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SACHIKO JUMAN ET AL: "Effects of Long-Term Oral Administration of Arachidonic Acid and Docosahexaenoic Acid on the Immune Functions of Young Rats", NUTRIENTS, vol. 5, no. 6, 29 May 2013 (2013-05-29), pages 1949-1961, XP055397243, DOI: 10.3390/nu5061949
Jeffrey Braverman: "Intralipid Therapy for Recurrent Pregnancy Loss - Controversies and Future Directions 34th Annual Meeting of the American Society for Reproductive Immunology June 2-5, 2014", , 2 June 2014 (2014-06-02), XP055397244, Retrieved from the Internet: URL:https://www.preventmiscarriage.com/documents/Intralipid-Therapy-for-Recurrent-Pregnancy-Loss-v19.pptx [retrieved on 2017-08-09]
ROUMEN G. ROUSSEV ET AL: "Natural Killer Cell Functional Activity Suppression By Intravenous Immunoglobulin, Intralipid and Soluble Human Leukocyte Antigen-G", AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY, vol. 57, no. 4, 1 April 2007 (2007-04-01), pages 262-269, XP055397185, US ISSN: 1046-7408, DOI: 10.1111/j.1600-0897.2007.00473.x cited in the application
YAMASHITA N ET AL: "Inhibition of natural killer cell activity by eicosapentaenoic acid in vivo and in vitro", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ELSEVIER, AMSTERDAM, NL, vol. 150, no. 1, 15 January 1988 (1988-01-15), pages 497-505, XP024842563, ISSN: 0006-291X, DOI: 10.1016/0006-291X(88)90548-7 [retrieved on 1988-01-15]
YAQOOB P ET AL: "Inhibition of natural killer cell activity by dietary lipids", IMMUNOLOGY LETTERS,

ELSEVIER BV, NL, vol. 41, no. 2-3, 1 July 1994 (1994-07-01) , pages 241-247, XP023786866, ISSN: 0165-2478,
DOI: 10.1016/0165-2478(94)90140-6 [retrieved on 1994-07-01]

DESCRIPTION

Field of the invention

[0001] The present invention relates to oral compositions for use in a method for the treatment of repeated spontaneous abortions (RSA) or repeated implantation failures (RIF) after in vitro fertilization (IVF) in sub-fertile human female subjects having increased number and/or cytotoxicity of NK cells.

Background of the invention

[0002] Natural Killer (NK) cells are a specific group of large granular lymphocytes (LGL) which play a major role in innate immune responses but are also important in influencing adaptive immune responses and in immune regulation (Herberman RB. Role of human natural killer cell responses in health and disease. Clin Diagn Lab Immunol 1994;1:125-33). They form 1-2% of lymphoid tissue found mainly in spleen. In the peripheral blood, NK cells make up 5-15% of all lymphocytes and 70-95% of LGLs (Stites DP, Terr AI, Parslow TG. Basic and clinical immunology. 8th edition, Appleton and Lange, Norwalk, Conn, 1994).

[0003] NK cells express on their surface a series of markers, the most characteristic of which are the molecules CD16 and CD56 (Caligiuri M. Human natural killer cells. Blood. 2008;112:461-9). Based on the relative expression of CD16 and CD56, NK cells can be subdivided into different sub-populations. The two major subsets are CD56dim CD16bright (low expression of CD56, high expression of CD16), and CD56bright CD16dim/- (high expression of CD56, low or no expression of CD16). CD56dim CD16bright cells, which represent at least 90% of all peripheral blood NK cells, have cytotoxic function. Upon activation, they release perforin and granzymes from their granules, while they move to inflammation sites in response to chemokines released during inflammation by endothelial and innate immune cells (Moretta A, Marcenaro E, Parolini S, Ferlazzo G, Moretta L. NK cells at the interface between innate and adaptive immunity. Cell Death Differ 2008;15:226-33). CD56bright CD16dim/- cells constitute the majority of NK cells in secondary lymphoid tissues, they are only weakly cytotoxic before activation, but, upon the effect of cytokines, they immediately produce large amounts of INF- γ and may have immunoregulatory properties (Poli A, Michel T, Theresine M, Andres E, Hentges F, Zimmer J, CD56bright natural killer (NK) cells: an important NK cell subset. Immunology 2009 Apr;126(4):458-65). These cells constitute the major cell population of endometrial leukocytes (CD56 eGL: endometrial granulocytes), possibly after local differentiation (Hiby SE1, King A, Sharkey AM, Loke YW, Human uterine NK cells have a similar repertoire of killer inhibitory and activatory receptors to those found in blood, as demonstrated by RT-PCR and sequencing, Mol Immunol. 1997 Apr;34(5):419-30).

[0004] NK-like cells (CD56^{bright} CD16^{dim/-}) are the dominant decidual cell population from the

first stages of pregnancy through the first trimester. There is evidence that their proliferation and differentiation is synchronized with the secretory phase of the menstrual cycle when estrogens and progesterone prepare the endometrium for a prospective pregnancy. During this phase, uterine stromal leukocytes increase highly (25% from 5%) as a result of NK cell influx from blood or other tissues or of reprogramming and differentiation of endometrial stromal cells to NK cells (Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med* 2007; 25:445-53. Santoni A, Carlino C, Gismondi A. Uterine NK cell development, migration and function. *Reprod Biomed Online* 2008; 16:202-10). If pregnancy occurs, NK-like cells increase rapidly and are distributed broadly throughout decidua found in close proximity to extravillous trophoblast (King A. Uterine leukocytes and decidualization. *Hum Reprod Update* 2000; 6:28-36). Due to their increased presence and direct contact with invading trophoblast, they have been considered as important for the establishment of normal pregnancy. There is evidence that, coincident with blastocyst implantation and decidualization, uterine NK cells become activated, they produce IFN- γ , perforin and other molecules, including angiogenetic factors, so that they may control trophoblast invasion through their cytotoxic activity, and also initiate vessel instability and remodelling of decidual arteries to increase the blood supply to the fetoplacental unit (Ashkar AA, Di Santo JP, Croy BA. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000;192:259-70). Furthermore, decidual NK cells may be involved in cytokine-mediated immunoregulation of the maternal immune response producing Th2-type cytokines and growth factors, which result in placental augmentation and local immunosuppression and immunomodulation (Clark D, Arck PC, Jallili R, et al. Psycho-Neuro-Cytokine/Endocrine pathways in immunoregulation during pregnancy. *Am J Reprod Immunol* 1996;35:330-7. Chaouat G, Tranchot Diallo J, Volumenier JL, et al. Immune suppression and Th1/Th2 balance in pregnancy revisited: A (very) personal tribute to Tom Wegmann. *Am J Reprod Immunol* 1997;37:427-34).

