COMPOSITIONS AND METHODS FOR ENHANCING BIOENERGETIC STATUS IN FEMALE GERM CELLS

CROSS-REFERENCE TO RELATED SUBJECT MATTER

This application claims benefit under 35 U.S.C. § 119(e) of the U.S. provisional application Ser. Nos. 61/502,840, filed June 29, 2011, and U.S. provisional application Ser. No. 61/600,529, filed February 17, 2012, the entire disclosures of which are incorporated herein by reference.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This invention was made with Government support under Grant No. NIH R37-AG012279 awarded by The National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Since the early 1950s, clinical management of problems associated with ovarian insufficiency and failure, including infertility due to aging or insults, has been restricted by the belief that the pool of oocytes set forth at birth is not amenable to replacement or renewal (Zuckerman, *Recent Prog Horm Res* 1951 6:63–108). In other words, any therapeutic intervention had to conform to manipulation of the existing stockpile of oocyte-containing follicles to produce a desired clinical outcome. In 2004, however, studies with mice challenged the idea of a fixed ovarian reserve of oocytes being endowed at birth (Johnson et al., *Nature* 2004 428:145–150). Based on results from several experimental approaches, it was concluded that ovaries of adult female mammals retain rare germline or oogonial stem cells (OSCs) that routinely produce new oocytes in a manner analogous to germline stem cell support of sperm production in the adult testis (Spradling, *Nature* 2004 428:133–134). Several years later, OSCs were successfully isolated from neonatal and adult mouse ovaries (Zou et al., *Nat Cell Biol* 2009 11:631–636; Pachiarotti et al., *Differentiation* 2010 79:159-170). Collectively, these investigations, along with several other reports from studies of mice (Johnson et al., *Cell* 2005 122:303–315; Wang et al., *Cell Cycle* 2010 9:339–349; Niikura et al., *Aging* 2010 2:999–1003) have conceptually validated the use of OSCs as agents for transplantation and as targets for new therapies to modulate ovarian function and female fertility (Tilly et al., *Biol Reprod* 2009 80:2–12; Tilly et al., *Mol Hum Reprod* 2009; 15:393–398). In addition, the identification of dormant OSCs in atrophic ovaries of aged mice, which spontaneously resume oocyte formation when exposed to a young adult ovarian environment, indicates that

namely OSCs and compositions obtained thereof (e.g., OSC cytoplasm or isolated mitochondria), in ooplasmic transplantation procedures would prevent mitochondrial heteroplasmy, and alleviate ethical and safety concerns currently associated with the procedure. Importantly, oocytes, which are prone to aging-associated defects, are not of high enough quantity or quality to be reliably used in such procedures.

Accordingly, it is desirable to restore the quality of aged oocytes, as well as to further enhance OSCs or improve derivatives thereof (e.g., cytoplasm or isolated mitochondria) for use in conducting a range of assisted reproductive technologies.

SUMMARY OF THE INVENTION

The present invention provides for the use of agents to enhance mitochondrial numbers, mitochondrial activity, cellular energy levels or cellular energy-producing potential (collectively referred to as "bioenergetic status") in oocytes, postnatal female germline stem cells (also referred to herein as OSCs) and/or preimplantation embryos prior to conducting and/or following methods of in vitro fertilization, or following exposure of ovaries, oocytes, OSCs and/or preimplantation embryos in vivo. In certain embodiments, agents for such uses include soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a Sirt1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof. These agents will be collectively referred to herein as "bioenergetic agents."

In one aspect, the invention provides a composition containing one or more of an oocyte, an oogonial stem cell (OSC) or the progeny of an OSC, and a bioenergetic agent (e.g., one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof).

In another aspect, the invention provides an isolated cell having enhanced mitochondrial function, where the cell is one or more of an oocyte, an oogonial stem cell (OSC) or the progeny of an OSC, where the cell has been contacted with a bioenergetic agent (one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof).

In yet another aspect, the invention provides a composition containing OSC mitochondria or oocyte mitochondria and a bioenergetic agent that is one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In still another aspect, the invention provides an isolated mitochondria, where the mitochondria has been contacted with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In another aspect, the invention provides an isolated cell-free composition containing OSC mitochondria or oocyte mitochondria and a bioenergetic agent that any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In another aspect, the invention provides a method of preparing an oocyte for in vitro fertilization (IVF), the method involving transferring a composition containing OSC mitochondria and a bioenergetic agent that is one or more of any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof, into an autologous oocyte, thereby preparing the oocyte for in vitro fertilization. In one embodiment, the composition containing OSC mitochondria is a purified preparation of mitochondria obtained from the OSC.

In yet another aspect, the invention provides an oocyte prepared according to the method of the previous aspect or any other aspect of the invention delineated herein.

In yet another aspect, the invention provides a method of in vitro fertilization, the method involving the steps of (a) incubating an OSC from a female subject with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan,

quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof; (b) obtaining a composition containing OSC mitochondria from the OSC; (c) transferring the composition into an isolated, autologous oocyte; and (d) fertilizing the autologous oocyte in vitro to form a zygote. In one embodiment, the method further involves transferring a preimplantation stage embryo derived from the zygote, into the uterus of a female subject. In one embodiment, step a) is optional and step b) further involves incubating the composition containing OSC mitochondria with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In still another aspect, the invention provides a method of preparing an oocyte for in vitro fertilization, the method involving transferring a composition containing oocyte mitochondria and a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof, into an autologous oocyte, thereby preparing the oocyte for in vitro fertilization. In one embodiment, the composition containing oocyte mitochondria is oocyte cytoplasm without a nucleus. In another embodiment, the composition containing oocyte mitochondria is a purified preparation of mitochondria obtained from the oocyte. In oen embodiment, the method further involves fertilizing the oocyte *in vitro* to form a zygote and transferring a preimplantation stage embryo derived from said zygote, into the uterus of the female subject. In another embodiment, the zygote and pre-implantation stage embryo is incubated with a bioenergetic agent selected from the group consisting of tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, nicotinic acid, fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a SIRT1 activator, a CD38 inhibitor, a compound of any one of formulas I-XV, and functional derivatives thereof.

In still another aspect, the invention provides an oocyte prepared according to the method of the previous aspect or any other aspect of the invention delineated herein.

In yet another aspect, the invention provides a method of in vitro fertilization, the method involving the steps of (a) incubating an oocyte from a female subject with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof; (b) obtaining a composition containing oocyte mitochondria from the oocyte; (c) transferring the composition into an isolated, autologous oocyte; and (d) fertilizing the autologous oocyte in vitro to form a zygote. In one embodiment, the method further involves transferring a preimplantation stage embryo derived from the zygote, into the uterus of a female subject. In another embodiment, step a) is optional and step b) further involves incubating the composition containing oocyte mitochondria with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In still another aspect, the invention provides a method of in vitro fertilization, the method involving the steps of: incubating an oocyte from a female subject with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof; and fertilizing the oocyte in vitro to form a zygote.

In still another aspect, the invention provides a composition containing a solution selected from the group consisting of cell culture medium, oocyte retrieval solution, oocyte washing solution, oocyte in vitro maturation medium, ovarian follicle in vitro maturation medium, oocyte in vitro fertilization medium, embryo culture medium, cleavage medium, vitrification solution, cryopreservation solution and embryo thawing medium and a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In still another aspect, the invention provides a method of improving fertility in a female subject, the method containing administering to the subject a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof, in an amount effective to improve oocyte and/or OSC quality, de novo production and/or ovulated oocyte yield, thereby improving fertility in the female subject. In one embodiment, the bioenergetic agent is systemically administered to the female subject. In another embodiment, the bioenergetic agent is locally administered to an ovary of the female subject. In yet another embodiment, the pregnancy outcomes of the female subject are improved compared to a reference standard.

In still another aspect, the invention provides a method of in vitro fertilization, the method containing the steps of:

(a) administering to a female subject a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereofin an amount effective to improve oocyte and/or OSC de novo production, quality and/or ovulated oocyte yield;

(b) obtaining an oocyte from the female subject; and

(c) fertilizing the oocyte in vitro to form a zygote. In one embodiment, the bioenergetic agent is systemically administered to the female subject. In another embodiment, the bioenergetic agent is locally administered to an ovary of the female subject. In another embodiment, step a) is conducted prior to steps b) and c) and/or after steps b) and c). In another embodiment, the method further involving step d) transferring a preimplantation stage embryo derived from the zygote, into the uterus of the female subject and continuing to administer to the female subject the bioenergetic agent. In another embodiment, step b) and/or step c) further contains incubating the oocyte with a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof.

In another embodiment, the pregnancy outcomes of the female subject are improved compared to a reference standard.

