

INCREASING TELOMERE LENGTH AND/OR SUPPRESSING TELOMERE SHORTENING

5 The present invention relates to telomeres, the treatment of telomere-associated disorders and/or to fertilisation.

10 Telomeres, the repetitive DNA elements that cap the ends of chromosomes, are crucial protectors of genome stability, and telomere length is a critical determinant of healthy aging and lifespan in mammals. Telomeres shorten with each somatic cell division, and once below a threshold length, cellular senescence is triggered, contributing to tissue dysfunction and pathology. Thus, long telomeres are associated with good health in old age and increased longevity, whereas shorter telomere length is a biomarker of aging and susceptibility to a gamut of aging-associated co-morbidities, particularly cardiovascular disease. There is a need for an effective treatment of telomere-associated disorders.

15 Furthermore, telomere length at birth is a principal determinant of lifetime telomere length—more so than environmental stressors that influence telomere attrition rate throughout the rest of life. Yet even though it is a major risk factor for chronic disease and early death, there is relatively limited understanding of the developmental mechanisms that establish neonatal telomere length.

20 Preimplantation embryo development is an exceptional circumstance where telomeric DNA is extended. Specifically, rapid telomere elongation occurs between fertilization and blastocyst formation. Telomere length achieved with the Inner Cell Mass (ICM), the pluripotent precursors to all foetal tissues, is considered to be the maximum set-point for that future individual, from which telomeres then continuously shorten during foetal development, childhood, and adulthood. Thus, the initial elongation and resetting mechanism that establishes ICM telomere length is essential for genome integrity and determines progenitor cell replicative capacity in offspring.

30 Mechanisms that regulate resetting of ICM telomere length during embryogenesis are unknown and there is a need to understand how maternal physiological factors impact the set-point. Shorter telomeres are observed in children of women with obesity or metabolic syndrome, or born to women of advanced reproductive age. Likewise, children conceived through the use of *in vitro* embryo culture have shorter telomeres than *in vivo* conceived peers.

Shorter telomeres in such children is problematic resulting in an increased risk of telomere-associated disorders, e.g. in later life.

The present invention overcomes one or more of the above-mentioned problems.

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The present inventors have found that a mitochondrial-targeted agent finds utility in the treatment of telomere-associated disorders.

10 The inventors have found that gametes and/or the fertilisation products thereof having an elevated level of mitochondrial reactive oxygen species are associated with deficiencies in telomere elongation during pre-implantation embryogenesis. Surprisingly, by contacting such a gamete and/or fertilisation product thereof with a mitochondrial-targeted agent, telomere length can be increased, thereby reversing (partially or completely) telomere elongation defects.

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Advantageously, the present inventors have shown, for the first time, that a mitochondrial-targeted agent increases telomere length (e.g. by promoting telomere elongation). For example, telomere length may be increased either by contacting the mitochondrial-targeted agent with a gamete (e.g. an oocyte) and/or fertilisation product thereof *in vitro* or by
20 administering the mitochondrial-targeted agent to a mammal before ovulation and/or fertilisation. In particular, telomere length may be increased either by contacting the mitochondrial-targeted agent with an oocyte and/or fertilisation product thereof *in vitro* or by administering the mitochondrial-targeted agent to a female mammal before ovulation and/or fertilisation.

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Thus, the present invention may find particular utility in the treatment of telomere-associated disorders by increasing telomere length during embryonic development of a subject.

30 Additionally or alternatively, the invention may allow for the production of improved embryos, with the capacity to develop into healthier offspring.

The invention may be particularly suitable for use with gametes from mammals with reduced fertility and/or for use in *in vitro* fertilisation where mitochondrial reactive oxygen species have been shown to be elevated in gametes (e.g. in oocytes).

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In one aspect, the invention provides a mitochondrial-targeted agent for use in treating a telomere-associated disorder. In a related aspect, there is provided a method for treating a telomere-associated disorder, comprising the administration of a mitochondrial-targeted agent. In another related aspect, there is provided the use of a mitochondrial-targeted agent in the manufacture of a medicament for treating a telomere-associated disorder.

In one aspect, the invention provides a mitochondrial-targeted agent for use in increasing telomere length. In a related aspect, the invention provides a method for increasing telomere length, comprising the administration of a mitochondrial-targeted agent. In another related aspect, the invention provides the use of a mitochondrial-targeted agent in the manufacture of a medicament for increasing telomere length.

In one aspect, the invention provides a mitochondrial-targeted agent for use in suppressing telomere shortening. In a related aspect, the invention provides a method for suppressing telomere shortening, comprising the administration of a mitochondrial-targeted agent. In another related aspect, the invention provides the use of a mitochondrial-targeted agent in the manufacture of a medicament for suppressing telomere shortening. By increasing telomere length (e.g. of a subject) and/or suppressing telomere shortening, a mitochondrial-targeted agent may promote healthy ageing and/or longevity. Healthy ageing may be ageing that is not associated with disorders caused by short telomeres, such as a telomere-associated disorder described herein. For example, a subject may be administered a mitochondrial-targeted agent (e.g. as a supplement) prophylactically to increase telomere length and/or suppress telomere shortening. Said administration may promote healthy ageing and/or longevity.

In one aspect, the invention provides a mitochondrial-targeted agent for use in increasing telomere length during embryonic development, comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent. In a related aspect, there is provided a method for increasing telomere length during embryonic development, comprising contacting a gamete and/or a fertilisation product thereof with a mitochondrial-targeted agent. In another related aspect, there is provided the use of a mitochondrial-targeted agent in the manufacture of a medicament for increasing telomere length during embryonic development, comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent. The contacting may be *in vivo*. For example, a gamete of a male or female mammal may be contacted with the mitochondrial-targeted agent by administering the mitochondrial-targeted agent to the male or female mammal that is producing, will produce,

and/or has produced the gamete. In a first example, a male or female mammal to which the mitochondrial-targeted agent has been administered may mate with a mammal of the opposite sex, which optionally may also have been administered the mitochondrial-targeted agent. During subsequent *in vivo* embryonic development, telomere length may be increased. In a
5 second example, the gamete may subsequently be used in a method of assisted reproduction. It may be in said method that embryonic development occurs and thus where the increase in telomere length occurs.

Preferably, at least a fertilisation product of a gamete is contacted by administering the
10 mitochondrial-targeted agent to a female subject comprising said fertilisation product.

In one aspect, the invention provides a (preferably *in vitro*) method for increasing telomere length during embryonic development, the method comprising contacting a gamete and/or a fertilisation product thereof with a mitochondrial-targeted agent *in vitro*.
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In one aspect, the invention provides the use of a mitochondrial-targeted agent (preferably *in vitro*) for increasing telomere length during embryonic development, the use comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent *in vitro*.
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In one aspect the invention provides a (preferably *in vitro*) method for producing an embryo (e.g. having increased telomere length), the method comprising contacting a gamete and/or a fertilisation product thereof with a mitochondrial-targeted agent *in vitro*.

25 The gamete and/or fertilisation product thereof contacted with a mitochondrial-targeted agent *in vitro* may be used in a method of assisted reproduction.

For example, a method or use may comprise:

- (a) contacting a gamete with a mitochondrial-targeted agent *in vitro*; and
- 30 (b) fertilising a gamete obtainable from a mammal of the opposite sex with the gamete to produce a fertilisation product; and
- (c) preferably culturing the fertilisation product (e.g. to produce an embryo).

Step (b) and (c) may also be carried out *in vitro*.

35 For example, a method or use may comprise:

- (a) contacting a fertilisation product of a gamete with a mitochondrial-targeted agent *in vitro*; and
- (b) culturing the fertilisation product (e.g. to produce an embryo).

Step (b) may also be carried out *in vitro*.

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Culturing of the fertilisation product may result in the production of an embryo having increased telomere length.

10 In one aspect, the invention provides a (preferably *in vitro*) method for increasing telomere length during embryonic development, the method comprising using a gamete that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, preferably in a method of *in vitro* fertilisation.

15 In one aspect, the invention provides the use of a mitochondrial-targeted agent (preferably *in vitro*) for increasing telomere length during embryonic development, comprising using a gamete that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, preferably in a method of *in vitro* fertilisation.

20 In one aspect the invention provides a (preferably *in vitro*) method for producing an embryo, the method comprising using a gamete that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, preferably in a method of *in vitro* fertilisation.

25 In one aspect, the invention provides a (preferably *in vitro*) method for increasing telomere length during embryonic development, the method comprising using a fertilisation product of a gamete, wherein the fertilisation product has been contacted with a mitochondrial-targeted agent, in a method of assisted reproduction.

30 In one aspect, the invention provides the use of a mitochondrial-targeted agent (preferably *in vitro*) for increasing telomere length during embryonic development, comprising using a fertilisation product of a gamete, wherein the fertilisation product has been contacted with a mitochondrial-targeted agent, in a method of assisted reproduction.

In one aspect the invention provides a (preferably *in vitro*) method for producing an embryo, the method comprising using a fertilisation product of a gamete, wherein the fertilisation

product has been contacted with a mitochondrial-targeted agent, in a method of assisted reproduction.

Preferably, a method or use described herein is an *in vitro* method or use.

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In a method or use described herein comprising contacting a gamete and/or a fertilisation product thereof with a mitochondrial-targeted agent *in vitro* or using a gamete and/or a fertilisation product thereof that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, it is preferred that the embryonic development and increase in telomere length occurs *in vitro*, however, said embryonic development and increase in telomere length may occur subsequently *in vivo*. In some instances, it is preferred that the fertilisation occurs *in vitro*. However, said fertilisation may occur subsequently *in vivo*.

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In one aspect, the invention provides an embryo obtainable by a method described herein.

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The term "obtainable" as used herein also encompasses the term "obtained".

The term "disorder" as used herein also encompasses a "disease".

Mitochondrial reactive oxygen species are representative of mitochondrial superoxide production. A gamete and/or fertilisation product thereof may preferably comprise an elevated level of mitochondrial reactive oxygen species, e.g. before contact with a mitochondrial-targeted agent of the invention. Said gamete and/or fertilisation product thereof may additionally comprise an elevated level of mitochondrial reactive oxygen species during contacting with the mitochondrial-targeted agent, but preferably not after contacting. A gamete and/or fertilisation product thereof comprising an elevated level of mitochondrial reactive oxygen species may have a level of mitochondrial reactive oxygen species that is at least 10%, 20%, 30%, 40%, 50%, 75%, or 100% higher than the level of mitochondrial reactive oxygen species of a reference gamete and/or fertilisation product thereof.

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It is preferred that a gamete comprise an elevated level of mitochondrial reactive oxygen species, e.g. before contact with a mitochondrial-targeted agent of the invention.

A reference gamete may be a gamete obtained from a mammal with normal or increased fertility. In other words, in one embodiment, said mammal is not one suffering from, or

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susceptible to, a condition that is associated with reduced fertility and/or reduced gamete quality. A reference gamete may be a gamete obtained from a mammal having a normal gamete production level and/or rate. A reference gamete may be a gamete obtained from a mammal having normal blood sugar levels and insulin levels and/or that has not been exposed to a high fat diet. A reference gamete may be a gamete obtained from a healthy mammal. A reference gamete may be a gamete obtained from a mammal that has not, and/or does not, smoke. Preferably, a reference gamete may be a gamete obtained from a mammal that is not overweight or obese. Most preferably, a reference gamete may be a gamete obtained from a mammal that: is not overweight or obese; does not have an abnormal gamete production level and/or rate; does not have polycystic ovary syndrome; does not have metabolic syndrome; does not have reduced fertility; does not have sub-fertility; does not have infertility; does not have ovarian dysfunction; does not have anovulation; does not have reduced ovulation rate; does not have pre-diabetes; does not have diabetes; does not have hyperandrogenism; does not have insulin resistance; does not have impaired glucose tolerance; does not have an elevated blood sugar level; does not have hyperinsulinemia; does not have dyslipidaemia; does not have low ovarian reserve; does not have premature ovarian failure; does not have ovarian ageing; does not have low sperm quality (e.g. low sperm motility, viability, and/or capacitation, poor sperm morphology and/or DNA integrity); does not have low sperm count; has not been exposed to a high fat diet; is not aged; has not experienced recurrent miscarriage; and/or has not, and/or does not, smoke.

A reference fertilisation product may be one produced from any of the above-described reference gametes.

Suitable methods for measuring levels of mitochondrial reactive oxygen species are described in the Examples herein, such as MitoSOX Red staining.

A gamete and/or fertilisation product thereof comprising an elevated level of mitochondrial reactive oxygen species may be a gamete and/or fertilisation product thereof that has been exposed to high levels of oxygen, e.g. during an *in vitro* fertilisation method. The term "high levels of oxygen" as used in this context refers to levels of oxygen that an embryo would not normally be exposed to *in vivo*, such as normal air, which comprises ~20% oxygen. In other words, the term "high levels of oxygen" may refer to a non-physiological oxygen level. Thus, a gamete and/or fertilisation product thereof comprising an elevated level of mitochondrial reactive oxygen species may be a gamete and/or fertilisation product thereof that has been

exposed to a non-physiological level of oxygen. By way of explanation, the oviduct is physiologically hypoxic.

5 A gamete and/or fertilisation product thereof comprising an elevated level of mitochondrial reactive oxygen species may be characterised by an altered epigenetic state (e.g. nuclear epigenetic state), e.g. prior to contacting with a mitochondrial-targeted agent according to the invention. The altered epigenetic state may be an abnormal epigenetic state. A gamete and/or fertilisation product thereof comprising an elevated level of mitochondrial reactive oxygen species may be characterised by altered (e.g. abnormal) DNA methylation levels, e.g. prior to
10 contacting or administration with a mitochondrial-targeted agent according to the invention. Said gamete and/or fertilisation product thereof may have an altered 5-methylcytosine (5mC) level and/or an altered 5-hydroxymethylcytosine (5hmC) level. Said gamete and/or fertilisation product thereof may have an increased 5-methylcytosine (5mC) level and/or a decreased 5-hydroxymethylcytosine (5hmC) level. In particular, a zygote may have an increased 5-methylcytosine (5mC) level and/or a decreased 5-hydroxymethylcytosine (5hmC) level prior to
15 contacting or administration with a mitochondrial-targeted agent according to the invention. A difference/alteration (e.g. increase or decrease) may be when compared to a reference gamete and/or reference fertilisation product (as defined herein).

20 A gamete and/or fertilisation product thereof may have a 5-methylcytosine (5mC) level that is at least 10%, 20%, 30%, 40% or 50% higher than the level of 5mC in a reference gamete and/or fertilisation product thereof (as defined herein).

A gamete and/or fertilisation product thereof may have 5-hydroxymethylcytosine (5hmC) level
25 that is at least 10%, 20%, 30%, 40% or 50% lower than the level of 5hmC in a reference gamete and/or fertilisation product thereof (as defined herein).

A gamete and/or fertilisation product thereof for use in the invention may additionally comprise one or more hallmarks of mitochondrial dysfunction. For example, a gamete and/or fertilisation
30 product thereof may have a reduced mitochondrial membrane potential, e.g. prior to (and optionally during) contacting with a mitochondrial-targeted agent according to the invention. A gamete and/or fertilisation product thereof comprising a reduced mitochondrial membrane potential may have a mitochondrial membrane potential that is at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the mitochondrial membrane potential of a reference gamete and/or
35 fertilisation product thereof (as defined herein).

Suitable methods for measuring mitochondrial membrane potential are described in the Examples herein, such as tetramethylrhodamine methyl ester perchlorate (TMRM) staining.

5 A telomere-associated disorder treated in accordance with the invention may be any telomere-associated disorder. A telomere-associated disorder may be a disorder associated with short telomeres (e.g. abnormally short telomeres). A telomere-associated disorder may be ageing (e.g. premature ageing), bone marrow failure, dyskeratosis congenita, acquired aplastic anaemia, pulmonary fibrosis (e.g. idiopathic pulmonary fibrosis), liver disease (e.g. associated
10 with or caused by dyskeratosis congenita and/or pulmonary fibrosis), cardiovascular disease, heart disease, cancer, neurodegeneration, inflammatory bowel disease, Barrett's oesophagus, and/or miscarriage (e.g. recurrent miscarriage).

Preferably, a telomere-associated disorder is ageing. Thus, in a preferred embodiment, a
15 mitochondrial-targeted agent of the invention treats ageing, such as premature ageing. In other words, a mitochondrial-targeted agent may function as an anti-ageing agent.

The term "treat" or "treating" as used herein encompasses prophylactic treatment (e.g. to prevent onset of a telomere-associated disorder) as well as corrective treatment (e.g.
20 treatment of a subject already suffering from a telomere-associated disorder). Preferably "treat" or "treating" as used herein means preventative treatment. The term "treat" or "treating" as used herein may refer to a telomere-associated disorder and/or a symptom thereof.

Treatment of a telomere-associated disorder may encompass treating at least one symptom
25 thereof. Thus, a telomere-associated disorder will preferably be considered treated when at least one symptom thereof is treated. A symptom may be considered treated if the severity of said symptom is reduced. Said reduction may be a reduction of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, preferably a 100% reduction. The severity of a symptom after treatment may be assessed by
30 comparison to the severity of the same symptom before treatment.

A mitochondrial-targeted agent of the invention may be administered in a therapeutically effective amount or a prophylactically effective amount. Preferably a mitochondrial-targeted agent of the invention is administered in a therapeutically effective amount.

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A “therapeutically effective amount” may be any amount of the mitochondrial-targeted agent, which when administered alone or in combination with another agent (preferably alone) is sufficient to effect such treatment. For example, a “therapeutically effective amount” may be any amount of the mitochondrial-targeted agent, which when administered alone or in combination with another agent (preferably alone) is sufficient to increase telomere length (e.g. of a subject and/or during embryonic development, such as subsequent embryonic development) and/or suppress telomere shortening. For example, a “therapeutically effective amount” may be any amount of the mitochondrial-targeted agent, which when administered alone or in combination with another agent (preferably alone) for treating said telomere-associated disorder (or a symptom thereof) is sufficient to effect such treatment of said telomere-associated disorder (or a symptom thereof).

A “prophylactically effective amount” may be any amount of the mitochondrial-targeted agent that, when administered alone or in combination with another agent (preferably alone), inhibits or delays the onset or reoccurrence of a disorder (or a symptom thereof) and/or of telomere shortening. In some embodiments, the prophylactically effective amount prevents the onset or reoccurrence of the disorder and/or of telomere shortening entirely. “Inhibiting” the onset may mean either lessening the likelihood of onset of the disorder (or symptom thereof) and/or of telomere shortening, preventing the magnitude of the peak effect of the disorder (or symptom thereof), and/or of telomere shortening and/or preventing the onset entirely.