[0005] Decidual NK cells appear to be the main cell population involved in cases of the so called alloimmune abortions where the embryo is recognized as foreign and is "rejected" by the mother (Labarrere CA. Allogeneic recognition and rejection reactions in the placenta. *Am J Reprod Immunol* 1989;21:94-9. Stern JJ, Coulam CB: Current status of immunologic recurrent pregnancy loss. *Curr Opin Obstet Gynecol* 1993;5:252-9). Under the influence of Th1-type cytokines they are stimulated to become classical NK cells expressing CD16 (CD56^{dim} CD16^{bright}), which can damage trophoblast either directly by releasing cytolytic substances or indirectly by producing inflammatory cytokines (King A, Wheeler R, Carter NP, et al. The response of human decidual leukocytes to IL-2. *Cell Immunol* 1992;141:409-42).

[0006] Clinical studies have demonstrated that women who tend to abort have increased numbers of NK cells of the conventional CD3-CD56+CD16+ type in the uterus. (Vassiliadou N, Bulmer JN: Immunohistochemical evidence for increased numbers of 'classic' CD57+ natural killer cells in the endometrium of women suffering spontaneous early pregnancy loss. *Hum Reprod* 1996;11:1569-74. Kwak JY, Beer AE, Kim SH, et al. Immunopathology of the implantation site utilizing monoclonal antibodies to natural killer cells in women with recurrent

pregnancy losses. Am J Reprod Immunol 1999;41:91-8), as well as increased blood NK subsets and NK cell activity, all of which have been associated with abortion of chromosomally normal embryos (Coulam CB, Goodman C, Roussev RG, et al. Systemic CD56+ cells can predict pregnancy outcome. Am J Reprod Immunol 1995;33:40-6 Aoki K, Kajiura S, Matsumoto Y, et al. Preconceptual natural killer cell activity as a predictor of miscarriage. Lancet 1995;345:1340-2).

[0007] Because of the above data, the increased numbers and/or cytotoxicity of peripheral blood NK cell (CD56^{bright} CD16^{dim/-}) are used in practice for the diagnosis of alloimmune abortions and as markers for the selection of women for immunotherapy (Coulam CB, Goodman C, Roussev RG, et al. Systemic CD56+ cells can predict pregnancy outcome. Am J Reprod Immunol 1995;33:40-6 Aoki K, Kajiura S, Matsumoto Y, et al. Preconceptual natural killer cell activity as a predictor of miscarriage. Lancet 1995;345:1340-2).

[0008] Classically, the main therapies to reduce detrimental NK cell activation and prevent abortions and/or RIF have been active immunization of the women using paternal or third party lymphocytes (Gafer U, Sredni B, Segal J, Kalechman Y. Suppressed cell-mediated immunity and monocyte and natural killer cell activity following allogeneic immunization of women with spontaneous recurrent abortion. J Clin Immunol 1997;17:408-19), and passive immunization in the form of intravenous administration of immunoglobulin G (IVIg) (Ruiz JE, Kwak JY, Baum L, et al. Intravenous immunoglobulin inhibits natural killer cell activity in vivo in women with recurrent spontaneous abortion. Am J Reprod Immunol 1996;35:370-5). Some known side effects or adverse events of Intravenous Immunoglobulin are: Aseptic meningitis, renal failure, thromboembolism, haematolytic reactions, anaphylactic reactions, lung disease, enteritis, dermatologic disorders and infectious diseases (Stiehm, 2013). An alternative treatment proposed during the last years to suppress NK cell functional cytotoxicity, is the intravenous administration of fat emulsions (ex, Intralipid®) comprising purified soya bean oil, purified egg phospholipid, glycerol anhydrous and water for injection which is usually used as a source of energy and of essential fatty acids in patients with nutritive or other disorders requiring parenteral nutrition (Natural killer cell functional activity suppression by intravenous immunoglobulin, Intralipid and soluble human leukocyte antigen-G. Am J Reprod Immunol 2007;57:262-9, Meng L, Lin J, Chen L, Wang Z, Liu M, Liu Y, Chen X, Zhu L). Jeffrey Braverman, "Intralipid Therapy for Recurrent Pregnancy Loss - Controversies and Future Directions 34 th Annual Meeting of the American Society for Reproductive Immunology June 2-5, 2014" discloses the treatment for recurrent pregnancy loss by the intravenous administration of Intralipid®. The treatment with Intralipid® reduces the cytotoxicity of the peripheral blood NK cells, but it does not reduce the number of peripheral blood NK cells. Furthermore intravenous infusion of Intralipid® may cause several side effects such as bladder pain, bloody or cloudy urine, chills, difficulty, burning, or painful urination, fever, frequent urge to urinate, lower back or side pain, sore throat and vomiting. Recent research has shown that intravenous administration of Intralipid® can cause hepatomegaly, jaundice, cholestasis, splenomegaly, thrombocytopenia, leukopenia and fat overload syndrome (<1% in clinical trials) (FDA, 2007).

[0009] Both the increased number and the increased cytotoxicity of peripheral blood NK cells can cause recurrent spontaneous abortions (Am J Reprod Immunol. 2007 Nov;58(5):447-59. Decline in number of elevated blood CD3(+) CD56(+) NKT cells in response to intravenous immunoglobulin treatment correlates with successful pregnancy, van den Heuvel MJ1, Peralta CG, Hatta K, Han VK, Clark DA; An Increased NK-cell percentage 5 days after the pregnancy termination could be a marker of immune aetiology of miscarriage, as the probability of an aborter with NK >12% to have an immune-mediated abortion is almost 90%. Am J Reprod Immunol. 2008 Apr;59(4):306-15. doi: 10.1111/j.1600-0897.2007.00547.x. Epub 2008 Feb 21).