In still another aspect, the invention provides a method of sustaining embryonic development in a pregnant female subject in need thereof, the method containing administering to the subject a therapeutically effective amount of a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof, thereby sustaining embryonic development in the pregnant female subject. In one embodiment, the bioenergetic agent is systemically administered to the female subject. In another embodiment, the bioenergetic agent is locally administered to an ovary of the female subject.

In still another aspect, the invention provides a method of restoring ovarian function in a female subject in need thereof, containing administering a therapeutically effective amount of a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof, thereby restoring ovarian function in the female subject.

In one embodiment, the bioenergetic agent is systemically administered to the female subject. In another embodiment, the bioenergetic agent is locally administered to an ovary of the female subject. In another embodiment, where the female subject has premature ovarian failure.

In still another aspect, the invention provides a method of preparing a tissue or cell thereof from a female subject for harvest, the method containing administering an effective amount of a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof to the female subject, thereby preparing the tissue or cell thereof from the female subject for harvest. In one embodiment, the tissue is ovary, ovarian follicle, bone marrow or peripheral blood.

In still another aspect, the invention provides a method of producing an oocyte, containing culturing a stem cell that is an OSC, embryonic stem cell, pancreatic stem cell, skin

stem cell or induced pluripotent stem cell (iPS cell) in the presence of a bioenergetic agent that is any one or more of one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof, under conditions sufficient to differentiate the stem cell into an oocyte.

In another aspect, the invention features a composition containing an isolated cell that is an oocyte, an oogonial stem cell (OSC) or the progeny of an OSC and a bioenergetic agent for use in *in vitro* fertilization.

In another aspect, the invention features an isolated cell having enhanced mitochondrial function relative to a reference, where the cell is an oocyte, an oogonial stem cell (OSC) or the progeny of an OSC, and where the cell has been contacted with a bioenergetic agent for use in *in vitro* fertilization.

In another aspect, the invention features a bioenergetic agent that is tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, nicotinic acid, fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, CD38 inhibitor, a compound of any one of formulas I-XV, or functional derivatives thereof for use in one or more of improving the fertility of a female, sustaining embryonic development in a pregnant female, restoring or increasing ovarian function in a a female, preparing a tissue or cell thereof from a female for harvest or preparing an oocyte.

In various embodiments of any of the above aspects or any aspect of the invention delineated herein, the bioenergetic agent is one or more of soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, resveratrol, DOI, hydroxytyrosol, pyrroloquinoline quinone, metformin, apigenin, luteolin, tyrphostin-8, berberine, a CD38 inhibitor, SRT-1720, a SIRT1 activator, a compound of any one of formulas I-XV, or functional derivatives thereof. In various embodiments of any of the above aspects or any aspect delineated herein, the bioenergetic agent is a compound shown in Figure 29.

In various embodiments of any of the above aspects or any aspect delineated herein, a composition of the invention further contains a solution selected from one or more of cell culture medium, oocyte retrieval solution, oocyte washing solution, oocyte in vitro maturation medium, ovarian follicle in vitro maturation medium, oocyte in vitro fertilization medium, vitrification solution and cryopreservation solution. In various embodiments, the composition contains ovarian tissue, ovarian follicles, bone marrow, umbilical cord blood or peripheral blood. In other embodiments, the OSC is an isolated non-embryonic stem cell that is mitotically

Figure 37 is a schematic diagram depicting pathways for the synthesis and degradation of NAD^+ in mammalian cells (Hassa, P. et al., Microbiol. Mol. Biol. Rev. September 2006 vol. 70 no. 3 789-829).

Figure 38 shows the structures of exemplary soluble precursors to NAD^+. These agents can be used to raise cellular NAD^+ levels and boost cellular energetics in damaged and/or aged cells.

Figures 39A-39C are graphs showing that nicotinamide mononucleotides raise cellular NAD^+ and NAD+/NADH in murine oogonial stem cells (OSCs). Oogonial stem cells were isolated from dissociated ovaries using a FACS based sorting protocol to purify OSCs free of contaminating oocytes (see Example 1). Cells were maintained in culture medium consisted of minimum essential medium α (MEMα), 10% FBS, 1 mM sodium pyruvate, 1 mM non-essential amino acids, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol (Sigma), 10 ng/ml–1 LIF (Millipore), 1X N-2 MAX Media Supplement (R&D) 10 ng/ml EGF (Epidermal growth factor, Recombinant human; Gibco), 40 ng/ml human GDNF (glial cell line-derived neurotrophic factor; R&D systems), 1 ng/ml human bFGF (basic fibroblast growth factor; Gibco Division of ThermoFisher Scientific, Waltham, MA, USA).

Figure 40 is a graph showing that nicotinamide mononucleotide increases mitochondrial DNA content in murine OSCs. Total cellular DNA was isolated from cells at the indicated time points using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. Mt DNA copy number was quantified using LightCycler 480 SYBR Green I Master (Roche Applied Science) using a Roche 480 PCR machine.

Figure 41 is a graph showing that nicotinamide mononucleotide increases spontaneous oocyte formation in cultured murine oogonial stem cells. For assessment of spontaneous oocyte formation, each well of a 24- well plate was seeded with 25,000 OSCs, and the number of oocytes formed and released into the medium per well was assessed the second day after seeding as well as the designated time points after NMN treatment.

Figure 42 is a graph showing that the NAD^+ precursor NMN raises NAD^+ levels *in vivo* in young and old mice. Cardiac [NAD^+] declines with age and is reversed by NMN treatment (n = 3; 200 mg.kg.d. I.P. for 1 week).

Figures 43A-D are graphs showing the restorative effects of an NAD^+ precursor (NMN) on mitochondrial function *in vivo*. The decline in mitochondrial function in skeletal muscle of 24-month old mice is completely reversed by NMN (nicotinamide mononucleotide) after only 1 week of treatment (Figures 43A, B). NMN is delivered by intraperitoneal (I.P.) injection and raises NAD^+ levels in brain, heart and skeletal muscle ~30-100%. NMN increases mitochondrial function in C2C12 cells in a SIRT1-dependent manner (Figures 43C, D). sh Ctl = scrambled shRNA, Sh SIRT1 = shRNA against SIRT1.

triazolyl, thiadiazolyl, isoquinolinyl, indazolyl, benzoxazolyl, benzofuryl, indolizinyl, imidazopyridyl, tetrazolyl, benzimidazolyl, benzothiazolyl, benzothiadiazolyl, benzoxadiazolyl, and indolyl.

The term "heterocyclic" as used herein, refers to organic compounds that contain at least at least one atom other than carbon (e.g., S, O, N) within a ring structure. The ring structure in these organic compounds can be either aromatic or non-aromatic. Some examples of heterocyclic moieties include, are not limited to, pyridine, pyrimidine, pyrrolidine, furan, tetrahydrofuran, tetrahydrothiophene, and dioxane. "Bioenergetic agents," as used herein, refer to agents that enhance mitochondrial numbers, mitochondrial activity, cellular energy levels or cellular energy-producing potential (bioenergetic status) in oocytes, OSCs and/or preimplantation embryos prior to conducting and/or following methods of in vitro fertilization, or following exposure of ovaries, oocytes, OSCs and/or preimplantation embryos in vivo. In particular, by enhancing mitochondrial numbers or activity, bioenergetic agents of the invention improve oocyte or OSC production and quality, for example, by preventing or decreasing aging-related increases in oocyte aneuploidy, chromosomal misalignment on the metaphase plate, meiotic spindle abnormalities, and/or mitochondrial dysfunction (aggregation, impaired ATP production). Bioenergetic agents include Sirt1 activators. Exemplary Sirt1 activators are listed in Table 1 (below).

Table 1: Sirt1 Activators

Compound:
Resveratrol (3,5,4'-Trihydroxy-*trans*-stilbene)
Butein (3,4,2',4'-Tetrahydroxychalcone)
Piceatannol (3,5,3',4'-Tetrahydroxy-*trans*stilbene)
Isoliquiritigen (4,2',4'-Trihydroxychalcone)
Fisetin (3,7,3',4'-Tetrahydroxyflavone)
5,7,3',4',5'-Pentahydroxyflavone
Luteolin (5,7,3',4'-Tetrahydroxyflavone)
3,6,3',4'-Tetrahydroxyflavone
Quercetin (3,5,7,3',4'-Pentahydroxyflavone)
7,3',4',5'-Tetrahydroxyflavone
Kaempferol (3,5,7,4'-Tetrahydroxyflavone)
6-Hydroxyapigenin (5,6,7,4'-Tetrahydroxyflavone; Scutellarein)

Preferred bioenergetic agents for such uses include, but are not limited to, soluble precursors to NAD^+ (e.g., tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, and nicotinic acid), fisetin, quercetin, hydroxytyrosol (4-(2-Hydroxyethyl)-1,2-benzenediol), pyrroloquinoline quinone (PQQ), metformin, apigenin, luteolin, tyrphostin-8, berberine a CD38 inhibitor, and SRT-1720, a compound of any one of formulas I-XV, and functional derivatives thereof.