A “prophylactically effective amount” may be any amount of the mitochondrial-targeted agent that, when administered alone or in combination with another agent (preferably alone), inhibits or delays the onset or reoccurrence of a telomere-associated disorder (or a symptom thereof). In some embodiments, the prophylactically effective amount prevents the onset or reoccurrence of the telomere-associated disorder entirely. “Inhibiting” the onset may mean either lessening the likelihood of onset of the telomere-associated disorder (or symptom thereof), preventing the magnitude of the peak effect of the telomere-associated disorder (or symptom thereof), and/or preventing the onset entirely.

A mitochondrial-targeted agent may be administered to a subject. A mitochondrial-targeted agent may be administered to a subject after the birth of the subject. In other words, the mitochondrial-targeted agent may be administered to a subject any time after the birth of the subject. For example, a mitochondrial-targeted agent may be administered during childhood and/or adulthood. Thus, a subject for treatment in accordance with the invention may be a child

(e.g. a human less than 18 years old) or an adult (e.g. a human of at least 18 years old). Preferably, the subject for treatment is an adult.

5 A "subject" as used herein is a mammal. A subject may be a non-murine mammal. A subject may be a livestock mammal, a domesticated mammal, such as a pet, or a wildlife species. A subject may be a human, a horse, a monkey, a cow (including a bull), a pig, a dog, a cat, a sheep, a goat, an elephant, a panda, a mouse, a rabbit, a rat, or other mammal. Preferably "subject" means a human subject, a horse, or a cow. Most preferably a subject is a human subject. A "subject" is preferably an adult subject, e.g. a human subject of at least 18 years
10 old. The terms "subject" and "patient" are used synonymously herein. A subject may be a subject having a telomere-associated disorder as defined herein.

A gamete and/or a fertilisation product thereof may be contacted with a mitochondrial-targeted agent, preferably wherein the gamete and/or the fertilisation product thereof (preferably the
15 gamete) comprises an elevated level of mitochondrial reactive oxygen species before contacting with a mitochondrial-targeted agent. Said contacting may be *in vivo* or *in vitro*. In the context of the medical uses and methods of treatment described herein, said contacting is preferably *in vivo*.

20 A gamete and/or a fertilisation product thereof may be contacted with a mitochondrial-targeted agent by administration of the mitochondrial-targeted agent to a mammal. The mammal may be a mammal that is producing, will produce, and/or has produced the gamete and/or that comprises the fertilisation product thereof (e.g. at the time of administration).

25 When administering to a mammal comprising the fertilisation product of a gamete, the mammal is a female mammal. Contacting a gamete (preferably an oocyte) and/or a fertilisation product thereof with a mitochondrial-targeted agent preferably increases telomere length during embryonic development (e.g. of a subject as described herein). By increasing telomere length during embryonic development (e.g. of a subject), a mitochondrial-targeted agent may treat a
30 telomere-associated disorder (e.g. of the subject).

The gamete may be comprised in the testes, ovaries, fallopian tubes, or uterus of the mammal. A mammal comprising a fertilisation product of a gamete encompasses a pregnant female mammal. The fertilisation product may be present in the fallopian tubes or uterus of the female
35 mammal, preferably in the uterus.

A gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent before, during, and/or after fertilisation. For example, a mitochondrial-targeted agent may be administered to a subject or a mammal before, during, and/or after fertilisation.

5 Preferably, before, during, and after fertilisation.

While a "subject" is a mammal, the terms "subject" and "mammal" are sometimes used herein in the alternative. This is because, in some examples, (e.g. increasing telomere length and/or suppressing telomere shortening and/or treating a telomere-associated disorder), the subject

10 may be the individual treated (a subject that develops from an embryo described herein), but the mitochondrial-targeted agent may be administered to a different mammal. The "different mammal" may be a mammal that is producing, will produce, and/or has produced a gamete and/or that comprises a fertilisation product thereof (e.g. at the time of administration) as described herein.

15 A mitochondrial-targeted agent may be administered to provide a blood or plasma concentration in the range of 1 nM to 500 μ M, 10 nM to 500 μ M, 100 nM to 500 μ M, 500 nM to 500 μ M, 1 μ M to 500 μ M, 1 μ M to 100 μ M, 5 μ M to 100 μ M, 10 μ M to 100 μ M, 50 μ M to 100 μ M, 1 μ M to 50 μ M, 5 μ M to 50 μ M, 10 μ M to 50 μ M, 1 μ M to 10 μ M, 5 μ M to 10 μ M, or 1 μ M to 5 μ M. A mitochondrial-targeted agent may be administered to provide a blood or plasma

20 concentration in the range of 0.1 μ M to 30 μ M, 0.5 μ M to 30 μ M, 1 μ M to 30 μ M, 5 μ M to 30 μ M, 10 μ M to 30 μ M, 20 μ M to 30 μ M, 0.1 μ M to 20 μ M, 0.5 μ M to 20 μ M, 1 μ M to 20 μ M, 5 μ M to 20 μ M, 10 μ M to 20 μ M, 0.1 μ M to 10 μ M, 0.5 μ M to 10 μ M, 1 μ M to 10 μ M, 5 μ M to 10 μ M, 0.1 μ M to 5 μ M, 0.5 μ M to 5 μ M, 1 μ M to 5 μ M, or 0.1 μ M to 0.5 μ M.

25 A mitochondrial-targeted agent may be administered to provide a blood or plasma concentration in the range of 1-25 μ M, e.g. 5-15 μ M, e.g. 10 μ M.

Preferably, a mitochondrial-targeted agent may be administered to provide a blood or plasma

30 concentration in the range of 5-100 μ M, such as 20-80 μ M. Said amounts may be particularly suitable for a human. Said amounts may be particularly relevant when the mitochondrial-targeted agent is BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

A mitochondrial-targeted agent may be administered to provide a blood or plasma concentration in the range of 175-19,000 ng/ml, 184.6-18,460 ng/ml, 250-10,000 ng/ml, 300-6,000 ng/ml, 1,000-3,000 ng/ml, or 1,200-2,500 ng/ml. For example, a mitochondrial-targeted agent may be administered to provide a blood or plasma concentration in the range of 1,400-2,200 ng/ml, e.g. 1,800-1,900 ng/ml. Said amounts may be particularly relevant when the mitochondrial-targeted agent is metformin and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

A mitochondrial-targeted agent may be administered in an amount ranging from one of the following selected ranges: 0.1 µg/kg to 1000 mg/kg, 0.5 µg/kg to 1000 mg/kg, 1 µg/kg to 1000 mg/kg, 1 µg/kg to 100 mg/kg; 1 µg/kg to 10 mg/kg; 1 µg/kg to 1 mg/kg; 1 µg/kg to 100 µg/kg; 1 µg/kg to 10 µg/kg; 10 µg/kg to 1000 mg/kg, 10 µg/kg to 100 mg/kg; 10 µg/kg to 10 mg/kg; 10 µg/kg to 1 mg/kg; 10 µg/kg to 100 µg/kg; 10 µg/kg to 1000 mg/kg, 100 µg/kg to 100 mg/kg; 100 µg/kg to 10 mg/kg; 100 µg/kg to 1 mg/kg; 1 mg/kg to 1000 mg/kg, 1 mg/kg to 100 mg/kg; 1 mg/kg to 10 mg/kg; 10 mg/kg to 1000 mg/kg; 10 mg/kg to 100 mg/kg; and 100 mg/kg to 1000 mg/kg body weight. A mitochondrial-targeted agent may be administered in an amount ranging from one of the following selected ranges: 0.1 mg/kg to 10 mg/kg, 0.5 mg to 10 mg/kg, 1 mg/kg to 10 mg/kg, 5 mg/kg to 10 mg/kg. 0.1 mg/kg to 5 mg/kg, 0.5 mg/kg to 5 mg/kg, 1 mg/kg to 5 mg/kg, 0.1 mg/kg to 1 mg/kg or 0.5 mg/kg to 1 mg/kg.

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A mitochondrial-targeted agent may be administered in an amount ranging from 50 mg/kg to 150 mg/kg, such as 100 mg/kg. Said amounts may be particularly relevant when the mitochondrial-targeted agent is BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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A mitochondrial-targeted agent may be administered in an amount of 0.1-100 mg/kg or 1-100 mg/kg, such as 2-50 mg/kg, e.g. 5-25 mg/kg. Said amounts may be particularly relevant when the mitochondrial-targeted agent is MitoQ and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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A mitochondrial-targeted agent may be administered in an amount of 1-600 mg/kg or 50-550 mg/kg, such as 100-500 mg/kg, e.g. 150-450 mg/kg. Said amounts may be particularly relevant when the mitochondrial-targeted agent is metformin and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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A mitochondrial-targeted agent may be administered once a day, twice a day, multiple times a day, or continuously.

5 A suitable administration period may be selected. A mitochondrial-targeted agent may be administered once or for a period of at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks. A mitochondrial-targeted agent may be administered for a continuous period.

10 A mitochondrial-targeted agent may be administered to a female mammal for a period starting before ovulation. For example, a mitochondrial-targeted agent may be administered to a female mammal before, during, and after ovulation. A mitochondrial-targeted agent may be administered to a female mammal for a period starting at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks (preferably at least 5 days, such as 4 days) before ovulation.

15 A mitochondrial-targeted agent may be administered in an amount ranging from one of the following selected ranges: 0.1 µg/kg/day to 1000 mg/kg/day, 0.5 µg/kg/day to 1000 mg/kg/day, 1 µg/kg/day to 1000 mg/kg/day, 1 µg/kg/day to 100 mg/kg/day; 1 µg/kg/day to 10 mg/kg/day; 1 µg/kg/day to 1 mg/kg/day; 1 µg/kg/day to 100 µg/kg/day; 1 µg/kg/day to 10 µg/kg/day; 10 µg/kg/day to 1000 mg/kg/day, 10 µg/kg/day to 100 mg/kg/day; 10 µg/kg/day to 10 mg/kg/day; 10 µg/kg/day to 1 mg/kg/day; 10 µg/kg/day to 100 µg/kg/day; 10 µg/kg/day to 1000 mg/kg/day, 100 µg/kg/day to 100 mg/kg/day; 100 µg/kg/day to 10 mg/kg/day; 100 µg/kg/day to 1 mg/kg/day; 1 mg/kg/day to 1000 mg/kg/day, 1 mg/kg/day to 100 mg/kg/day; 1 mg/kg/day to 10 mg/kg/day; 10 mg/kg/day to 1000 mg/kg/day; 10 mg/kg/day to 100 mg/kg/day; and 100 mg/kg/day to 1000 mg/kg/day body weight. A mitochondrial-targeted agent may be administered in an amount ranging from one of the following selected ranges: 0.1 mg/kg/day to 10 mg/kg/day, 0.5 mg/kg/day to 10 mg/kg/day, 1 mg/kg/day to 10 mg/kg/day, 5 mg/kg/day to 10 mg/kg/day, 0.1 mg/kg/day to 5 mg/kg/day, 0.5 mg/kg/day to 5 mg/kg/day, 1 mg/kg/day to 5 mg/kg/day, 0.1 mg/kg/day to 1 mg/kg/day, or 0.5 mg/kg/day to 1 mg/kg/day.

30 A mitochondrial-targeted agent may be administered in an amount ranging from 50 mg/kg/day to 150 mg/kg/day, such as 100 mg/kg/day. Said amounts may be particularly relevant when the mitochondrial-targeted agent is BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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A mitochondrial-targeted agent may be administered in an amount of 0.1-100 mg/kg/day or 1-100 mg/kg/day, such as 2-50 mg/kg/day, e.g. 5-25 mg/kg/day. Said amounts may be particularly relevant when the mitochondrial-targeted agent is MitoQ and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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A mitochondrial-targeted agent may be administered in an amount of 1-600 mg/kg/day or 50-550 mg/kg/day, such as 100-500 mg/kg/day, e.g. 150-450 mg/kg/day. Said amounts may be particularly relevant when the mitochondrial-targeted agent is metformin and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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Preferably, a mitochondrial-targeted agent may be administered in an amount of 10-1000 ng, 50-500 ng, or 100-300 ng. More preferably, a mitochondrial-targeted agent may be administered in an amount of 150-250 ng (e.g. in an amount of 200 ng). Said amount is preferably administered per day, most preferably orally. Said amounts may be particularly

15 suitable for a human. Said amounts may be particularly relevant when the mitochondrial-targeted agent is BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

15

A mitochondrial-targeted agent may be administered in a suitable form. In this regard, the

20 terms "administering" (and the like) includes administering the agent, or administering a prodrug, or a derivative, that will form an effective amount of the desired mitochondrial-targeted agent *in vivo*. The terms include routes of administration that are systemic (e.g., via injection such as intravenous injection, orally in a tablet, pill, capsule, or other dosage form useful for systemic administration), and topical (e.g., creams, solutions, pastes, ointment, including

25 solutions such as mouthwashes, for topical oral administration).

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A mitochondrial-targeted agent is preferably administered orally. A mitochondrial-targeted agent may be administered intravenously. A mitochondrial-targeted agent may be administered via injection, such as intravenous injection. A mitochondrial-targeted agent may

30 be administered by direct introduction to the lungs. A mitochondrial-targeted agent may be administered by nebulized administration, by aerosolized administration or by being instilled into the lung. A mitochondrial-targeted agent may be administered parenterally. A mitochondrial-targeted agent may be administered by implant. A mitochondrial-targeted agent may be administered subcutaneously (e.g. via subcutaneous injection), intratracheal

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administration, parenteral administration, intraarticularly, rectally, intranasally, intraocularly, vaginally, or transdermally. Methods of administration are known in the art.

"Intravenous administration" is the administration of substances directly into a vein.

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"Oral administration" is a route of administration where a substance is taken through the mouth, and includes buccal, sublabial and sublingual administration, as well as enteral administration. Typical forms for the oral administration of therapeutic agents includes the use of tablets or capsules.

10

A mitochondrial-targeted agent may be administered alone or may be delivered in a mixture with other therapeutic agents and/or agents that, for example, enhance, stabilise or maintain the activity of the agent. An administration vehicle may be used (e.g., pill, tablet, implant, injectable solution, etc.) that comprises both the mitochondrial-targeted agent and an additional agent. A mitochondrial-targeted agent may be administered and additional agent(s).

15

The present invention may also encompass combination therapy. In other words, the present invention may, in some embodiments, comprise co-administration of a mitochondrial-targeted agent and a further agent. In this regard, another drug or treatment modality may be administered in conjunction with the mitochondrial-targeted agent. This combination therapy can be sequential therapy, where the treatment is with a first treatment modality and then the other, or the two or more treatment modalities may be given simultaneously.

20

"Co-administering" or "co-administration" refers to the administration of two or more therapeutic agents together at one time. The two or more therapeutic agents can be co-formulated into a single dosage form or "combined dosage unit" or formulated separately and subsequently combined into a combined dosage unit, typically for intravenous administration or oral administration.

25

When administered, the therapeutically effective dosage of an agent may vary depending upon the particular agent utilized, the mode of administration, the condition, and severity thereof, as well as the various physical factors related to the subject being treated. The dosages are expected to vary with route of administration, and the nature of the agent administered and any other agents administered. In certain embodiments, the mitochondrial-targeted agent may be administered in escalating doses and/or repeated doses.

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A mitochondrial-targeted agent may be administered as a continuous release formulation. A mitochondrial-targeted agent may be administered as an immediate release formulation. The term "immediate release formulation" is a formulation which is designed to quickly release a therapeutic agent in the body over a shortened period of time. Immediate release formulations are known in the art. A mitochondrial-targeted agent may be administered as a sustained release formulation. The term "sustained release formulation" is a formulation which is designed to slowly release a therapeutic agent over an extended period of time. Sustained release formulations are known in the art.

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A mitochondrial-targeted agent may be administered as a pharmaceutically acceptable salt. In this regard, the term "pharmaceutically acceptable salt" refers to acid addition salts or metal complexes which are commonly used in the pharmaceutical industry. Metal complexes include zinc, iron, and the like. Suitable acids for use in the preparation of pharmaceutically acceptable salts may include acetic acid, 2,2-dichloroacetic acid, acylated amino acids, adipic acid, alginic acid, ascorbic acid, L-aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, boric acid, (+)-camphoric acid, camphorsulfonic acid, (+)-(1S)-camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, cinnamic acid, citric acid, cyclamic acid, cyclohexanesulfamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxy-ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, D-gluconic acid, D-glucuronic acid, L-glutamic acid, oxo-glutaric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, hydroiodic acid, (+)-L-lactic acid, (±)-DL-lactic acid, lactobionic acid, lauric acid, maleic acid, (-)-L-malic acid, malonic acid, (±)-DL-mandelic acid, methanesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, nitric acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, perchloric acid, phosphoric acid, L-pyroglutamic acid, saccharic acid, salicylic acid, 4-amino-salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tannic acid, (+)-L-tartaric acid, thiocyanic acid, p-toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, suberic acid, valeric acid and the like.

30

Suitable bases for use in the preparation of pharmaceutically acceptable salts, may include inorganic bases, such as magnesium hydroxide, calcium hydroxide, potassium hydroxide, zinc hydroxide, or sodium hydroxide; and organic bases, such as primary, secondary, tertiary, and quaternary, aliphatic and aromatic amines, including L-arginine, benethamine, benzathine, choline, deanol, diethanolamine, diethylamine, dimethylamine, dipropylamine,

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diisopropylamine, 2-(diethylamino)-ethanol, ethanolamine, ethylamine, ethylenediamine, isopropylamine, N-methyl-glucamine, hydrabamine, 1H-imidazole, L-lysine, morpholine, 4-(2-hydroxyethyl)-morpholine, methylamine, piperidine, piperazine, propylamine, pyrrolidine, 1-(2-hydroxyethyl)-pyrrolidine, pyridine, quinuclidine, quinoline, isoquinoline, secondary amines, triethanolamine, trimethylamine, triethylamine, N-methyl-D-glucamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, tromethamine, and the like.

Most preferably, a mitochondrial-targeted agent may be administered as part of a pharmaceutical composition. A pharmaceutical composition may be particularly suitable for *in vivo* use. A pharmaceutical composition may comprise an effective amount (e.g. a prophylactically or therapeutically effective amount) of a mitochondrial-targeted agent. A pharmaceutical composition may further comprise a pharmaceutically acceptable carrier and/or suitable excipient(s). The mitochondrial-targeted agent may be present in an amount to provide a suitable dose (e.g. a blood or plasma concentration) described herein. A pharmaceutical composition may be suitable for administration by any one or more of the administration routes described herein. Preferably, a pharmaceutical composition is an oral pharmaceutical composition.