[0010] It has been shown that the measurement of peripheral blood NK cells at the time of abortion is valuable and due to the significant role of elevated NK cells number. (Paparistidis N1, Papadopoulou C, Chioti A, Papaioannou D, Tsekoura C, Keramitsoglou T, Kontopoulou-Antonopoulou V, Agapitos E, Balafoutas C, Varla-Leftherioti M).

[0011] A number of studies have shown that the reduction of peripheral NK cells number and activity results to an important improvement in live birth rate that rises in very high levels Am J Reprod Immunol. 2006 Mar;55(3):232-9; High levels of peripheral blood NK cells in women suffering from recurrent spontaneous abortion are reverted from high-dose intravenous immunoglobulins (Perricone R1, Di Muzio G, Perricone C, Giacomelli R, De Nardo D, Fontana L, De Carolis C. Am J Reprod Immunol. 2010 Mar 1;63(3):263-5. doi: 10.1111/j.1600-0897.2009.00790.x. Epub 2010 Jan 8; CD3-CD56+CD16+ natural killer cells and improvement of pregnancy outcome in IVF/ICSI failure after additional IVIG-treatment. Heilmann L1, Schorsch M, Hahn T. Am J Reprod Immunol. 2007 Apr;57(4):262-9; Natural killer cell functional activity suppression by intravenous immunoglobulin, intralipid and soluble human leukocyte antigen-G.. Roussev RG1, Ng SC, Coulam CB.(Am J Reprod Immunol. 2007 Nov;58(5):447-59; Decline in number of elevated blood CD3(+) CD56(+) NKT cells in response to intravenous immunoglobulin treatment correlates with successful pregnancy. Van den Heuvel MJ1, Peralta CG, Hatta K, Han VK, Clark DA).

[0012] The above mentioned treatments, have a number of drawbacks and disadvantages. For example, they require repeated hospitalizations of the patients as well as permanent doctor's surveillance. Furthermore, since the patients are hospitalized, they may be exposed to hospital-acquired infections. In addition, these methods are associated with the risks and side effects of intervention treatments (including life-threatening ones such sepsis from human derived products, hyper-coagulation from fat emulsion infusions). In addition, these treatments are associated with serious side effects.

[0013] The present invention addresses these issues and provides a number of advantages compared to the prior art.

Summary of the invention

[0014] The present invention provides oral compositions comprising a combination of fatty

acids or esters thereof, for use by women who experience recurrent spontaneous abortions (RSA) or repeated implantation failures (RIF) in the in vitro fertilization (IVF) setting and have increased number and/or cytotoxicity of peripheral blood NK cells.

[0015] The compositions of the present invention comprise as active ingredients a combination of fatty acids or esters thereof and they are administered in certain amounts and over a certain period of time.

[0016] Accordingly, the present invention aims to prevent immune-mediated sub-fertility in a considerably significant cohort of couples facing related problems, which apart from the family status, depriving them the opportunity to give birth to a child, could have also considerable social, psychological and economical impact.

Detailed description of the invention

[0017] It has now surprisingly been found that an oral composition comprising a mixture of fatty acids or esters thereof administered in certain amounts and over a certain period of time reduces the number and cytotoxicity of peripheral NK cells in women experiencing recurrent spontaneous abortions (RSA) or repeated implantation failures (RIF) and having increased numbers of peripheral blood NK cells and/or increased peripheral blood NK cell cytotoxicity.

[0018] Thus, the present invention provides an oral composition comprising as active ingredients a combination of fatty acids or esters thereof, for use in a method for the treatment of recurrent spontaneous abortions or repeated implantation failures in a human female subject having 12% or more of peripheral blood NK cells in total peripheral blood cells and/or wherein the cytotoxicity of the peripheral blood NK cells of the human female subject is at least 10% higher than the mean cytotoxicity of a control population of healthy human females of reproductive age with no reproductive failures and who have given birth to at least one child, wherein the composition is orally administered for 30 to 60 days and wherein the total amount of fatty acids or esters thereof administered per day is from 0,10 g/kg of body weight to 0,70 g/kg of body weight, calculated as fatty acids.

[0019] The present invention also provides a method for the treatment of recurrent spontaneous abortions or repeated implantation failures in a human female subject having 12% or more of peripheral blood NK cells in total peripheral blood cells and/or wherein the cytotoxicity of the peripheral blood NK cells of the human female subject is at least 10% higher than the mean cytotoxicity of a control population of healthy human females of reproductive age with no reproductive failures and who have given birth to at least one child, comprising administering to the human female subject an oral composition comprising as active ingredients a combination of fatty acids or esters thereof, wherein the composition is orally administered for 30 to 60 days and wherein the total amount of fatty acids or esters thereof administered per day is from 0,10 g/kg of body weight to 0,70 g/kg of body weight, calculated as fatty acids.

[0020] In order to evaluate the NK cell cytotoxicity, several methods can be applied. Although ⁵¹Cr release is the classical method for determining cytotoxicity, alternate methods are available and several other are in developmental stages. In laboratories where the use of radioactive isotopes is not desirable or permissible, or when using tumor target cells that do not readily take up the ⁵¹Cr label, test systems that utilize enzymes and colorimetric or fluorometric dyes could be employed. Today the most common method for the determination of NK cell activity is flow cytometry, a method which can be modified regarding the use of the reagents, nevertheless the result remains the same. (Determination of natural killer cell function by flow cytometry. Kimberly I. Kane et al, Clinical and diagnostic laboratory immunology, may 1996, p. 295-300; Evaluation of a Flow Cytometry-Based Assay for Natural Killer Cell Activity in Clinical Settings, R. Valiathan et al. 2011 The Authors. Scandinavian Journal of Immunology).

[0021] A person skilled in the art appreciates that the NK cytotoxicity normally may be inconsistent within an individual because of the many possible immunological and endocrine influences on NK cells. Therefore, minor changes in NK activity may be expected when repeatedly testing healthy individuals. A different control population could provide different mean NK cytotoxicity values. In order to have a statistically correct result the more the control samples are the better. Nevertheless, the present invention is applied to human female subjects having cytotoxicity of the peripheral blood NK cells at least 10% higher than the mean cytotoxicity of a control population of healthy human females of reproductive age with no reproductive failures, such as miscarriages or unsuccessful IVFs, and who have given birth to at least one child.