DOI (2,5-dimethoxy-4-iodo-phenylisopropylamine) is a phenylalkylamine that has been characterized as a 5-HT2-selective agonist.

Fisetin (2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one) is described, for example, by Herzig, *Monatshefte für Chemie* 1891 12: 177–90; Gábor et al.,. *Nature* 1966 212 (5067): 1273; and Maher et al., *PLoS ONE* 2011 6 (6): e21226, each of which is incorporated by reference. Quercetin is described, for example, by Bentz, *The Journal of Young Investigators: Appalachian State University*. [Online] April 1, 2009, which is incorporated herein by reference.

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is described, for example, by Takaoka, *Nippon Kagaku Kaishi* 1939 60: 1090-1100; Hathway et al., *Biochemical Journal* 1959 72: 369-374; and Nonomuraet al., *Yakugaku Zasshi* 1963 83: 988-990, each of which is incorporated by reference.

Pyrroloquinoline quinone (4,5-Dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) is described, for example, by Hauge *J Biol Chem* 1964 239: 3630–9; Anthony et al., *Biochem J* 1967 104: 960–9; Salisbury et al., *Nature* 1979 280: 843–4; Westerling et al., *Biochem Biophys Res Commun* 1979 87: 719–24; Ameyama *FEBS Lett* 1981 130: 179–83, each of which is incorporated by reference.

Metformin (N,N-dimethylimidodicarbonimidic diamide) is described, for example, by Werner. *J Chem Soc, Transactions* 1921 121:1790–5; Shapiro et al.,. *J Am Chem Soc.* 1959 81:2220–5; *Patent FR 2322860* 1975 French; and *Pharmaceutical Manufacturing Encyclopedia (Sittig's Pharmaceutical Manufacturing Encyclopedia)*. 3rd ed. Vol. 3. Norwich, NY: William Andrew; 2007, each of which is incorporated by reference.

Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) is described, for example, by *Merck Index*, 11th Edition, 763, which is incorporated herein by reference.

Luteolin (2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone) is described, for example, by Mann *Secondary Metabolism* 1992 (2nd ed.). Oxford, UK: Oxford University Press. pp. 279–280; and López-Lázaro *Mini Rev Med Chem* 2009 9: 31–59.

Tyrphostin-8 (2-[(4-hydroxyphenyl)methylidene]propanedinitrile) is described, for example, by Martin; *Biochem. Pharmacol.* 1998 56: 483; Wolbring, et al.; *J. Biol. Chem.* 1994 269: 22470; Stanley, et al., *J. Immunol.* 1990 145: 2189, and Gazit, et al., *J. Med. Chem.* 1989 32: 2344 .

associated with cell adhesion, migration and differentiation. During hypoblast formation, SSEA-1 positive cells can be identified in the blastocoel and hypoblast and later in the germinal crescent. SSEA-1 functions in the early germ cell and neural cell development. (D'Costa et al., *Int J. Dev. Biol.* 1999 43(4):349-356; Henderson et al., *Stem Cells* 2002 20:329-337). In specific embodiments, expression of SSEAs in female germline stem cells may arise as the cells differentiate. SSEAs useful in the invention include SSEA-1, -2, -3, and -4.

The term "autologous" as used herein refers to biological compositions obtained from the same subject. In one embodiment, the biological composition includes OSCs, OSC-derived compositions and oocytes. Accordingly, in conducting methods of the invention, the female germ cell cytoplasm or mitochondria used for transfer and the recipient oocyte into which the aforementioned compositions are transferred are obtained from the same subject.

The term "increase" as used herein generally means an increase of at least 5%, for example an increase by at least about 10%, or at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase (i.e. substantially above levels of detection), or any increase between 5-100% as compared to a reference level, as that term is defined herein, and as determined by a method that achieves statistical significance ($p < 0.05$).

The term "isolated" as used herein refers to an OSC, mitochondrion or composition derived from an OSC (e.g., cytoplasm, mitochondrial preparation), which has been physically separated or removed from its natural biological environment. An isolated OSC, mitochondrion or composition need not be purified. The biological sample can include, for example, bone marrow, peripheral blood, umbilical cord blood, ovary or spleen or cells obtained from bone marrow, peripheral blood, ovary or spleen. Preferably, the composition comprises at least 50%, 75%, 85%, 90%, 95% or 100% of the cell type or organelle of interest relative to other cell types or organelles.

As used herein, the term "low ovarian reserve" as it relates to humans refers to a woman who exhibits a circulating Follicle Stimulating Hormone (FSH) level greater than 15 IU/ml in a "day 3 FSH test," as described in Scott et al., *Fertility and Sterility,* 1989 51:651-4, or a circulating Anti-Mullerian Hormone (AMH) level less than 0.6 ng/ml, or an antral follicle count less than 7 as measured by ultrasound.

The term "exogenous" as used herein refers to transferred cellular material (e.g., mitochondria) that is removed from one cell and transferred into another cell. Preferably, the cells and transferred materials are autologous. For example, OSC derived mitochondria that have

been transferred into an oocyte, even if both are derived from the same subject, would be exogenous.

The term "prodrug" includes compounds with moieties which can be metabolized *in vivo*. Generally, the prodrugs are metabolized *in vivo* by esterases or by other mechanisms to active drugs. Examples of prodrugs and their uses are well known in the art (See, *e.g.*, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19). The prodrugs can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Hydroxyl groups can be converted into esters *via* treatment with a carboxylic acid. Examples of prodrug moieties include substituted and unsubstituted, branch or unbranched lower alkyl ester moieties, (*e.g.*, propionoic acid esters), lower alkenyl esters, di-lower alkyl-amino lower-alkyl esters (*e.g.*, dimethylaminoethyl ester), acylamino lower alkyl esters (*e.g.*, acetyloxymethyl ester), acyloxy lower alkyl esters (*e.g.*, pivaloyloxymethyl ester), aryl esters (phenyl ester), aryl-lower alkyl esters (*e.g.*, benzyl ester), substituted (*e.g.*, with methyl, halo, or methoxy substituents) aryl and aryl-lower alkyl esters, amides, lower-alkyl amides, di-lower alkyl amides, and hydroxy amides. Preferred prodrug moieties are propionoic acid esters and acyl esters. Prodrugs which are converted to active forms through other mechanisms *in vivo* are also included.

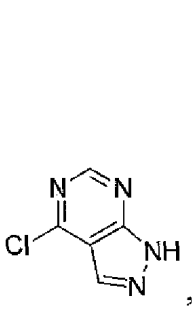
Compounds that increase the activity of sirtuins, e.g., SIRT1, are referred to as "SIRT1 activators." Exemplary compounds are listed in Tables 1, 2A, and 2B, and are described, e.g., in WO 05/002672, WO 05/002555, US 20050136537, US 20060025337, WO 2005/065667 and WO 2007/084162, and include polyphenols, e.g. plant polyphenols.

The term "reduced" or "reduce" or "decrease" as used herein generally means a decrease of at least 5%, for example a decrease by at least about 10%, or at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (i.e. substantially absent or below levels of detection), or any decrease between 5-100% as compared to a reference level, as that term is defined herein, and as determined by a method that achieves statistical significance ($p < 0.05$).

A "subject" is a vertebrate, including any member of the class *Mammalia*, including humans, domestic and farm animals, and zoo, sports or pet animals, such as mouse, rabbit, pig, sheep, goat, cattle and higher primates.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean " includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is

In certain embodiments, the invention relates to any one of the compositions, tissues, cells (e.g., OSCs, oocytes), or methods described herein, wherein the bioenergetic agent is selected from the group consisting of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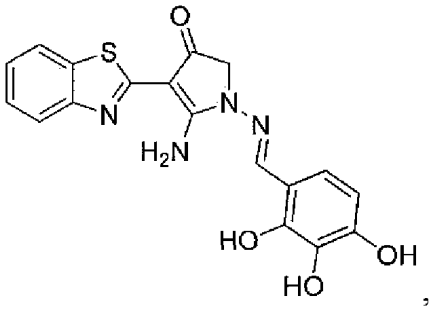
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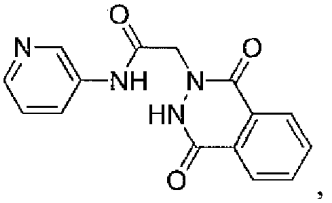
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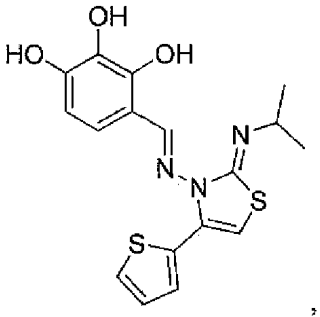
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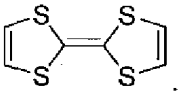
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In certain embodiments, the invention relates to any one of the compositions, tissues, cells (e.g., OSCs, oocytes), or methods described herein, wherein the bioenergetic agent is selected from the group consisting of α-lineolic acid, lineolic acid, stearic acid, elaidic acid, arachidonic acid, oleic acid, and palmitoleic acid.