A carrier may be chosen based on various considerations including the route of administration, the agent(s) being delivered and the time course of delivery of the agents. The term "pharmaceutically acceptable carrier" refers to a substantially inert solid, semi-solid or liquid filler, diluent, excipient, encapsulating material or formulation auxiliary of any type. An example of a pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known in the art. Some examples of materials which may serve as pharmaceutically acceptable carriers include sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; detergents such as TWEEN 80; buffering agents such as magnesium hydroxide and aluminium hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as colouring agents, releasing

agents, coating agents, sweetening, flavouring and perfuming agents, preservatives and antioxidants can also be present.

Suitable excipients for use in the form of a tablet include a tablet with a tablet core and a film coat as follows: tablet core - maize starch, pregelatinised starch, sodium starch glycollate, povidone, glycerol dibehenate, magnesium stearate; film coat – hypromellose, glycerol triacetate, talc, titanium dioxide (E171), iron oxide yellow (E172), iron oxide red (E172), ethylcellulose. A tablet comprising a mitochondrial-targeted agent may include an amount of the agent as a hydrate and a tablet core with maize starch, pregelatinised starch, sodium starch glycollate, povidone, glycerol dibehenate, and magnesium stearate, and a film coat with hypromellose, glycerol triacetate, talc, titanium dioxide (E171), iron oxide yellow (E172), iron oxide red (E172), and ethylcellulose.

A mitochondrial-targeted agent administered, or present in a pharmaceutical composition, may be a pharmaceutically acceptable hydrate. A hydrate is a solid adduct containing both the parent compound (e.g., the anhydrate of a drug or excipient) and water.

A pharmaceutical composition as described herein may comprise other therapeutic agents and/or agents that enhance, stabilise or maintain the activity of the mitochondrial-targeted agent.

Oral formulations as described herein may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Surface modifying agents may include nonionic and anionic

surface modifying agents. Representative examples of surface modifying agents may include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminium silicate, and triethanolamine. Oral formulations
5 may utilise standard delay or time-release formulations to alter the absorption of the peptides. The oral formulation may also comprise a mitochondrial-targeted agent in water or a fruit juice, containing appropriate solubilizers or emulsifiers as needed. Oral formulations are known in the art and may be formulated by a skilled person.

10 Formulations for the administration of aerosol forms are known in the art and may be formulated by a skilled person.

A composition may also be administered parenterally (such as directly into the joint space) or intraperitoneally. For example, solutions or suspensions of agents in a non-ionised form or as
15 a pharmacologically acceptable salt may be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations typically contain a preservative to prevent the growth of microorganisms. Parenteral formulations are known in the art and may be formulated by a skilled person.

20 Pharmaceutical forms suitable for injectable use may include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier may be a solvent or dispersion medium containing, for example, water, isotonic saline, ethanol, polyol (e.g., glycerol, propylene glycol and liquid
25 polyethylene glycol), suitable mixtures thereof, and vegetable oils. Injectable formulations are known in the art and may be formulated by a skilled person.

Compositions suitable for intravenous administration are known in the art and may be formulated by a skilled person. For example, isotonic saline may be used in an intravenous
30 composition containing an antagonist.

Transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using a mitochondrial-targeted agent as described
35 herein, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches,

suspensions, solutions, and suppositories (rectal and vaginal). Transdermal administration may also be accomplished through the use of a transdermal patch containing the mitochondrial-targeted agent and a carrier that is inert to the active compound, is nontoxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semi-permeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Transdermal formulations are known in art and may be formulated by a skilled person.

A pharmaceutical composition may also be administered by way of a suppository. Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used. Suppository formulations are known in the art and may be formulated by a skilled person.

Additional numerous various excipients, dosage forms, dispersing agents and the like may be suitable for use in connection with administration and/or the formulation of a mitochondrial-targeted agent into medicaments or pharmaceutical compositions. Formulations are known and described in, for example, Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, which is incorporated herein by reference in its entirety.

A gamete may be sperm. Sperm may be obtainable from a male mammal. The term "sperm" as used herein refers to the male reproductive cell or a spermatozoon, and includes one or more spermatozoa.

Where a gamete is sperm, a sperm may be contacted with a mitochondrial-targeted agent by administration of the mitochondrial-targeted agent to a male mammal. The male mammal may be one that is producing, will produce, and/or has produced the sperm at the time of administration.

A gamete is preferably an oocyte. An oocyte may be obtainable from a female mammal. An oocyte may be part of a cumulus oocyte complex. In certain embodiments, the oocyte is a denuded oocyte. Methods for removing cumulus cells from a cumulus oocyte complex are known. The term "oocyte" as used herein includes an oocyte alone or an oocyte in association
5 with one or more other cells, such as an oocyte as part of a cumulus oocyte complex.

Where a gamete is an oocyte, an oocyte may be contacted with a mitochondrial-targeted agent by administration of the mitochondrial-targeted agent to a female mammal.

10 A mammal may be a donor. Thus, a gamete for use in the invention may be obtainable therefrom. A donor may be a sperm donor or an oocyte donor.

The mitochondrial targeted agent may be administered to a mammal and, subsequently, following fertilisation with a gamete obtainable from said mammal (e.g. in a different mammal)
15 telomere length may be increased during embryonic development. For example, a male mammal may be administered the mitochondrial-targeted agent and said male may mate with a female mammal and, subsequently, following fertilisation of an oocyte of said female by sperm of said male, telomere length may be increased during embryonic development. In another example, a male mammal may be administered the mitochondrial-targeted agent,
20 sperm of said male obtained and used in an assisted reproductive method, and telomere length may be increased during embryonic development during said method. For example, a female mammal may be administered the mitochondrial-targeted agent and said female may mate with a male mammal and, subsequently, following fertilisation of an oocyte of said female by sperm of said male, telomere length may be increased during embryonic development. In
25 another example, a female mammal may be administered the mitochondrial-targeted agent, an oocyte of said female obtained and used in an assisted reproductive method, and telomere length may be increased during embryonic development during said method.

A mammal may be a non-murine mammal. A mammal may be a livestock mammal, a
30 domesticated mammal, such as a pet, or a wildlife species. Preferably, the mammal is a female mammal. A mammal may be a human, a horse, a monkey, a cow (including a bull), a pig, a dog, a cat, a sheep, a goat, an elephant, a panda, a mouse, a rabbit, a rat, or other mammal. Preferably a mammal is a human, a horse, or a cow. Most preferably a mammal is a human. The mammal is preferably an adult mammal, e.g. a human of at least 18 years old.

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A gamete and/or fertilisation product thereof may be a non-murine mammalian gamete and/or fertilisation product thereof. A gamete and/or fertilisation product thereof may be a livestock mammalian, a domesticated mammalian (e.g. pet), or a wildlife species gamete and/or fertilisation product thereof. A gamete and/or fertilisation product thereof may be a human,
5 horse, monkey, cow (including a bull), pig, dog, cat, sheep, goat, elephant, panda, mouse, rabbit, or rat gamete and/or fertilisation product thereof. Preferably, the gamete and/or fertilisation product thereof is a human, horse or cow gamete and/or fertilisation product thereof. Most preferably, the gamete and/or fertilisation product thereof is a human gamete and/or fertilisation product thereof.

10

A mammal preferably has reduced fertility. A gamete for use in the invention is preferably obtainable from a mammal having reduced fertility. In other words, said mammal may be suffering from, or susceptible to, a condition that is associated with reduced fertility and/or reduced gamete quality. Said mammal may: be overweight or obese, have an abnormal
15 gamete production level and/or rate; have polycystic ovary syndrome; metabolic syndrome; have reduced fertility; have sub-fertility; have infertility; have ovarian dysfunction; have anovulation; have reduced ovulation rate; have pre-diabetes; have diabetes; have hyperandrogenism; have insulin resistance; have impaired glucose tolerance; have an abnormal blood sugar level (e.g. an elevated blood sugar level); have hyperinsulinemia;
20 dyslipidaemia; have low ovarian reserve; have premature ovarian failure; have ovarian ageing; have low sperm quality (e.g. low sperm motility, viability, and/or capacitation, poor sperm morphology and/or DNA integrity); have low sperm count; be one exposed to a high fat diet; be aged; have not, and/or does not, smoke; and/or may be one that has experienced recurrent miscarriage.

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A mammal may be aged. A gamete for use in the invention may be obtainable from an aged mammal. An aged human mammal may be at least 30, 35, 40 or 45 years old, such as 35-48 years old, e.g. 38-45 years old. An aged human mammal may be greater than 30, 35, 40 or 45 years old. The mammal may be suffering from, or susceptible to, a condition of ageing that is
30 associated with reduced fertility.

A mammal may be a mammal that smokes and/or that has smoked. A gamete for use in the invention may be obtainable from a mammal that smokes and/or that has smoked. The mammal may be suffering from, or susceptible to, a condition state associated with reduced
35 fertility associated with smoking.

A mammal may be overweight. A gamete for use in the invention may be obtainable from a mammal that is overweight. An overweight human mammal may have a body mass index (BMI) of greater than 25 kg/m² up to 30 kg/m².

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A mammal may be obese. A gamete for use in the invention may be obtainable from a mammal that is obese. An obese human mammal may have a body mass index (BMI) of greater than 30 kg/m².

10 In some instances, a gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent at one or more stages of development. For example, a gamete and a subsequently-formed fertilisation product thereof may be contacted with a mitochondrial-targeted agent. Said contact may be continuous or semi-continuous, preferably continuous.

15 A fertilisation product of a gamete may be a zygote or embryo. Preferably, the fertilisation product is a zygote. The term "zygote" is used herein to refer to a fertilised cell comprising both sperm and oocyte DNA prior to the 2-cell cleavage stage. The term "zygote" is thus intended to encompass a fertilised cell before, during, or after syngamy. "Syngamy" is defined as the fusion of the sperm and oocyte pronuclei. The pronuclear stages of development, including
20 PN5, occur prior to syngamy.

A gamete and/or zygote may be contacted with a mitochondrial-targeted agent. It is preferred that such contact is prior to syngamy. More preferably, contact is prior to PN5, more preferably prior to PN5 only. Said contact may therefore comprise contact (e.g. continuous contact) of the
25 mitochondrial-targeted agent with the gamete and subsequently-formed zygote up to PN5. Most preferably, contact takes place between fertilisation and PN5 (e.g. continuously between fertilisation and PN5).

Advantageously, the inventors have found that contact of the mitochondrial-targeted agent
30 (especially BGP-15) prior to syngamy is particularly effective at increasing telomere length and/or treating a telomere-associated disorder.

An "embryo" is used herein to refer to the stage of development starting with the 2-cell cleavage. An embryo may be at any stage including at the 2-cell cleavage stage, 4-cell
35 cleavage stage, 8-cell cleavage stage, morula, and blastocyst, as well as at intervening stages.

An embryo may exhibit normal developmental kinetics or abnormal kinetics, as well as species-specific developmental morphology. An embryo may be a blastocyst stage embryo. An embryo may be a pre-blastocyst stage embryo.

- 5 Preferably, an embryo is a pre-implantation stage embryo. A pre-implantation stage embryo may be an embryo between the 2-cell cleavage stage and the blastocyst stage (e.g. including the 2-cell cleavage stage and the blastocyst stage). *In vivo* in humans, implantation typically takes place approximately 8-10 days following conception.

- 10 A gamete may be contacted with a mitochondrial-targeted agent. Preferably, only the gamete is contacted with the mitochondrial-targeted agent.

- A fertilisation product of a gamete may be contacted with the mitochondrial-targeted agent between the 8-cell stage and up to (preferably before) the blastocyst stage of embryonic development and/or implantation.
- 15

The term "between the 8-cell stage" is intended to include the 8-cell stage.

The term "up to" as used herein encompasses "up to and including".

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A fertilisation product of a gamete may be contacted with the mitochondrial-targeted agent after the 8-cell stage and up to (preferably before) the blastocyst stage of embryonic development and/or implantation.

- 25 A fertilisation product of a gamete may be contacted with the mitochondrial-targeted agent between the morula stage and up to (preferably before) the blastocyst stage of embryonic development and/or implantation.

- A fertilisation product of a gamete may be contacted with the mitochondrial-targeted agent after the morula stage and up to (preferably before) the blastocyst stage of embryonic development and/or implantation.
- 30

- Preferably a fertilisation product of the gamete is contacted with the mitochondrial-targeted agent between the 8-cell stage and before the blastocyst stage of embryonic development only and/or implantation.
- 35

A mitochondrial-targeted agent may be administered to a female mammal before implantation of a fertilisation product of a gamete. A mitochondrial-targeted agent may be administered to a female mammal comprising a fertilisation product between the 8-cell stage and up to (preferably before) implantation. A mitochondrial-targeted agent may be administered to a female mammal comprising a fertilisation product after the 8-cell stage and up to (preferably before) implantation. A mitochondrial-targeted agent may be administered to a female mammal comprising a fertilisation product between the morula stage and up to (preferably before) implantation. A mitochondrial-targeted agent may be administered to a female mammal comprising a fertilisation product after the morula stage and up to (preferably before) implantation.

An embryo described herein preferably has increased telomere length after having carried out the invention. The telomere length may be increased when compared to an embryo obtained via different methodology, e.g. where a gamete and/or fertilisation product thereof has not been contacted with a mitochondrial-targeted agent of the invention. Said embryo may have a telomere length that is at least 1%, 5%, 10%, 20%, 30%, 40%, or 50% longer. Telomere length may be increased in heart and/or liver cells of the embryo.

An embryo described herein preferably develops into a foetus having increased telomere length. The telomere length may be increased when compared to a foetus obtained via different methodology, e.g. where a gamete and/or fertilisation product thereof has not been contacted with a mitochondrial-targeted agent of the invention. Said foetus may have a telomere length that is at least 1%, 5%, 10%, 20%, 30%, 40%, or 50% longer. Telomere length may be increased in heart and/or liver cells of the foetus.

An inner cell mass (ICM) of an embryo described herein may exhibit increased telomere length.

Suitable methods for assaying telomere length (e.g. qPCR) are described in the present Examples (see Figure 4).

Typically, as part of the natural ageing process, telomere length decreases over time (so-called telomere loss). A mitochondrial-targeted agent may suppress said decrease (i.e. may suppress telomere shortening). Any such suppression may be partial or complete. In other words, a mitochondrial-targeted agent may prevent or slow a decrease in telomere length of a subject

or of a cell. After contacting with or administering a mitochondrial-targeted agent, the telomere may be at least 1%, 5%, 10%, 20%, 30%, 40% or 50% longer, e.g. when: (i) compared to the length of the telomere of an equivalent cell that has not been contacted with the mitochondrial-targeted agent; and/or (ii) compared to the length of the telomere of an equivalent subject that has not been administered the mitochondrial-targeted agent.

A mitochondrial-targeted agent may increase telomere length of a subject or of a cell. The increase of telomere length may be achieved by promoting telomere elongation. Preferably, the telomere length may be increased during embryonic development of a subject, e.g. by promoting telomere elongation during embryonic development of the subject. An increase may be an increase of at least 1%, 5%, 10%, 20%, 30%, 40% or 50% in telomere length, e.g. when: (i) compared to the length of the telomere of the subject prior to administration of the mitochondrial-targeted agent; (ii) compared to the length of the telomere of a cell prior to contacting with the mitochondrial-targeted agent; (iii) compared to the length of the telomere of an equivalent cell that has not been contacted with the mitochondrial-targeted agent; and/or (iv) compared to the length of the telomere of an equivalent subject that has not been administered the mitochondrial-targeted agent. Likewise, telomere elongation may be increased by at least 1%, 5%, 10%, 20%, 30%, 40% or 50%, e.g. when: (i) compared to the length of the telomere of the subject prior to administration of the mitochondrial-targeted agent; (ii) compared to the length of the telomere of the cell prior to contacting with the mitochondrial-targeted agent; (iii) compared to the length of the telomere of an equivalent cell that has not been contacted with the mitochondrial-targeted agent; and/or (iv) compared to the length of the telomere of an equivalent subject that has not been administered the mitochondrial-targeted agent.

A "mitochondrial-targeted agent" may be any agent that functionally interacts with one or more mitochondrion of a cell to achieve its therapeutic effect. A mitochondrial-targeted agent may physically interact with one or more mitochondrion of a cell. Preferably, a mitochondrial-targeted agent is capable of reducing the level of mitochondrial reactive oxygen species in a cell, preferably wherein the cell is a cell with an elevated level of mitochondrial reactive oxygen species. Said reduction may be a reduction of at least 5%, 10%, 20%, 30%, 40%, 50%, 75% or 100% in the level of mitochondrial reactive oxygen species of a cell, e.g. when: (i) compared to the level of mitochondrial reactive oxygen species of the cell prior to contact with, or administration of, the mitochondrial-targeted agent; and/or (ii) compared to the level of mitochondrial reactive oxygen species of an equivalent cell that has not been contacted with,

or administered, the mitochondrial-targeted agent. A mitochondrial-targeted agent may increase the mitochondrial membrane potential of a cell (preferably present in an embryo, such as an embryo at morula stage or later, and/or preferably wherein the cell is a cell with a reduced mitochondrial membrane potential) by at least 5%, 10%, 20%, 30%, 40% or 50%, e.g. when:

5 (i) compared to the mitochondrial membrane potential of the cell prior to contact with, or administration of, the mitochondrial-targeted agent; and/or (ii) compared to the mitochondrial membrane potential of an equivalent cell that has not been contacted with, or administered, the mitochondrial-targeted agent. A mitochondrial-targeted agent may alter an epigenetic state (e.g. nuclear epigenetic state) of a cell, such as DNA methylation levels of a gamete and/or

10 fertilisation product thereof, preferably wherein the cell is a cell with altered (e.g. abnormal) epigenetic state. For example, a mitochondrial-targeted agent may alter DNA methylation levels of a zygote, preferably wherein the zygote is a zygote with altered (e.g. abnormal) DNA methylation levels. A mitochondrial-targeted agent may reduce 5-methylcytosine (5mC) level and/or increase 5-hydroxymethylcytosine (5hmC) levels of a gamete and/or fertilisation

15 product thereof. For example, a mitochondrial-targeted agent may reduce 5-methylcytosine (5mC) levels by at least 10%, 20%, 30%, 40% or 50%, e.g. when: (i) compared to the 5-methylcytosine (5mC) levels of a cell prior to contact with, or administration of, the mitochondrial-targeted agent; and/or (ii) compared to the 5-methylcytosine (5mC) levels of an

20 equivalent cell that has not been contacted with, or administered, the mitochondrial-targeted agent. For example, a mitochondrial-targeted agent may increase 5-hydroxymethylcytosine (5hmC) levels by at least 10%, 20%, 30%, 40% or 50%, e.g. when: (i) compared to the 5-hydroxymethylcytosine (5hmC) levels of a cell prior to contact with, or administration of, the mitochondrial-targeted agent; and/or (ii) compared to the 5-hydroxymethylcytosine (5hmC) of an equivalent cell that has not been contacted with, or administered, the mitochondrial-targeted

25 agent.