[0022] According to the present invention, the term "recurrent spontaneous abortions (RSA)" is defined as two or more spontaneous losses of pregnancy prior to the 20th gestational week of pregnancy.

[0023] According to the present invention, the term "repeated implantation failures (RIF)" is defined as the absence of implantation after two repetitive unsuccessful cycles of in vitro fertilization (IVF), or intracytoplasmic sperm injection (ICSI) or frozen embryo replacement cycles.

[0024] According to the present invention, increased cytotoxicity of peripheral blood NK cells is defined as any killing activity which is at least 10% higher than the mean cytotoxicity of a control population of healthy women of reproductive age with no reproductive failures, such as miscarriages or unsuccessful IVFs, and who have given birth to at least one child,

[0025] The term "treatment of recurrent spontaneous abortions or repeated implantation failures" means a reduction of the risk of miscarriage of a treated subject compared to the risk she would have been exposed to had she not been treated, as well as an improvement of pregnancy outcome.

[0026] According to the present invention, the term "fatty acid" means an aliphatic monocarboxylic acid having a saturated or unsaturated chain, commonly comprising from 4 to 28 carbon atoms.

[0027] According to the present invention, the term "long chain fatty acid" means a fatty acid having an aliphatic chain of 13 or more carbon atoms.

[0028] According to the present invention, the term "medium chain fatty acid" means a fatty acid having an aliphatic chain of 6 to 12 carbon atoms.

[0029] According to the present invention, the term "short chain fatty acid" means a fatty acid having an aliphatic chain of less than 6 carbon atoms.

[0030] The compositions of the present invention comprise a combination of fatty acids or esters thereof. The fatty acids include short chain, medium chain or long chain, saturated or unsaturated fatty acids. Preferably, the compositions of the present invention comprise long chain fatty acids or long chain and medium chain fatty acids.

[0031] Examples of saturated long chain fatty acids include palmitic, stearic, myristic, and arachidic acid.

[0032] Examples of monounsaturated long chain fatty acids include oleic, docosahexaenoid (DHA), eicosapentaenoic (EPA), palmitoleic, myristoleic and sapienic acid.

[0033] Examples of polyunsaturated long chain fatty acids include linoleic, α -linolenic and γ -linolenic acid.

[0034] Examples of medium chain fatty acids include lauric, capric and caprilic acid.

[0035] Examples of short chain fatty acids include butanoic, 2-methylpropanoic, pentanoic and 3-methylbutanoic acid.

[0036] The fatty acid esters of the compositions of the present invention are esters of fatty acids with aliphatic alcohols, such as C_1 - C_6 alcohols, glycerides, i.e. monoglycerides, diglycerides, or triglycerides, phospholipids, such as phosphatidic acids or phosphoglycerides, sphingolipids, saccharolipids, waxes and mixtures thereof. Preferably, the fatty acid esters are glycerides, phospholipids, sphingolipids or saccharolipids and mixtures thereof. More preferably, the fatty acid esters are glycerides.

[0037] According to the present invention, the term "calculated as fatty acids" means that when the composition comprises fatty acid esters, their amounts have to be converted to those of the corresponding fatty acids in order to determine the quantity of the oral composition to be administered.

[0038] Preferably, the compositions of the present invention comprise at least one saturated, at least one monounsaturated and at least one polyunsaturated long chain fatty acid or an ester thereof.

[0039] Preferably, at least 50% by weight of the active ingredients in the compositions of the present invention, calculated as fatty acids, are long chain fatty acids or esters thereof. More preferably, at least 65% by weight of the active ingredients in the compositions of the present invention, calculated as fatty acids, are long chain fatty acids or esters thereof. Even more preferably, at least 97% by weight of the active ingredients in the compositions of the present invention, calculated as fatty acids, are long chain fatty acids or esters thereof.

[0040] The fatty acids of the compositions of the present invention act as intracellular and intercellular mediators having various effects on immune and inflammatory responses where NK cells are involved. Fatty acids are involved through several mechanisms in the modulation of the immune system including (i) membrane fluidity; (ii) production of lipid peroxides; (iii) eicosanoid synthesis; and (iv) influence on gene regulation. It is possible that fatty acid immunomodulation occurs not only by the individual, but also by the collective action of these factors.

[0041] The compositions of the present invention may comprise additional active ingredients, such as vitamins, metal ions or amino acids. When these active ingredients are present, their total amount is preferably less than 6% of the total amount of active ingredients. Examples of vitamins include vitamins B1, B2, B3, B6, D, myo-inositol, inositol and folic acid. Metal ions may include for example selenium, magnesium, copper, zinc or manganese. Amino acids may include naturally occurring amino acid, such as L-arginine or L- glutamine. The vitamins, metal ions or amino acids do not contribute directly to a decrease in the number and/or the cytotoxic activity of the NK cells but contribute to the body's immune defenses by supporting physical barriers, cellular immunity and antibody production. Nevertheless there is a belief that the steroid hormone 1,25-dihydroxyvitamin D3 {1,25-(OH)₂ -D3} has immunosuppressive action.

[0042] The presence of vitamins in the compositions of the present invention exhibits an immune modulating and homeostatic effect to the environment where the NK cells are found. The aim is to create a favorable milieu and contribute to the improvement of the whole reproductive process, having a modulation of the maternal response by keeping the immune system in balance. However, increased level of vitamins may boost the immune system and increase the effect of the NK cells. For this reason, when vitamins are present in the compositions of the present invention, their total amount is preferably less than 4% of the total amount of active ingredients.

[0043] The compositions of the present invention may have any form suitable for oral administration. Such forms are well known to a person skilled in the art and they include liquid forms, such as oral solutions, oral suspensions or oral emulsions, oral gels or solid forms, such as tablets, capsules or sachets. The compositions of the present invention can be administered as nutritional supplements.

[0044] In addition to the active ingredients, the compositions of the present invention may comprise one or more pharmaceutically acceptable excipients well known in the art, such as binders, diluents, disintegrants, suspending agents, lubricants, solvents, emulsifiers, thickening agents, buffering agents, preservatives, sweeteners, colouring agents, flavouring agents and the like.

