Cell Tissue Res (2012) 349:795–808
DOI 10.1007/s00441-012-1444-2

REVIEW

Transmission, inheritance and replication of mitochondrial DNA in mammals: implications for reproductive processes and infertility

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Received: 17 February 2012 / Accepted: 26 April 2012 / Published online: 14 June 2012

Abstract The mitochondrial genome contributes key proteins to the electron-transfer chain, which through oxidative phosphorylation, generates the vast majority of cellular ATP. This maternally inherited genome is transmitted to subsequent generations through the oocyte. Its transmission, inheritance and replication are strictly regulated so that fully mature cells can be appropriately populated with mitochondrial DNA once they mature into adult cells. However, gametes do not always acquire the appropriate numbers of mitochondrial DNA copy; this often renders them inappropriate for successful fertilisation outcome. Furthermore, the number of assisted reproductive technologies that can overcome problems associated with infertility and that can provide enhanced genetic outcomes for the offspring is increasing. However, such techniques could also have a detrimental effect on offspring survival. If we are to introduce these technologies into in vitro fertilisation clinics and animal production, then we first need to validate their use carefully.

Keywords Mitochondrial DNA · Inheritance · Transmission · Replication · Gamete

Introduction

The transmission, inheritance and replication of mammalian mitochondrial DNA (mtDNA) are unique to a genome that invaded eukaryotic cells billions of years ago. Since then, the mitochondrial genome has built up a symbiotic relationship with its host whereby it contributes to the energy-generating process in return for its continued propagation through replication, which is mediated by chromosomal genes. Gametes, which possess mtDNA in highly different formats from those of somatic cells, have developed a mechanism for the transmission of mtDNA from one generation to the next. This results in a non-Mendelian pattern to inheritance whereby only the oocyte passes mtDNA onto the next generation. These key factors associated with mtDNA imply that assisted reproductive technologies used for treating human infertility, preventing the transmission of mutant mtDNA from one generation to the next, generating enhanced livestock and rescuing endangered species might contravene the strict modes of transmission, inheritance and replication of mtDNA.

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Why is mtDNA important?

Nearly all mammalian cells possess mitochondria, which are the energy-generating powerhouses of the cell. The biochemical process that generates the vast majority of cellular energy, known as ATP (Pfeiffer et al. 2001), is oxidative phosphorylation (OXPHOS), which takes place in the electron-transfer chain (Fig. 1). Importantly, aerobic cells, such as neurons and skeletal muscle, are highly dependent on OXPHOS-derived ATP to perform their specialised functions (Moyes et al. 1998). The electron-transfer chain (see Fig. 1) is the only cellular apparatus that has its proteins encoded by two distinct genomes, namely the chromosomal genome and the double-stranded circular mitochondrial genome (mtDNA; Anderson et al. 1981; see Fig. 2), located within the mitochondrion. The mitochondrial genome is 16.6 kb in size in the human