Thus, in one aspect, the invention provides a mitochondrial-targeted agent for use in altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, preferably comprising contacting the cell with the mitochondrial-targeted agent. In a related aspect, there is provided a method

30 for altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, the method comprising contacting the cell with a mitochondrial-targeted agent (e.g. via administration to a subject and/or mammal as described herein). In another related aspect, there is provided the use of a mitochondrial-targeted agent in the manufacture of a medicament for altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, preferably comprising contacting the cell with the

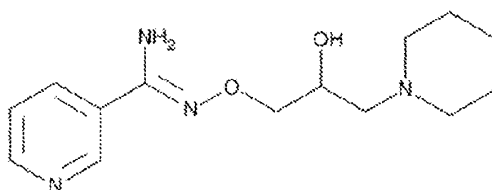
35 mitochondrial-targeted agent. In the foregoing aspects, the contacting is preferably *in vivo*.

Contacting may be achieved by administering the mitochondrial-targeted agent to a subject and/or mammal as described herein. In another aspect, there is provided, a (preferably *in vitro*) method for altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, the method comprising contacting the cell with a mitochondrial-targeted agent *in vitro*. In another aspect, there is provided a (preferably *in vitro*) use of a mitochondrial-targeted agent for altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, comprising contacting the cell with the mitochondrial-targeted agent *in vitro*. The epigenetic state is preferably a DNA methylation level as described above. Such alteration is preferably reversal (including partial or complete reversal) of an abnormal epigenetic state. The cell may be gamete and/or fertilisation product thereof. Preferably the cell is a cell with an altered (e.g. abnormal) epigenetic state. More preferably, a mitochondrial-targeted agent may alter DNA methylation levels of a zygote, preferably wherein the zygote is a zygote with altered (e.g. abnormal) DNA methylation levels.

A mitochondrial-targeted agent may comprise (or consist of) mitoquinone mesylate (MitoQ) and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof. For example, a mitochondrial agent may be MitoQ or an acceptable prodrug, solvate, salt, tautomer, stereoisomer, or racemate thereof. A mitochondrial-targeted agent may be MitoQ. MitoQ (Mitoquinone Mesylate: [10-(4,5-Dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl](triphenyl)phosphonium methanesulfonate is a ubiquinone moiety linked to a lipophilic triphenylphosphonium cation by a 10-carbon alkyl chain that preferentially accumulates in mitochondria (M. P. Murphy, R. A. Smith, Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annu Rev Pharmacol Toxicol* 47, 629-656 (2007)). MitoQ is commercially available from MitoQ Ltd, New Zealand. MitoQ may have CAS number 845959-50-4.

A mitochondrial-targeted agent may comprise (or consist of) metformin and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof. For example, a mitochondrial agent may be metformin or an acceptable prodrug, solvate, salt, tautomer, stereoisomer, or racemate thereof. A mitochondrial-targeted agent may be metformin. Metformin (1,1-Dimethylbiguanide) may be present as a HCl salt (1,1-Dimethylbiguanide hydrochloride). The HCl salt of metformin have CAS number 1115-70-4. Said salt of metformin is commercially available from Sigma-Aldrich (D150959).

Preferably, a mitochondrial-targeted agent comprises (or consists of) BGP-15 (O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime):

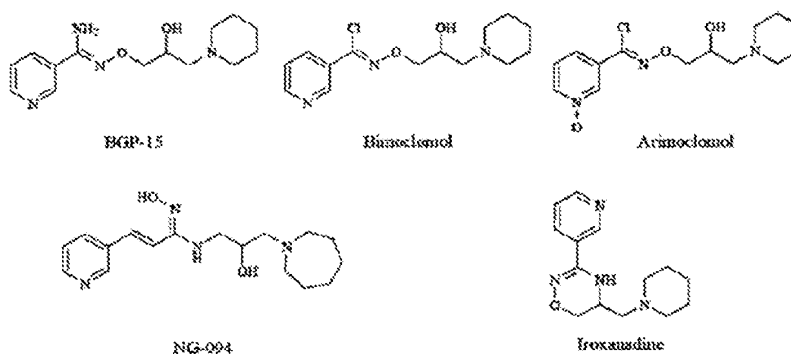


and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or
 5 racemate thereof. For example, a mitochondrial-targeted agent may be BGP-15 or an
 acceptable prodrug, solvate, salt, tautomer, stereoisomer, or racemate thereof. Most
 preferably, the mitochondrial-targeted agent is BGP-15.

BGP-15 is commercially available (typically as a suitable salt) or can be synthesized by a
 10 method known in the art. Examples of commercial sources of BGP-15 include Hangzhou
 Molcore Biopharmatech Co Ltd, Sigma-Aldrich (Product # B4813 SIGMA) and Cayman
 Chemicals (Product # 17503). BGP-15 may have CAS number 66611-37-8.

A derivative of BGP-15 may comprise (or consist of) bimoclomol, arimoclomol, NG-94,
 15 iroxadine, and/or a pharmaceutically acceptable derivative, prodrug, solvate, salt, tautomer,
 stereoisomer, and/or racemate of any of the aforementioned. For example, a mitochondrial
 agent may be bimoclomol, arimoclomol, NG-94, or iroxadine or an acceptable prodrug,
 solvate, salt, tautomer, stereoisomer, or racemate thereof.

20 The chemical structures of bimoclomol, arimoclomol, NG-94, iroxadine are as follows:



The above compounds may be synthesised by a method known in the art or are commercially available.

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Bimocloamol ((3Z)-N-(2-hydroxy-3-piperidin-1-ylpropoxy)pyridine-3-carboximidoyl chloride) may be synthesized by a method known in the art, for example as described in Hungarian Patent No. 207988 (1988). Arimocloamol (3-[chloro({[(2R)-2-hydroxy-3-(piperidin-1-yl)propoxy]imino})methyl]pyridin-1-ium-1-olate) may be synthesized by a method known in the art, for example as described in international patent application WO0179174 or in *Tetrahedron: Asymmetr.* 2012, 23: 1564-1570. NG-094 may be synthesized by a method known in the art. Iroxanaadine may be synthesised by a method known in the art and is commercially available from 360 Reagent.

15 In some instances, more than one different type of mitochondrial-targeted agent may be employed in the invention. For example, the invention may employ the use of at least two, three, or four different types of mitochondrial-targeted agents. The term "different type(s) of mitochondrial-targeted agents" is intended to encompass an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate of a given mitochondrial-targeted agent, as well as a structurally unrelated mitochondrial-targeted agent. In some instances, different types of mitochondrial-targeted agents are structurally unrelated.

A mitochondrial-targeted agent, gamete, and/or fertilisation product thereof may be used in, or be part of, an assisted reproductive method or technology. Likewise, an *in vitro* method or use described herein may be an assisted reproductive method or use. Examples of assisted reproductive technologies include artificial insemination (also known as intra-uterine insemination), *in vitro* fertilization (IVF), gamete intrafallopian transfer (GIFT), placement of oocytes and sperm into the fallopian tube), zygote intrafallopian transfer (ZIFT), tubal embryo transfer (TET), peritoneal oocyte and sperm transfer (POST), intracytoplasmic sperm injection (ICSI), testicular sperm extraction (TESE), and microsurgical epididymal sperm aspiration (MESA). Assisted reproductive technologies are known in the art, for example as described in Textbook of Assisted Reproduction: Laboratory and Clinical Perspectives (2003) Editors Gardner, D. K., Weissman, A., Howies, CM., Shoham, Z. Martin Dunits Ltd, London, UK; and Gordon, I. (2003) Laboratory Production of Cattle Embryos 2nd Edition CABI Publishing, Oxon, UK.

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In some instances, a mitochondrial-targeted agent, gamete, and/or fertilisation product thereof may be used in, or be part of, *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), or intra-uterine insemination (IUI). In some instances, an *in vitro* method or use described
5 herein comprises *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

IVF relates to the fertilization of an oocyte *in vitro*, wherein the oocyte isolated from a mammal is typically incubated in liquid media with sperm to allow fertilization of the oocyte. In some instances, fertilisation of the oocyte by the sperm may occur greater than 24 hours, but usually
10 not later than 60 hours, after the oocyte collection step, such that maturity of the oocyte is at a sufficient stage to maximise the success of subsequent steps in the IVF procedure.

Methods for performing assisted reproduction technologies in both humans and non-human mammals are known in the art.
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By increasing telomere length in accordance with the present invention, the developmental competence of a gamete and/or fertilisation product (e.g. an embryo) may be improved. For example, said improvement may be: an ability of a gamete to produce a zygote that has completed cleavage fertilization within the optimal timeframe ('on-time') for that species; the
20 ability of a two cell embryo to complete blastocyst development within the optimal timeframe ('on-time') for that species; and/or the ability of a blastocyst to initiate hatching.

In some aspects, a gamete and/or fertilisation thereof is contacted with a mitochondrial-targeted agent *in vitro*. In an *in vitro* method or use, a gamete and/or fertilisation thereof is
25 preferably contacted with a mitochondrial-targeted agent *in vitro*.

A gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent in any suitable manner for the mitochondrial-targeted agent to function. For example, contacting may occur in a liquid medium, exposing to a precursor mitochondrial-targeted agent
30 that is altered or metabolised to an active agent, or exposing to a mitochondrial-targeted agent that induces the expression of a further agent.

A gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent before, during, and/or after *in vitro* fertilisation. Preferably, before, during, and after *in vitro* fertilisation.
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A gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent for at least 10 minutes, 30 minutes, 1 hour, 2 hours 3 hours, 10 hours, 15 hours, 24 hours, 2 days, 4 days, 5 days, or 10 days. A gamete and/or fertilisation product thereof may
5 be contacted with a mitochondrial-targeted agent for up to 10 days, 5 days, 4 days, 2 days, 24 hours, 15 hours, 10 hours, 3 hours, 2 hours or 1 hour. A gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent for 1-10 days or 5-8 days.

A gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted
10 agent for 24 hours or less, 18 hours or less, 12 hours or less, 6 hours or less, 5 hours or less, 4 hours of less, 3 hours or less, 2 hours of less, or 1 hour or less. Preferably, a gamete and/or fertilisation product thereof may be contacted with a mitochondrial-targeted agent for 10 minutes to 24 hours, 30 minutes to 24 hours, 1-20 hours, or 2-15 hours or 3-10 hours, preferably 5-7 hours, such as 6 hours.

15 Where the gamete is sperm, said sperm may be present in a seminal fluid, or a diluted form thereof. The sperm may be enriched for a particular characteristic, such as motility, viability, vitality, capacitation, increased or higher DNA integrity, or reduced or lower DNA quality. For example, the sperm may be sorted, for example by density gradient centrifugation, by a swim-
20 up technique, or by flow cytometry. Methods for sorting sperm are known in the art. The sperm may be present in a sperm preparation medium. Sperm preparation media are known in the art and commercially available. In certain embodiments, the sperm is present in a sperm washing medium. Sperm may be contacted with the mitochondrial-targeted agent prior to, during and/or after capacitation. The sperm may be contacted with the mitochondrial-targeted
25 agent prior to, during and/or after maturation.

A gamete and/or fertilisation product thereof may be present in an in vitro fertilization (IVF) medium. IVF media are known in the art and commercially available.

30 A gamete and/or fertilisation product thereof may be present in a freezing or cryopreservation medium. Freezing/cryopreservation medium are known in the art and commercially available.

A gamete and/or fertilisation product thereof may be contacted with the mitochondrial-targeted agent at a concentration in the range of 1 nM to 500 μ M, 10 nM to 500 μ M, 100 nM to 500 μ M,
35 500 nM to 500 μ M, 1 μ M to 500 μ M, 1 μ M to 100 μ M, 2 μ M to 100 μ M, 5 μ M to 100 μ M, 10 μ M

to 100 μM , 20 μM to 100 μM , 50 μM to 100 μM , 1 μM to 50 μM , 2 μM to 100 μM , 5 μM to 50 μM , 10 μM to 50 μM , 20 μM to 50 μM , 1 μM to 20 μM , 2 μM to 20 μM , 5 μM to 20 μM , 5 μM to 10 μM , 1 μM to 5 μM , 2 μM to 5 μM , or 1 μM to 2 μM .

- 5 Preferably, a gamete and/or fertilisation product thereof may be contacted with the mitochondrial-targeted agent at a concentration in the range of 1-25 μM , more preferably 5-15 μM , e.g. 10 μM . Said amounts may be particularly relevant when the mitochondrial-targeted agent is BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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The mitochondrial-targeted agent may be present in a composition comprising the same. A gamete and/or fertilisation product thereof may be contacted with the composition comprising the mitochondrial-targeted agent at a concentration in the range of 1 nM to 500 μM , 10 nM to 500 μM , 100 nM to 500 μM , 500 nM to 500 μM , 1 μM to 500 μM , 1 μM to 100 μM , 2 μM to 100 μM , 5 μM to 100 μM , 10 μM to 100 μM , 20 μM to 100 μM , 50 μM to 100 μM , 1 μM to 50 μM , 2 μM to 100 μM , 5 μM to 50 μM , 10 μM to 50 μM , 20 μM to 50 μM , 1 μM to 20 μM , 2 μM to 20 μM , 5 μM to 20 μM , 5 μM to 10 μM , 1 μM to 5 μM , 2 μM to 5 μM , or 1 μM to 2 μM .

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Preferably, a gamete and/or fertilisation product thereof may be contacted with the composition comprising the mitochondrial-targeted agent at a concentration in the range of 1-25 μM , more preferably 5-15 μM , e.g. 10 μM . Said amounts may be particularly relevant when the mitochondrial-targeted agent is BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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A gamete and/or fertilisation product thereof may be contacted with the composition comprising the mitochondrial-targeted agent at a concentration in the range of 175-19,000 ng/ml, 184.6-18460 ng/ml, 250-10,000 ng/ml, 300-6,000 ng/ml, 1,000-3,000 ng/ml, or 1,200-2,500 ng/ml. For example, a gamete and/or fertilisation product thereof may be contacted with the composition comprising the mitochondrial-targeted agent at a concentration in the range of 1,400-2,200 ng/ml, e.g. 1,800-1,900 ng/ml. Said amounts may be particularly relevant when the mitochondrial-targeted agent is metformin and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof.

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The composition may be a medium. The composition may be a gamete preparation medium.

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Such media are known in the art and are commercially available. For example, a gamete

preparation media may be used for collection of gametes, washing of gametes and/or isolation of gametes. The composition may be an *in vitro* fertilization medium. IVF media are known in the art and commercially available. Such IVF media, typically comprise the following components: calcium chloride; gentamicin sulphate; glucose; human (or animal) albumin solution; magnesium sulphate; potassium chloride; sodium bicarbonate; sodium chloride; sodium phosphate; sodium pyruvate; and synthetic serum replacement. The composition may be a gamete and/or fertilisation product thereof freezing or cryopreservation medium. Again, such media are known in the art and are commercially available. The composition may be a freezing or cryoprotection medium.

10

Preferably, a composition comprising a mitochondrial-targeted agent is a pharmaceutical composition as described herein.

A gamete and/or fertilisation product thereof treated in accordance with the method may be characterised by an improved developmental competence. The term "developmental competence" as used herein may comprise one or more of: (i) the ability and/or likelihood of the gamete to produce an embryo (for example, upon fertilization of an oocyte or by other mechanisms, such as parthenogenic activation); (ii) one or more of the ability, likelihood and rate of an oocyte to progress through blastocyst development upon formation of an embryo; and (iii) the quality of the embryo (for example, as determined by morphological and/or biochemical assessments) achieved upon the production of an embryo from the oocyte. Methods for determining the developmental competence are known in the art.

For example, improved developmental competence may encompass improved progression through blastocyst development, an increased ability to progress through blastocyst development, an increased fertilization rate, an increased likelihood of progression through blastocyst development, an increased rate of forming a two cell embryo, an increased rate of forming a four cell embryo, an increased rate of forming a blastocyst, an increased rate of progressing through blastocyst development, and/or an increased rate of blastocyst hatching.

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Reference herein to a difference/alteration, such as an increase or decrease, is preferably reference to a statistically significant difference/alteration. Statistical significance may be determined by a method disclosed in the Examples.

Embodiments related to the various uses of the invention (e.g. the mitochondrial-targeted agent for use) are intended to be applied equally to alternative methods and/or uses, and *vice versa*.

5 **SEQUENCE HOMOLOGY**

Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, e.g., segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art. Global methods align sequences from the beginning
 10 to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, e.g., CLUSTAL W, see, e.g., Julie D. Thompson et al., CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position- Specific Gap Penalties and Weight Matrix Choice, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative
 15 refinement, see, e.g., Osamu Gotoh, Significant Improvement in Accuracy of Multiple Protein. Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments, 264(4) J. Mol. Biol. 823-838 (1996). Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, e.g., Match-box, see, e.g., Eric Depiereux and Ernest Feytmans, Match-Box: A
 20 Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences, 8(5) CABIOS 501 -509 (1992); Gibbs sampling, see, e.g., C. E. Lawrence et al., Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment, 262(5131) Science 208-214 (1993); Align-M, see, e.g., Ivo Van Walle et al., Align-M - A New Algorithm for Multiple Alignment of Highly Divergent Sequences, 20(9) Bioinformatics:1428-1435 (2004).

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Thus, percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-19, 1992.

30 The "percent sequence identity" between two or more nucleic acid sequences is a function of the number of identical positions shared by the sequences. Thus, % identity may be calculated as the number of identical nucleotides divided by the total number of nucleotides, multiplied by 100. Calculations of % sequence identity may also take into account the number of gaps, and the length of each gap that needs to be introduced to optimize alignment of two or more
 35 sequences. Sequence comparisons and the determination of percent identity between two or

more sequences can be carried out using specific mathematical algorithms, such as BLAST, which will be familiar to a skilled person.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 20 ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide the skilled person with a general dictionary of many of the terms used in this disclosure.

10

This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation.

15

The headings provided herein are not limitations of the various aspects or embodiments of this disclosure.

Other definitions of terms may appear throughout the specification. Before the exemplary embodiments are described in more detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be defined only by the appended claims.

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Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within this disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within this disclosure, subject to any specifically excluded limit in the stated range. Where the stated

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range includes one or both of the limits, ranges excluding either or both of those included limits are also included in this disclosure.

