publicly available from the FDA http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm105852.htm.

Although the use of autologous mitochondria from somatic cells would avoid mitochondrial heteroplasmy, the mitochondria of somatic cells also suffer from age-related loss of mitochondrial function, due to decreased mitochondrial numbers (biogenesis), diminished mitochondrial activity (production of ATP, which is the main source of energy for cells) and/or accumulation of mitochondrial mtDNA mutations and deletions. Therefore, for women of advanced maternal age, no significant benefit would have been expected from transferring mitochondria derived from autologous somatic cells into oocytes. Moreover, a variety of stem cells are known to possess low mitochondrial activity (Ramalho-Santos et al., *Hum Reprod Update*. 2009 (5):553-72) and, therefore, adult stem cells were not thought to be viable sources of high activity mitochondria.

SUMMARY OF THE INVENTION

The present ~~invention~~disclosure is based, in part, upon the surprising discovery that the mammalian female germline stem cells or oogonial stem cells (OSCs), which are present in the somatic tissue of the ovary, contain mitochondria with the highest known ATP-generating capacity of all stem cell types evaluated, and containing mtDNA having a reduced amount of accumulated mutations, including, in some cases, non-detectable levels of a common mtDNA deletion known to accumulate with age in somatic cells.

In one aspect, there is provided a method of preparing an oocyte for in vitro fertilization (IVF) or artificial insemination, the method comprising transferring a composition comprising i) oogonial stem cell (OSC) mitochondria, or ii) mitochondria isolated from a progeny of an OSC, the OSC isolated from ovarian tissue, into an autologous oocyte, thereby preparing said oocyte for IVF or artificial insemination, wherein the OSC is an isolated non-embryonic stem cell that is mitotically competent and expresses Vasa, Oct-4, Dazl, Stella and optionally a stage-specific embryonic antigen.

In another aspect, there is provided a method of in vitro fertilization, said method comprising the steps of: a) providing a composition comprising i) mitochondria isolated from an OSC, or ii) mitochondria isolated from a progeny of an OSC, the OSC isolated from ovarian tissue; b) transferring the composition into an isolated, autologous oocyte; and c) fertilizing the autologous oocyte in vitro to form a zygote, wherein the OSC is an isolated non-embryonic stem cell that is mitotically competent and expresses Vasa, Oct-4, Dazl, Stella and optionally a stage-specific embryonic antigen, and wherein the step of transferring the composition and fertilizing the autologous oocyte are performed simultaneously in vitro.

In another aspect, there is provided a method for increasing the ATP-generating capacity of an oocyte, said method comprising the steps of: a) providing a composition comprising mitochondria isolated from at least one OSC or at least one progeny of an OSC that is autologous to the oocyte, the at least one OSC isolated from ovarian tissue; and b) injecting the composition comprising mitochondria into the oocyte, wherein the OSC is an isolated non-embryonic stem cell that is mitotically competent and expresses Vasa, Oct-4, Dazl, Stella and optionally a stage-specific embryonic antigen, and wherein the composition comprises mitochondria that have been isolated by centrifugation or by mitochondrial membrane potential-dependent cell sorting, and wherein the OSC or progeny of an OSC produces at least 5-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell.

In one ~~aspect~~embodiment, ~~the invention provides~~disclosed herein is a method of preparing an oocyte for *in vitro* fertilization (IVF) or artificial insemination. The method comprises transferring a composition comprising OSC mitochondria, or mitochondria obtained from a progeny of an OSC, into an autologous oocyte, thereby preparing the oocyte for *in vitro* fertilization or artificial insemination.

In some embodiments, the OSC is an isolated non-embryonic stem cell that is mitotically competent and expresses Vasa, Oct-4, Dazl, Stella and optionally a stage-specific embryonic antigen (SSEA) (e.g., SSEA-1, -2, -3, and -4). The OSC can be obtained from ovarian tissue, or non-ovarian tissue/sources, such as, *e.g.*, bone marrow or blood, *e.g.*, peripheral and umbilical cord blood.