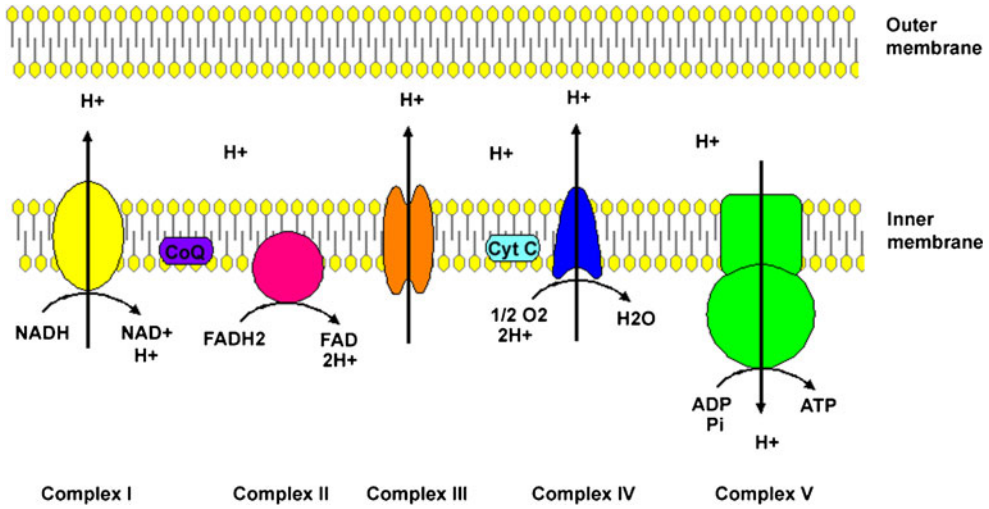

Fig. 1 The electron-transfer chain. Electrons are produced by NADH and $FADH_2$ and transferred to Complexes I and II, respectively. They then transfer to Complex III and onto coenzyme Q. Cytochrome c is an electron acceptor, which then donates to Complex IV. As the electrons are transferred, the energy released is utilised by Complexes I, III and IV to generate an electrochemical gradient. This is achieved by pumping protons across the mitochondrial inner membrane. Complex V then uses the membrane potential to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. Complex I (NADH dehydrogenase; ND), Coenzyme Q (CoQ), Complex II (succinate dehydrogenase), Complex III (cytochrome c reductase), Cytochrome C (CytC), Complex IV (cytochrome C oxidase; COX), Complex V (ATPase)

(Anderson et al. 1981), 16.2 kb in the mouse (Bibb et al. 1981) and 16.7 kb in the pig (Ursing and Arnason 1998).

MtDNA consists in a heavy (H) and a light (L) strand, which encodes 13 of the more than 90 subunits of the electron-transfer chain and 22 tRNAs and two rRNAs. The coding genes in the electron-transfer chain consist of seven subunits of NADH dehydrogenase (Complex I), one subunit of cytochrome c reductase (Complex III), three subunits of cytochrome C oxidase (Complex IV) and two subunits of the ATP synthase (Complex V; Fig. 2). Only Complex II has none of its subunits encoded by mtDNA. Unlike replication, the mitochondrial genome contributes to the process of its translation by encoding some of the required tRNAs and rRNAs. This highlights the semi-autonomous nature of mtDNA, whereby the cell is highly dependent on this genome for ATP production, whereas the mitochondrial genome cannot survive without the influence of

