

DESCRIPTION

Title of Invention: ANTI-AGING AGENT FOR FEMALES

5 **Technical Field**

[0001] The present invention relates to an anti-aging agent for females.

Background Art

10 [0002] In women, the aging of reproductive tissues such as the ovaries, ovarian follicles, and ova proceeds with the aging process, and pregnancy and childbirth become difficult. In the infertility treatment of such women in whom pregnancy is difficult, hormone replacement therapy is often performed for the induction of ovulation. However, the growth and the ovulation of ovarian follicles which are uniformly
15 mature are difficult, and symptoms called the ovarian hyperstimulation syndrome (OHSS) are developed as adverse reactions, and when these deteriorate, there is also a risk of lapsing into respiratory failure or circulatory failure. Therefore, a safe and effective therapy has been desired in infertility treatment (Non Patent Literature 1).

20 [0003] Decrease in the concentrations of female hormones such as estrogen with the aging progression of reproductive tissues in women leads to menopausal disorder mainly in women in their late forties and fifties. Even some women in their thirties and middle forties may develop irregular menstruation besides symptoms of the
25 above-mentioned menopausal disorder due to stress or overwork. This is also called pre-menopausal disorder and reduces the QOLs of women

markedly like menopausal disorder. Although hormone replacement therapy is mainly performed also in the treatment of these menopausal disorder and pre-menopausal disorder, there are problems with a carcinogenic possibility and adverse reactions such as metrorrhagia, vomiting, headache, and liver malfunction. While every woman can develop pre-menopausal disorder or menopausal disorder, it is very important especially in Japan, in which aging is proceeding, for women to stay healthy from pre-menopause to climacteric. Therefore, a safe and effective therapy for pre-menopausal disorder or menopausal disorder has been desired in the same way as infertility treatment (Non Patent Literature 2).

[0004] The aging of female reproductive tissues is a problem not only in humans but also in domestic animals such as bovines and pigs. For example, although a cycle from the mating of mother pigs to the delivery is 2.5 times per year in the pig raising industry, the litter sizes of mother pigs decrease, and the reproductive performance also deteriorates due to an increase in reproductive disorder such as miscarriage with the aging process. Therefore, the development of a method for suppressing the progression of aging of female reproductive tissues also as to non-human animals in addition to humans has been desired.

Citation List

Non Patent Literature

[0005] Non Patent Literature 1 : Acta Obstetrica et Gynaecologica Japonica, 1998, vol. 50, No. 6, N135-N138

Non Patent Literature 2 : JIM, 1999, vol. 9, No. 8, 688-691

Summary of Invention

Technical Problem

5 [0006] An object of the present invention is to provide an anti-aging agent for the ovaries, ovarian follicles, and ova (hereinafter, these may also be collectively referred to as "female reproductive tissues.").

Solution to Problem

10 [0007] The present inventors have found that pyrroloquinoline quinone or a salt thereof suppresses the progression of aging of female reproductive tissues and promotes the production of estradiol. The present invention is based on this new finding.

[0008] The present invention provides, for example, the following
15 inventions.

[1]

An anti-aging agent for an ovary, an ovarian follicle, or an ovum, comprising: pyrroloquinoline quinone or a salt thereof.

[2]

20 An inhibiting agent or an improving agent for fibrosis of a female reproductive tissue, comprising: pyrroloquinoline quinone or a salt thereof.

[3]

25 A quality enhancing agent for an ovum, comprising: pyrroloquinoline quinone or a salt thereof.

[4]

An improving agent or a preventing agent for irregular menstruation, comprising: pyrroloquinoline quinone or a salt thereof.

[5]

5 An improving agent or a preventing agent for an ovarian hyperstimulation syndrome, comprising: pyrroloquinoline quinone or a salt thereof.

[6]

An estrogen production promoting agent, comprising: pyrroloquinoline quinone or a salt thereof.

10 [7]

The agent according to [6] for improving or preventing female symptoms derived from lack of estrogen.

[8]

15 The agent according to [6] for improving or preventing female pre-menopausal disorder or menopausal disorder.

[9]

The agent according to [6] for improving or preventing a female menopausal symptom.

[10]

20 The agent according to any one of [1] to [9] for use in a non-human animal.

[11]

25 A method for suppressing progression of aging of an ovary, an ovarian follicle, or an ovum by ingesting a composition comprising pyrroloquinoline quinone or a salt thereof.

[2-1]

The agent according to [1] for suppressing or improving fibrosis of a female reproductive tissue.

[2-2]

The agent according to [1] for enhancing quality of an ovum.

5 [2-3]

The agent according to [1] for improving or preventing irregular menstruation.

[2-4]

10 The agent according to [1] for improving or preventing an ovarian hyperstimulation syndrome.

[2-5]

The agent according to [1] for promoting production of estrogen.

[2-6]

15 The agent according to [2-5] for improving or preventing female symptoms derived from lack of estrogen.

[2-7]

The agent according to [2-5] for improving or preventing female pre-menopausal disorder or menopausal disorder.

20 [2-8]

The agent according to [2-5] for improving or preventing a female menopausal symptom.

Advantageous Effects of Invention

25 [0009] According to the present invention, an anti-aging agent for female reproductive tissues can be provided.

Brief Description of Drawings

[0010] [Figure 1] Figure 1 is a graph showing the concentration of AMH in the blood in female mice in Test Example 1.

5 [Figure 2] Figure 2(A) is a graph showing an estrous cycle when a pyrroloquinoline quinone disodium salt is not administered to a female aging model mouse in Test Example 2. Figure 2(B) is a graph showing an estrous cycle when a pyrroloquinoline quinone disodium salt was administered to a female aging model mouse in Test Example 2. In
10 Figure 2, M indicates metestrus, WE indicates weak estrus, E indicates estrus, P indicates proestrus, and D indicates diestrus.