In alternate embodiments, one or more of the following bioenergetic agents is specifically excluded from the methods of the invention: tryptophan, quinolinic acid, nicotinamide mononucleotide, nicotinamide riboside, nicotinic acid, fisetin, quercetin, hydroxytyrosol, pyrroloquinoline quinone (PQQ), metformin, apigenin, luteolin, tyrphostin-8, berberine, SRT-1720, and

Prior to isolation of OSCs, adult ovarian cortical tissue can be obtained using a minor laparoscopic procedure known in the art to collect a small (e.g., 3x3x1 mm) ovarian biopsy, which is then processed for OSC isolation. *See* Gook et al., *Hum Reprod* 2004 20:72-78. Isolation of human OSCs from adult ovarian cortical tissue can be performed as described in Example 1, Figure 1 or as previously described. *See*, for example, paragraph 0116 of U.S. Patent Pub. No. 20060010508, filed as U.S. Application Serial No. 11/131,114 on May 17, 2005 and Zou et al., *Nat Cell Biol* 2009 11:631-636. OSCs can also be obtained from non-ovarian sources, such as bone marrow or peripheral blood. Bone marrow and peripheral blood derived OSCs can be isolated by standard means known in the art for the separation of stem cells from, for example, the marrow or blood (e.g., cell sorting). Optionally, the isolation protocol includes generation of a kit+/lin- fraction that is depleted of hematopoietic cells. Additional selection means based on the unique profile of gene expression (e.g., Vasa, Oct-4, Dazl, Stella, Fragilis) can be employed to further purify populations of cells to an extent where they become substantially free of the biological sample from which they were obtained (e.g. bone marrow, peripheral blood, umbilical cord blood). For example, the methods described in Example 1, Figure 1 have been applied to a mononuclear fraction of blood cells and bone marrow cells to obtain the purified OSCs from non-ovarian sources. Briefly, cells were incubated with a rabbit anti-VASA antibody (COOH-Antibody 577-716) for 20 minutes (ab13840; Abcam, Cambridge, MA), washed, and incubated with goat anti-rabbit IgG conjugated to allophycocyanin (APC) for 20 minutes, and washed again. Labeled cells in the eluate were isolated by fluorescence-activated cell sorting (FACS) using a BD Biosciences FACSAria II cytometer (Harvard Stem Cell Institute, Boston, MA), gated against negative (unstained and no primary antibody) controls. Propidium iodide was added to the cell suspension just prior to sorting for dead cell exclusion. Results obtained using cell surface expression of Vasa to isolate OSCs from non-ovarian sources are provided in Figures 14 and 15, where the FACS–based germ cell purification of bone marrow and peripheral blood preparations from adult female mice during estrus of the female reproductive cycle is shown. Other antibodies for use in isolation methods include those described in U.S. Patent Nos. 7,884,193, 7,226,994 and 6,875,854, the contents of which are incorporated herein by reference.

Preparation of Oocyte and OSC Derived Compositions and Methods of Transfer

Methods for the preparation and transfer of purified mitochondria are known in the art and can be carried out as previously described. *See*, for example, Perez et al., *Cell Death Differ* 2007 14:524-533 and Perez et al., *Nature* 2000 , 403:500-1, the contents of which are expressly incorporated herein by reference. Briefly, OSCs and/or oocytes can be isolated and cultured as described above. Optionally, OSCs and/or oocytes can be isolated and cultured in the presence

the transcervical transfer of several 2-6 cell (day 2) or 8-16 (day 3) preimplantation embryos to the fundus of the uterus (4-5 preimplantation embryos provides optimal success).

Procedures for in vitro fertilization are also described in U.S. Pat. Nos., 6,610,543 6,585,982, 6,544,166, 6,352,997, 6,281,013, 6,196,965, 6,130,086, 6,110,741, 6,040,340, 6,011,015, 6,010,448, 5,961,444, 5,882,928, 5,827,174, 5,760,024, 5,744,366, 5,635,366, 5,691,194, 5,627,066, 5,563,059, 5,541,081, 5,538,948, 5,532,155, 5,512,476, 5,360,389, 5,296,375, 5,160,312, 5,147,315, 5,084,004, 4,902,286, 4,865,589, 4,846,785, 4,845,077, 4,832,681, 4,790,814, 4,725,579, 4,701,161, 4,654,025, 4,642,094, 4,589,402, 4,339,434, 4,326,505, 4,193,392, 4,062,942, and 3,854,470, the contents of which are specifically incorporated by reference for their description of these procedures.

Alternatively, patients may elect to have the oocyte, optionally comprising exogenous, autologous OSC mitochondria, reimplanted and fertilized in vivo using Intrauterine Insemination (IUI). IUI is a well-known process that involves preparing and delivering a highly concentrated amount of active motile sperm directly through the cervix into the uterus. There are several techniques available for preparing the sperm for IUI. First, sperm is separated from seminal fluid. One method of sperm separation is known as "Density Gradient Separation". In this technique, motile sperm are separated from dead sperm and other cells through the use of viscous solution. After preparation, the sperm concentrate is placed through the cervix into the uterus by using a thin, flexible catheter and fertilization of the reimplanted oocyte follows.

Culture Medium

Physiologically compatible solutions can be formulated or supplemented with effective amounts of bioenergetic agents or functional derivatives thereof for conducting the methods of the invention (e.g., IVF, cryopreservation, gamete preparation, cell and/or embryo washing or culture). Cell culture medium, embryo culture medium and cryopreservation solutions, for example, are well known in the art and can be formulated or supplemented as needed using standard methods known in the art for the preparation of physiological solutions. Commercially available medium for the preparation and handling of gametes for in vitro fertilization includes G-IVF™ PLUS, available from Invitrolife, which is a bicarbonate buffered medium containing human serum albumin and gentamicin as an antibacterial agent. SAGE Media™ maturation medium for oocytes contains sodium chloride, potassium chloride, sodium bicarbonate, glucose, sodium pyruvate, phenol red, gentamicin, nonessential and essential amino acids, magnesium sulfate, sodium phosphate, calcium chloride, D-calcium pantothenate, chlorine chloride, folic acid, i-inositol, nicotinamide, pyridoxine, HCL, riboflavin, and thiamine, supplemented with a final concentration of 75 mIU/ml FSH and 75 mIU/ml LH supplemented with a final concentration of 75 mIU/ml FSH and 75 mIU/ml LH. Media products for freezing and