In other embodiments, the composition comprising OSC mitochondria, or mitochondria obtained from a progeny of an OSC, is the cytoplasm of the cells without a nucleus.

In yet other embodiments, the composition comprising OSC mitochondria or mitochondria obtained from the progeny of an OSC is a purified preparation. In certain embodiments, the purified preparation does not contain or is at least about 85%, 90%, 95% free of OSCs, OSC progeny and/or non-functional mitochondria.

In some embodiments, the composition comprises $1x10^3$ to $5x10^4$ mitochondria.

In other embodiments, the OSC or progeny of an OSC produces at least 5-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In yet other embodiments, the OSC or progeny of an OSC produces at least 10-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In still other embodiments, the OSC or progeny of an OSC produces at least 50-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain, embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In other embodiments, the OSC or progeny of an OSC produces at least 100-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the oocyte is obtained from a human female of advanced maternal age. In other embodiments, the oocyte is obtained from a human female with low ovarian reserve.

In some embodiments, the composition comprises mitochondria that have been isolated by centrifugation. In other embodiments, the composition comprises mitochondria that have been isolated by mitochondrial membrane potential-dependent cell sorting.

In some embodiments, the composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC, is the cytoplasm of the cells without a nucleus. In other embodiments, the composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC is a purified preparation of mitochondria.

In another ~~aspect~~embodiment, ~~the invention provides~~there is disclosed ~~an~~ composition comprising isolated OSC mitochondria, or mitochondria obtained from a progeny of an OSC.

In some embodiments, the composition is at least about 85%, 90%, 95% free of cells or non-functional mitochondria.

In some embodiments, the composition comprises $1x10^3$ to $5x10^4$ mitochondria.

In other embodiments, the OSC or progeny of an OSC produces at least 5-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the OSC or progeny of an OSC produces at least 10-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In other embodiments, the OSC or progeny of an OSC produces at least 50-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the OSC or progeny of an OSC produces at least 100-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the OSC or progeny of an OSC is obtained from a human female of advanced maternal age. In other embodiments, the OSC or progeny of an OSC is obtained from a human female with low ovarian reserve.

In some embodiments, the composition comprises mitochondria that have been isolated by centrifugation. In other embodiments, the composition comprises mitochondria that have been isolated by mitochondrial membrane potential-dependent cell sorting.

In yet another ~~aspect~~embodiment, ~~the invention provides~~there is disclosed a composition comprising at least one isolated mitochondrion obtained from an OSC or at least one progeny of an OSC.

In some embodiments, the composition comprises $1x10^3$ to $5x10^4$ mitochondria.

In other embodiments, the OSC or progeny of an OSC produces at least 5-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the OSC or progeny of an OSC produces at least 10-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In other embodiments, the OSC or progeny of an OSC produces at least 50-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the OSC or progeny of an OSC produces at least 100-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the OSC or progeny of an OSC is obtained from a human female of advanced maternal age. In other embodiments, the OSC or progeny of an OSC is obtained from a human female with low ovarian reserve.

In some embodiments, the composition comprises mitochondria that have been isolated by centrifugation. In other embodiments, the composition comprises mitochondria that have been isolated by mitochondrial membrane potential-dependent cell sorting.

In another ~~aspect~~embodiment, ~~the invention provides~~there is disclosed an oocyte prepared in accordance with any of the methods described above.

In yet another ~~aspect~~embodiment, the ~~invention~~disclosure provides an oocyte comprising exogenous, autologous OSC mitochondria or mitochondria obtained from a progeny of an OSC.

In yet another ~~aspect~~embodiment, the ~~invention~~disclosure provides a method of in vitro fertilization. The method comprises the steps of: a) obtaining a composition comprising i) mitochondria obtained from an OSC, or ii) mitochondria obtained from a progeny of an OSC; b) transferring the composition into an isolated, autologous oocyte; and c) fertilizing the autologous oocyte *in vitro* to form a zygote. In an embodiment, the method further comprises transferring the zygote, or a preimplantation stage embryo derived from the zygote, into the uterus or oviduct of a female subject.