[Figure 3] Figure 3(A) is a graph showing the relationship between time and the concentration of estradiol in blood when eCG (pregnant mare serum gonadotropin) was administered to immature female mice to
15 which a pyrroloquinoline quinone disodium salt was administered or not administered in Test Example 4. Figure 3(B) is a graph showing the relationship between time and the concentration of progesterone in blood when eCG (pregnant mare serum gonadotropin) and hCG (human chorionic gonadotropin) were administered to immature female mice to
20 which a pyrroloquinoline quinone disodium salt was administered or not administered in Test Example 4.

[Figure 4] Figure 4(A) is a photograph of the ovary stained with PSR before a pyrroloquinoline quinone disodium salt was administered to a female aging model mouse in Test Example 5 (at a magnification of 20).
25 Figure 4(B) is a photograph of the ovary stained with PSR after the pyrroloquinoline quinone disodium salt was administered to a female

aging model mouse for 2 weeks in Test Example 5 (at a magnification of 20). Figure 4(C) and Figure 4(D) are figures traced from Figure 4(A) and Figure 4(B), respectively, and shaded regions show the stained regions.

5

Description of Embodiments

[0011] Hereinafter, embodiments for implementing the present invention will be described in detail. However, the present invention is not limited to the following embodiments.

10 [0012] [1. Anti-aging agent for ovaries, ovarian follicles, or ova]

An anti-aging agent for the ovaries, ovarian follicles, or ova according to the present embodiment contains pyrroloquinoline quinone or a salt thereof (also referred to as an "(A) ingredient.>").

15 [0013] Pyrroloquinoline quinone or a salt thereof produces the effect of suppressing the elimination of AMH (anti-Mullerian hormone) or a decrease in AMH in a female. Here, AMH is a hormone used as an index to the aging of female reproductive tissues (especially the ovaries), and it is known that as the aging of female reproductive tissues proceeds, the concentration of AMH in blood decreases.

20 Pyrroloquinoline quinone or a salt thereof produces the effect of suppressing or improving the fibrosis of female reproductive tissues (such as the ovaries, ovarian follicles, and ova, especially the ovaries). As one embodiment of the present invention, an anti-aging agent for the ovaries, ovarian follicles, or ova and an inhibiting agent or an improving agent for the fibrosis of female reproductive tissues containing
25 pyrroloquinoline quinone or a salt thereof are therefore provided. The

term "anti-aging" mentioned here means that women become rejuvenated, the progression of aging of women is suppressed, women can maintain high QOLs even though the women are of advanced ages, and women can live vividly and youthfully even though the women are of advanced ages. The term "advanced age" mentioned here refers to their thirties or older, preferably their forties or older, and more preferably their fifties or older. Examples of the aging of female reproductive tissues (especially ovary tissues) include an ovarian reserve (the number of secondary follicles which remain in the ovaries). Since, as mentioned above, pyrroloquinoline quinone or a salt thereof has the effect of suppressing the elimination of AMH or a decrease in AMH, pyrroloquinoline quinone or a salt thereof also has the effect of enhancing the ovarian reserve besides.

[0014] Pyrroloquinoline quinone or a salt thereof produces the effect of enhancing the quality of ova. As one embodiment of the present invention, a quality enhancing agent for ova containing pyrroloquinoline quinone or a salt thereof is therefore provided. Here, the phrase "enhancing the quality of ova" refers to maintaining or enhancing the fertilization rate of discharged ova while increasing the number of the discharged ova, and the fertilization rate mentioned here is a proportion of the number of fertilized ova based on the total number of the discharged ova. As one embodiment of the present invention, an agent for increasing the number of discharged ova and maintaining or enhancing the fertilization rate of the discharged ova containing pyrroloquinoline quinone or a salt thereof is therefore provided. The phrase "enhancing the quality of ova" refers to maintaining or enhancing

the development rate while maintaining or enhancing the fertilization rate of ova, and the development rate mentioned here refers to the proportion of the number of fertilized ova which have become blastocyst stage embryos based on the number of the fertilized ova. As one embodiment of the present invention, an agent for maintaining or enhancing the fertilization rate of ova and maintaining or enhancing the development rate containing pyrroloquinoline quinone or a salt thereof is therefore provided.

[0015] Pyrroloquinoline quinone or a salt thereof produces the effect of improving or preventing irregular menstruation. As one embodiment of the present invention, an improving agent or a preventing agent for irregular menstruation containing pyrroloquinoline quinone or a salt thereof is therefore provided. Here, the term "irregular menstruation" means a state in which the cycles of menstruation in females (ovulation in female non-human animals without menstruation) are abnormal. For example, in humans, the normal cycle of menstruation is supposed to be 25 to 38 days, and when the cycle of menstruation is 24 days or less or 39 days or more, or when menstruation does not occur for 3 months or more (in the case of amenorrhea), the diagnosis of irregular menstruation is made.

[0016] Pyrroloquinoline quinone or a salt thereof produces the effect of promoting the production of estrogens (including estrone, estradiol, and estriol, preferably estradiol). As one embodiment of the present invention, an estrogen production promoting agent containing pyrroloquinoline quinone or a salt thereof is therefore provided. By promoting the production of estrogen, pyrroloquinoline quinone or a salt

thereof also produces the effect of replenishing female hormones in addition to the effect of improving or preventing female symptoms derived from a lack of estrogen, the effect of improving or preventing female menopausal symptoms, the effect of improving or preventing female pre-menopausal disorder, or the effect for improving or preventing female menopausal disorder. As one embodiment of the present invention, an improving agent or a preventing agent for female symptoms derived from a lack of estrogen, an improving agent or a preventing agent for female menopausal symptoms, or an improving agent or a preventing agent for female pre-menopausal disorder or female menopausal disorder containing pyrroloquinoline quinone or a salt thereof is therefore provided.