containment of human blastocysts include SAGE Media™ equilibration solution and vitrification solution available from SAGE In Vitro Fertilization, Inc. Ovarian follicle maturation medium is also well known in the art and described, for example, by Telfer et al., *Hum Reprod* 2008 23:1151-1158, the contents of which are expressly incorporated herein by reference. The SAGE Media™ equilibration solution is a MOPS buffered solution of modified HTF containing nonessential and essential amino acids, gentamicin sulfate (0.01 g/L), 7.5% (v/v) each of DMSO and ethylene glycol and 12 mg/mL human albumin. The vitrification solution is a MOPS buffered solution of modified human tubal fluid (HTF) containing nonessential and essential amino acids, gentamicin sulfate (0.01 g/L), and 15% (v/v) each of DMSO and ethylene. SAGE blastocyst medium is formulated for use with in vitro fertilization procedures involving the culture of human embryos from the compaction phase on day 3 of development to the blastocyst stage and consists of sodium chloride, potassium chloride, potassium phosphate, magnesium sulfate, calcium lactate, sodium bicarbonate, glucose, sodium pyruvate, taurine, glutathione, alanyl-glutamine, L-asparagine, L-aspartic acid, glycine, L-proline, L-serine, L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, L-valine, D-calcium pantothenate, choline chloride, folic acid, I-inositol, nicotinamide, pyridoxine, riboflavin, thiamine, gentamicin and phenol red. SAGE cleavage medium is formulated for use with in vitro fertilization procedures involving the culture of cleavage stage human embryos and consists of sodium chloride, potassium chloride, magnesium sulfate, calcium lactate, sodium bicarbonate, glucose, sodium pyruvate, alanyl-glutamine, taurine, L-asparagine, L-aspartic acid, glycine, L-proline, L-serine, sodium citrate, EDTA, gentamicin and phenol red. SAGE fertilization medium is formulated for use with in vitro procedures involving the fertilization of human oocytes and consists of sodium chloride, potassium chloride, magnesium sulfate, potassium phosphate, calcium lactate, sodium bicarbonate, glucose, sodium pyruvate, alanyl-glutamine, taurine, L-Asparagine, L-Aspartic acid, glycine, L-Proline, L-Serine, sodium citrate, EDTA, gentamicin and phenol red. Several products are commercially available from LifeGlobal® Group LLC, USA, including solutions for embryo washing and handling (consisting of sodium chloride, potassium chloride, calcium chloride, potassium phosphate, magnesium sulfate, sodium bicarbonate, glucose, lactate NA salt, sodium pyruvate, amino acids, EDTA, gentamicin phenol red, and HEPES, optionally enriched with selected non-essential amino acids); oocyte retrieval and washing (consisting of sodium chloride, potassium chloride, calcium chloride, potassium phosphate, magnesium sulfate, sodium bicarbonate, glucose, lactate NA salt, sodium pyruvate, gentamicin, phenol red, and HEPES); embryo culture from day 1 to the blastocyst stage (consisting of sodium chloride, potassium chloride, calcium chloride, potassium phosphate, magnesium sulfate, sodium bicarbonate, glucose, lactate na salt, sodium pyruvate, amino acids, EDTA, gentamicin and phenol red); maintenance of embryos during

the biopsy procedure (consisting of sodium chloride, potassium chloride, potassium phosphate, sodium bicarbonate, glucose sodium lactate, sodium pyruvate, amino acids, EDTA, phenol red, gentamicin sulfate, HEPES, sucrose and human serum albumin); embryo freezing (consisting of sodium chloride, calcium chloride, potassium chloride, potassium phosphate, magnesium chloride, sodium phosphate, and human serum albumin) and embryo thawing (consisting of sodium chloride, calcium chloride, potassium chloride, potassium phosphate, magnesium chloride, sodium phosphate, human serum albumin and optionally 1,2-propanediol and sucrose). Early Cleavage Media™ (ECM®) is available from Irvine Scientific and is intended for use in culturing human gametes during fertilization (IVF) and growth of embryos through day 3 of development. This solution consists of glucose sodium, pyruvate sodium, lactate (d/l), sodium chloride, potassium chloride, magnesium sulfate, calcium chloride, sodium bicarbonate, alanyl-glutamine, taurine, sodium citrate, EDTA, disodium, dehydrate, phenol red, gentamicin, and sulfate. A culture medium for human gametes and embryos during fertilization and growth of embryos up to day 5/6 of development is also available from Irvine Scientific and consists of sodium chloride, potassium chloride, potassium phosphate, calcium chloride, magnesium sulfate, sodium bicarbonate, sodium pyruvate, glucose, sodium lactate, EDTA, dipeptide, alanyl-glutamine, phenol red, gentamicin, alanine, asparagines, aspartic acid, glutamic acid, glycine, proline, serine, arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine.

Accordingly, appropriate selection or formulation of medium for use in conducting assisted reproductive technologies (e.g., IVF) is routinely practiced by physicians and clinical laboratories. An effective amount of any one or more of the bioenergetic agents or functional derivatives thereof can be added to the medium of interest either prior to, during or following protocols conducted in connection with assisted reproductive technologies, which are well known in the art. The concentration, time and other conditions used will be optimized to achieve maximum exposure of the bioenergetic agents or functional derivatives thereof to the desired tissues or cells of interest, including, but not limited to, ovarian tissue, oocytes, OSCs or derivatives thereof (e.g., cytoplasm or isolated mitochondria) or preimplantation embryos. Tissues and/or cells of interest can be treated, ex vivo, using medium comprising bioenergetic agents or functional derivatives thereof prior to or during procedures associated with assisted reproductive technologies known in the art including, but not limited to, oocyte or OSC maturation and collection, ovarian follicle maturation, ovarian tissue or ovarian cell grafting, ovarian tissue or ovarian cell transplantation, cryopreservation, in vitro fertilization, as well as the culture of human oocytes, zygotes and preimplantation embryos.

The present invention also provides methods of producing an oocyte, comprising culturing a stem cell, including but not limited to, an OSC, embryonic stem cell, skin stem cell,

pancreatic stem cell, and induced pluripotent stem cell (iPS cell) in the presence of a bioenergetic agent or a functional derivative thereof, under conditions sufficient to differentiate the stem cell into an oocyte.

Stem cells can undergo self-renewing cell division to give rise to phenotypically and genotypically identical daughters for an indefinite time and ultimately can differentiate into at least one final cell type. Stem cells are defined as cells that have extensive, and perhaps indefinite, proliferation potential that differentiate into several cell lineages, and that can repopulate tissues upon transplantation. The quintessential stem cell is the embryonic stem (ES) cell, as it has unlimited self-renewal and multipotent differentiation potential. These cells are derived from the inner cell mass of the blastocyst, or can be derived from the primordial germ cells from a post-implantation embryo (embryonic germ cells or EG cells). ES and EG cells have been derived from mouse, non-human primates and humans. When introduced into mouse blastocysts or blastocysts of other animals, ES cells can contribute to all tissues of the mouse (animal). When transplanted in post-natal animals, ES and EG cells generate teratomas, which again demonstrates their multipotency.

Somatic stem cells have been identified in most organ tissues. Accordingly, in some embodiments, the stem cells useful for the oocyte differentiation and/or maturation culture methods described herein include, but are not limited to OSCs, mesenchymal stem cells, bone-marrow derived stem cells, hematopoietic stem cells, chrondrocyte progenitor cells, skin stem cells (e.g., epidermal stem cells), gastrointestinal stem cells, neural stem cells, hepatic stem cells, adipose-derived mesenchymal stem cells, pancreatic progenitor and/or stem cells, hair follicular stem cells, endothelial progenitor cells and smooth muscle progenitor cells.

An "induced pluripotent stem (iPS) cell" is a cell that exhibits characteristics similar to embryonic stem cells (ESCs) including, for example, unlimited self-renewal in vitro, a normal karyotype, a characteristic gene expression pattern including stem cell marker genes like Oct3/4, Sox2, Nanog, alkaline phosphatase (ALP) and stem cell-specific antigen 3 and 4 (SSEA3/4), and the capacity to differentiate into specialized cell types (Hanna et al., *Science* 2007 318: 1920-1923; Meissner A. et al. *Nat Biotechnol* 2007 25(10): 1177-81, Okita K. et al. *Nature* 2007 448(7151): 313-7, Takahashi K. et al. *Cell* 2007 131(5): 861-72, Wernig M. et al. *Nature* 2007 448(7151): 318-24, Yu J. et al. *Science* 2007 318(5858): 1917-20, and Park, I. H. et al. *Nature* 2008 451(7175): 141-6. The state of the art generation of iPS cells from fibroblast cultures has been described in Takahashi, Okita, Nakagawa, Yamanaka Nature Protocols (2007) 2(12).

Conditions for the differentiation and/or maturation of cells, including stem cells, progenitor cells and reprogrammed cells into oocytes are known in the art and are described, for example, by Danner S. et al. *Mol Hum Reprod.* 2007 Jan;13(1):11-20, Dyce P.W. et al. *PLoS One.* 2011;6(5), Dyce P.W. et al. *Stem Cells Dev.* 2011 May;20(5):809-19, Linher K. et al. *PLoS*

Polym. Ed. 1995 7:623-645; as biodegradable and injectable gel formulations, see, e.g., Gao Pharm. Res. 1995 12:857-863; or, as microspheres for oral administration, see, e.g., Eyles J. Pharm. Pharmacol. 1997 49:669-674.

In practicing this invention, the pharmaceutical compounds can be parenterally administered, such as by intravenous (IV) administration or administration into a body cavity or directly into the ovary. These formulations can comprise a solution of active agent dissolved in a pharmaceutically acceptable carrier. Acceptable vehicles and solvents that can be employed are water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils can be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These solutions are sterile and generally free of undesirable matter. These formulations may be sterilized by conventional, well known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. For IV administration, the formulation can be a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a suspension in a nontoxic parenterally-acceptable diluent or solvent, such as a solution of 1,3-butanediol. The administration can be by bolus or continuous infusion (e.g., substantially uninterrupted introduction into a blood vessel for a specified period of time).