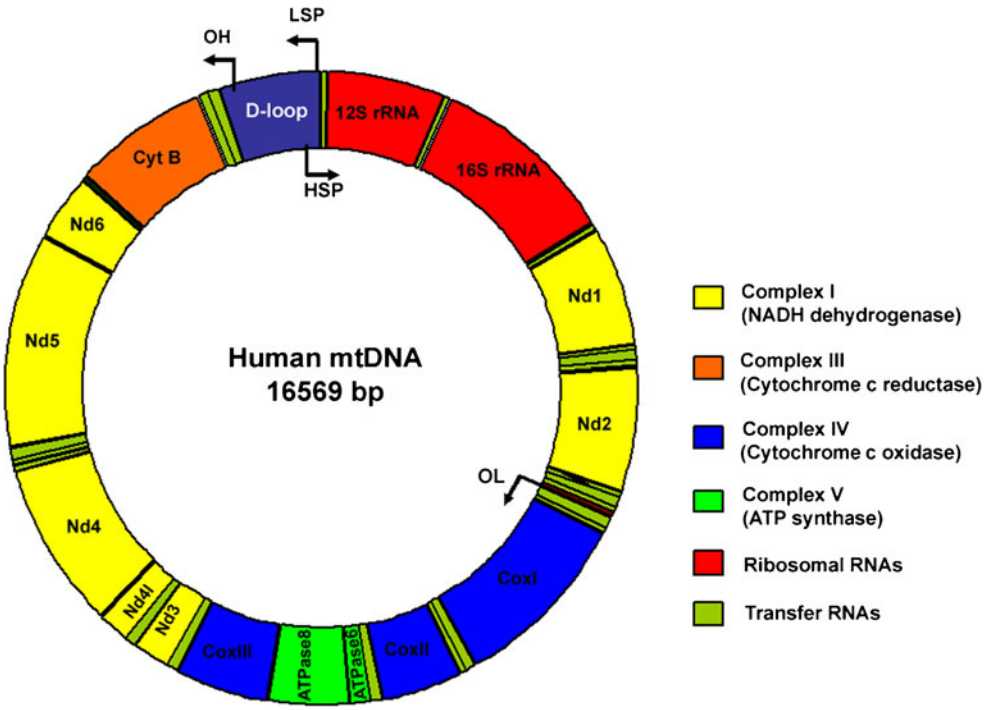

Fig. 2 The mammalian mtDNA genome encodes 13 of the subunits of the electron-transfer chain. These are associated with Complex I (*Nd1*, *Nd2*, *Nd3*, *Nd4*, *Nd4l*, *Nd5* and *Nd6*), Complex III (*CytB*), Complex IV (*CoxI*, *CoxII*, *CoxIII*) and Complex V (*ATPase6*, *ATPase8*). MtDNA also encodes two rRNAs (*12S rRNA*, *16S rRNA*) and 22 tRNAs. The Displacement Loop (*D-loop*) is the primary control region, which is the location of the H-strand promoter region (*HSP*), the L-strand promoter region (*LSP*) and the origin of H-strand replication (*OH*). A secondary control region of 30 bp is located between ND2 and COXI and is the site of the origin of L-strand replication (*OL*)

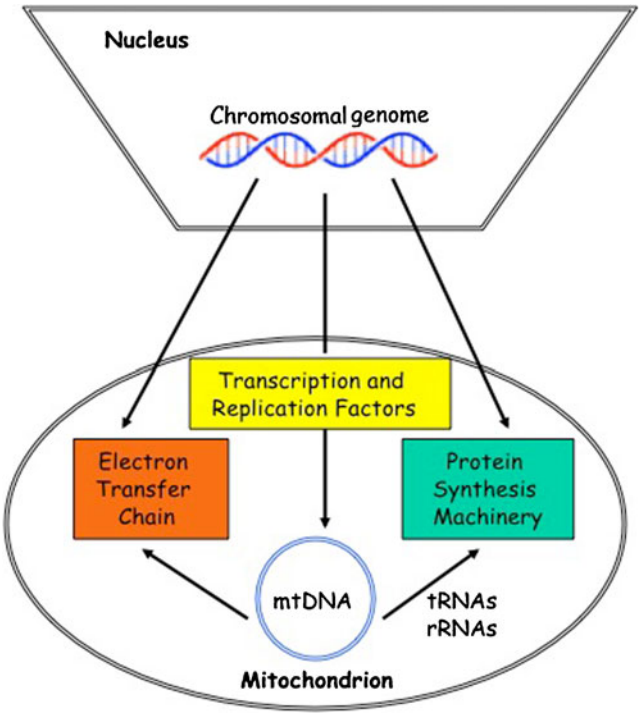

Fig. 3 Relationship between the cell and the mitochondrial genome. Cells are dependent on the mitochondrial genome to generate energy, ATP, through the electron-transfer chain. In turn, the cell contributes chromosomal-encoded factors to the electron-transfer chain and mediates transcription, replication and translation of the mitochondrial genes

chromosomal genes (Fig. 3). The mitochondrial genome also has one non-coding region, the Displacement Loop (D-Loop), which interacts with nuclear-encoded factors that transcribe and replicate mtDNA.

Defects to mtDNA

Normally, all of the thousands of copies of mtDNA within an individual are wild-type (WT) and identical, i.e. homoplasmic (Birky 1995). However, mutant and WT mtDNA molecules can coexist in a state described as heteroplasmy. Over 100 point mutations and large-scale deletions have been identified (Schaefer et al. 2008). These are found in encephalomyopathies, deafness and blindness, cardiomyopathies and endocrinopathies, which, although occurring at all ages, are usually diagnosed in neonates and children.