[0017] Pyrroloquinoline quinone or a salt thereof produces the effect of improving or preventing the ovarian hyperstimulation syndrome. As one embodiment of the present invention, an improving agent or a preventing agent for the ovarian hyperstimulation syndrome containing pyrroloquinoline quinone or a salt thereof is therefore provided. Here, the term "ovarian hyperstimulation syndrome" means symptoms resulting from ascites or pleural effusion as a result of the swelling of the ovary due to an excessive stimulus of an ovulation inducer; and in serious cases, blood is thickened, and complications such as renal insufficiency and thrombosis are also caused. Examples of specific symptoms of the ovarian hyperstimulation syndrome include abdominal bloat, nausea, rapid body weight gain, a decrease in urine volume, abdominal pain, and diarrhea. Pyrroloquinoline quinone or a salt thereof also produces the effect of improving or preventing symptoms

derived from the ovarian hyperstimulation syndrome and complications of the ovarian hyperstimulation syndrome by improving or preventing the ovarian hyperstimulation syndrome. The agents containing pyrroloquinoline quinone or a salt thereof according to the above-mentioned embodiments are also collectively referred to as "agents according to the present embodiments."

[0018] Herein, the term "menopause" means about 10 years around climacteric, and in the case of humans, a period of their late forties to fifties corresponds to the menopause. Herein, the term "pre-menopause" means a period which is prior to the above-mentioned menopause and in which females may manifest the below-mentioned symptoms of the pre-menopausal disorder, and in the case of humans, a period of their thirties to middle forties corresponds to the pre-menopause.

[0019] Examples of the symptoms of the "female menopausal disorder" include the feeling of choking, orthostatic dizziness, dizziness, numbness in the hands or the feet, and stiffness in the hands or the feet, dropsy, the feeling of fatigue, tinnitus, facial flash, hot flash, perspiration, palpitation, excitement, insomnia, irritation, touchiness, emotional instability, the feeling of depression, weepiness, a decreased motivation, the feeling of uneasiness, headache, stiffness in the shoulders, chest pain, lumbago, arthralgia, the cold in the hands or the feet, itching, dryness in the skin or the eyes, nausea, loss of appetite, abdominal pain, constipation, diarrhea, frequent urination, sexual dysfunction, and unpleasantness in the external genitalia.

[0020] Examples of the "female symptoms derived from a lack of

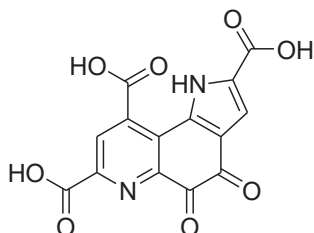
estrogen" include symptoms described in "1. HRT ni kitaisareru sayou, kouka (Action and effect expected from HRT)" in "Hormone replacement therapy guideline 2017 edition" edited by Japan Society of Obstetrics and Gynecology besides menstrual disorder, autonomic imbalance, neuropsychiatric symptoms, the atrophy symptom of the urogenital apparatus, hyperlipemia, arteriosclerosis, high blood pressure, apoplexy, osteoporosis, a symptom of a disease in bone quantity, atrophy of skin, arthralgia, dry mouth, change in taste, sleep disorder, and the above-mentioned symptoms of "female menopausal disorder."

[0021] Examples of the symptoms of the "female pre-menopausal disorder (female juvenile menopausal disorder)" include menstrual irregularity and amenorrhea besides the above-mentioned symptoms of the "female menopausal disorder".

[0022] The term "female menopausal symptoms" refers to the above-mentioned symptoms of the "female menopausal disorder" and symptoms of the "female pre-menopausal disorder (female juvenile menopausal disorder)". However, the symptoms thereof can be manifested not only in the "menopause" and the "pre-menopause" but also from earlier ages (for example, in their teens from which menstruation begins, in the case of humans). The "female menopausal symptoms" may be mild symptoms among these specific symptoms mentioned above. Examples include states in which there are subjective symptoms such as hot flash and perspiration, but the numbers of occurrence are small and states in which the severity of symptoms such as depression and stiffness in the shoulders is low.

[0023] Pyrroloquinoline quinone is a known compound represented by the following formula:

[Chemical Formula 1]



5 [0024] Examples of the salt of pyrroloquinoline quinone include alkali metal salts, alkaline-earth metal salts, and ammonium salts. Examples of the alkali metal salt include a sodium salt, a potassium salt, and a lithium salt. Examples of the alkaline-earth metal salts include a calcium salt and a magnesium salt.

10 [0025] As pyrroloquinoline quinone or a salt thereof, an alkali metal salt of pyrroloquinoline quinone is preferable, a sodium salt of pyrroloquinoline quinone is more preferable, and a pyrroloquinoline quinone disodium salt is further preferable.

15 [0026] Commercially available pyrroloquinoline quinone or a salt thereof can also be used. As pyrroloquinoline quinone or a salt thereof, for example, fermented soybeans, soybeans, cocoa powder, cacao mass, cacaos, parsley, green peppers, or the like, which contains pyrroloquinoline quinone or a salt thereof, may be used.

20 [0027] For use in humans, it is preferable that the total content of the (A) ingredient in the agent according to the present embodiment be 0.01 to 30% by weight, it is more preferable that the total content be 0.2 to 10% by weight, it is further preferable that the total content be 1 to 5% by weight, and it is particularly preferable that the total content be 1.3 to

3% by weight based on the total amount of the agent according to the present embodiment from the viewpoint of the stability of the preparation to be administered. For use in non-human animals, it is preferable that the total content of the (A) ingredient in the agent according to the present embodiment be 0.01 to 0.3% by weight, and it is more preferable that the total content be 0.02 to 0.2% by weight based on the total amount of the agent according to the present embodiment.

[0028] In the agent according to the present embodiment, the intake of the (A) ingredient per day can vary depending on the state (body weight, age, sex, and the like) of the individual that ingests the agent, the formulation, and the like; however, when the agent is used for humans, the intake is preferably 7 to 100 mg, more preferably 10 to 40 mg, and further preferably 20 to 25 mg; and when the agent is used for non-human animals, the intake is preferably 1 to 30 mg/kg and more preferably 2 to 20 mg/kg from the viewpoint of the ease of ingestion as a dose, the viewpoint of the efficacy of physiological action based on the (A) ingredient, and the viewpoint of safety.