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a mitochondrial-targeted agent” includes a plurality of such candidate agents and reference to “the mitochondrial-targeted agent” includes reference to one or more mitochondrial-targeted agents and equivalents thereof known to those skilled in the art, and so forth.

10

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto.

15 **BRIEF DESCRIPTION OF THE DRAWINGS**

Embodiments of the invention will now be described, by way of example only, with reference to the following Figures and Examples. Many of the Figures submitted herein are better understood in colour. The colour versions of the drawings are part of the application as filed and the right to present colour images of the drawings in later proceedings is hereby reserved.

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Figure 1. Telomere length in male and female embryos generated by IVF (*in vitro* fertilization) or *in vivo* (mating). ICM telomere length in male (n=30) and female (n=15) embryos (A) generated by IVF. Telomere length in IVF-derived compared to *in vivo*-derived 2-cell embryos (B; n=157-176) and ICMs (C; n=13-22). Violin plots represent the population distributions, and horizontal lines are mean $\pm$ SEM. Data was log transformed for statistical analysis using an unpaired t-test.

Figure 2. Telomere elongation during pre-implantation embryogenesis is impaired by oxidative stress. Mouse oocytes underwent in vitro fertilization (IVF) and embryos were collected at specific developmental stages at the times indicated (A). Telomere length (telomere/ Rn18S) was measured by qPCR. Telomere length in individual oocytes/ embryos (B; n=44-77). A whole blastocyst (C; upper) or isolated Inner Cell Mass (ICM) after immunosurgery (C; lower [all green]) to show purity of ICM cells (green, OCT4⁺) without TE cells (red, CDX2⁺). Telomere length in dissected ICM and TE from the same blastocysts (D; n=51). Telomere length in ICM from day 5 (n=16) and day 6 (n=21) blastocysts (E). MII oocytes were fertilized by IVF and

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cultured *in vitro* at either 5% or 20% oxygen (F). Telomere length in individual 8-cell embryos (G), morulae (H), and ICMs (H, I); n=16-47/ group. Zygotes (6h post-IVF) were labelled with mitochondrial superoxide (mtROS) indicator MitoSox Red (plus DNA stain Hoechst-3342 (blue)) and corrected total fluorescence determined (J; n=19/ group). Zygotes (4h post-IVF), 8-cells, morulae and blastocysts were stained with mitochondrial membrane potential (MMP) indicator TMRM (red) (plus Hoechst-3342 (blue)) (K; left). Corrected fluorescence was determined (right; n=4-41/ group). Zygotes (10h post-IVF) were immuno-labelled with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (L; left). Fluorescence intensity was measured and expressed as the ratio in paternal versus maternal pronuclei (L; n=14 5% O₂; n=16 20% O₂). Violin plots show population distribution of telomere length, and horizontal lines are mean $\pm$ SEM (B, D, E, G-I). qPCR data was log transformed for statistical analysis. Statistical tests were: linear mixed-effects model (B); paired t-test (D), unpaired t-test (E, G, I, J, L); two-way ANOVA (H); or one-way ANOVA (K); different letters indicate p<0.05; *p<0.05, ***p=0.0004, ****p<0.0001. Representative images shown, scale bar: 20 μ m (C, J, K, L).

Figure 3. Pronuclei transfer between control and rotenone-exposed zygotes. *In vivo* fertilized zygotes were collected approximately 8 hours post-fertilization, and labelled with MitoSOX Red (MSR) mtROS indicator (and DNA stain Hoechst-3342; blue) (A) and red fluorescence (CTCF) was determined (A; n=25 control and n=18 rotenone). Schematic representation of pronuclear transfer between zygotes and subsequent embryo culture (B). Zygotes (18h post-hCG) were obtained from female mice that had consumed control or rotenone-containing (150ppm) diet for 3 weeks prior to hormone stimulation and mating. Zygotic pronuclei and cytoplasm were recombined to produce 4 embryo types derived either from control or rotenone-exposed zygotes only (pronuclei were transferred between zygotes from the same group) or a combination of pronuclei and cytoplasm derived from control and rotenone-exposed zygotes. Embryo development after pronuclear transfer was assessed at the 2-cell (C) and blastocyst (D) stages, as well as proportion of hatching blastocysts (E).

Figure 4. Telomere length qPCR assay. To enable robust measurement of telomere DNA per cell in individual embryos, a novel qPCR assay was developed. The use of a reference gene enabled normalization of changes in cell number with development. (A) qPCR assay design for quantification of telomere and Rn18S sequences. Individual oocytes or embryos are collected in 1 μ L, and 9 μ L of lysis solution added to give a sample volume of 10 μ L and used for telomere and Rn18S analysis. The sensitivity of the telomere assay was validated using

MII oocytes (n=15) and 2-cell (2C) and blastocyst stage (n=12) embryos generated by IVF; with the expected decrease in Ct values with increasing cell number demonstrated for both Rn18S (B), and telomere (C). Specifically, a doubling in cell number from 1 (MII oocyte) to 2 (2-cell embryo) was associated with a 1 cycle decrease in Ct value for Rn18S, while a blastocyst was associated with a 6 cycle lower Ct value than a 2-cell. Efficiency of primers and linearity of sequence amplification with input DNA concentration was validated (D, E). DNA was extracted from one whole mouse ovary and serially diluted (1:2) to produce concentrations of 6ng/μL, 3ng/μL, 1.5ng/μL, 0.75ng/μL, and 0.375ng/μL which were used to demonstrate efficiency of the telomere primers (D), and Rn18S reference gene primers, with 36B4 as a comparator (E) under identical cycling conditions. To test for linearity, DNA concentration was log10 transformed for data plotting with Cycle Threshold (Ct) values, with r^2 values shown. Each data point is derived from triplicate PCR reactions. Each primer pair showed a high degree of linearity, with correlation coefficients consistently above 0.995 (D, E). Comparison of multi-copy reference gene Rn18S to that of the single-copy gene 36B4, showed the Cycle Threshold (Ct) of detection decreased by a value of 1 with each halving of input DNA concentration for both Rn18S and 36B4 primers, and correlation coefficients were above 0.995 for both primer sets (E), demonstrating the suitability of Rn18S as a reference gene. The telomere: reference gene ratio for 36B4 and Rn18S was calculated and linear correlation analysis performed (F) demonstrating the suitability of both methods for analysis of samples where DNA amount is not limiting (i.e. foetal tissues). Data presented as mean ± SEM.

Figure 5. High oxygen culture impairs foetal development following embryo transfer. A: Telomere length was assessed in ICMs derived from blastocysts that were 'fresh' (i.e. continuous culture from fertilization) or vitrified at the morula stage and then thawed before culture to the blastocyst stage, to confirm that vitrification did not impact embryo telomere elongation (A; n=25 fresh and 42 vitrified embryos at 5% O₂, and 27 fresh and 36 vitrified embryos at 20% O₂). qPCR data was log transformed for statistical analysis using one-way ANOVA; *p<0.02. Shaded areas represent the population distributions, and horizontal lines are mean ± SEM (A). Blastocyst stage embryos were transferred to pseudo-pregnant recipient females (n=4 females for 5% O₂ and n=5 females for 20% O₂ embryos) at day 2.5 of pregnancy and fetuses collected at day 18.5 (B). The number of uterine sites where an embryo had implanted was counted and expressed as a percentage of the embryos transferred to give implantation rate (C). Whether the implantation resulted in a foetus or resorption was noted (D). The proportion of resulting fetuses from total embryos transferred was calculated (E), as

well as the number of foetuses per recipient (F). Embryo transfer and foetal outcomes and foetal tissue length were assessed using unpaired t-test where $*p < 0.025$.

Figure 6. Oocyte rotenone exposure does not affect foetal outcomes following embryo transfer. A: Telomere length was assessed in ICMs derived from blastocysts that were 'fresh' (i.e. continuous culture from fertilization) or vitrified at the morula stage and then thawed before culture to the blastocyst stage, to confirm that vitrification did not impact embryo telomere elongation (A; n=31 fresh and n=28 vitrified control embryos, and n=34 fresh and n=27 vitrified rotenone-exposed embryos). ICM qPCR data was log transformed for statistical analysis using one-way ANOVA; $*p < 0.03$. Shaded areas represent the population distributions, and horizontal lines are mean $\pm$ SEM (A). Blastocysts were transferred to pseudo-pregnant recipient females (n=9 females for each embryo type) at day 2.5 of pregnancy and foetuses were collected at day 18.5 for analysis (B). The number of uterine sites where an embryo had implanted was counted and expressed as a percentage of the embryos transferred to give implantation rate (C). Whether the implantation resulted in a foetus or resorption was noted (D). The proportion of resulting foetuses from total embryos transferred was calculated (E), as well as the number of foetuses per recipient (F). Foetal characteristics (n=31 from control and n=30 from rotenone-exposed embryos) including weight (G) and foetal sex (H) were analysed. Telomere length in foetal hearts (see Figure 9G) and foetal livers (I) were analysed via qPCR, and telomere length between the two tissue types compared (J), as well as between male ('M') and female ('F') embryos (K). Embryo transfer and foetal outcomes (C-H), foetal liver telomere length (I), and tissue telomere length differences between male and female embryos in the same group (K) were assessed using unpaired t-test. Comparison of foetal tissue types analysed using paired t-test between tissues of the same foetus; $****p < 0.0001$ (J). Violin plots represent the population distributions, and horizontal lines are mean $\pm$ SEM (A, I-K).

Figure 7. Rotenone exposure prior to natural conception reduces foetal heart telomere length. A cohort of female mice that had consumed either control or rotenone diet for three weeks were paired (1:1) with a male, and upon presence of a vaginal copulatory plug, were transferred to control diet to limit gestational rotenone exposure. Foetuses were collected on day 18.5 of pregnancy for analysis (A; n=33 control and n=40 rotenone foetuses). Foetal number per female (B), foetal weight (C) and foetal sex (D) were determined. Telomere length in foetal hearts (E) and foetal livers (F) were analysed via qPCR, and telomere length between the two tissue types compared (G), as well as between male and female foetuses (H). Foetal outcomes (B-D), foetal heart and liver telomere length (E, F), and tissue telomere length

differences between male and female embryos in the same group (H) were assessed using unpaired t-test; * $p < 0.02$. Comparison of foetal tissue types analysed using paired t-test between tissues of the same foetus; **** $p < 0.0001$ (G). Violin plots represent the population distributions, and horizontal lines are mean $\pm$ SEM (E-H). These data showing normal
 5 implantation rates yet a specific reduction in foetal heart telomere length are consistent with the results of experiments where embryos from oocytes of rotenone-fed mice were conceived by IVF and transferred to surrogates for pregnancy (see Figure 9G and Figure 6).

Figure 8. High oxygen culture alters zygotic epigenetic reprogramming. Zygotes were
 10 collected 10 hours after fertilization and immune-labelled with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies. Representative images are shown (A). Pronuclei size was measured (B) and fluorescent signal intensity levels were quantified for 5mC (C) and 5hmC (D) in maternal and paternal pronuclei ($n=14$ 5% O_2 and 16
 20% O_2 zygotes). Data presented as signal intensity for maternal (Mat.) and paternal (Pat.)
 15 pronuclei on the left axis, and the ratio of the fluorescence signal in paternal versus maternal pronuclei of the same zygote on the right axis. Data analysed using paired t-test for comparisons between maternal and paternal pronuclei of the same oocytes, and unpaired t-test for pronuclei comparisons and ratio comparisons between groups. For B, $p < 0.0001$; for C,D * $p < 0.05$, ** $p < 0.002$.

Figure 9. Oocyte mitochondrial dysfunction reduces embryo and foetal telomere length, via nuclear modifications, that is reversible with BGP-15. Mice were exposed to rotenone (150ppm in chow) for three weeks before ovulation, IVF and assessments of embryos and fetuses (A). Zygotes were labelled with MitoSox Red (and DNA stain Hoechst-3342) and fluorescence
 25 measured (B; $n=18$ control and $n=16$ rotenone). Zygotes were co-stained with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (C). Fluorescent signal intensity was quantified for 5mC (left) and 5hmC (right) in maternal and paternal pronuclei ($n=15$ control; $n=12$ rotenone) and presented as the ratio in paternal versus maternal pronuclei. Zygotes and 8-cell embryos were labelled with MMP indicator TMRM (and
 30 Hoechst-3342) and red fluorescence measured (D; $n=8-22$ / group). Telomere length in individual MII oocytes, 8-cells, blastocysts (E; $n=45-86$ /group), and ICMs (F; $n=38-42$). IVF-conceived blastocysts from rotenone-treated (or control) mice were transferred to surrogate females for gestation, and foetal tissues collected at day 18.5 of pregnancy for qPCR telomere length analysis of heart (G; $n=36$ control, $n=35$ rotenone fetuses). Pronuclei were transferred

between zygotes derived from control or rotenone-exposed females in each possible combination, and reconstructed embryos cultured to blastocyst (H). ICMs from blastocysts were analysed for telomere length by qPCR (I). MitoSox Red in zygotes (J, n=15-20) and telomere length in isolated ICMs (K, n=31-44) from embryos derived from mice fed rotenone and treated with BGP-15 (100mg/kg by i.p. injection, or saline vehicle) for 4 days prior to gonadotropin-stimulated ovulation. Violin plots show population distribution, and horizontal lines are mean $\pm$ SEM (E-G, I, K). qPCR data was log transformed for statistical analysis. Statistical tests were: unpaired t-test (B, C, F, G), or one-way ANOVA (D, E, I, J, K); different letters indicate $p < 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Representative images shown, scale bar: 20 μ m (B, C, D, J).

Figure 10. Maternal rotenone exposure alters zygotic epigenetic reprogramming. Zygotes were collected 10 hours after fertilization and immuno-labelled with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (A). Pronuclei size was measured (B) and fluorescent signal intensity levels were quantified for 5mC (C) and 5hmC (D) in maternal and paternal pronuclei (n=12-15 zygotes per group). Data presented as signal intensity for maternal (Mat.) and paternal (Pat.) pronuclei on the left axis, and the ratio of the fluorescence signal in paternal versus maternal pronuclei of the same zygote on the right axis. Data analysed using paired t-test for comparisons between maternal and paternal pronuclei of the same oocyte, and unpaired t-test for pronuclei comparisons and ratio comparisons between groups. For B, $p < 0.02$; for C,D * $p < 0.05$, ** $p = 0.0038$, *** $p = 0.0003$, **** $p < 0.0001$.

Figure 11. Advanced maternal age impairs embryo telomere elongation that is restored by preconception treatment with mitochondria-acting therapeutics. Female mice that were reproductively aged (12 months old) and young (3-4 months) controls were treated with BGP-15 (100mg/kg) or saline vehicle before ovulation, IVF and embryo assessments (A). Zygotes were labelled with MitoSox Red (and Hoechst-3342 (blue)); and red fluorescence measured (B; n=10-21). Zygotes were co-stained with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (C). Fluorescent signal intensity was quantified for 5mC (left) and 5hmC (right) in maternal and paternal pronuclei (n=12-20) and presented as the ratio in paternal versus maternal pronuclei. Morulae were stained with TMRM (red) and Hoechst-3342 (blue) and red fluorescence measured (D; n=8-33). Telomere length per cell in individual MII oocytes of young vs 12 month old mice (E); as well as oocytes from 12 month old mice that were nulliparous vs multiparous (F). Telomere length per cell in

individual 8-cell embryos (G), whole blastocysts (H) and isolated ICM (I). $n \geq 10$ oocytes/embryos per group, $n=12-19$ ICMs. Metformin (2mg/mL) or MitoQ (150 μ M) was administered in drinking water to reproductively aged females for two weeks prior to gonadotropin stimulation and IVF of ovulated oocytes. Telomere length in individual ICMs (J; young $n=47$, aged $\pm$ treatment $n=16-25$). Violin plots show population distribution, and horizontal lines are mean $\pm$ SEM. qPCR data was log transformed for statistical analysis. Statistical tests were: one-way ANOVA (B-D, G-J) or unpaired t-test (E, F); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Representative images shown, scale bar: 20 μ m (B, C, D).

Figure 12. Advanced maternal age alters zygotic epigenetic reprogramming that is modified by BGP-15 treatment. Zygotes from young, aged, and aged females treated with BGP-15 (Aged+B) were collected 10 hours after in vitro fertilization and immuno-labelled with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (A). Pronuclei size was measured (B) and fluorescent signal intensity levels were quantified for 5mC (C) and 5hmC (D) in maternal and paternal pronuclei ($n=12-20$ zygotes per group). Data presented as signal intensity for maternal (Mat.) and paternal (Pat.) pronuclei on the left axis, and the ratio of the fluorescence signal in paternal versus maternal pronuclei of the same zygote on the right axis. Data analysed using paired t-test for comparisons between maternal and paternal pronuclei of the same zygote, and one-way ANOVA for pronuclei comparisons and ratio comparisons between groups, * $p < 0.03$, ** $p < 0.005$, *** $p = 0.0001$, **** $p < 0.0001$.

Figure 13. Maternal obesity impairs embryo and foetal telomere elongation that is restored by treatment with mitochondria-acting therapeutics at fertilization. Obese female mice ($>36g$) and lean littermate controls were treated with BGP-15 (100mg/kg) or saline vehicle prior to ovulation, IVF and analysis of embryos and foetal tissues (A). Zygotes were labelled with MitoSox Red and Hoechst-3342 (blue) and red fluorescence measured (B; $n=10-17$). Zygotes were co-stained with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (C). Fluorescent signal intensity was quantified ($n=17-33$) and presented as the ratio in paternal versus maternal pronuclei (C). Oocytes, 8-cell embryos and morulae were stained with TMRM (red) and Hoechst-3342 (blue) (D). Red fluorescence was determined for oocytes (E; $n=16-32$), 8-cells (F; $n=14-28$), and morulae (G; $n=7-17$). Telomere length per cell was assessed by qPCR in individual MII oocytes (H), 8-cell embryos (I), whole blastocysts (J) and ICM (K). $n \geq 16$ oocytes/embryos, $n=17-31$ ICMs. Metformin (2mg/mL) or MitoQ (150 μ M) was administered in drinking water to obese females for two weeks prior to gonadotropin stimulation and IVF of ovulated oocytes. Telomere length in individual ICMs (L;

n=37-57). Blastocysts from oocytes of lean, obese, or obese BGP-15-treated (100mg/kg) mice were transferred to surrogate females for gestation and foetal tissues collected at day 14.5 of pregnancy for qPCR telomere length analysis of liver and heart (M; n=6-17). Ovulated oocytes from lean or obese mice were cultured in media containing 10µM BGP-15 (or vehicle) from fertilization (N-P). MitoSox Red in zygotes (N; n=15-23), MMP in morulae (O; n=17-18), and telomere length in ICM (P; n=24-43). Violin plots show population distribution, and horizontal lines are mean ± SEM (H-M, P). qPCR data was log transformed for statistical analysis. Data analysed using one-way ANOVA; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 14. Maternal obesity results in alterations to zygotic epigenetic reprogramming that are reversed by BGP-15 treatment. Zygotes from lean, obese (Ob) and obese females treated with BGP-15 (Ob+B) were collected 10 hours after *in vitro* fertilization and immuno-labelled with anti-5-methylcytosine (5mC; red) and anti-5-hydroxymethylcytosine (5hmC; green) antibodies (A). Pronuclei size was measured (B) and fluorescent signal intensity levels were quantified for 5mC (C) and 5hmC (D) in maternal and paternal pronuclei (n=17-33 zygotes per group). Data presented as signal intensity for maternal (Mat.) and paternal (Pat.) pronuclei on the left axis, and the ratio of the fluorescence signal in paternal versus maternal pronuclei of the same zygote on the right axis. Data analysed using paired t-test for comparisons between maternal and paternal pronuclei of the same zygote, and one-way ANOVA for pronuclei comparisons and ratio comparisons between groups, *p<0.034, **p<0.008, ***p<0.0002, ****p<0.0001.