The pharmaceutical compounds and formulations of the invention can be lyophilized. The invention provides a stable lyophilized formulation comprising a composition of the invention, which can be made by lyophilizing a solution comprising a pharmaceutical of the invention and a bulking agent, e.g., mannitol, trehalose, raffinose, and sucrose or mixtures thereof.

The compositions and formulations of the invention can be delivered by the use of liposomes. By using liposomes, particularly where the liposome surface carries ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the active agent into target cells *in vivo*. See, e.g., U.S. Patent Nos. 6,063,400; 6,007,839; Al-Muhammed J. Microencapsul. 1996 13:293-306; Chron. Curr. Opin. Biotechnol. 1995 6:698-708; Ostro Am. J. Hosp. Pharm. 1989 46:1576-1587.

method (2-ΔΔCT) using actin as an internal control. Primers were designed using the IDT software (IDT).

Several bioenergetic agents known to inhibit CD38 produced activity above the baseline (DMSO alone) and were scored as a positive result, including apigenin, luteoline, tyrphostin 8, beberine and SRT-1720. Accordingly, these bioenergetic agents were shown to increase mitochondrial parameters in a beneficial way.

Bioenergetic agents, including apigenin, luteolin, berberine, and tyrphostin 8, were shown to raise NAD^+ levels and increase mitochondrial parameters in a beneficial way. In one embodiment, such agents are useful in enhancing female germ cell bioenergetics for the treatment of female infertility associated with chemotherapy, aging and premature ovarian failure.

Example 10: FACS-based Isolation of Mitochondria

As described in this Example, FACS-based methods can be employed to isolate the total mitochondrial population in a tissue. In addition, FACS-based methods for mitochondrial isolation can employ dual-labeling using two different fluorescent dyes (mitochondrial membrane potential (MMP)-dependent and MMP-independent) to isolate only the functional (e.g., actively respiring) mitochondrial population or quantitate the ratio of functional to total mitochondria in a tissue, cell, lysed cell or fraction derived thereof.

The non-oxidation dependent MitoTracker Green FM (Invitrogen M7514) mitochondrial tracking probe, which indicates mitochondrial mass, was prepared and utilized as described below. MitoTracker stock solution (1-5mg/ml dissolved in anhydrous dimethylsulfoxide (DMSO)) was diluted in serum free growth medium to reach a working concentration of between 25-500nM. Freshly isolated or thawed OSCs were pelleted by centrifugation at 300 x g for 5 minutes. The supernatant was aspirated and the cell pellet was resuspended in 200 µl of the diluted MitoTracker stock solution.

Cells were incubated at 37°C for 45 minutes, washed in pre-warmed (37°C) serum free growth medium and pelleted by centrifugation at 300 x g for 5 minutes (alternatively, cells can be lysed prior to incubation with a probe of interest). Supernatant was aspirated and cells were resuspended in 100 µl mitochondrial lysis buffer and transferred to a FACS sort tube for lysis by mechanical permeabilization using rapid osmotic shock. Following lysis, cells were equilibrated cells on ice for 15-30 minutes, incubated in 200 µl (minimum volume) ice cold PBS and vortexed. As shown in Figure 36, three distinct populations were observed: residual M7514 positive cells (Cells MT +), high fluorescent mitochondria (functional, Mito MT high), and low expressing mitochondria (non-functional, Mito MT Low). The ratio of functional to non-functional mitochondria post lysis was approximately 1:1 (1552 mitochondria 743 were gated as

to detect components of the mitochondrial complex, for example, using the commercially available OXPHOS Complexes Detection cocktail (MitoSciences).

Example 12: Mitochondrial Isolation Using Sucrose Gradient Separation

The protocol employs the following reagents, which are commercially available: n-dodecyl-β-D-maltopyranoside (Lauryl maltoside; MitoSciences MS910), Phosphate buffered saline (PBS), Sucrose solutions 15, 20, 25, 27.5, 30 and 35 %, double distilled water, a protease inhibitor cocktail (MitoSciences), and 13 x 51 mm polyallomer centrifuge tubes (Beckman 326819).

The sucrose gradient separation procedure is a protein subfractionation method optimized for mitochondria. This method resolves a sample into at least 10 fractions. It is possible to separate solubilized whole cells into fractions of much lower complexity but when analyzing already isolated mitochondria the fractions are even more simplified. The sucrose gradient separation technique is designed for an initial sample volume of up to 0.5 ml at 5 mg/ml protein. Therefore 2.5 mg or less of total protein should be used. For larger amounts, multiple gradients can be prepared or larger scale
gradients are made.

The sample is solubilized in a non-ionic detergent. It has been determined that at this protein concentration mitochondria are completely solubilized by 20 mM n-dodecyl-β-D-maltopyranoside (1% w/v lauryl maltoside). The key to this solubilization process is that the membranes are disrupted while the previously Membrane embedded multi-subunit OXPHOS complexes remain intact, a step necessary for the density based sucrose separation procedure described herein. One important exception is the pyruvate dehydrogenase enzyme (PDH). In order to isolate PDH at a protein concentration of 5 mg/ml mitochondria, the required detergent concentration is only 10 mM (0.5 %) lauryl maltoside. The PDH enzyme should also be centrifuged at lower speeds, a centrifugal force of 16 000 g is maximum for the PDH complex.

To a mitochondrial membrane suspension at 5 mg/ml protein in PBS, lauryl maltoside is added to a final concentration of 1 %. This is mixed well and incubated on ice for 30 minutes. The mixture is then centrifuged at 72,000 g for 30 minutes. A Beckman Optima benchtop ultracentrifuge is recommended for small sample volumes. However, at a minimum a benchtop microfuge, on maximum speed (e.g., about 16 000 g) should suffice. After centrifugation, the supernatant is collected and the pellet discarded. A protease inhibitor cocktail is added to the sample, which is maintained on ice until centrifugation is performed. In samples very rich in mitochondria the cytochromes in complexes III and IV may give the supernatant a brown color, which is useful when checking the effectiveness of the following separation.

fractions are appropriate however collecting more fractions which are thus smaller in volume is also possible (e.g. 20 x 0.25 ml fractions). The fractions are stored at – 80°C until analysis. collected fractions are analyzed to determine mitochondrial integrity using any of the methods described herein (e.g., in Example 10, 11) or known in the art.

Example 13: Agents that Increase NAD^+ levels Increase Oocyte Production

As shown in Figure 37, NAD^+ is synthesized via three main pathways: (i) the NAD Salvage pathway (via NAMPT and NMNAT1-3 from nicotinamide, NAM to NMN to NAD^+); (ii) from tryptophan, via the de novo pathway, via quinolinic acid (Figure 38); and (iii) from nicotinamide riboside, a molecule found in milk and other food products (Figure 38). Until recently, NAD^+ was regarded simply as a coenzyme, carrying electrons from one reaction to another. It has now been discovered that NAD^+ is a primary signal for low caloric intake, coordinating the activities of major metabolic pathways, in large part, by stimulating the activity of sirtuins. Two critical downstream mediators are SIRT1 (a nuclear sirtuin) and SIRT3 (a mitochondrial sirtuin), which act synergistically to increase respiration and fatty acid oxidation in heart and skeletal muscle in response to fasting and exercise. In oogonial stem cells, SIRT1 controls the expression of a critical transcription factor that regulates the differentiation of oogonial stem cells into oocytes.

During aging, however, NAD^+ levels in the nucleus and mitochondria decline, reducing the activity of these two sirtuins and severely compromising mitochondrial function. NAD^+ levels can be increased by incubating cells with a NAD precursor such as NMN (e.g. Figure 39), by injecting or otherwise delivering an NAD precursor to cells in vivo (Figure 42), by increasing the expression of genes that synthesize NAD^+ e.g. NAMPT, NMNAT1-3; or by inhibiting NAD degradation, via PARPs or CD38 inhibition. (see Figure 37CD38 inhibitors include, but are not limited to those listed herein above in Tables 2A and 2B and are described by Dong M. et al. *Org. Biomol. Chem.* 2011 (9): 3246-3257 and Kellenberger E. et al. *Bioorg Med Chem Lett.* 2011 21(13):3939-42, the contents of which are incorporated herein by reference. Increasing NAD+ in cells can be achieved by other methods, such as applying substrates for the TCA cycle (e.g. Pyruvate, fatty acids) The following experiments were carried out to determine whether genes and small molecules that raise NAD^+ levels and/or activate the sirtuins delay or reverse the effects of aging and cell stress/damage on female fertility *in vivo* and *in vitro*.