[0029] The agent according to the present embodiment may further contain polyamine (also referred to as a "(B) ingredient") and a crude drug (also referred to as a "(C) ingredient"). By adding such ingredients, the effect of the present invention is produced more remarkably.

[0030] Polyamine is aliphatic hydrocarbon containing two or more primary amino groups in a molecule. Specific examples of the polyamine include putrescine, cadaverine, ethylenediamine, trimethylenediamine, hexamethylenediamine, spermidine, caldine,

homospermidine, aminopropylcadaverine, spermine, thermine,
 thermospermine, canavalmine, aminopentyl norspermidine,
 aminopropylhomospermine, canavalmine, homospermine,
 caldopentamine, homocaldopentamine, aminopropylcanavalmine,
 5 bis(aminopropyl)homospermine, bis(aminopropyl)norspermine,
 aminobutylcanavalmine, aminopropylhomospermine, homopentamine,
 caldohexamine, homocaldohexamine, thermohexamine, and
 homothermohexamine. As the polyamine, spermidine, spermine, and
 putrescine are preferable, spermidine and spermine are more preferable,
 10 and spermidine is further preferable from the viewpoint of the ease of
 utilization.

[0031] Commercially available polyamine can also be used. The
 polyamine may be used alone or in combination of two or more. As
 the polyamine, polyamine containing compositions such as extract from
 15 a plant such as soybean (preferably soybean germs), wheat (preferably
 wheat germs), or rice (preferably rice germs), extract from fish and
 shellfish (preferably milt), and dry sake yeast (also called a "polyamine
 composition") can also be used. When the polyamine composition is
 the extract from a plant, water, organic solvent such as ethanol, a mixed
 20 solvent thereof, or the like can be used as an extracting solvent. As the
 polyamine composition, the extract from a plant is preferable, and the
 extract from soybean (preferably soybean germs) is more preferable
 from the viewpoint of the ease of utilization. Examples of the
 commercial polyamine composition include "SOYPOLYA" (produced
 25 by Combi Corporation), "ORYZA POLYAMINE-P" (produced by
 Oryza Oil & Fat Chemical Co., Ltd.), "ORYZA POLYAMINE-LC"

(produced by Oryza Oil & Fat Chemical Co., Ltd.),
"PHYTOPOLYAMINE-S" (produced by TOYOBO CO., LTD.),
"PHYTOPOLYAMINE-SP" (produced by TOYOBO CO., LTD.), and
"Elion SP" (produced by MITSUBISHI GAS CHEMICAL COMPANY,
5 INC.).

[0032] The content of the (B) ingredient in the agent according to the
present embodiment is not particularly limited, and is suitably set
depending on the type of the (B) ingredient, the types and the contents
of other blended ingredients, the formulation, and the like. As the
10 content of the (B) ingredient, for example, it is preferable that the total
content of the (B) ingredient be 0.001 to 10% by weight, it is more
preferable that the total content be 0.01 to 0.1% by weight, and it is
further preferable that the total content be 0.02 to 0.05% by weight
based on the total amount of the agent according to the present
15 embodiment from the viewpoint of the stability of the preparation and
the viewpoint of reducing the influence of a bad odor which the (B)
ingredient has.

[0033] When the agent according to the present embodiment contains a
polyamine composition as the (B) ingredient, the content of the
20 polyamine composition is not particularly limited, and is suitably set
depending on the type of the polyamine composition, the types and the
contents of other blended ingredients, the formulation, and the like. As
the content of the polyamine composition, for example, it is preferable
that the total content of the polyamine composition be 5 to 35% by
25 weight, it is more preferable that the total content be 10 to 30% by
weight, and it is further preferable that the total content be 14 to 23% by

weight based on the total amount of the agent according to the present embodiment from the viewpoint of the stability of the preparation, the viewpoint of the efficacy of the physiological action based on the (B) ingredient, the viewpoint of safety, and the viewpoint of reducing the influence of a bad odor which the (B) ingredient has.

[0034] The content ratio of the (B) ingredient to the (A) ingredient in the agent according to the present embodiment is not particularly limited, and is suitably set depending on the types of the (A) ingredient and the (B) ingredient, the types and the contents of other blended ingredients, the forms of formulations, and the like. As the content ratio of the (B) ingredient to the (A) ingredient, for example, it is preferable that the total content of the (B) ingredient be 0.001 to 0.1 parts by weight, it is more preferable that the total content of the (B) ingredient be 0.01 to 0.03 parts by weight, and it is further preferable that the total content of the (B) ingredient be 0.015 to 0.02 parts by weight based on a total content of 1 part by weight of the (A) ingredient contained in the agent according to the present embodiment from the viewpoint of further raising the effect of the present invention.

[0035] When the agent according to the present embodiment contains a polyamine composition as the (B) ingredient, the content ratio of the polyamine composition to the (A) ingredient is not particularly limited, and is suitably set depending on the types of the (A) ingredient and the polyamine composition, the types and the contents of other blended ingredients, the forms of formulation, and the like. As the content ratio of the polyamine composition to the (A) ingredient, for example, it is preferable that the total content of the polyamine composition be 5 to

15 parts by weight, and it is more preferable that the total content of a polyamine composition be 7 to 10 parts by weight based on a total content of 1 part by weight of the (A) ingredient contained in the agent according to the present embodiment from the viewpoint of further enhancing the effect of the present invention.

