group. The role of the endometrium and embryo in human implantation. Hum Reprod Update 2007;13:365–77.
[5] Halvorson LM. Reproductive endocrinology. In: Schorge JO, Schaffer JI, Halvorson LM, Hoffman BL, Bradshaw KD, Cunningham FG, editors. Williams gynecology. 1st ed. New York: McGraw-Hill Companies; 2008. p. 681–4.
[6] Merviel P, Challier JC, Carbillon L, Foidart JM, Uzan S. The role of integrins in human embryo implantation. Fetal Diagn Ther 2001;16:364–71.
[7] Ma WG, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc Natl Acad Sci USA 2003;100:2963–8.
[8] Kimber SJ, Spanswick C. Blastocyst implantation: the adhesion cascade. Semin Cell Dev Biol 2000;11:77–92.
[9] Holman RT. The slow discovery of the importance of omega 3 essential fatty acids in human health. J Nutr 1998;128(2 Suppl):427S–33S.
[10] Burtis CA, Ashwood ER. Clinical chemistry. Philadelphia: WB Saunders Company; 1994.
[11] Simopoulus AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 1991;54:438–63.
[12] Holub J. Clinical nutrition: 4. Omega-3 fatty acids in cardiovascular care. CMAJ 2002;166:608–15.
[13] Cook ME, Miller CC, Park Y, Pariza MW. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poult Sci 1993;72:1301–5.
[14] Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very long chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:e39–44.
[15] Borja-Hart NL, Marino J. Role of omega-3 fatty acids for prevention or treatment of perinatal depression. Pharmacotherapy 2010;30:210–6.
[16] Calder PC. Long-chain n-3 fatty acids and inflammation: potential application in surgical and trauma patients. Braz J Med Biol Res 2003;36:433–46.
[17] Cilaker Micili S, Ergur BU, Ozogul C, Sarıoğlu S, Bağrıyanık HA, Tuğyan K, et al. Effects of lipoic acid in an experimentally induced hypertensive and diabetic rat model. Clin Exp Hypertens 2013;35:373–81.
[18] Cilaker Micili S, Göker A, Sayın O, Akokay P, Ergür BU. The effect of lipoic acid on wound healing in a full thickness uterine injury model in rats. J Mol Hist 2013;44:339–45.
[19] Rashid NA, Lalitkumar S, Lalitkumar PG, Gemzell-Danielsson K. Endometrial receptivity and human embryo implantation. Am J Reprod Immunol 2011;66(Suppl 1):23–30.
[20] Uauy R, Dangour AD. Nutrition in brain development and aging: role of essential fatty acids. Nutr Rev 2006;64(5 Pt 2):S24–33. discussion S72–91.
[21] Church MW, Jen KL, Jackson DA, Adams BR, Hotra JW. Abnormal neurological responses in young adult offspring caused by excess omega-3 fatty acid (fish oil) consumption by the mother during pregnancy and lactation. Neurotoxicol Teratol 2009;31:26–33.
[22] Carlson SE, Colombo J, Gajewski BJ, Gustafson KM, Mundy D, Yeast J, et al. DHA supplementation and pregnancy outcomes. Am J Clin Nutr 2013;97:808–15.
[23] Nikas G. Pinopodes as markers of endometrial receptivity in clinical practice. Hum Reprod 1999;14(Suppl 2):99–106.
[24] Nikas G, Develioglu OH, Toner JP, Jones Jr HW. Endometrial pinopodes indicate a shift in the window of receptivity in IVF cycles. Hum Reprod 1999;14:787–92.
[25] Aghajanova L, Stavreus-Evers A, Nikas Y, Hovatta O, Landgren BM. Coexpression of pinopodes and leukemia inhibitory factor, as well as its receptor, in human endometrium. Fertil Steril 2003;79(Suppl 1):808–14.
[26] Lessey BA, Castelbaum AJ. Integrins and implantation in the human. Rev Endocr Metab Disord 2002;3:107–17.
[27] Abe E, Tanaka H, Ishimi Y, Miyaura C, Hayashi T, Nagasawa H, et al. Differentiation-inducing factor purified from conditioned medium of mitogen-treated spleen cell cultures stimulates bone reabsorption. Proc Natl Acad Sci USA 1986;83:5958–62.