Oogonial stem cells were isolated from dissociated ovaries using a FACS based sorting protocol to purify OSCs free of contaminating oocytes (for details, see Example 1). Cells were maintained in culture medium consisted of minimum essential medium α (MEMα), 10% FBS, 1 mM sodium pyruvate, 1 mM non-essential amino acids, 2 mM L-1-glutamine, 0.1 mM β-mercaptoethanol (Sigma), 10 ng/ml–1 LIF (Millipore), 1X N-2 MAX Media Supplement (R&D)

10 ng/ml EGF (Epidermal growth factor, Recombinant human; Gibco Division of ThermoFisher Scientific, Waltham, MA, USA), 40 ng/ml human GDNF (glial cell line-derived neurotrophic factor; R&D systems), 1 ng/ml human bFGF (basic fibroblast growth factor; Gibco Division of ThermoFisher Scientific, Waltham, MA, USA)

For all experiments 25,000 cells were plated in each well of a 24 well plate. Cells were allowed to attach for twenty-four hours and then were treated with NMN (β-Nicotinamide mononucleotide; Sigma). Unless otherwise stated, NMN was added twice to the cells, first at twelve hours and then again at six hours prior to analysis (12 + 6 h). Mitochondrial DNA Copy number was analyzed as follows. Total cellular DNA was isolated from cells at the indicated time points using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. Mitochondrial DNA copy number was quantified using LightCycler 480 SYBR Green I Master (Roche Applied Science) using the following primers on a Roche 480 PCR machine.

MT- ND2:
F: AAGGGATCCCACTGCACATA
R: AGTCCTCCTCATGCCCCTAT

RPS18 Nuclear
F: CCAGAGGTTGCATTTTCCCAAG
R: TAAGGCCGATAAGGCAAACGAA

Following treatment with NMN, NAD^+/NADH levels were measured according to the manufacturer's instructions using the NAD/NADH Quantitation Kit (Biovision) Raising NAD^+ levels in cells and *in vivo* dramatically increased mitochondrial function and mitochondrial content, which is generally recognized as a major determinant of female fertility, metabolic health, brain function, cardiovascular health and glucose metabolism/type II diabetes. OSCs and oocytes treated with an NAD precursor (e.g. nicotinamide riboside i.e., "NMN", see Figure 38) had increased NAD^+, NAD^+:NADH, and mitochondrial DNA content (Figure 40).

To determine whether increasing NAD^+ levels had an effect on oocyte production, spontaneous oocyte formation was assayed. Each well of a 24- well plate was seeded with 25,000 OSCs. The number of oocytes formed and released into the medium per well was assessed the second day after seeding as well as the designated time points after NMN treatment. NMN treatment increased the rate of egg formation (EFA) (,Figures 37, 38, and 41). Based on these results, compounds and genes that increase NAD^+ *in vivo* or *in vitro* are expected to reduce or reverse infertility associated with mitochondrial damage, energetic defects, and aging of the

ovary in female subjects. NMN treatment is also expected to enhance the function of OCSs, oocytes, granulose cells, and blood vessels in the ovary.

NMN and other compounds that increase NAD^+ levels are useful for increasing fertility or otherwise reducing or reversing infertility in a female subject. In one embodiment, such compounds are delivered to a subject orally, by intraperitoneal injection (IP), or subcutaneously to increase the probability that the subject will conceive and deliver healthy offspring. Systemic administration of the NAD^+ precursor NMN raised NAD^+ levels *in vivo* in young and old mice. Cardiac [NAD^+] declines with age. This decline in $NAD^+$was reversed by NMN treatment (n = 3; 200 mg.kg.d. I.P. for 1 week)(Figure 42). NMN treatment also had a restorative effect on mitochondrial function (Figure 43). In another embodiment, NMN and other compounds that increase NAD^+ levels are delivered to an OSC, oocyte, blastocyst, sperm, or isolated mitochondria in vitro. For example, such compounds are delivered to a germ cell prior to, during or following IVF. In another embodiment, a compound of the invention is used to enhance the yield or preservation of mitochondria from an OSC, oocyte, blastocyst, sperm, or isolated mitochondria in vitro.

Example 14: Oral Intake of Apigenenin, Luteolin, and SRT1720 Improve Quality of Oocytes in Aged Females

Female mice (8 months old, strain C57BL/6) were maintained on a 12:12 light:dark cycle and provided ad-libitum access to water and food. Conditions within rooms were maintained at 21° ± 1 °C with 50% ± 20% relative humidity. Mice were placed on the experimental diets at 8.5 months of age and were maintained for 3 months on the diets. All diets were custom made, ordered from Research Diets: OpenStandard Diet (20 kcal% Protein, 15 kcal% Fat and 65 kcal% Carbohydrate) and the experimental groups consisted of regular OpenStandard Diet, OpenStandard Diet + Apigenin at 0.5g/kg of body weight, OpenStandard Diet + Luteolin at 0.5g/kg, and OpenStandard Diet + SRT-1720 at 2g/kg. Food intake was measured weekly and average weight gain was assessed every two weeks. Each experimental group consisted of 12 randomly allocated mice. When mice were euthanized an additional group of 3-month old C57BL/6 females (3 M) were used as positive controls. In all groups, oocyte numbers were assessed following hormonal stimulation for superovulation.

It was determined that 11.5 month control mice in this study ovulate very few if any oocytes, and apigenenin, luteolin, and SRT-1720 all increase oocyte yield (Figure 44). The 12M control group fed OpenStandard Diet failed to ovulate a sufficient number of oocytes for subsequent analyses (approximately 1.3 oocytes/mouse, n=12 mice). Because of the extremely low yield of oocytes from the aged control females, historical data for oocyte yield from 12-month-old C57BL/6 females (referred to as "Hist 12 M"; from Selesniemi et al., *Proc Natl Acad*

CLAIMS

1. A cell culture medium for culturing an oocyte or an oogonial stem cell (OSC) comprising a CD38 inhibitor and a nicotinamide adenine dinucleotide (NAD) precursor, wherein the OSC is obtained from ovarian tissue or is the progeny of an OSC obtained from ovarian tissue, is an isolated non-embryonic stem cell, is mitotically competent and expresses Vasa, Oct-4, Dazl and Stella and, optionally, a stage-specific embryonic antigen,

wherein the CD38 inhibitor is a compound selected from the group consisting of 1-[(2-Acetoxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-[(2-Benzyloxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Methoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Nitro-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(3-Trifluoromethyl-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridiniumchloride, 1-{[2-(8'-Quinolyloxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1,2-Dimethoxy-ethylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butyne-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-hexamethylene-bis-N,N'-3-(aminocarbonyl)- pyridinium dichloride, (*E*)-1-{[4-(8'-Quinolyloxy)but-2-enyloxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-6-(aminocarbonyl)-quinolinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-4-amino-pyridinium chloride, luteolinidin, kuromanin, luteolin, delphinidin, pelargonidin, malvidin, quercetagetinidin, peonidin, myricetin, cyanidin, diosmetinidin, quercetin, robinetin, petunidin, fisetin, fisetinidin, quercetagetin, *rac*-taxifolin, *rac*-catechin, piceatannol, resveratrol, apigenin, tyrphostin-8, berberine and SRT-1720,

wherein the CD38 inhibitor is present in said medium at a concentration ≥ 25μM and the NAD precursor is present in said medium at a concentration ≥ 100μM, and

wherein said concentrations of the CD38 inhibitor and the NAD precursor are effective to enhance the bioenergetic status of the oocyte or the OSC cultured in said medium.

2. The cell culture medium of claim 1,

wherein the CD38 inhibitor is selected from the group consisting of apigenin, luteolin, fisetin, tyrphostin-8, berberine and SRT-1720; and

wherein the NAD precursor is selected from the group consisting of nicotinamide mononucleotide, nicotinamide riboside and nicotinic acid.

3. The cell culture medium of claim 1, wherein said concentrations of the CD38 inhibitor and NAD precursor present in said medium are effective to increase at least one selected from the group consisting of the number of functional mitochondria in, the mitochondrial energy of, and the cellular energy of the oocyte or the OSC cultured in said medium.

4. The cell culture medium of claim 1, wherein the medium further comprises ovarian tissue, ovarian follicles, bone marrow, umbilical cord blood or peripheral blood.

5. The cell culture medium of claim 1, further comprising oocyte retrieval solution, oocyte washing solution, oocyte *in vitro* maturation medium, ovarian follicle *in vitro* maturation medium, oocyte *in vitro* fertilization medium, vitrification solution or cryopreservation solution.