In some embodiments, the composition comprises $1x10^3$ to $5x10^4$ mitochondria.

In other embodiments, the OSC or progeny of an OSC produces at least 5-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In yet other embodiments, the OSC or progeny of an OSC produces at least 10-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In still other embodiments, the OSC or progeny of an OSC produces at least 50-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain, embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In other embodiments, the OSC or progeny of an OSC produces at least 100-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the oocyte is obtained from a human female of advanced maternal age. In other embodiments, the oocyte is obtained from a human female with low ovarian reserve.

In some embodiments, the composition comprises mitochondria that have been isolated by centrifugation. In other embodiments, the composition comprises mitochondria that have been isolated by mitochondrial membrane potential-dependent cell sorting.

In some embodiments, the at least one OSC is obtained from ovarian tissue. In other embodiments, the at least one OSC is obtained from a non-ovarian tissue.

In some embodiments, the non-ovarian tissue is blood. In other embodiments, the non-ovarian tissue is bone marrow.

In some embodiments, the composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC, is the cytoplasm of the cells without a nucleus. In other embodiments, the composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC is a purified preparation of mitochondria.

In yet another ~~aspect~~embodiment, the ~~invention~~disclosure provides a method of isolating a population of functional mitochondria from at least one OSC, or at least one progeny of an OSC. The method comprises the steps of incubating a composition comprising at least one OSC, or at least one progeny of an OSC, with a mitochondrial tracking probe under conditions sufficient to bind the probe to the functional mitochondria and sorting the functional mitochondria from the non-functional mitochondria, thereby isolating the population of functional mitochondria from at least one OSC, or at least one progeny of an OSC. In some embodiments, non-functional mitochondria are excluded from the population of functional mitochondria.

In some embodiments, the mitochondrial tracking probe is a non-oxidation dependent probe. In some embodiments, the mitochondrial tracking probe is an accumulation dependent probe. In some embodiments, the mitochondrial tracking probe is a reduced oxidative state probe. In some embodiments, the sorting step includes fluorescence-activated cell sorting.

In yet another ~~aspect~~embodiment, the ~~invention~~disclosure provides a method of identifying a population of functional mitochondria obtained from at least one OSC, or at least one progeny of an OSC. The method comprises the steps of: a) incubating a composition comprising at least one OSC, or at least one progeny of an OSC, with a fluorescent reduced oxidative state probe and a fluorescent accumulation dependent probe under conditions sufficient to bind the fluorescent reduced oxidative state probe to functional mitochondria in the composition and bind the fluorescent accumulation dependent probe to total mitochondria in the composition; b) obtaining a composition comprising the functional mitochondria using fluorescence-activated cell sorting, wherein the composition excludes non-functional mitochondria; c) determining the amount of functional mitochondria and the amount of total mitochondria; and d) calculating the ratio of functional mitochondria to total mitochondria; and e) determining whether the ratio is greater than about 0.02, thereby identifying a population of functional mitochondria obtained from at least one OSC, or at least one progeny of an OSC.

In some embodiments, the fluorescent accumulation dependent probe can fluoresce in one portion of the spectrum (e.g., green). In other embodiments, the fluorescent reduced oxidative state probe can fluoresce in a different portion of the spectrum (e.g., red).

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a composition comprising functional mitochondria obtained according to a method comprising the steps of: a) incubating a composition comprising at least one OSC, or at least one progeny of an OSC, with a fluorescent reduced oxidative state probe and a fluorescent accumulation dependent probe under conditions sufficient to bind the fluorescent reduced oxidative state probe to functional mitochondria in the composition and bind the fluorescent accumulation dependent probe to total mitochondria in the composition; and b) obtaining a composition comprising the functional mitochondria using

fluorescence-activated cell sorting, wherein the composition excludes non-functional mitochondria.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides a kit comprising a composition comprising isolated OSC mitochondria or mitochondria obtained from a progeny of an OSC and instructions for use. In one embodiment, the composition is at least about 85%, 90%, 95% free of cells or non-functional mitochondria.