[0036] When the (B) ingredient contains spermidine and spermine in the agent according to the present embodiment, the weight ratio of spermine to spermidine is not particularly limited, and is suitably set depending on the types of the (A) ingredient and (B) ingredient, the types and the contents of other blended ingredients, the forms of formulations, and the like. As the weight ratio of spermine to spermidine, for example, it is preferable that the weight ratio of spermine to spermidine be 0.01 to 10, it is more preferable that the weight ratio be 0.1 to 2, and it is further preferable that the weight ratio be 0.2 to 0.5 from the viewpoint of further enhancing the effect of the present invention.

[0037] Although, in the agent according to the present embodiment, the intake of the (B) ingredient per day can vary depending on the states (body weight, age, sex, and the like) of the individual that ingests the agent, the formulation, and the like, the intake is preferably 0.001 to 20 mg, more preferably 0.01 to 2 mg, further preferably 0.1 to 0.7 mg, and particularly preferably 0.3 to 0.4 mg from the viewpoint of the efficacy of the physiological action based on the (B) ingredient, the viewpoint of safety, and the viewpoint of reducing the influence of the bad odor which the (B) ingredient has.

[0038] When the agent according to the present embodiment contains

the polyamine composition as the (B) ingredient, the intake of the polyamine composition per day can vary depending on the states (body weight, age, sex, and the like) of the individual that ingests the agent, the formulation, and the like; however, the intake is preferably 50 to 400 mg, more preferably 100 to 300 mg, further preferable 200 to 250 mg, and particularly preferably 201 to 220 mg from the viewpoint of the ease of ingestion as a dose, the viewpoint of the efficacy of the physiological action based on the (B) ingredient, the viewpoint of safety, and the viewpoint of reducing the influence of the bad odor which the (B) ingredient has.

[0039] The crude drug means all or a part of a natural product such as a plant, an animal, or a mineral itself or this subjected to simple processing such as drying for medicinal use. In the agent according to the present embodiment, examples of the form of the crude drug include crude drugs themselves (raw crude drugs); crude drug powders obtained by drying and pulverizing raw crude drugs; and crude drug extracts obtained by extracting raw crude drugs or crude drug powders with water, an organic solvent such as ethanol, a mixed solvent thereof, or the like. Among these, the crude drug extracts are preferable from the viewpoint of producing the effect of the present invention more remarkably. The crude drug extracts may be extracted liquids themselves (tinctures, fluid extracts, or the like), may be diluted or concentrated extracted liquids (soft extracts or the like), or may be powders or pastes into which the dried extracted liquids are prepared after extracted liquids are dried (dried extracts).

[0040] Specific examples of the crude drug extracts include ginger

extract, maca extract, glycyrrhiza extract, Mongolian milkvetch extract, citrus unshiu peel extract, peony extract, ginseng extract, turmeric extract, *Cistanche salsa* extract, cinnamon extract, Siberian ginseng extract, hawthorn extract, rehmannia root extract, Japanese angelica root extract, saw palmetto extract, ginkgo leaf extract, bupleurum root extract, cimicifuga rhizome extract, dioscorea rhizome extract, jujube extract, atractylodes rhizome extract, *Atractylodes lancea* rhizome extract, *Fomes japonicus* extract, pilose antler extract, tongkatali extract, French coast pine bark extract, European oak extract, wild yam extract, diosgenin-containing yam extract, hesperidium extract, garlic extract, Siberian ginseng extract, *Mucuna Pruriens* extract, gardenia extract, shilajit extract, plant worm mycelium powder, black ginger extract, black pepper extract, pomegranate seed extract, chest tree extract, black cohosh extract, and common dandelion extract. As the crude drug extract, maca extract, ginseng extract, saw palmetto extract, ginger extract, tongkatali extract, French coast pine bark extract, European oak extract, wild yam extract, diosgenin-containing yam extract, pomegranate seed extract, and chest tree extract are preferable, and maca extract and ginseng extract are more preferable from the viewpoint of the stability of the preparation.

[0041] Commercially available crude drugs may be used. The crude drugs may be used alone or in combination of two or more.

[0042] The content of the (C) ingredient in the agent according to the present embodiment is not particularly limited, and is suitably set depending on the type of the (C) ingredient, the types and the contents of other blended ingredients, the forms of formulations, and the like.

As the content of the (C) ingredient, for example, it is preferable that the total content of the (C) ingredient be 0.1 to 70% by weight, it is more preferable that the content be 1 to 10% by weight, and it is further preferable that the content be 2 to 4% by weight based on the total amount of the agent according to the present embodiment from the viewpoint of producing the effect by the present invention more remarkably.

[0043] The content ratio of the (C) ingredient to the (A) ingredient in the agent according to the present embodiment is not limited, and is suitably set depending on the types of the (A) ingredient and (C) ingredient, the types and the contents of other blended ingredients, the formulation, and the like. As the content ratio of the (C) ingredient to the (A) ingredient, for example, it is preferable that the total content of the (C) ingredient be 0.1 to 3 parts by weight, it is more preferable that the total content be 0.5 to 2.5 parts by weight, and it is further preferable that the total content be 1 to 1.5 parts by weight based on a total content of 1 part by weight of the (A) ingredient contained in the agent according to the present embodiment from the viewpoint of further raising the effect of the present invention.

[0044] The content ratio of the (C) ingredient to the (B) ingredient in the agent according to the present embodiment is not particularly limited, and is suitably set depending on the types of the (B) ingredient and (C) ingredient, the types and the contents of other blended ingredients, the formulation, and the like. As the content ratio of the (C) ingredient to the (B) ingredient, for example, it is preferable that the total content of the (C) ingredient be 50 to 250 parts by weight, it is

more preferable that the total content be 80 to 200 parts by weight, and it is further preferable that the total content be 100 to 170 parts by weight based on a total content of 1 part by weight of the (B) ingredient contained in the agent according to the present embodiment from the viewpoint of further raising the effect of the present invention.