6. A composition comprising an isolated oocyte or oogonial stem cell (OSC) according to claim 1, and a CD38 inhibitor and an NAD precursor,

wherein the CD38 inhibitor is a compound selected from the group consisting of 1-[(2-Acetoxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-[(2-Benzyloxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Methoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Nitro-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(3-

Trifluoromethyl-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridiniumchloride, 1-{[2-(8'-Quinolyloxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1,2-Dimethoxy-ethylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butyne-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-hexamethylene-bis-N,N'-3-(aminocarbonyl)- pyridinium dichloride, (*E*)-1-{[4-(8'-Quinolyloxy)but-2-enyloxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-6-(aminocarbonyl)-quinolinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-4-amino-pyridinium chloride, luteolinidin, kuromanin, luteolin, delphinidin, pelargonidin, malvidin, quercetagetinidin, peonidin, myricetin, cyanidin, diosmetinidin, quercetin, robinetin, petunidin, fisetin, fisetinidin, quercetagetin, *rac*-taxifolin, *rac*-catechin, piceatannol, resveratrol, apigenin, tyrphostin-8, berberine and SRT-1720,

wherein the CD38 inhibitor is present in said composition at a concentration ≥ 25µM and the NAD precursor is present in said composition at a concentration ≥ 100µM, and

wherein said concentrations of the CD38 inhibitor and the NAD precursor are effective to enhance the bioenergetic status of the oocyte or the OSC.

7. The composition of claim 6,

wherein the CD38 inhibitor is selected from the group consisting of apigenin, luteolin, fisetin, tyrphostin-8, berberine and SRT-1720; and

wherein the NAD precursor is selected from the group consisting of nicotinamide mononucleotide, nicotinamide riboside and nicotinic acid.

8. The composition of claim 6, wherein the composition further comprises ovarian tissue, ovarian follicles, bone marrow, umbilical cord blood or peripheral blood.

9. A composition comprising a mitochondrial preparation selected from the group consisting of a purified preparation of oocyte mitochondria and a nucleus-free preparation of oocyte cytoplasm, and a CD38 inhibitor and an NAD precursor, wherein

the mitochondria are isolated from an oocyte or an oogonial stem cell (OSC) according to claim 1,

wherein the CD38 inhibitor is is a compound selected from the group consisting of 1-[(2-Acetoxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-[(2-Benzyloxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Methoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Nitro-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(3-Trifluoromethyl-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridiniumchloride, 1-{[2-(8'-Quinolyloxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1,2-Dimethoxy-ethylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butyne-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-hexamethylene-bis-N,N'-3-(aminocarbonyl)- pyridinium dichloride, (*E*)-1-{[4-(8'-Quinolyloxy)but-2-enyloxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-6-(aminocarbonyl)-quinolinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-4-amino-pyridinium chloride, luteolinidin, kuromanin, luteolin, delphinidin, pelargonidin, malvidin, quercetagetinidin, peonidin, myricetin, cyanidin, diosmetinidin, quercetin, robinetin, petunidin, fisetin, fisetinidin, quercetagetin, *rac*-taxifolin, *rac*-catechin, piceatannol, resveratrol, apigenin, tyrphostin-8, berberine and SRT-1720,

wherein the CD38 inhibitor is present in said composition at a concentration ≥ 25µM and the NAD precursor is present in said composition at a concentration ≥ 25µM, and

wherein said concentrations of the CD38 inhibitor and the NAD precursor are effective to enhance the bioenergetic status of the mitochondrial preparation.

10. The composition of claim 10,

wherein the CD38 inhibitor is selected from the group consisting of apigenin, luteolin, fisetin, tyrphostin-8, berberine and SRT-1720; and

wherein the NAD precursor is selected from the group consisting of nicotinamide mononucleotide, nicotinamide riboside and nicotinic acid.

11. A method of preparing an oocyte or oogonial stem cell for use in *in vitro* fertilization comprising:

culturing an oocyte or an oogonial stem cell (OSC) according to claim 1 in a medium comprising a CD38 inhibitor and an NAD precursor,

wherein the CD38 inhibitor is a compound selected from the group consisting of 1-[(2-Acetoxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-[(2-Benzyloxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Methoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Nitro-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(3-Trifluoromethyl-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridiniumchloride, 1-{[2-(8'-Quinolyloxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1,2-Dimethoxy-ethylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butyne-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-hexamethylene-bis-N,N'-3-(aminocarbonyl)- pyridinium dichloride, (*E*)-1-{[4-(8'-Quinolyloxy)but-2-enyloxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-6-(aminocarbonyl)-quinolinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-4-amino-pyridinium chloride, luteolinidin, kuromanin, luteolin, delphinidin, pelargonidin, malvidin, quercetagetinidin, peonidin, myricetin, cyanidin, diosmetinidin, quercetin, robinetin, petunidin, fisetin, fisetinidin, quercetagetin, *rac*-taxifolin, *rac*-catechin, piceatannol, resveratrol, apigenin, tyrphostin-8, berberine and SRT-1720,

wherein the CD38 inhibitor is present in the medium at a concentration of concentration ≥ 25μM and the NAD precursor is present in the medium at a concentration of concentration ≥ 100μM, and

wherein said concentrations of the CD38 inhibitor and the NAD precursor are effective to enhance the bioenergetic status of the oocyte or oogonial stem cell cultured in said medium.

12. The method of claim 11,

wherein the CD38 inhibitor is selected from the group consisting of apigenin, luteolin, fisetin, tyrphostin-8, berberine and SRT-1720; and

wherein the NAD precursor is selected from the group consisting of nicotinamide mononucleotide, nicotinamide riboside and nicotinic acid.

13. A method of preparing a preimplantation zygote for use *in vitro* fertilization comprising:

culturing said preimplantation zygote in a medium comprising a CD38 inhibitor and an NAD precursor,

wherein the CD 38 inhibitor is a compound selected from the group consisting of 1-[(2-Acetoxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-[(2-Benzyloxyethoxy)methyl]-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Methoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Nitro-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(3-Trifluoromethyl-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridiniumchloride, 1-{[2-(8'-Quinolyloxy)ethoxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1,2-Dimethoxy-ethylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butylene-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-butyne-bis-N,N'-3-(aminocarbonyl)-pyridinium dichloride, 1,4-Dimethoxy-hexamethylene-bis-N,N'-3-(aminocarbonyl)- pyridinium dichloride, (*E*)-1-{[4-(8'-Quinolyloxy)but-2-enyloxy]methyl}-3-(aminocarbonyl)-pyridinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-6-(aminocarbonyl)-quinolinium chloride, 1-{[2-(4-Phenoxy-phenoxy)ethoxy]methyl}-3-(aminocarbonyl)-4-amino-pyridinium chloride, luteolinidin, kuromanin, luteolin, delphinidin, pelargonidin, malvidin, quercetagetinidin, peonidin, myricetin, cyanidin, diosmetinidin, quercetin, robinetin, petunidin, fisetin, fisetinidin, quercetagetin, *rac*-taxifolin, *rac*-catechin, piceatannol, resveratrol, apigenin, tyrphostin-8, berberine and SRT-1720,

wherein the CD38 inhibitor is present in said medium at a concentration ≥ 25µM and the NAD precursor is present in said medium at a concentration ≥ 25µM, and

wherein said concentrations of the CD38 inhibitor and the NAD precursor are effective to enhance the bioenergetic status of the preimplantation zygote cultured in said medium.

14. The method of claim 15,

wherein the CD38 inhibitor is selected from the group consisting of apigenin, luteolin, fisetin, tyrphostin-8, berberine and SRT-1720; and

wherein the NAD precursor is selected from the group consisting of nicotinamide mononucleotide, nicotinamide riboside and nicotinic acid.

15. The method of claim 11 or claim 13, wherein the medium is selected from the group consisting of cell culture medium, oocyte retrieval solution, oocyte washing solution, oocyte *in vitro* maturation medium, ovarian follicle *in vitro* maturation medium, oocyte *in vitro* fertilization medium, vitrification solution and cryopreservation solution.

16. The method of claim 11 or claim 13, wherein the medium further comprises ovarian tissue, ovarian follicles, bone marrow, umbilical cord blood or peripheral blood.

17. The method of claim 11, wherein the oocyte or the OSC is mammalian.

18. The method of claim 17, wherein the oocyte or the OSC is from a human female.

19. The method of claim 18, wherein the human female is selected from the group consisting of selected from the group consisting of females of advanced maternal age, females suffering from oocyte-related infertility and females with low ovarian reserve.

20. The method of claim 11, wherein the oocyte is used for *in vitro* fertilization.