[0045] The compositions can be produced by processes well known to the person skilled in the art.

[0046] The compositions of the present invention are administered for 30 to 60 days. Preferably, the compositions are administered for 40 to 50 days.

[0047] The compositions of the present invention may be administered once per day or more than once per day, e.g. twice per day. Nevertheless, according to the present invention the total amount of fatty acids or esters thereof administered per day is from 0,10 g/kg of body weight to 0,70 g/kg of body weight, calculated as fatty acids. Preferably, the total amount of fatty acids or esters thereof administered per day is from 0,20 g/kg of body weight to 0,50 g/kg of body weight, calculated as fatty acids.

[0048] The compositions of the present invention specifically decrease the number and cytotoxicity of the peripheral blood NK cells without overall suppression of the immune system.

[0049] With the present invention, both the NK cell's cytotoxicity and the NK cell percentage in total peripheral blood cells remain at low levels for 12-14 weeks after the discontinuation of the treatments, achieving a prolonged protection and prevention of RSA or RIF in sub-fertile women having increased number of peripheral blood NK cells and/or increased peripheral blood NK cells activity. This represents a great improvement over intravenous administration of Intralipid®, in which the effect ceases after 6-9 weeks from the discontinuation of the treatment (Acacio B, Ng SC, Coulam CB., Am J Reprod Immunol. 2008 Sep;60(3):258-63. doi: 10.1111/j.1600-0897.2008.00621.x. Duration of intralipid's suppressive effect on NK cell's functional activity Roussev RG). Furthermore, the treatment with Intralipid® reduces the cytotoxicity of the peripheral blood NK cells, but it is not known to reduce the number of peripheral blood NK cells. In contrast, the present invention reduces both the number and the cytotoxicity of the peripheral blood NK cells. In addition, intravenous infusion of Intralipid® may cause several side effects such as bladder pain bloody or cloudy urine, chills, difficulty, burning, or painful urination, fever, frequent urge to urinate, lower back or side pain, sore throat and vomiting, whereas the present invention does not show these side effects.

[0050] In contrast to the treatments of the prior art, in which intravenous or subcutaneous (lymphocyte immunization) infusions are applied, with the present invention the compositions are administered orally and its components are digested through the digestive tract and follow the normal process of food consumption and absorption. In this way, the treatment of RSA or RIF becomes easier and safer in comparison to the treatments of the prior art. Furthermore,

with the present invention there is no need for hospitalization or permanent surveillance by doctors and protects women from exposure to hospital-acquired infections. In addition, the present invention is not associated with the risks and side effects of intervention treatments. Furthermore, with the present invention the reduction of the numbers and cytotoxicity of the peripheral blood NK cells last longer after the treatment is discontinued and a prolonged protection is achieved in comparison to the treatments of the prior art.

Examples

[0051] Examples 1-4 illustrate the effect of compositions of the present invention in the reduction of the number peripheral NK cells and their activity (cytotoxicity). The compositions were administered to healthy women of reproductive age (between 25 - 46 years old) experiencing RSA or RIF and having increased number of peripheral blood NK cells and increased peripheral blood NK cells activity. Blood samples from all women were tested at day zero before the initiation of the treatment, at day 45 and at day 70 depending on the case. All of them had a routine check 12 weeks after the discontinuation of the treatment.

[0052] The determination of the peripheral blood samples for NK cell percentage in total lymphoid cells and NK cell activity was performed by flow - cytometry using previously described techniques. (Determination of natural killer cell function by flow cytometry. Kimberly I. Kane et al, Clinical and diagnostic laboratory immunology, may 1996, p. 295-300; Evaluation of a Flow Cytometry-Based Assay for Natural Killer Cell Activity in Clinical Settings, R. Valiathan et al. 2011 The Authors. Scandinavian Journal of Immunology). This method gives the percentage of target cells killed by natural cytotoxic cells present in PBL. Two fluorescent dyes are used to discriminate between effector and target cells and between live and dead target cells. One is a green fluorescent dye, 3,3'-Diocadecyloxacarbocyanine perchlorate (DiO) which is used to label the plasma membranes of K562, a human erythroleukaemic tumor cell line used as the target population. The second dye propidium iodide (PI), a membrane impermeable, red fluorescent dye, which is added during the assay when targets cells membranes were disrupted by NK-like cells. Intact target cells unaffected by effectors were single positive (and exhibited only green fluorescence), while targets killed by effectors bearing disrupted membranes are double positive (and exhibited green as well as red fluorescence). Effector and target cells are added in tubes to create different effector-to-target (E:T) ratios, 1:50, 1:25 and 1:12,5. In the following examples the results are presented at E:T ratios 50:1. (Natural killer cell functional activity suppression by intravenous immunoglobulin, intralipid and soluble human leukocyte antigen-G. Roussev RG1, Ng SC, Coulam CB. Am J Reprod Immunol. 2007 Apr;57(4):262-9.). Control samples were healthy women of reproductive age with no reproductive failures such as miscarriages or unsuccessful IVFs and who have given birth to at least one child. Based upon the healthy control population, mean NK cytotoxicity was 19% in 50:1 ratio.

[0053] Changes in NK cell cytotoxicity and number from day 0 to day 70 were measurable by the above mentioned assay. During the administration of the compositions of the invention,

baseline mean percentage NK cell activity and number was significantly decreased after 45 days and then declined close to its baseline levels after 70 days. A repeated measurement after the administration of the oral compositions showed significant decrease on both the number as well as the cytotoxicity of the NK cells.

[0054] In the examples below, the following abbreviations are used:

LCFA - Long chain fatty acids

SFA - Saturated fatty acids

MUFA - Monounsaturated fatty acids

PUFA - Polyunsaturated fatty acids

MCFA - Medium chain fatty acids.

Example 1

[0055] A group of 15 women was treated once per day with oral composition 1 in the form of oral solution comprising the active ingredients shown in Table 1a. The fatty acids of the composition were in the form of glycerides. The amount of the administered composition was such that the total amount of fatty acids administered per day was 0,25g/kg of body weight. Blood samples were collected and tested prior to and 45 days after the initiation of the treatment.