Figure 15. Exposure to BGP-15 during fertilization and embryo culture attenuates superoxide production in a high oxygen environment. Oocytes underwent fertilization at 5% O₂ or 20% O₂ in media containing 10µM BGP-15 (+) or media with the equivalent volume of vehicle (-). At 6 hours post-fertilization, zygotes were labelled with MitoSox Red (MSR) superoxide (mtROS) indicator, (and DNA stain Hoechst-3342; blue); and red fluorescence (CTCF) was determined (n=19-20). Data analysed using one-way ANOVA **** p<0.0001.

Figure 16. Exposure to BGP-15 during fertilization and embryo culture attenuates superoxide production in zygotes derived from rotenone-exposed mice. Oocytes derived from control or rotenone-exposed females underwent *in vitro* fertilization in media containing 10µM BGP-15 (+) or media with the equivalent volume of vehicle (-). At 6 hours post-fertilization, zygotes were labelled with MitoSox Red (MSR) superoxide (mtROS) indicator (and DNA stain Hoechst-3342; blue) and red fluorescence (CTCF) was determined (n=15-18). Data analysed using one-way ANOVA ****p<0.0001.

SEQUENCE LISTING

- 5 SEQ ID NO: 1 (Telomere Forward Primer)
 CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT
- SEQ ID NO: 2 (Telomere Reverse Primer)
 GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT
- 10 SEQ ID NO: 3 (Rn18S Forward Primer)
 AGA AAC GGC TAC CAC ATC CAA
- SEQ ID NO: 4 (Rn18S Reverse Primer)
 CCT GTA TTG TTA TTT TTC GTC ACT ACC T
- 15 SEQ ID NO: 5 (SRY Forward Primer)
 AAG CGC CCC ATG AAT GCA TT
- SEQ ID NO: 6 (SRY Reverse Primer)
 TCC CAG CTG CTT GCT GAT CT
- 20 SEQ ID NO: 7 (36B4 Forward Primer)
 ACT GGT CTA GGA CCC GAG AAG
- 25 SEQ ID NO: 8 (36B4 Reverse Primer)
 TCA ATG GTG CCT CTG GAG ATT

EXAMPLES**MATERIALS AND METHODS****Animals, hormone treatment, and drug administration**

All animal experiments were approved by the University of Adelaide's Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Female CBA.F1 (CBA/CaH x C57/BL6Arc) mice at 6-7 weeks of age, and males at 6-8 weeks of age were obtained from the University of Adelaide's Laboratory Animal Services. Mice were maintained in 12h/12h light/dark conditions and given water and 10% fat rodent chow *ad libitum*. For all drug treatments or dietary manipulations, mice were randomly assigned to experimental groups.

Obese or reproductively aged female mice, as well as lean young controls were generated from the same colony (C57BL/6JSfdAnu-Alms1bbb/Apb mouse strain maintained as heterozygous breeding pairs), termed "Blobby" mice, and fed an identical standard chow diet as in (T. Umehara *et al.*, Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. *Science advances* 8, eabn4564 (2022), L. L. Wu *et al.*, Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development* (Cambridge, England) 142, 681-691 (2015)). Obese mice were homozygous (bbb/bbb) for the 'Blobby' mutation of the Alms1 gene which results in hyperphagia and profound obesity even when maintained on a standard mouse chow diet (L. L. Wu *et al.*, Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development* (Cambridge, England) 142, 681-691 (2015)). Females were deemed obese when they weighed at least 36g, which occurred at 4-5 months of age, and wild-type littermates were used in parallel as lean controls. Reproductively aged females were wild-type or heterozygous (+/+ or bbb/+) at 12 months of age and young females (3-4 months old) were used in parallel as young controls.

Rotenone (#R8875) was sourced from Sigma and rotenone diet was prepared and used as in (O. A. Lozoya *et al.*, Single Nucleotide Resolution Analysis Reveals Pervasive, Long-Lasting DNA Methylation Changes by Developmental Exposure to a Mitochondrial Toxicant. *Cell reports* 32, 108131 (2020)). Specifically, control diet was a modified diet of AIN-93G (TD.97184), and Rotenone Diet was formulated at 150ppm rotenone in TD.97184 meal diet by Teklad/Envigo exactly as for (O. A. Lozoya *et al.*, Single Nucleotide Resolution Analysis Reveals Pervasive, Long-Lasting DNA Methylation Changes by Developmental Exposure to a

Mitochondrial Toxicant. Cell reports 32, 108131 (2020)). CBA.F1 mice were randomly assigned to control or rotenone diet for three weeks as per previous experiments (T. Umehara *et al.*, Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. Science advances 8, eabn4564 (2022)). This dose of rotenone is demonstrated to decrease Complex I- and Complex II-driven respiration without altering oxidative phosphorylation subunit abundance or causing overt physiological effects (O. A. Lozoya *et al.*, Single Nucleotide Resolution Analysis Reveals Pervasive, Long-Lasting DNA Methylation Changes by Developmental Exposure to a Mitochondrial Toxicant. Cell reports 32, 108131 (2020)).

BGP-15 ([O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime]), CAS 66611-37-8; Hangzhou Molcore Biopharmatech Co Ltd) was injected intra-peritoneally (ip) at 100mg/kg of bodyweight in saline for 4 days, starting the day before PMSG injection (as in (T. Umehara *et al.*, Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. Science advances 8, eabn4564 (2022)), L. L. Wu *et al.*, Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. Development (Cambridge, England) 142, 681-691 (2015)). Controls were injected with 0.9% saline vehicle at the equivalent volume for body weight.

Metformin (1,1-Dimethylbiguanide hydrochloride, D150959, Sigma-Aldrich) was provided to mice at 2mg/mL in drinking water *ad libitum* for 2 weeks, before PMSG and hCG treatment (as in (T. Umehara *et al.*, Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. Science advances 8, eabn4564 (2022)).

MitoQ (Mitoquinone Mesylate: [10-(4,5-Dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl](triphenyl)phosphonium methanesulfonate) is a ubiquinone moiety linked to a lipophilic triphenylphosphonium cation by a 10-carbon alkyl chain that preferentially accumulates in mitochondria (M. P. Murphy, R. A. Smith, Targeting antioxidants to mitochondria by conjugation to lipophilic cations. Annu Rev Pharmacol Toxicol 47, 629-656 (2007)). MitoQ (generously donated by MitoQ Ltd, New Zealand) was delivered at 150µM in drinking water *ad libitum* for 2 weeks, before PMSG and hCG treatment (as in (T. Umehara *et al.*, Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. Science advances 8, eabn4564 (2022)).

Female mice were given pregnant mare serum gonadotropin (PMSG, #493-10, Lee BioSolutions) at 5IU/12g body weight, followed by an equivalent dose of human chorionic gonadotropin (hCG, Pregnyl) 47.5h later, each via intraperitoneal (ip) injection.

5 **Derivation of oocytes, early embryos, and blastocysts**

For Meiosis II (MII) oocyte collection, mice were culled via cervical dislocation 15h post-hCG administration, and ovaries and oviducts collected and placed in pre-warmed (37°C) α MEM-HEPES handling media, supplemented with 1% FCS. Ovulated cumulus oocyte complexes (COCs) were isolated by opening the oviducts using a 30G needle. Cumulus cells were completely removed from MII oocytes via treatment with hyaluronidase (Seikagaku, #100741) for 5-10 minutes at 37°C, followed by aspiration using a glass pipette pulled to an appropriate diameter.

To generate embryos via *in vitro* fertilization (IVF), ovulated COC clusters were gently washed twice in pre-warmed (37°C) fertilization media (Vibro Fertilization; Cook Australia, Brisbane, Australia), before being placed in a 100 μ L fertilization drop containing the equivalent of 10 μ L of capacitated sperm from a male of proven fertility (referred to as 'fertilization time'), before being returned to the incubator (37°C, 5% O₂, 6% CO₂) for 4h. Fertilized oocytes were then cleaned of all excess sperm and cumulus cells via gentle aspiration and transferred to a culture dish containing cleave media (Vibro Cleave; Cook Australia, Brisbane, Australia, 10 embryos per 20 μ L cleave media drop) and returned to the incubator until 24h post-fertilization time. At this time, the number of embryos that successfully reached the 2-cell stage were scored. For *in vitro* BGP-15 treatment of oocytes and embryos, BGP-15 stock at 0.5M in sterile H₂O was diluted to 10 μ M in the respective media (fertilization media or cleave media). For controls, the equivalent volume of sterile H₂O was added to the media. For high oxygen (20% O₂) experiments, the fertilization incubation and subsequent culture in cleave media were conducted at 37°C, 20% O₂, 6% CO₂.

The number of ovulated oocytes per mouse and embryo on-time development was monitored in every experiment to ensure collection of embryos at the precise stage. 4-cell embryos were collected at 39h post-fertilization, 8-cell at 55h, morula at 77h, and blastocysts at 96h postfertilization time. Morulae collected for analysis were all of a similar cell number, and blastocysts were all at a similar developmental stage (late-expanded or early hatching) and morphologically normal.

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For *in vivo* embryos, female mice were housed (1:1) with male mice of proven fertility after hCG administration and then separated after 16 hours. To collect 2-cell embryos, females were culled via cervical dislocation 24h later (40h post-hCG) and 2-cell embryos dissected from the oviduct. To collect blastocysts, females were culled 82h post-hCG and embryos flushed from the uterus, using a 1cc syringe with pre-warmed α MEM-HEPES + 1% FCS handling media. Telomere length in embryos derived via IVF were directly compared to those conceived by *in vivo* fertilization (i.e. mating) and found to be not different (Figure 1B, C).

Isolation of blastocyst cell populations

- Collection of paired Inner Cell Mass (ICM) and Trophectoderm (TE) cell populations (Figure 2D) was by manual dissection. The zona pellucida was removed using Acid Tyrode's Embryomax solution (Millipore, #MR-004D) and blastocysts transferred to individual 5 μ L pre-warmed (37°C) drops of α MEM handling media with 1% FCS in a 50 x 9mm petri dish (Falcon, #351006). Blastocysts were manually separated into their two cell populations using the Eppendorf TransferMan 4r system, and a standard holding pipette with a 15 μ M inner diameter and 120 μ M outer diameter (The Pipette Company, #LHC-ID15) and biopsy pipette with a 30 μ M tip (OD) and 90° bevel (The Pipette Company, #LBC-OD30-BA90). Cell populations were separated and frozen individually per blastocyst, but paired for analysis.
- Immunosurgery was used to isolate a purified ICM population (see Figure 2C). Blastocysts had zona pellucidae removed via treatment with Acidic Tyrode's Embryomax solution, washed twice in cleave media and transferred to cleave media containing 20% heat-inactivated rabbit antimouse serum (Sigma, #M5774). Following a 1h incubation (37°C, 5% O₂, 6% CO₂), blastocysts were washed three times in cleave media and transferred to cleave media containing 20% guinea pig serum (Sigma, #G9774) and incubated for 15 minutes. Blastocysts were washed three times in cleave media and incubated for a further 30 minutes in this media. Lysed TE cells were removed by gently aspirating ICMs in a finely drawn glass pipette.

Zygotic pronuclear transfer

- Female mice that consumed control or rotenone-containing diet for three weeks were given PMSG and hCG (as above) and housed (1:1) with an 8-week old CBA.F1 male to allow mating. 18 hours post-hCG (approximately 6h post-fertilization) females were humanely killed and presumptive zygotes collected, fully denuded of cumulus cells, transferred to cleave media and placed in the incubator for 1.5-2h (approximately 8h post-fertilization). After this, zygotes were incubated in cleave media containing 1 μ g/mL Cytochalasin D (Sigma, #C2618) and 0.3 μ g/mL

Nocodazole (Sigma, #SML1665) for 20 minutes. Zygotes were then transferred to α MEM-HEPES handling media containing 60 μ g/mL BSA, 1mg/mL PVP, and Cytochalasin D and Nocodazole at the above concentrations. Both pronuclei were removed from a zygote by micropipette (Eppendorf, #5195000079) followed by a small volume of inactivated Sendai
5 Virus (Cosmo Bio, USA; ~3000 hemagglutinating units/ml) being drawn into the pipette. The virus and pronuclei were injected into the peri-vitelline space of a second enucleated one-cell embryo (see Figure 3B). Reconstructed embryos were washed in cleave media to remove inhibitors, and cultured in cleave media as above to the blastocyst stage. Blastocyst development rates were similar to non-reconstructed embryos and not different between
10 groups (Figure 3C-E).

Telomere and Rn18S qPCR using DNA from individual oocytes and embryos

Individual oocytes or embryos for qPCR analysis were washed three times in 1x phosphate buffered saline (PBS) containing 1mg/mL polyvinylpyrrolidone (PVP, Sigma), and transferred
15 in 1 μ L to a 0.5mL PCR tube (Axygen, #PCR-05-C), snap frozen in liquid nitrogen (LN₂), and stored at -80°C until use. DNA was extracted by adding 9 μ L of lysis buffer (50mM Tris-HCl pH 8.0, 1mM EDTA, 200 μ g/mL Proteinase K, 0.5% Tween-20) to each sample (final volume of 10 μ L) and heating to 55°C for 2h, followed by 95°C for 10 minutes. Samples were cooled to 4°C and briefly centrifuged. ICM samples were further diluted with an additional 10 μ L of sterile
20 H₂O to give a total volume of 20 μ L.

To generate 'calibrator' DNA for use as a standard within every assay, a whole ovary from a 6 week old PMSG- and hCG-stimulated CBA female mouse was collected and total DNA extracted using the QIAmp DNA Micro Kit (Qiagen, #56304) 'Isolation of Genomic DNA from
25 Small Volumes of Blood' protocol, according to manufacturer's instructions. DNA concentration and purity were quantified by NanoDrop One Microvolume UV-Vis Spectrophotometer and diluted to 2.5ng/ μ L.

qPCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, #4367659), standard MicroAmp Optical 96-well Reaction Plates (Life Technologies, #4306737), and custom made primers (Sigma), using an Applied Biosystems 7900HT Fast Real-Time PCR system or Quantstudio 12K Flex (ThermoFisher). Cycling conditions for all
30 primers were set at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15s, 60°C annealing for 30s and extension at 72°C for 30s, followed by standard melt curve cycling. Two replicate
35 reactions were performed for each sample and each primer pair, using 2 μ L of sample per

reaction (see Figure 4D). The final reaction for each well was 10 μ L Power SYBR Green PCR Master Mix, 0.5 μ L each of forward and reverse primer (stock at 10 μ M), 2 μ L of sample, and sterile H₂O to a final volume of 20 μ L. Telomere primer sequences were: telomere forward 5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3' (SEQ ID NO: 1),
 5 telomere reverse 5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3' (SEQ ID NO: 2) (R. J. Callicott, J. E. Womack, Real-time PCR assay for measurement of mouse telomeres. *Comparative medicine* 56, 17-22 (2006)). Reference gene primer sequences were: Rn18S forward 5'-AGA AAC GGC TAC CAC ATC CAA-3' (SEQ ID NO: 3), Rn18S reverse 5'-CCT GTA TTG TTA TTT TTC GTC ACT ACC T-3' (SEQ ID NO: 4) (C. de
 10 Frutos *et al.*, Spermatozoa telomeres determine telomere length in early embryos and offspring. *Reproduction* (Cambridge, England) 151, 1-7 (2016)). To minimize plate-to-plate variation, Rn18S and telomere reactions for each sample were run on the same plate, and calibrator DNA included on every plate. Telomere relative to Rn18S copies was calculated using the $2^{-\Delta\Delta C_t}$ method, where the ΔC_t of the calibrator (telomere C_t - Rn18S C_t) was
 15 subtracted from the ΔC_t of the sample (telomere C_t - Rn18S C_t) to give the $\Delta\Delta C_t$.

Embryo sex determination qPCR was performed using the same reagents and systems as above, with two reactions performed for every sample. The final reaction for each sample consisted of 10 μ L Power SYBR Green PCR Master Mix, 0.5 μ L each of forward and reverse
 20 primer (SRY primers, stock at 10 μ M, forward sequence 5'-AAG CGC CCC ATG AAT GCA TT-3' (SEQ ID NO: 5), reverse sequence 5'-TCC CAG CTG CTT GCT GAT CT-3' (SEQ ID NO: 6)), 4 μ L of sample, and sterile H₂O to a final volume of 20 μ L. To confirm qPCR product size, reactions were run on a 4% agarose (Promega, #V3125t) gel with Gel Red (Biotium, #41003). Each sample had 2.5 μ L of 6x loading dye (New England Bio Labs, #B70245) added and 10 μ L
 25 run per lane using a 100bp ladder (ThermoFisher Scientific, #SM0243) for reference. Gels were run at 100V for 1h and imaged via Biorad Gel Doc EZ Imager with Image Lab software. Male embryos were distinguished by a PCR product at 105bp. Telomere length was not different between ICM from male and female embryos (see Figure 1A), thus, unless specifically stated, male and female embryos were grouped for analysis.

30

MitoSOX Red (MSR) staining

Zygotes were collected and incubated for 20 minutes at 37°C in handling media containing 60 μ g/mL BSA, 1mg/mL PVP, 5 μ M MitoSOX Red (Invitrogen, #M36008) with Hoescht-3342. Samples were washed briefly in handling media before being mounted in pre-warmed handling
 35 media (containing BSA and PVP, as above) and imaged using an Olympus FV3000 Confocal

Microscope. Images were acquired using the same confocal microscope settings with the operator blinded to treatment group. Total cell fluorescence was calculated and adjusted for background fluorescence using Image J to give the corrected total cell fluorescence (CTCF).