[0045] As long as the effect of the present invention is not impaired, the agent according to the present embodiment can contain pharmacologically active ingredients or physiologically active ingredients other than the (A) ingredient, the (B) ingredient, and the (C) ingredient. Specific example of such pharmacologically active ingredients or physiologically active ingredients include ubiquinone (coenzyme Q10), vitamin B1, vitamin B2, vitamin B6, folic acid, vitamin B12, vitamin C, vitamin D, vitamin A, vitamin E, niacin, calcium pantothenate, taurine, carnosine, anserine, balenine, citrulline, γ -aminobutyric acid, valine, leucine, isoleucine, glycine, arginine, ornithine, carnitine, glutamic acid, glutamine, creatine, carnitine, luteolin, quercetin, genistin, cyanidin, resveratrol, diosgenin, isoflavone aglycon, isoflavone, lipoic acid, zinc, iron, calcium, selenium, astaxanthin, zeaxanthin, β -carotene, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, placental extract, pycnogenol (proanthocyanidin), and equol. As the pharmacologically active ingredients or the physiologically active ingredients, ubiquinone (coenzyme Q10), vitamin B12, vitamin C, vitamin E, astaxanthin, zinc, carnitine, resveratrol, isoflavone, equol, pycnogenol (proanthocyanidin), and folic acid are preferable, and ubiquinone (coenzyme Q10), astaxanthin, zinc, resveratrol, isoflavone,

equol, pycnogenol (proanthocyanidin), and folic acid are more preferable from the viewpoint of further enhancing the effect of the present invention. The pharmacologically active ingredients or the physiologically active ingredients may be used alone or in combination of two or more.

[0046] As long as the effect of the present invention is not impaired, the agent according to the present embodiment can contain various additives besides the above-mentioned ingredients. Specific examples of such additives include excipients, binders, disintegrators, lubricants, coloring agents, flavoring agents, smell correcting agents, sugars, sugar alcohols and polyhydric alcohols, highly sweet sweeteners, oils and fats, emulsifiers, thickeners, acidulants, and fruit juices. Examples of the excipients include lactose, white sugar, sodium chloride, glucose, starch, gelatin, calcium carbonate, kaolin, crystalline cellulose, and silicic acid.

Examples of the binders include water, ethanol, propanol, simple syrup, glucose liquid, starch liquid, gelatin liquid, cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl starch, methyl cellulose, ethyl cellulose, calcium phosphate, and polyvinylpyrrolidone. Examples of the disintegrators include dry starch, sodium alginate, agar powder, sodium hydrogencarbonate, calcium carbonate, sodium lauryl sulfate, stearic acid monoglyceride, and lactose. Examples of the lubricants include talc, stearates, borax, and polyethylene glycol. Examples of the flavoring agents include white sugar, bitter orange peel, citric acid, and tartaric acid. Examples of the sugars include sugars such as sucrose, isomerized sugar, glucose, fructose, palatinose, trehalose, lactose, and

xylose. Examples of the sugar alcohols and the polyhydric alcohols include sorbitol, xylitol, erythritol, lactitol, palatinit, reduced syrup, reduced maltose syrup, glycerin, and propylene glycol. Examples of the highly sweet sweeteners include aspartame, stevia, acesulfame potassium, and sucralose. Examples of the oils and fats include safflower oil, grape seed oil, sunflower oil, olive oil, corn oil, sesame oil, soybean oil, rapeseed oil, and perilla oil. Examples of the emulsifiers include sucrose fatty acid esters, glycerin fatty acid esters, and lecithin. Examples of the thickeners include carrageenan, xanthan gum, guar gum, pectin, and locust bean gum. Examples of the acidulants include citric acid, lactic acid, and malic acid. Examples of the fruit juices include lemon fruit juice, orange fruit juice, and berry fruit juices. The additives may be used alone or in combination of two or more. It is preferable that the additives contain oils and fats from the viewpoint of the stability of the (A) ingredient and the (B) ingredient at the time of preparation.

[0047] The dosage form of the agent according to the present embodiment is not particularly limited, and examples include agents for internal use such as tablets (including orally disintegrating tablets, chewable tablets, and troche tablets), granules, powdered drugs, soft capsules, hard capsules, troches, jellies, or liquid medicines (including suspensions, emulsions, and syrups); agents for external use such as ointments, suppositories, patches, and sprays; and injections. Among these, it is preferable that the dosage form be an agent for internal use, and it is more preferable that the dosage be a tablet, granules, a soft capsule, or a hard capsule from the viewpoint of handleability and the

viewpoint of further enhancing the effect of the present invention.

[0048] The agent according to the present embodiment can be used, for example, as an ingredient of drug products, quasi drugs, cosmetics, and foods and drinks (beverages and foods). The agent according to the present embodiment can also be used, for example, as pharmaceutical preparations, quasi drug preparations, foods for specified health uses, foods with nutrient function claims, foods for the aged, foods for special use, foods with function claims, supplements, preparations for foods (for example, confectionery tablets), and products obviously recognized as food. The agent according to the present embodiment can also be used, for example, as a drug for animals and an additive for feeds.

[0049] When the agent according to the present embodiment is used as foods, the foods may be foods obtained by blending the (A) ingredient and other ingredients, if needed, into foods in general. Examples of such foods include solid foods such as cookies, biscuits, snack foods, jelly, gummi candy, chocolate, chewing gum, candy, and cheese; and liquid foods such as nutritional drinks, juice, tea beverages, coffee beverages, and milk beverages.

[0050] The agent according to the present embodiment can be suitably used for subjects (for example, humans; and non-human animals such as bovines, pigs, horses, sheep, goats, dogs, rabbits, mice, rats, guinea pigs, Mongolian gerbils, hamsters, and ferrets) that require the action of suppressing the progression of aging of female reproductive tissues. As the subject, humans are preferable from the viewpoint of further enhancing the effect of the present invention. Among non-human animals, bovines, pigs, and horses are preferable, and pigs are more

preferable from the viewpoint of further enhancing the effect of the present invention.