Table 1a

% Active substances			
LCFA 97,64 %	SFA 14,74 %	Miristic acid	0,20%
		Palmitic acid	4,50%
		Stearic acid	10,04%
	MUFA 23,39 %	Oleic acid	21,39%
		Docosahexaenoid acid (DHA)	0,50%
		Eicosapentaenoic acid(EPA)	1,50%
	PUFA 59,51 %	Linoleic acid	49,27%
		γ -linolenic	0,50%
		α -linolenic	9,74%
Vitamins 1,64 %		B1	0,02%
		B2	0,02%
		B3	0,02%

% Active substances		
	B6	0,01%
	D3 (15 µg-600 IU)	0,48%
	Folic Acid	0,03%
	Myo-inositol	1,06%
Metal ions 0,10 %	Cooper	0,01%
	Zinc	0,02%
	Magnesium	0,06%
	Manganium	0,01%
Aminoacids	L-Glutamin	0,62%

[0056] The results of the numbers and activity of peripheral blood NK cells are shown in Table 1b.

Table 1b : (45days)

Subjects 15	Day 0	Day 45	Mean Decline %	Day 0	Day 45	Mean activity Decline %
	NK% number (mean)	NK% number (mean)		NK Activity	NK Activity	
	20,25	13,59		46,26	18,54	

[0057] The average percentage of peripheral blood NK cells in total peripheral blood cells was 20,25 on day 0 before the treatment and 13,59 on day 45 after the beginning of the treatment. This difference was statistically significant (p value <0,0001).

[0058] The average activity of peripheral blood NK cells at 50:1 ratio was 46,26 on day 0 before the treatment and 18,54 on day 45 after the beginning of the treatment. This difference was statistically significant (p value <0,0001).

[0059] Five women of the above-mentioned group continued the treatment for a total of 70 days. They were tested at day 70 for peripheral blood NK cell numbers and activity. The results are shown in Table 1c.

Table 1c : 70das

Subjects 5	Day 0	Day 70	Mean Decline %	Day 0	Day 70	Mean activity Decline %
	NK% number (mean)	NK% number (mean)		NK Activity	NK Activity	
	18,42	11,45		46,54	13,60	

[0060] The average percentage of peripheral blood NK cells in total peripheral blood cells was

18,42 at day 0 before the treatment and 11,45 at 70 days after the beginning of the treatment. This difference was statistically significant (p value <0,0001).

[0061] The average activity of peripheral blood NK cells at 50:1 ratio was 46,54 at day 0 before the treatment and 13,60 at 45 days after the beginning of the treatment. This difference was statistically significant (p value <0,0001).

[0062] All women treated with this composition are currently pregnant.

Example 2

[0063] A group of 6 women were tested at day 0 and at day 70 for NK cell number and activity after administration of oral composition 2 once per day. The composition was in the form of oral solution comprising the active ingredients shown in Table 2a. The fatty acids of the composition were in the form of glycerides. The amount of the administered composition was such that the total amount of fatty acids administered per day was 0,25g/kg of body weight.

Table 2a

% active substances			
LCFA 97,84 %	SFA 11,29 %	Miristic acid	3,05%
		Palmitic acid	6,84%
		Steatic acid	1,40%
	MUFA 35,85 %	Oleic acid	28,85%
		Docosahexaenoid acid (DHA)	1,80%
		PUFA 50,70 %	Eicosapentaenoic acid(EPA)
Linoleic acid			29,28%
γ-linolenic			18,69%
α-linolenic			2,73%
MCFA 2%		Capric acid	0,50%
		Caprylic acid	0,50%
		Lauric acid	1,00%
Vitamins 0,16 %		D3	0,10%
		Folic acid	0,03%
		Myo-inositol	0,03%

[0064] The results of the numbers and activity of peripheral blood NK cells are shown in Table 2b.

Table 2b

Subjects	Day 0	Day 70	Mean	Day 0	Day 70	Mean
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6	NK % number (mean)	NK% number (mean)	Decline %	NK % Mean Activity	NK % Mean Activity	activity Decline %
	18,45	12,80	30,62	43,32	19	56,14

[0065] The average percentage of peripheral blood NK cells in total peripheral blood cells was 18,45 on day 0 before the treatment and 12,80 on day 70 after the beginning of the treatment. This difference was statistically significant (p value 0,001).

[0066] The average activity of peripheral blood NK cells at 50:1 ratio was 43,32 on day 0 before the treatment and 19, on day 70 after the beginning of the treatment. This difference was statistically significant (p value 0,001).

Example 3

[0067] In this group 6 patient's blood samples were collected and tested prior to and at 45 days after administration of oral composition 3 once per day. The composition was in the form of oral solution comprising the active ingredients shown in Table 3a. The fatty acids of the composition were in the form of glycerides. The amount of the administered composition was such that the total amount of fatty acids administered per day was 0,25g/kg of body weight.

Table 3a

% active substances			
LCFA 100 %	SFA 25%	Miristic acid	10%
		Palmitic acid	10%
		Steatic acid	5%
	MUFA 50 %	Oleic acid	22%
		Docosahexaenoid acid (DHA)	6%
		Eicosapentaenoic acid(EPA)	19%
		Miristoleic acid	0,2%
		Palmitoleic acid	2,6%
		Sapienic acid	0,2%
	PUFA 25%	Linoleic acid	12%
		γ-linolenic	5%
		α-linolenic	8%

[0068] The results of the numbers and activity of peripheral blood NK cells are shown in Table 3b.

Table 3b

Subjects 6	Day 0	Day 45	Mean Decline%	Day 0	Day 45	Mean activity Decline %
	NK % number (mean)	NK% number (mean)		NK % Activity Day 0	NK % Activity Day 70	
	17,84	12,40		36	16,6	

[0069] The average percentage of peripheral blood NK cells in total peripheral blood cells was 17,84 on day 0 before the treatment and 12,40 on day 45 after the beginning of the treatment. This difference was statistically significant (p value <0,001).

[0070] The average activity of peripheral blood NK cells at 50:1 ratio was 36 on day 0 before the treatment and 16,6 on day 45 after the beginning of the treatment. This difference was statistically significant (p value 0,001).

[0071] Three out of the six women treated with this composition have given birth and 2 are pregnant.