5 **Mitochondrial membrane potential assay**

Fully denuded MII oocytes or fertilized zygotes, or embryos at the indicated developmental stage were washed twice in pre-warmed (37°C) handling media containing 60µg/mL BSA, 1mg/mL PVP and incubated for 30 minutes at 37°C in handling media containing BSA and PVP, as above, and 25µM TMRM (Tetramethylrhodamine methyl ester perchlorate; Sigma, #T5428) with 1:250 Hoescht-33342 added for the final 15 min. Samples were washed briefly in handling media before being mounted in pre-warmed handling media (as above) and imaged using a Cell Voyager CV1000 spinning disc confocal (Yokogawa) with a 40x objective. Images were acquired using the same confocal microscope settings with the operator blinded to treatment group. Total cell fluorescence was calculated and adjusted for background fluorescence using Image J to give the corrected total cell fluorescence (CTCF).

Pronuclear cytosine methylation immunofluorescence

Fertilized zygotes (stage PN3 to PN4) were collected 10h after fertilization. The zona pellucida was dissolved using Acid Tyrode's Embryomax solution (Millipore, #MR-004D), followed by a brief wash in phosphate buffered saline (PBS) containing 1mg/ml polyvinylpyrrolidone (PVP, Sigma) (PBS-PVP). Zygotes were fixed in 3.7% PFA-PBS for 20 minutes at room temperature (RT), followed by two five-minute washes in PBS-PVP, then permeabilized in 0.2% Triton X-100 (in PBS) for 10 minutes at room temperature, followed by three five-minute washes in PBS-PVP. Zygotes were incubated in 4N HCl solution for 10 minutes at RT, and washed thoroughly in 0.05% Tween-20 in PBS (PBS-T). Zygotes were blocked overnight at 4°C in blocking solution (1% BSA, 0.02% Triton X-100 in PBS), then incubated overnight at 4°C with mouse anti-5mC (1:200, Biorad, #ab10805), and rabbit anti-5hmC (1:600, Active Motif, #39769), diluted in blocking solution. Following three ten-minute washes in PBS-T, samples were incubated for one hour at RT, while protected from light, with Alexa Fluor 594-conjugated goat anti-mouse (1:500, Invitrogen) and Alexa Fluor 488-conjugated goat anti-rabbit secondary antibodies (1:1000, Invitrogen). Samples were mounted on slides with Prolong™ Diamond Antifade Mountant (Invitrogen, #P36965) and examined using a Cell Voyager CV1000 spinning disc confocal (Yokogawa) with a 40x objective. Images were acquired using the same confocal microscope settings with the operator blinded to treatment group. Pronuclear size and staining intensity was calculated using ImageJ software. Mean signal intensity was calculated first by

subtracting the signal from the cytoplasmic area (representing background staining), then dividing by the area of the pronuclei. To quantify the normal differential in 5mC and 5hmC between the male and female pronuclei as in (L. Han *et al.*, Embryonic defects induced by maternal obesity in mice derive from Stella insufficiency in oocytes. *Nature genetics* 50, 432-442 (2018)), the signal intensity of each marker was also expressed as a ratio of the values within the paternal versus maternal pronucleus of the same zygote.

Transfer of blastocyst embryos to pseudo-pregnant female mice

Embryos from high oxygen culture or *in vivo* rotenone experiments were vitrified at the morula stage to later be thawed, cultured to blastocyst and transferred to pseudopregnant females. To determine whether embryo vitrification impacts ICM telomere length, vitrified morulae were thawed and cultured overnight prior to ICM collection and analysis. ICM telomere length was not different between those derived from fresh or vitrified morulae, regardless of oxygen culture conditions (Figure 5) or pre-conception rotenone exposure (Figure 6A).

Mice were humanely killed by cervical dislocation on day 18.5 of pregnancy. Implantation sites were counted and outcomes (i.e. foetus or resorption) documented. Foetal weight, crown to rump length, placenta weight, and foetal sex were recorded. Foetuses were humanely euthanized and multiple tissues collected (heart, liver, kidney, tail, gonads, brain) and individually snap frozen in liquid N₂ and stored at -80°C until use. Embryos cultured at 20% O₂ exhibited reduced implantation potential compared to those cultured at 5% O₂ (Figure 5) and thus fetal tissues from the two groups could not be directly compared and were not examined further. Embryos from mice fed rotenone exhibited identical implantation rates as those from control mice (Figure 6, Figure 7).

Experiments involving the obese mouse model used embryos generated by IVF and cultured at atmospheric oxygen to the blastocyst stage followed immediately by uterine transfer to surrogate females as previously described in (L. L. Wu *et al.*, Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development (Cambridge, England)* 142, 681-691 (2015)). Briefly, female mice confirmed to have mated with a sterile vasectomized male were considered day 0.5 of pregnancy and selected as embryo recipients. At day 2.5 of pseudopregnancy, 7-10 fresh (i.e. continuously cultured) blastocysts were transferred to uteri (3-5 blastocysts/horn) of each pseudopregnant mouse anesthetized by i.p. injection of Avertin (0.5 mg/g body weight, Sigma-Aldrich, St. Louis, MO). Analgesia Carprofen (5 mg/kg) (Rimadyl

Pfizer) was injected subcutaneously once after the surgery. Foetal tissues were collected at day 14.5 of pregnancy, and foetal tissue was extracted as in (L. L. Wu *et al.*, Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development (Cambridge, England)* 142, 681-691 (2015)).

In vivo-conceived fetuses

To generate naturally conceived (*in vivo*) fetuses, females that had been on rotenone or control diet for 3 weeks were housed (1:1) with 8-week old CBA.F1 males and presence of a copulatory plug was considered day 0.5 of pregnancy. Males were not exposed to the rotenone diet (i.e. given control diet), and all females were placed onto control diet after presence of a copulatory plug to restrict rotenone exposure to the pre-conception period. Foetuses were collected on day 18.5 of gestation, and foetal number, weight, and sex ratios were not different (Figure 7). Placenta weight, crown to rump length, and foetal to placenta weight ratio were also not different. Foetal tissue collection was as above.

Foetal tissue DNA extraction and qPCR

Foetal heart and liver tissues were lysed overnight at 55°C with constant shaking (100rpm) in 250µL of lysis buffer (20mM EDTA pH 8.0, 50mM Tris pH 8.0, 120mM NaCl, 1% SDS) with 5µL of Proteinase K (10mg/mL). The following morning, 250µL of 4M ammonium acetate was added, briefly vortexed, then incubated for 15 minutes at RT with shaking, then a further 10 minutes without. Samples were centrifuged at 10,000rpm for 10 minutes and 400µL of supernatant removed and added to a clean tube. 800µL of 100% ethanol was added and briefly vortexed prior to centrifugation at 10,000rpm for 8 minutes to pellet the DNA. Ethanol was removed and the pellet was washed using 500µL of 70% ethanol. Excess ethanol was removed and DNA was resuspended by incubation at 55°C with shaking (100rpm) in 300µL of sterile H₂O. DNA was quantified using a ThermoFisher Nanodrop One UV-Vis spectrophotometer and diluted to 4ng/µL with sterile water for use.

Telomere length was analysed in foetal tissues via qPCR as described above using a Quantstudio 12K Flex (ThermoFisher). Telomere and reference gene (Rn18S) primer sequences were as above. Foetal tissues from oocytes of obese (and lean control) mice were analysed using the reference gene 36B4. Primer sequences were: 36B4 Forward: 5' ACT GGT CTA GGA CCC GAG AAG 3' (SEQ ID NO: 7); and 36B4 Reverse: 5' TCA ATG GTG CCT CTG GAG ATT 3' (SEQ ID NO: 8) as in (R. J. Callicott, J. E. Womack, Real-time PCR assay for measurement of mouse telomeres. *Comparative medicine* 56, 17-22 (2006)). Cycling

conditions were as above. The final reaction for each well was 10 μ L Power SYBR Green PCR Master Mix, 0.2 μ L each of forward and reverse primer (stock at 10 μ M), 4 μ L of sample, and sterile H₂O to a final volume of 20 μ L, and two replicate reactions were performed for each sample. Telomere relative to reference gene copies (T/S ratio) was calculated using the
5 formula $1/(\text{telomere Ct}/\text{reference Ct})$ as in (R. J. Callicott, J. E. Womack, Real-time PCR assay for measurement of mouse telomeres. Comparative medicine 56, 17-22 (2006)).

Telomere length was compared between heart and liver tissues and revealed that telomeres are significantly longer in liver than in heart, and not different between males and females,
10 whether the foetuses were derived via IVF and embryo transfer (Figure 6J, K), or *in vivo* conception (Figure 7G, H).

Statistical analysis

Results are presented as mean $\pm$ SEM. qPCR data is displayed as mean $\pm$ SEM overlaid on a
15 violin plot to illustrate the distribution of data points. All data points represent independent biological replicates. Analysis of normality showed that telomere data required log transformation for statistical analysis. Statistical analysis was performed using Graph Pad Prism version 8 for Windows (GraphPad Software Inc., La Jolla, CA) and SPSS Statistics 26 (IBM, Armonk, NY). Paired two-tailed t-test, unpaired two-tailed t-test, one-way and two-way
20 analysis of variance (ANOVA, comparison of means), and linear-mixed effects models were used as indicated and statistical significance was considered at P-value <0.05.

EXAMPLE 1

Telomere elongation between 8-cell and blastocyst formation is blunted by oxidative 25 stress

Preimplantation development involves complete reprogramming of the parental nuclear DNA between the time of fertilization and the formation of the ICM; however the kinetics of telomere elongation are not well characterized. To comprehensively map telomere length dynamics across this phase of embryogenesis, mouse MII stage oocytes were fertilized *in vitro* and
30 collected at precise developmental milestones between 2-cell (24h post-fertilization) and blastocyst formation (96h post-fertilization) (Figure 2A). Telomere DNA was measured in individual oocytes and embryos and normalized to a reference gene, to account for increases in cell number, using a validated quantitative PCR-based assay (Figure 4). Telomere lengthening was detected by the 2-cell stage (Figure 2B) and again at the 8-cell stage (Figure
35 2B). Between the 8-cell and blastocyst stages, telomere elongation was rapid (occurring within

~40h) and of greater magnitude (Figure 2B). This is consistent with the well-documented telomerase-mediated elongation that occurs between the morula to blastocyst stages. Telomere length in isolated ICM (Figure 2C) was measured to verify telomere elongation in the embryo proper, compared to the trophectoderm (TE, the placental precursor) of individual
5 blastocysts. This confirmed that telomere length is longer in the ICM (Figure 2D). There was no further change in ICM telomere length at day 6 of culture (Figure 2E), the time of normal implantation, confirming that ICM telomere length in day 5 blastocysts is the maximum achieved during pre-implantation development.

10 Oxidative stress is a critical determinant in telomere stability. Specifically, because they are GC-rich, telomeres are particularly sensitive to oxidative stress-induced telomere attrition. In contrast, the potential impact of oxidative stress on telomere elongation during preimplantation embryogenesis is not known. Thus, oxidative stress was induced during embryo culture and impact on telomere length examined to better understand the regulation of telomere elongation
15 kinetics. Mouse MII oocytes were exposed to 20% O₂ (similar to ambient air) during *in vitro* fertilization (IVF) and embryo culture (Figure 2F), to induce oxidative stress. These were compared with embryos fertilized and cultured at 5% O₂ (which better mimics the female reproductive tract and thus is the gold-standard for mouse and human *in vitro* embryo culture). Telomere length in 8-cell embryos was not affected by culture in 20% O₂ (Figure 2G), indicating
20 no deficiency in the earliest phase of elongation (and no acceleration of telomere attrition). However, by the morula stage, embryos cultured in high oxygen exhibited shorter telomeres (Figure 2H). The normal increase in telomere length per cell between the morula stage and ICM was blunted in embryos exposed to the oxidative 20% O₂ culture conditions (Figure 2H). Validation in a larger cohort of embryos clearly demonstrated significantly reduced telomeric
25 DNA per cell in ICM from embryos cultured at high oxygen (Figure 2I). These results indicate a previously uncharacterized link between oxidative stress and telomere biology. Specifically, in contrast to the well documented mechanism of ROS-induced telomere shortening, it has now been shown that oxidative stress impairs embryo telomere elongation, specifically at the morula and blastocyst stages of development.

30 Mitochondria are a major site of ROS production and more recently, mitochondria have been identified as an intracellular source of metabolites and enzymes that influence epigenetic reprogramming and zygotic genome activation, processes that directly control developmental gene expression networks. It was therefore tested whether mitochondrial dysfunction may be

- a potential mechanistic link between oxidative stress and impaired telomere lengthening. Mitochondrial ROS (mtROS; reflective of mitochondrial superoxide production) and mitochondrial membrane potential (MMP; an indicator of oxidative phosphorylation) were measured to characterize the effects of 20% O₂-induced oxidative stress on mitochondrial
- 5 bioenergetics. At 6h following IVF, mtROS were markedly increased in zygotes exposed to 20% O₂ (Figure 2J). In contrast, MMP, detected using TMRM potentiometric dye, was not altered in zygotes or 8-cell embryos, but the marked increase in MMP seen at the morula and blastocyst stage was significantly blunted in embryos cultured in 20% O₂ (Figure 2K).
- 10 To examine whether these disruptions to oocyte mitochondrial activity are associated with zygotic nuclear changes, global methylation status was measured by immunostaining as a general assessment of epigenetic reprogramming status in pronuclear stage zygotes (10h post-IVF). Paternal pronuclei (the larger of the two) preferentially stained for 5-hydroxymethylcytosine (5hmC), the oxidized form of 5-methylcytosine (5mC) generated during
- 15 Tet-mediated demethylation, as expected (Figure 2L, Figure 8). In zygotes exposed to 20% O₂, the differential in 5hmC abundance between the paternal and maternal pronucleus was reduced (Figure 2L, Figure 8). This indicates that oxidative stress alters zygotic nuclear processes, including potentially epigenetic reprogramming or transcriptional activation. Thus, disruptions to mitochondrial function and/or pronuclear reprogramming could be responsible
- 20 for the impaired telomere lengthening during late-stage preimplantation development.

EXAMPLE 2

Telomere length in offspring is regulated by oocyte mitochondria and zygotic nuclear reprogramming

- 25 To directly test whether mitochondrial function regulates telomere elongation during embryogenesis, mitochondrial bioenergetic activity was specifically targeted using a Complex I inhibitor (rotenone) and measured telomere length in oocytes, 8-cell embryos and blastocysts. A low dose of rotenone (150ppm in the diet, as in (O. A. Lozoya *et al.*, Single Nucleotide Resolution Analysis Reveals Pervasive, Long-Lasting DNA Methylation Changes
- 30 by Developmental Exposure to a Mitochondrial Toxicant. *Cell reports* 32, 108131 (2020))), was provided to 6 week old mice for three weeks prior to IVF under identical conditions as controls (Figure 9A). At 6h post-IVF, zygotes from oocytes of rotenone-exposed females exhibited dramatically elevated mtROS (Figure 9B). Disrupted 5mC/5hmC epigenetic patterning was also apparent in zygotes from oocytes of mice that were fed rotenone (Figure 9C, Figure 10),

indicating that disrupted oocyte mitochondrial activity directly impacts pronuclear reprogramming events. Rotenone-induced disruption to MMP persisted in 8-cell embryos (Figure 9D).

5 Telomere length was shorter in MII oocytes from rotenone-exposed females compared to controls (Figure 9E), likely due to ROS-mediated telomere attrition during folliculogenesis. This deficit was, however, reversed by the 8-cell stage (Figure 9E), again suggesting that the earliest phase of telomere elongation is not influenced by mitochondrial bioenergetics or oxidative stress. Although some telomere lengthening occurred between the 8-cell and
10 blastocyst stages in embryos from oocytes of rotenone-exposed females, blastocyst telomeres were significantly shorter (Figure 9E), specifically within the ICM (Figure 9F). These results demonstrate that direct perturbation of oocyte mitochondrial function prior to fertilization impairs later telomere elongation capacity, specifically between the 8-cell and blastocyst stages of pre-implantation development, an identical phenotype as embryos exposed to *in vitro*
15 oxidative stress.

Blastocysts derived from control or rotenone-exposed females were transferred to uteri of surrogates (to provide identical gestational environments) and fetuses examined near the time of birth (see Figure 6) to determine whether the deficiency in ICM telomere length
20 persisted in offspring. Telomeres were shorter in the hearts of offspring (Figure. 9G), mirroring the relative differences in the ICM. These data demonstrate that relative differences in telomere length in the pluripotent stem cells of the blastocyst persist through foetal development. Further, because offspring of both groups were conceived under identical conditions for fertilization, embryo culture and gestation, the differential molecular signals that determined
25 telomere length at the time of birth were present within the ovulated egg.

To dissect the roles of mitochondria versus nuclear events in regulating telomere elongation in ICM, we conducted reciprocal pronuclear transfers. At the 1-cell stage (~8h post-fertilization), pronuclei from zygotes of either rotenone-fed or control mice were micro-injected
30 into enucleated cytoplasts of rotenone-exposed or control zygotes, resulting in four types of reconstructed embryos which were then cultured to the blastocyst stage (Figure 9H; Figure 3). ICM of embryos reconstructed from rotenone-exposed pronuclei plus rotenone-exposed cytoplast had shorter telomeres than those of embryos reconstructed from components of controls (Figure 9I), consistent with the relative differences in non-reconstructed embryos
35 (Figure 9F). Telomere length was also reduced in embryos reconstructed from pronuclei of

rotenone-exposed zygotes, but not in those receiving cytoplasm of rotenone-exposed zygotes (Figure 9I), demonstrating that the nuclear material contains the molecular signals leading to impaired telomere elongation potential. Thus, disrupted mitochondrial function in the oocyte at fertilization causes pronuclear epigenetic changes prior to the first mitotic cleavage that are responsible for impaired telomere elongation in ICM at the blastocyst stage.

It was investigated whether it was possible to restore ICM telomere length through modulation of oocyte mitochondrial activity. BGP-15 was used, a hydroxamic acid compound which improves mitochondrial bioenergetics and prevents fragmentation in contexts of induced oxidative stress, and exhibits therapeutic utility in pre-clinical pathologies involving mitochondrial dysfunction. Mice fed rotenone were treated with BGP-15 (100mg/kg by i.p. injection) or saline vehicle for 4 days prior to gonadotropin stimulated ovulation (as in (T. Umehara *et al.*, Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. *Science advances* 8, eabn4564 (2022), L. L. Wu *et al.*, Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development (Cambridge, England)* 142, 681-691 (2015)) and IVF under identical conditions (Figure 9A). This pre-conception BGP-15 treatment significantly reduced mtROS levels in zygotes of rotenone-fed mice (Figure 9J). BGP-15 treatment of rotenone-fed females also mitigated the defect in ICM telomere length (Figure 9K). Cumulatively, these data indicate that ICM telomere length is tightly coupled to oocyte mtROS levels at fertilization, and that this biology is modifiable to influence the telomere set-point.