[28] Gearing DP, Gough NM, King JA, Hilton DJ, Nicola NA, Simpson RJ, et al. Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). EMBO J 1987;6:3995–4002.
[29] Bhatt H, Brunet LJ, Stewart CL. Uterine expression of leukaemia inhibitory factor coincides with the onset of blastocyst implantation. Proc Natl Acad Sci USA 1991;88:11408–12.

[30] Nachtigall M, Kliman HJ, Feinberg RF, Olive DL, Engin O, Arici A. The effect of luekemia inhibitory factor (LIF) on trophoblast differentiation: a potential role in human implantation. J Clin Endocrinol Metab 1996;81:801–6.
[31] Serafini P, Rocha AM, Osório CT, da Silva I, Motta EL, Baracat EC. Endometrial leukemia inhibitory factor as a predictor of pregnancy after in vitro fertilization. Int J Gynaecol Obstet 2008;102:23–7.
[32] Mikolajczyk M, Skrzypczak J, Szymanowski K, Wirstlein P. The assessment of LIF in uterine flushing—a possible new diagnostic tool in states of impaired fertility. Reprod Biol 2003;3:259–70.
[33] Fouladi-Nashta AA, Jones CJ, Nijjar N, Mohamet L, Smith A, Chambers I, et al. Characterization of the uterine phenotype during the peri-implantation period for LIF-null, MF1 strain mice. Dev Biol 2005;281:1–21.
[34] Kimber SJ. Leukaemia inhibitory factor in implantation and uterine biology. Reproduction 2005;130:131–45.
[35] Zhao M, Chang C, Liu Z, Chen LM, Chen Q. Treatment with low-dose aspirin increased the level LIF and integrin β3 expression in mice during the implantation window. Placenta 2010;31:1101–5.
[36] Albert E. The extracellular matrix in development. In: Nikolas Z, editor. Organization of the early vertebrate embryo. New York: Plenum Press; 1995. p. 149–67.
[37] Mulholland J, Aplin JD, Ayad S, Hong L, Glasser SR. Loss of collagen type VI from rat endometrial stroma during decidualization. Biol Reprod 1992;46:1136–43.
[38] Zagris N, Stavridis V. The expression of the genes for laminin in the early embryo. In: Zagris N, editor. Organization of the early vertebrate embryo. New York: Plenum Press; 1995. p. 169–83.
[39] Hashmi S, Marinkovich MP. Molecular organization of the basement membrane zone. Clin Dermatol 2011;29:398–411.
[40] Hay ED. Interaction of embryonic cell surface and cytoskeleton with extracellular matrix. Am J Anat 1982;165:1–12.
[41] Ekblom P. Extracellular matrix in animal development. Role of extracellular matrix in animal development—an introduction. Experientia 1995;51(9–10):851–2.
[42] Clark EA, Brugge JS. Integrins and signal transduction pathways: the road taken. Science 1995;268:233–9.
[43] MacIntyre DM, Lim HC, Ryan K, Kimmins S, Small JA, MacLaren LA. Implantation-associated changes in bovine uterine expression of integrins and extracellular matrix. Biol Reprod 2002;66:1430–6.
[44] Guillomot M. Changes in extracellular matrix components and cytokeratins in the endometrium during goat implantation. Placenta 1999;20:339–45.
[45] Potts M, Psychoyos A. Évolution de l'ultrastructure des relations ovoendométriales sous l'influence de l'oestrogène, chez la ratte en retard expérimental de nidation [Evolution of the ultrastructure of the ovoendometrial connections under the influence of estrogen in the rat during experimental retardation of nidation]. C R Acad Sci Hebd Seances Acad Sci D 1967;264:370–3 [in French].
[46] Nikas G. Endometrial receptivity: changes in cell-surface morphology. Semin Reprod Med 2000;18:229–35.
[47] Sarani SA, Ghaffari-Novin M, Warren MA, Dockery P, Cooke ID. Morphological evidence for the “implantation window” in human luminal endometrium. Hum Reprod 1999;14:3101–6.