Example 4

[0072] In this group 8 patient's blood samples were collected and tested prior to and 45 days after administration of oral composition 4 once per day. The composition was in the form of oral solution comprising the active ingredients shown in Table 4a. The fatty acids of the composition were in the form of glycerides. The amount of the administered composition was such that the total amount of fatty acids administered per day was 0,25g/kg of body weight.

Table 4a

% active substances			
LCFA 65 %	SFA 6,43 %	Miristic acid	1,00%
		Palmitic acid	3,00%
		Stearic acid	1,40%
	MUFA 45,71 %	Oleic acid	45,71%
	PUFA 12,86 %	Linoleic acid	10,03%
		γ-linolenic acid	1,93%
		α-linolenic acid	1,93%
MCFA 30,71 %	Capric acid		5,00 %
	Caprylic acid		8,71 %
	Lauric acid		17,00 %
Vitamins 3,06 %		D3	0,80%

	B1	0,11%
	B2	0,11%
	B3	0,13%
	B6	0,11%
	Folic Acid	0,10%
	Inositol	1,7%
Metal ions 0,83 %	Cooper	0,10%
	Zinc	0,13%
	Magnesium	0,50%
	Manganium	0,10%
Aminoacids 1,04 %	L-Glutamin	0,54%
	L-Arginine	0,50%

[0073] The results of the numbers of peripheral blood NK cells are shown in Table 4b.

Table 4b

Subjects 8	Day 0	Day 45	Mean Decline
	NK % number (mean)	NK% number (mean)	
	18,97	14,55	

[0074] The average percentage of peripheral blood NK cells in total peripheral blood cells was 18,97 on day 0 before the treatment and 14,55 on day 45 after the beginning of the treatment. This difference was statistically significant (p value <0,017).

[0075] Five of the eight women treated with this oral composition have given birth to healthy children, 2 are currently pregnant after the 25th week and 1 has aborted.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **HERBERMAN RB** Role of human natural killer cell responses in health and disease *Clin Diagn Lab Immunol*, 1994, vol. 1, 125-33 [0002]
- **Basic and clinical immunology** **STITES DPTERR ALPARSLOW TG** Appleton and Lange Conn 1994 0000 [0002]
- **CALIGIURI M** Human natural killer cells *Blood*, 2008, vol. 112, 461-9 [0003]
- **MORETTA A MARCENARO E PAROLINI S FERLAZZO G MORETTA LNK** cells at the interface between innate and adaptive immunity *Cell Death Differ*, 2008, vol. 15, 226-33 [0003]
- **POLI A MICHEL T THERESINE MANDRES E HENTGES F ZIMMER JCD56** bright natural killer (NK) cells: an important NK cell subset *Immunology*, 2009, vol. 126, 4458-65 [0003]
- **HIBY SE1 KING ASHARKEY AM LOKE YW** Human uterine NK cells have a similar repertoire of killer inhibitory and activatory receptors to those found in blood, as demonstrated by RT-PCR and sequencing *Mol Immunol*, 1997, vol. 34, 5419-30 [0003]
- **GELLERSEN B BROSENS I BROSENS J J** Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives *Semin Reprod Med*, 2007, vol. 25, 445-53 [0004]
- **SANTONI A CARLINO C GISMONDI A** Uterine NK cell development, migration and function *Reprod Biomed Online*, 2008, vol. 16, 202-10 [0004]
- **KING A** Uterine leukocytes and decidualization *Hum Reprod Update*, 2000, vol. 6, 28-36 [0004]
- **ASHKAR AADI SANTO J PCROY BA** Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy *J Exp Med*, 2000, vol. 192, 259-70 [0004]
- **CLARK DARCK PC JALLILI R et al.** Psycho-Neuro-Cytokine/Endocrine pathways in immunoregulation during pregnancy *Am J Reprod Immunol*, 1996, vol. 35, 330-7 [0004]
- **CHAOUAT G TRANCHOT DIALLO J VOLUMENIE JL et al.** Immune suppression and Th1/Th2 balance in pregnancy revisited: A (very) personal tribute to Tom Wegmann *Am J Reprod Immunol*, 1997, vol. 37, 427-34 [0004]
- **LABARRERE CA** Allogeneic recognition and rejection reactions in the placenta *Am J Reprod Immunol*, 1989, vol. 21, 94-9 [0005]
- **STERN J J COULAM CB** Current status of immunologic recurrent pregnancy loss *Curr Opin Obstet Gynecol*, 1993, vol. 5, 252-9 [0005]
- **KING AWHEELER R CARTER NP et al.** The response of human decidual leukocytes to IL-2 *Cell Immunol*, 1992, vol. 141, 409-42 [0005]
- **VASSILIADOU N BULMER J N** Immunohistochemical evidence for increased numbers of 'classic' CD57+ natural killer cells in the endometrium of women suffering spontaneous early pregnancy loss *Hum Reprod*, 1996, vol. 11, 1569-74 [0006]
- **KWAK JYBEER AEKIM SH et al.** Immunopathology of the implantation site utilizing monoclonal antibodies to natural killer cells in women with recurrent pregnancy losses *Am J Reprod Immunol*, 1999, vol. 41, 91-8 [0006]