EXAMPLE 3

Maternal aging or obesity impairs telomere elongation in ICM that is reversible by restoring oocyte mitochondrial function

Two physiological states that influence oocyte mitochondrial function are aging and obesity. It was investigated whether telomere elongation is impacted in these contexts and reversible using BGP-15 to restore mitochondrial function. Female mice that were reproductively aged (12 months old, which extrapolates to 38-45 years old in women) or young (3-4 months old) were treated with BGP-15 (100mg/kg/day for 4 days) or vehicle and ovulated oocytes underwent IVF under identical conditions (Figure 11A). Ovulated oocytes of the aged mice exhibited high levels of mtROS that were reduced in females treated with BGP-15 (Figure 11B). Zygotes from aged females exhibited a trend towards altered relative levels of 5mC and 5hmC in paternal versus maternal pronuclei; while in those given pre-conception treatment

with BGP-15, ratios were the same as the zygotes of young mice (Figure 11C). Mitochondrial membrane potential (MMP) was reduced in morulae from the older mice; and this deficit was normalized in embryos of females given pre-conception BGP-15 treatment (Figure 11D).

- 5 Ovulated (MII) oocytes from old mice had shorter telomeres (Figure 11E), and interestingly, oocyte telomere length was significantly shorter in nulliparous females (that experienced continuous ovarian cycling) compared to littermates that were maintained in breeding pairs and continuously pregnant (Figure 11F). Thus, in animals of the exact same age, limiting the number of oestrous cycles appears to have a protective effect on oocyte telomere length.
- 10 Following IVF, 8-cell embryos from old and young mice had no differences in telomere length (Figure 11G), indicating a normalization of the defect in oocytes. However, blastocysts derived from aged mice had shorter telomeres than those from young mice (Figure 11H) indicating a deficiency in the later phase of lengthening. BGP-15 treatment of aged mice (for 4 days prior to ovulation) restored telomere length in blastocysts (Figure 11H). Telomere length in the ICM
- 15 population mirrored that in whole blastocysts, with maternal reproductive aging leading to reduce ICM telomere length, and BGP-15 treatment restoring this deficit (Figure 11I).

Given the notable effects of BGP-15 to restore ICM telomere length, two other pharmaceutical agents characterized to improve mitochondrial bioenergetics were tested. MitoQ is a

20 mitochondria-targeted CoQ10 ubiquinone available over-the-counter; while, metformin is commonly prescribed for the treatment of insulin resistance, including in women seeking pregnancy. Reproductively old (12 months) female mice were administered either metformin (2mg/mL) or MitoQ (150µM) in their drinking water for two weeks prior to gonadotropin-stimulated ovulation and IVF under standard conditions. Treatment of aged females with

25 metformin restored ICM telomere length, such that it was indistinguishable from embryos derived from young females (Figure 11J). These results further substantiate that telomere elongation capacity during pre-implantation development is regulated by maternal factors contained within the ovulated oocyte, particularly mitochondrial activity; and provide proof-of-concept that this biology can be targeted to influence embryo telomere length.

30 The impact of maternal obesity on offspring telomere elongation kinetics was examined in mice that are obese due to hyperphagia compared with lean littermates, and in mice treated for 4 days prior to ovulation with BGP-15; with ovulated oocytes from all groups undergoing IVF under identical conditions (Figure 13A). High mtROS (Figure 13B) and altered 5mC/5hmC

35 pronuclear epigenetic patterns (Figure 13C, Figure 14) were observed in zygotes of obese

mice, and normalized by pre-conception treatment with BGP-15. Mitochondrial membrane potential (MMP) was reduced in 8-cell embryos and morulae and this deficit was normalized in embryos of females given pre-conception BGP-15 treatment (Figure 13D-G).

- 5 Telomere length per cell was reduced in MII oocytes from obese mice when compared to lean, but not influenced by pre-conception treatment with BGP-15 (Figure 13H). At the 8C stage, telomere length was not different between any of the groups (Figure 13I) similar to the normalization in embryos exposed to other mitochondrial stressors (i.e. 20% O₂, rotenone, aging). At the blastocyst stage, telomeres were shorter in embryos derived from obese mice compared to those from lean controls (Figure 13J), a deficiency apparent in the ICM (Figure 13K). BGP-15 treatment of obese mice restored blastocyst telomere length (Figure 13J), including in the ICM (Figure 13K), such that it was similar to lean controls. Exactly as for maternal reproductive aging, obese female mice were administered either metformin (2mg/mL) or MitoQ (150µM) in drinking water for two weeks prior to gonadotropin-stimulated ovulation and IVF. Both treatments resulted in restored ICM telomere length, to levels similar to embryos derived from lean mice (Figure 13L). Cumulatively, these data demonstrate that physiological disruptions to oocyte mitochondria (via aging or obesity) lead to impaired telomere elongation and reduced telomere length in the ICM of offspring.
- 20 Blastocysts derived from oocytes of lean mice, obese mice, and obese mice treated with BGP-15 (*in vivo* for 4 days) were transferred to identical lean surrogates and telomere length analysed in day 14.5 foetal tissues. Telomere length was shorter in both heart and liver of fetuses derived from oocytes of obese females (Figure 13M), demonstrating that relative differences in the ICM (Figure 13K) were maintained through foetal development. Further, fetuses from oocytes of obese females treated with pre-conception BGP-15 had increased telomere length in both heart and liver compared to offspring from untreated obese controls (Figure 13M). Thus, maternal obesity leads to fundamental defects in the oocyte that impair telomere elongation in the ICM, a deficiency in the telomere set-point that persists through gestation. This molecular change is preventable by systemic treatment with a mitochondria-targeted therapeutic in the days prior to ovulation.
- 30

To further interrogate the developmental timeframes that influence telomere elongation capacity, it was tested whether direct acute manipulation of mitochondrial bioenergetics at fertilization, via *in vitro* administration of BGP-15, could restore blastocyst telomere lengthening. When oocytes from lean control or obese mice were treated with BGP-15 (10µM)

in the IVF and embryo culture media, mtROS levels were reduced within 6h in zygotes from oocytes of obese mice (Figure 13N). Identical responses to BGP-15 were observed in zygotes generated from oocytes exposed to 20% O₂ (Figure 15) or from oocytes of rotenone-fed mice (Figure 16), substantiating the efficacy of BGP-15 to directly influence mtROS production.

5 Treatment of oocytes from obese mice with BGP-15 during IVF and embryo culture also normalized MMP at the morula stage (Figure 13O). Most notably, this *in vitro* BGP-15 treatment normalized the deficiency in ICM telomere length in blastocysts (Figure 13P). These results demonstrate that telomere elongation capacity can be acutely regulated even from the time of fertilization. This information, combined with the results from the pronuclear transfer

10 experiments (Figure 9H-I), identifies the time between fertilization and PN5 (~10h post-IVF) as the key developmental window during which mitochondrial-pronuclear signals determine telomere elongation capacity 4 days later, establishing the ICM telomere set-point.

Conclusions

15 Thus, new findings have been made about the mechanisms that control telomere regeneration during early life and the resetting of offspring telomere length. Specifically, between fertilization and the first zygotic cell division, optimal mitochondrial function and epigenetic remodelling are coupled to induce telomere elongation during the second half of preimplantation development and determine the ICM telomere set-point. Via this process, relative differences in telomere

20 length at birth, a major determinant of lifetime health and longevity, are established within the first few days following fertilization. These findings have important clinical and public health implications. They provide a potential mechanistic explanation for the occurrence of shorter telomeres in some infants and identify oxidative stress during culture to blastocyst as a possible basis for shorter telomeres in IVF-conceived babies. More broadly, they substantiate

25 that maternal health and environmental conditions at the time of conception have long term consequences: that now include influencing that offspring's susceptibility (or resilience) to aging and aging-associated diseases in later life.

A different regulatory communication between the two organelles has been identified; wherein

30 appropriate mitochondrial bioenergetics facilitate telomere elongation capacity. Appropriate regulation of mitochondrial metabolism is also central to establishing embryonic stem cell pluripotency and subsequent cell fate acquisition; while telomere elongation is a hallmark and requisite component of nuclear reprogramming. Thus, the identification of this new connection between mitochondria and telomeres during *in vivo* embryogenesis may also extend to other

35 contexts of cellular reprogramming and stem cell biology. Without wishing to be bound by

theory, it is believed that the molecular communication cues between mitochondrial activity at fertilization and the telomere elongation machinery that is active days later are likely to involve mtROS (or other mitochondrial metabolites) signalling to nuclear effectors that actively reprogram DNA epigenetic marks and histone modifications to regulate subsequent gene expression.

These findings provide new evidence that the peri-conception period, between ovulation and syngamy, is a critical developmental phase when maternal environmental signals are transmitted to the next generation. They reveal a new mechanism by which oocyte mitochondria respond acutely to maternal physiological cues, and set in motion specific nuclear responses that direct the offspring's lifetime health trajectory. The demonstrated plasticity of oocyte mitochondrial responses, and our identification of specific pharmaceuticals compounds that can modulate them during preconception and immediately following fertilization, means that there are therapeutic opportunities to optimize this biology which is a major determinant of chronic disease risk.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A mitochondrial-targeted agent for use in treating a telomere-associated disorder.
- 5 2. A method for treating a telomere-associated disorder, comprising the administration of a mitochondrial-targeted agent.
3. Use of a mitochondrial-targeted agent in the manufacture of a medicament for treating a telomere-associated disorder.
- 10 4. The mitochondrial-targeted agent for use, method or use according to any one of the preceding claims, comprising administering the mitochondrial-targeted agent to a subject having the telomere-associated disorder after birth of the subject.
- 15 5. The mitochondrial-targeted agent for use, method or use according to claim 4, wherein the subject is a child or an adult subject, preferably an adult subject.
6. A mitochondrial-targeted agent for use in increasing telomere length or suppressing telomere shortening.
- 20 7. A method for increasing telomere length or suppressing telomere shortening, comprising the administration of a mitochondrial-targeted agent.
8. Use of a mitochondrial-targeted agent in the manufacture of a medicament for increasing telomere length or suppressing telomere shortening.
- 25 9. The mitochondrial-targeted agent for use, method or use according to any one of claims 6-8, comprising administering the mitochondrial-targeted agent to a subject after birth of the subject.
- 30 10. The mitochondrial-targeted agent for use, method or use according to claim 9, wherein the subject is a child or an adult subject, preferably an adult subject.

11. The mitochondrial-targeted agent for use, method or use according to any one of claims 6-10, wherein increasing telomere length or suppressing telomere shortening treats a telomere-associated disorder.
- 5 12. The mitochondrial-targeted agent for use, method or use according to any one of claims 1-3, 6-8 or 11, comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent.
- 10 13. The mitochondrial-targeted agent for use, method or use according to any one of claims 1-3, 11, or 12, wherein the telomere-associated disorder is prevented and/or treated by increasing telomere length during embryonic development of a subject.
- 15 14. The mitochondrial-targeted agent for use, method or use according to any one of claims 1-5 or 11-13, wherein the telomere-associated disorder is ageing (e.g. premature ageing), bone marrow failure, dyskeratosis congenita, acquired aplastic anaemia, pulmonary fibrosis (e.g. idiopathic pulmonary fibrosis), liver disease (e.g. associated with or caused by dyskeratosis congenita and/or pulmonary fibrosis), cardiovascular disease, heart disease, cancer, neurodegeneration, inflammatory bowel disease, Barrett's oesophagus, and/or miscarriage (e.g. recurrent miscarriage).
- 20 15. A mitochondrial-targeted agent for use in increasing telomere length during embryonic development, comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent.
- 25 16. A method for increasing telomere length during embryonic development, comprising contacting a gamete and/or a fertilisation product thereof with a mitochondrial-targeted agent.
- 30 17. Use of a mitochondrial-targeted agent in the manufacture of a medicament for increasing telomere length during embryonic development, comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent.
- 35 18. The mitochondrial-targeted agent for use, method or use according to any one of claims 12-17, wherein the gamete and/or fertilisation product thereof is contacted with the mitochondrial-targeted agent by administration of the mitochondrial-targeted agent to a

female mammal comprising the gamete and/or fertilisation product thereof, wherein the gamete is an oocyte.

19. The mitochondrial-targeted agent for use, method or use according to any one of claims 5 12-17, wherein the gamete is contacted with the mitochondrial-targeted agent by administration of the mitochondrial-targeted agent to a male mammal that is producing, will produce, and/or has produced the gamete, wherein the gamete is sperm.
20. The mitochondrial-targeted agent for use, method or use according to claim 18 or 19, 10 wherein an embryo subsequently produced by said gamete and/or fertilisation product thereof has increased telomere length.
21. The mitochondrial-targeted agent for use, method or use according to any one of claims 18-20, wherein the mammal has reduced fertility. 15
22. A method for increasing telomere length during embryonic development, the method comprising contacting a gamete and/or a fertilisation product thereof with a mitochondrial-targeted agent *in vitro*.
- 20 23. Use of a mitochondrial-targeted agent for increasing telomere length during embryonic development, the use comprising contacting a gamete and/or a fertilisation product thereof with the mitochondrial-targeted agent *in vitro*.
24. A method for producing an embryo, the method comprising contacting a gamete and/or 25 a fertilisation product thereof with a mitochondrial-targeted agent *in vitro*.
25. The method or use according to any one of claims 22-24, wherein the gamete is used in a method of *in vitro* fertilisation.
- 30 26. A method for increasing telomere length during embryonic development, the method comprising using a gamete and/or a fertilisation product thereof that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, preferably in a method of *in vitro* fertilisation.

27. Use of a mitochondrial-targeted agent for increasing telomere length during embryonic development, comprising using a gamete and/or a fertilisation product thereof that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, preferably in a method of *in vitro* fertilisation.
- 5 28. A method for producing an embryo, the method comprising using a gamete and/or a fertilisation product thereof that has been contacted with a mitochondrial-targeted agent in a method of assisted reproduction, preferably in a method of *in vitro* fertilisation.
- 10 29. The method or use according to any one of claims 26-28, wherein the gamete has been contacted with the mitochondrial-targeted agent by administering the mitochondrial-targeted agent to a mammal.
30. The method or use according to any one of claims 26-28, wherein the gamete has been
15 contacted with the mitochondrial-targeted agent *in vitro*.
31. The method or use according to any one of claims 22-30, wherein the gamete is an oocyte obtained from a female mammal with reduced fertility.
- 20 32. The method or use according to any one of claims 22-30, wherein the gamete is sperm obtained from a male mammal with reduced fertility.
33. The mitochondrial-targeted agent for use, method or use according to claim 18-21, or 31-32, wherein the mammal:
25 (a) is an aged mammal, preferably wherein the mammal is a human of at least 30, 35 or 40 years old; and/or
(b) is an overweight or obese mammal, preferably wherein the mammal is a human having a body mass index (BMI) of greater than 25 kg/m².
- 30 34. The mitochondrial-targeted agent for use, method or use according to any one of claims 12-33, wherein the fertilisation product of the gamete is a zygote or embryo, preferably a zygote.
- 35 35. The mitochondrial-targeted agent for use, method or use according to any one of claims 12-34, wherein at least the gamete and the fertilisation product of the gamete, wherein the

fertilisation product of the gamete is a zygote, are contacted with the mitochondrial-targeted agent (preferably prior to syngamy), preferably wherein only the gamete and the zygote are administered or contacted with the mitochondrial-targeted agent, most preferably prior to syngamy.

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36. The mitochondrial-targeted agent for use, method or use according to any one of claims 12-35, wherein the fertilisation product of the gamete is a zygote and wherein at least the zygote is contacted with the mitochondrial-targeted agent before PN5, preferably wherein the fertilisation product of the gamete is a zygote and only the zygote is contacted with the mitochondrial-targeted agent before PN5.

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37. The mitochondrial-targeted agent for use, method or use according to any one of claims 12-35, wherein the fertilisation product of the gamete is contacted with the mitochondrial-targeted agent between the 8-cell stage and before the blastocyst stage of embryonic development and/or implantation, preferably wherein the fertilisation product of the gamete is contacted with the mitochondrial-targeted agent between the 8-cell stage and before the blastocyst stage of embryonic development and/or implantation only.

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38. The mitochondrial-targeted agent for use, method or use according to any one of claims 12-37, wherein the gamete and/or the fertilisation product thereof comprises an elevated level of mitochondrial reactive oxygen species before contact (e.g. contacting) with the mitochondrial-targeted agent.

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39. The mitochondrial-targeted agent for use, method or use according to any one of the preceding claims, wherein the mitochondrial-targeted agent is:

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(a) BGP-15 and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof;

(b) mitoquinone mesylate (MitoQ) and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof; or

30

(c) metformin and/or an acceptable derivative, prodrug, solvate, salt, tautomer, stereoisomer, and/or racemate thereof .

40. The mitochondrial-targeted agent for use, method or use according to any one of the preceding claims, wherein the mitochondrial-targeted agent is:

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(a) BGP-15 or a derivative thereof;

- (b) mitoquinone mesylate (MitoQ) or a derivative thereof; or
- (c) metformin or a derivative thereof.

41. The mitochondrial-targeted agent for use, method or use according to any one of claims
5 13-40, wherein the embryo develops into a fetus having increased telomere length.
42. An embryo obtainable by the method according to any one of claims 24-25 or 28-41.
43. A mitochondrial-targeted agent for use in altering an epigenetic state (e.g. nuclear
10 epigenetic state) of a cell, preferably comprising contacting the cell with the mitochondrial-targeted agent.
44. A method for altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, the
15 method comprising contacting the cell (e.g. *in vivo*) with a mitochondrial-targeted agent.
45. Use of a mitochondrial-targeted agent in the manufacture of a medicament for altering
an epigenetic state (e.g. nuclear epigenetic state) of a cell, preferably comprising
contacting the cell with the mitochondrial-targeted agent.
- 20 46. A method for altering an epigenetic state (e.g. nuclear epigenetic state) of a cell, the
method comprising contacting the cell with a mitochondrial-targeted agent *in vitro*.
47. Use of a mitochondrial-targeted agent for altering an epigenetic state (e.g. nuclear
25 epigenetic state) of a cell, comprising contacting the cell with the mitochondrial-targeted
agent *in vitro*.