[0051] [2. Method for suppressing progression of aging of ovaries, ovarian follicles, or ova]

5 Pyrroloquinoline quinone or a salt thereof has the action of suppressing the progression of aging of the ovaries, ovarian follicles, or ova. As one embodiment of the present invention, a method for suppressing the progression of aging of the ovaries, ovarian follicles, or ova by the ingestion of the composition containing pyrroloquinoline
10 quinone or a salt thereof is therefore provided. The ovaries, the ovarian follicles, or the ova may be the ovaries, the ovarian follicles, or the ova of humans, or may be the ovaries, the ovarian follicles, or the ova of non-human animals such as bovines, pigs, horses, sheep, goats, dogs, rabbits, mice, rats, guinea pigs, Mongolian gerbils, hamsters, and
15 ferrets. It is preferable that the ovaries, the ovarian follicles, or the ova be the ovaries, the ovarian follicles, or the ova of humans from the viewpoint of further enhancing the effect of the present invention. Among the ovaries, the ovarian follicles, or the ova of non-human animals, the ovaries, the ovarian follicles, or the ova of bovines, pigs, or
20 horses are preferable, and the ovaries, the ovarian follicles, or the ova of pigs are more preferable from the viewpoint of further enhancing the effect of the present invention.

[0052] The type, the content, the intake per day, and the like of pyrroloquinoline quinone or a salt thereof, the types, the contents, the
25 intakes per day, and the like of the other ingredients in the composition, and the formulation of the composition, and the like in these

embodiments are as described in [1. Anti-aging agent for ovaries, ovarian follicles, or ova].

Examples

5 [0053] Hereinafter, the present invention will be described more specifically based on the Examples and the like. However, the present invention is not limited to the following Examples. All the following Test Examples were implemented with the approval of Animal Experiment Ethics Committee of Hiroshima University.

10 [0054] [Test Example 1: Influence of the administration of pyrroloquinoline quinone on AMH (anti-Mullerian hormone) in blood in female mice]

A PQQ disodium salt (2 mg/kg/day) in drinking water was administered to 6-month-old Leydig cell-specific NRG1 deficient female mice for 2 weeks (n = 5). Only water was administered to 15 6-month-old Leydig cell-specific NRG1 deficient male mice (KO; control) and 6-month-old wild type mice (WT mice) for 2 weeks. Then, the blood was collected, and the concentrations of AMH (anti-Mullerian hormone), which was a follicle marker, were measured using an AMH ELISA kit (Elabscience Biotechnology Inc., Bethesda, 20 MD) in accordance with the directions for the kit. The results are shown in Figure 1. The lives of Leydig cell-specific NRG1 deficient female mice are short, and the age of 6 months in the mice is equivalent to the age of the forties in humans.

25 [0055] As shown in Figure 1, while the concentrations of AMH decreased in the control female mice to which the PQQ disodium salt

was not administered as compared with the WT mice, the concentrations of AMH increased in the female mice to which the PQQ disodium salt was administered as compared with the WT mice. That is, it was confirmed that the ovary tissues were rejuvenated by the ingestion of the PQQ disodium salt.

[0056] [Test Example 2: Influence of administration of pyrroloquinoline quinone on estrous cycle in female mice]

The PQQ disodium salt (2 mg/kg/day) in drinking water was continuously administered to a 6-month-old Leydig cell-specific NRG1 deficient female mouse (KO). A female mouse to which only water was administered was used as a control. The estrous cycles of the mice were investigated by vaginal smear inspection. The vaginal smear inspection was performed as follows. The inside of the vagina was perfused with physiological saline solution, and the slide glass was coated with the perfusate. After drying, the perfusate was fixed with methanol and subjected to Giemsa staining. This was observed under an inverted microscope (manufactured by KEYENCE CORPORATION), the case where only white blood cells were seen was determined as diestrus, the case where only nucleate epithelial cells were seen was determined as proestrus, the case where only anucleate epithelial cells were seen was determined as estrus, the case where anucleate epithelial cells and white blood cells coexist, and more anucleate epithelial cells were seen was determined as weak estrus, and the case where anucleate epithelial cells and white blood cells coexist, and more white blood cells were seen was determined as metestrus. The results are shown in Figure 2.

[0057] As shown in Figure 2, while in the control female mouse to which the PQQ disodium salt was not administered, the weak estrus was always maintained, and periodic change in estrus was not seen; in the female mouse to which the PQQ disodium salt was administered, it was confirmed that periodical change in estrus was resumed from around 14 days after the initiation of the administration. That is, it was confirmed that the abnormalities of the ovulation cycle of the female mouse were improved by administering the PQQ disodium salt.

[0058] [Test Example 3: Influence of administration of pyrroloquinoline quinone on ova]

A PQQ disodium salt (2 mg/kg/day) in drinking water was administered to 3-week-old immature female mice (n = 5). Female mice to which only water was administered were used as controls. When the administration was started and 2 days passed, 4 IU of eCG (pregnant mare serum gonadotropin) which induced the growth of ovarian follicles was administered, and 5 IU of hCG (human chorionic gonadotropin) was subsequently administered 48 hours thereafter. Then, the number of the discharged ova of each female mouse was measured. The oviducts were collected from the sacrificed mouse, discharged ova-cumulus cell complexes were collected by incising the oviduct ampullas, and the number of the discharged ova was counted by observing the complexes under a stereomicroscope. In vitro fertilization was performed using the discharged ova, and the fertilization rate and the development rate were calculated. Here, the fertilized ova refers to cleaved ova 24 hours after fertilization, and the fertilization rate refers to the proportion of the number of ova

succeeding in fertilization based on the total number of the discharged mature ova. The development rate refers to the proportion of the number of fertilized ova which have become blastocyst stage embryos based on the number of the fertilized ova. The results obtained by expressing the numbers of the discharged ova and the fertilization rates in terms of the average values $\pm$ the standard deviations were shown in Table 1. The age of 3 weeks in mice is equivalent to the age of the teens in humans, at which menstruation begins.