- **COULAM CBGOODMAN CROUSSEV RG et al.**Systemic CD56+ cells can predict pregnancy outcomeAm J Reprod Immunol, 1995, vol. 33, 40-6 [0006] [0007]
- **AOKI KKAJIURA SMATSUMOTO Y et al.**Preconceptual natural killer cell activity as a predictor of miscarriageLancet, 1995, vol. 345, 1340-2 [0006] [0007]
- **GAFTER USREDNI BSEGAL JKALECHMAN Y**Suppressed cell-mediated immunity and monocyte and natural killer cell activity following allogeneic immunization of women with spontaneous recurrent abortionJ Clin Immunol, 1997, vol. 17, 408-19 [0008]
- **RUIZ JEKWAK JYBAUM L et al.**Intravenous immunoglobulin inhibits natural killer cell activity in vivo in women with recurrent spontaneous abortionAm J Reprod Immunol, 1996, vol. 35, 370-5 [0008]
- **MENG LLIN JCHEN LWANG ZLIU MLIU YCHEN XZHU L**Natural killer cell functional activity suppression by intravenous immunoglobulin, Intralipid and soluble human leukocyte antigen-GAm J Reprod Immunol, 2007, vol. 57, 262-9 [0008]
- **JEFFREY BRAVERMAN**Intralipid Therapy for Recurrent Pregnancy Loss - Controversies and Future Directions 34 th Annual Meeting of the American Society for Reproductive Immunology, 2014, [0008]
- Am J Reprod Immunol, 2007, vol. 58, 5447-59 [0009]
- Am J Reprod Immunol, 2008, vol. 59, 4306-15 [0009]
- Am J Reprod Immunol, 2006, vol. 55, 3232-9 [0011]
- **PERRICONE R1DI MUZIO GPERRICONE CGIACOMELLI RDE NARDO DFONTANA LDE CAROLIS C**Am J Reprod Immunol, 2010, vol. 63, 3263-5 [0011]
- **HEILMANN L1SCHORSCH MHAHN T**Am J Reprod Immunol, 2007, vol. 57, 4262-9 [0011]
- **VAN DEN HEUVEL MJ1PERALTA CGHATTA KHAN VKCLARK DA**Am J Reprod Immunol, 2007, vol. 58, 5447-59 [0011]
- **KIMBERLY I.KANE et al.**Clinical and diagnostic laboratory immunology, 1996, 295-300 [0020]
- **R. VALIATHAN et al.**Evaluation of a Flow Cytometry-Based Assay for Natural Killer Cell Activity in Clinical SettingsThe Authors. Scandinavian Journal of Immunology, 2011, [0020] [0052]
- Duration of intralipid's suppressive effect on NK cell's functional activity**ACACIO BNG SCCOULAM CB.**Am J Reprod ImmunolRoussev RG20080900vol. 60, 258-63 [0049]
- **KIMBERLY I.KANE et al.**Determination of natural killer cell function by flow cytometryClinical and diagnostic laboratory immunology, 1996, 295-300 [0052]
- **ROUSSEV RG1NG SCCOULAM CB**Natural killer cell functional activity suppression by intravenous immunoglobulin, intralipid and soluble human leukocyte antigen-GAm J Reprod Immunol, 2007, vol. 57, 4262-9 [0052]

Patentkrav

- 1.** Oral sammensætning omfattende en kombination af fedtsyrer eller estere deraf som aktive ingredienser, til anvendelse i en fremgangsmåde til behandlingen af tilbagevendende spontane aborter eller gentagne mislykkede implantationer hos en kvinde med 12% eller flere perifere NK-blodceller af totale perifere blodceller, og/eller hvor cytotoxiciteten af de perifere NK-blodceller hos kvinden er mindst 10% højere end den gennemsnitlige cytotoxicitet af en kontrolpopulation med raske kvinder i den reproduktive alder uden reproduktive problemer, og som har født mindst et barn, hvor sammensætningen indgives oralt i 30 til 60 dage, og hvor den totale mængde af fedtsyrer eller estere deraf indgivet per dag er fra 0,10 g/kg legemsvægt til 0,70 g/kg legemsvægt, beregnet som fedtsyrer.
- 2.** Oral sammensætning til anvendelse ifølge krav 1, hvor den totale mængde af fedtsyrer eller estere deraf indgivet per dag er fra 0,20 g/kg legemsvægt til 0,50 g/kg legemsvægt, beregnet som fedtsyrer.
- 3.** Oral sammensætning til anvendelse ifølge krav 1 eller 2, hvor sammensætningen indgives i 40 til 50 dage.
- 4.** Oral sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 3, hvor fedtsyrerne er valgt fra gruppen bestående af fedtsyrer med en alifatisk kæde med 13 eller flere carbonatomer, fedtsyrer med en alifatisk kæde med 6 til 12 carbonatomer, fedtsyrer med en alifatisk kæde med færre end 6 carbonatomer og blandinger deraf.
- 5.** Oral sammensætning til anvendelse ifølge krav 4, hvor fedtsyrerne med en alifatisk kæde med 13 eller flere carbonatomer er valgt fra gruppen bestående af palmitinsyre, stearinsyre, myristinsyre, arachinsyre, oleinsyre, docosahexaenoidsyre, eicosapentaensyre, palmitoleinsyre, myristoleinsyre, sapiensyre, linolsyre, α -linolsyre, γ -linolsyre og blandinger deraf.
- 6.** Oral sammensætning til anvendelse ifølge krav 4, hvor fedtsyrerne med en alifatisk kæde med 6 til 12 carbonatomer er valgt fra gruppen bestående af

laurinsyre, caprinsyre, caprylsyre og blandinger deraf.

7. Oral sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor sammensætningen omfatter mindst en mættet, mindst en mono-umættet og mindst en poly-umættet fedtsyre med en alifatisk kæde med 13 eller flere carbonatomer eller estere deraf.

8. Oral sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor mindst 50 vægtprocent af de aktive ingredienser i sammensætningen, beregnet som fedtsyrer, er fedtsyrer med en alifatisk kæde med 13 eller flere carbonatomer eller estere deraf.

9. Oral sammensætning til anvendelse ifølge krav 8, hvor mindst 65 vægtprocent af de aktive ingredienser i sammensætningen, beregnet som fedtsyrer, er fedtsyrer med en alifatisk kæde med 13 eller flere carbonatomer eller estere deraf.

10. Oral sammensætning til anvendelse ifølge krav 9, hvor mindst 97 vægtprocent af de aktive ingredienser i sammensætningen, beregnet som fedtsyrer, er fedtsyrer med en alifatisk kæde med 13 eller flere carbonatomer eller estere deraf.

11. Oral sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 10, hvor fedtsyreesterne er valgt fra gruppen bestående af monoglycerider, diglycerider, triglycerider, phospholipider, sphingolipider, saccharolipider og blandinger deraf.

12. Oral sammensætning til anvendelse ifølge krav 11, hvor fedtsyreesterne er valgt fra gruppen bestående af monoglycerider, diglycerider, triglycerider og blandinger deraf.

13. Oral sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 12, hvor sammensætningen yderligere omfatter et eller flere vitaminer, metalioner, aminosyrer eller blandinger deraf.