Yet another ~~aspect~~embodiment of the ~~invention~~disclosure provides a kit comprising at least one isolated mitochondrion obtained from an OSC or at least one progeny of an OSC and instructions for use.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides a method for increasing the ATP-generating capacity of an oocyte. The method comprises the steps of: a) obtaining a composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC that is autologous to the oocyte; and b) injecting the composition of mitochondria into the oocyte.

In some embodiments, the composition comprises $1x10^3$ to $5x10^4$ mitochondria.

In other embodiments, the OSC or progeny of an OSC produces at least 5-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In yet other embodiments, the OSC or progeny of an OSC produces at least 10-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In still other embodiments, the OSC or progeny of an OSC produces at least 50-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain, embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In other embodiments, the OSC or progeny of an OSC produces at least 100-fold more ATP per fg mtDNA than an ovarian somatic cell or mesenchymal stem cell. In certain embodiments, the ovarian somatic cell or mesenchymal stem cell is autologous.

In some embodiments, the oocyte is obtained from a human female of advanced maternal age. In other embodiments, the oocyte is obtained from a human female with low ovarian reserve.

In some embodiments, the composition comprises mitochondria that have been isolated by centrifugation. In other embodiments, the composition comprises mitochondria that have been isolated by mitochondrial membrane potential-dependent cell sorting.

In some embodiments, the at least one OSC is obtained from ovarian tissue. In other embodiments, the at least one OSC is obtained from a non-ovarian tissue.

In some embodiments, the non-ovarian tissue is blood. In other embodiments, the non-ovarian tissue is bone marrow.

In some embodiments, the composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC, is the cytoplasm of the cells without a nucleus. In other embodiments, the composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC is a purified preparation of mitochondria.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides an oocyte prepared by a method comprising the steps of: a) obtaining a composition comprising mitochondria obtained from at least one OSC or at least one progeny of an OSC that is autologous to the oocyte; and b) injecting the composition of mitochondria into the oocyte.

Yet another ~~aspect~~embodiment of the ~~invention~~disclosure provides a composition of mitochondria obtained from at least one OSC or at least one progeny of an OSC, wherein the composition comprises a population of mitochondria in which greater than about 75%, 85%, 90%, or about 99% of the mitochondria are high ATP-generating capacity mitochondria.

In still another ~~aspect~~embodiment, the ~~invention~~disclosure provides compositions comprising a population of mitochondria in which less than about 5% to about 25% of the mtDNA comprises a deletion mutation within nucleotides 8470-13447 of the mitochondrial genome, and methods pertaining to such compositions.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims. Thus, other aspects of the invention are described in the following disclosure and are within the ambit of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference.

Figure 1 depicts validation of a fluorescence-activated cell sorting (FACS)-based protocol for OSC isolation. In Figure 1a, immunofluorescence analysis of VASA expression (with DAPI counterstain) is shown in adult mouse ovaries using antibodies against the NH_2 or COOH terminus of VASA (scale bars, 50 μm). In Figure 1b, immunomagnetic sorting of dispersed mouse ovaries or isolated oocytes is shown using antibodies against the NH_2 or COOH terminus of VASA. Fraction 1 contains cells plus beads prior to separation, Fraction 2 is a wash or flow-through fraction (non-immunoreactive) and Fraction 3 is a bead fraction (VASA-positive cells). In Figure 1c, FACS analysis of live or permeabilized cells from dispersed mouse ovaries using antibodies against the NH_2 or COOH terminus of VASA is shown. Viable VASA-positive cells are only detected with the COOH antibody (dashed box) whereas permeabilization enables isolation of VASA-positive cells using the NH_2 antibody (dashed box). In Figure 1d, permeabilization of viable VASA-positive cells (dashed box) obtained with the COOH antibody