[0059] [Table 1]

	Control	PQQ disodium salt
The number of discharged ova	40.41 ± 1.12	60.21 ± 1.77
The number of fertilized ova	26.63 ± 1.29	41.67 ± 1.48
Fertilization rate (%)	65.77 ± 2.10	69.41 ± 0.77
Development rate (%)	80.42 ± 2.44	88.09 ± 1.56

[0060] As shown in Table 1, in the female mice to which the PQQ disodium salt was administered, the in vitro fertilization rate was maintained while the number of the discharged ova increased, and the development rate was improved while the in vitro fertilization rate was maintained as compared with the control female mice to which the PQQ disodium salt was not administered. That is, it was confirmed that the quality of ova was improved by administering the PQQ disodium salt.

[0061] [Test Example 4: Influence of administration of pyrroloquinoline quinone on concentration of female hormones in blood]

A PQQ disodium salt (2 mg/kg/day) in drinking water was administered to 3-week-old immature female mice. Female mice to

which only water was administered were used as controls. When the administration was started, and 2 days passed, 4 IU of eCG (pregnant mare serum gonadotropin) which induced the growth of ovarian follicles was administered, and 5 IU of hCG (human chorionic gonadotropin) was subsequently administered 48 hours thereafter. The serum of each female mouse was collected over time from directly before the administration of eCG, and the concentration of estradiol (E2) in blood, which was a follicle growth marker, and the concentration of progesterone (P4), which was a corpus luteum marker, were measured using a commercial kit of Endocrine Technologies, Inc. (Newark, CA). The results are shown in Figure 3.

[0062] As shown in Figure 3(A), in the female mice to which the PQQ disodium salt was administered, the concentration of estradiol in blood increased over time as compared with the control female mice to which the PQQ disodium salt was not administered. That is, it was confirmed that the production of estradiol was promoted by the administration of the PQQ disodium salt. It is widely known that estradiol is one of female hormones widely used in hormone replacement therapy, and is used for the treatment of menopausal disorder or improvement in menopausal symptoms. That is, it was confirmed that pyrroloquinoline quinone or a salt thereof was useful as an improving agent or a preventing agent for female symptoms derived from a lack of estrogen and an improving agent or a preventing agent for female pre-menopausal disorder or menopausal disorder. Women in pre-menopause or menopause can reach climacteric without pain thereby.

As shown in Figure 3(B), a great difference between female mice to which the PQQ disodium salt was administered and control female mice to which the PQQ disodium salt was not administered in increase in the concentration of progesterone in blood was not seen. It is known that progesterone is related to the thickening of blood in the ovarian hyperstimulation syndrome. That is, it was confirmed that since pyrroloquinoline quinone or a salt thereof lightened the production of progesterone while promoting the production of estradiol, pyrroloquinoline quinone or a salt thereof was also useful as a preventing agent for the ovarian hyperstimulation syndrome.

[0063] [Test Example 5: Influence of the administration of pyrroloquinoline quinone on fibrosis of ovaries]

A PQQ disodium salt (2 mg/kg/day) in drinking water was administered to a 6-month-old Leydig cell-specific NRG1 deficient female mouse (KO) for 2 weeks. Then, an extracted ovary was stained with PSR and observed at a magnification of 20 using an inverted microscope (manufactured by KEYENCE CORPORATION). The results are shown in Figure 4.

[0064] As shown in Figure 4, since the insides of interstitial cells were stained red as a whole in the mouse before the administration of the PQQ disodium salt, the deposition of collagen, namely the fibrosis of the ovary, was confirmed. The atrophy of interstitial ovarian follicles was also seen simultaneously. Meanwhile, it was confirmed that, in the mouse to which the PQQ disodium salt was administered for 2 weeks, the red of the interstitial cells disappeared, and the fibrosed interstitial cells were recovered to the healthy state. It is known that

the fibrosis of interstitial cells of the ovaries proceeds due to the aging process. That is, it was confirmed that ovary tissues were rejuvenated by the ingestion of a PQQ disodium salt. Since vesicular ovarian follicles were seen, and it was confirmed that atrophy of ovarian follicles was also improved, it was confirmed that the quality of ova was improved.

[0065] From the results of Test Examples 1 to 5, it can be said that the ingestion of pyrroloquinoline quinone or a salt thereof enables maintaining the QOLs of women, which tend to be deteriorated due to the abnormalities of the minds and bodies which occur as the climacteric becomes closer, and enables women to live vividly and youthfully even though the women are of advanced ages.

CLAIMS

- [Claim 1] An anti-aging agent for an ovary, an ovarian follicle, or an ovum, comprising: pyrroloquinoline quinone or a salt thereof.
- 5 [Claim 2] An inhibiting agent or an improving agent for fibrosis of a female reproductive tissue, comprising: pyrroloquinoline quinone or a salt thereof.
- [Claim 3] A quality enhancing agent for an ovum, comprising: pyrroloquinoline quinone or a salt thereof.
- 10 [Claim 4] An improving agent or a preventing agent for irregular menstruation, comprising: pyrroloquinoline quinone or a salt thereof.
- [Claim 5] An improving agent or a preventing agent for an ovarian hyperstimulation syndrome, comprising: pyrroloquinoline quinone or a salt thereof.
- 15 [Claim 6] An estrogen production promoting agent, comprising: pyrroloquinoline quinone or a salt thereof.
- [Claim 7] The agent according to claim 6 for improving or preventing female symptoms derived from lack of estrogen.
- [Claim 8] The agent according to claim 6 for improving or preventing female pre-menopausal disorder or menopausal disorder.
- 20 [Claim 9] The agent according to claim 6 for improving or preventing a female menopausal symptom.
- [Claim 10] The agent according to any one of claims 1 to 9 for use in a non-human animal.
- 25 [Claim 11] A method for suppressing progression of aging of an ovary, an ovarian follicle, or an ovum by ingesting a composition

comprising pyrroloquinoline quinone or a salt thereof.