As one of the most common inherited metabolic disorders, 1 in 10,000 of the population are affected by mtDNA disease and another 1 in 6000 are at risk (Schaefer et al. 2008). Furthermore, 1 in ~200 are carrying a known mtDNA point mutation (Elliott et al. 2008; Manwaring et al. 2007; Vandebona et al. 2009), indicating that the real prevalence of mtDNA disease is likely to be in the range of 1 in 1000 to 1 in 5000. The specific diseases arising from single point mutations are the most well-characterised mtDNA diseases. These include: mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like syndrome (MELAS; Kobayashi et al. 1990), neuropathy, ataxia and retinitis pigmentosa (NARP; Holt et al. 1990) and Leber's hereditary optic neuropathy (Wallace et al. 1988), which are all associated with mutations located in the coding genes, whereas myoclonic epilepsy with ragged-red fibres syndrome (MERRF) is associated with a mutation in a tRNA (Shoffner et al. 1990). Large-scale deletions, such as the 4977-bp deletion, include the loss of several coding and tRNA genes and are indicative of, for example, Kearns-Sayre syndrome (Schon et al. 1989).

The severity of the phenotype is usually determined by the degree of mutant to WT ratio, i.e. mutant load, within the affected tissue. In MERRF, mtDNA mutant loading of over 85% is typical (Boulet et al. 1992). In the case of NARP, high mutant loads modify the phenotype from a mild neuromuscular disorder to a severe or even fatal encephalopathy called Leigh syndrome (Schaefer et al. 2008). Lower levels (~50%) of a point mutation in the brain, otherwise causing MELAS, can also lead to Leigh syndrome (Kirby et al. 2003). A point mutation in a tRNA gene with a mutant load of <25% can also lead to severe multisystem disorder and respiratory chain deficiency (Sacconi et al. 2008). Therefore, markedly different levels of mutant load can lead to the onset of mtDNA disease.

Defects in sperm mtDNA

Unlike mature somatic cells and oocytes, spermatozoa possess few mitochondria. They have between 22 to 28 mitochondria, which are located in the midpiece in a helical manner (Otani et al. 1988). Somatic cells and oocytes are vastly different from spermatozoa, as they have larger numbers of mitochondria that are present within their cytoplasm and that communicate with the nucleus prior to cell division and then disperse into the peripheral regions of the cytoplasm at other stages of the cell cycle (Lee et al. 2007). Interestingly, the helical arrangement of mitochondria is only indicative of mature sperm. During spermatogenesis, the mitochondria are present within the cytoplasm in a manner similar to those in somatic cells (de Sousa Lopes and Roelen 2010). Nevertheless, as for somatic cells and oocytes, sperm mitochondria possess mtDNA.

The maternally inherited mtDNA mutations have been demonstrated to have an effect on sperm quality and motility (Folgerø et al. 1993; Spiropoulos et al. 2002). Interestingly, Folgerø et al. (1993) have demonstrated that the effects of such mutations can be overcome by supplementing the sperm with a metabolite (succinate) that can circumvent the mutation to Complex I of the electron-transfer chain. Although the 4977-bp "common" deletion, which excises about a third of the mitochondrial genome resulting in the loss of five genes, has been argued to be representative of poor semen and poor

sperm quality (Kao et al. 1995), others have not been able to demonstrate this in poor semen or poor sperm samples (Cummins et al. 1998; St John et al. 2001). However, it is evident that multiple large-scale deletions can affect sperm and semen quality (Lestienne et al. 1997; Reynier et al. 1997). In each case, these rearrangements to the mitochondrial genome affect the quality of the semen with affected men being diagnosed as having poor sperm motility (asthenozoospermic), poor sperm morphology (teratozoospermia), poor sperm counts (oligozoospermia), combinations of these or even all three symptoms. Furthermore, naturally occurring mtDNA variants, which are associated with specific populations of individuals arising from the same maternal lineage that give rise to mtDNA haplotypes, can also have an affect on sperm motility (Ruiz-Pesini et al. 2000). The other mtDNA association with sperm and semen quality is the number of mtDNA copies in a sperm sample. For instance, poor quality sperm, surprisingly, have more copies of mtDNA than sperm from men with normal semen parameters (Amaral et al. 2007).

Defects in oocyte mtDNA

A number of studies have also analysed oocyte mitochondrial genomes for rearrangements (Blok et al. 1997). However, many of these have been in the context of the segregation (discussed below) of known mtDNA defects related to mitochondrial disease. Nevertheless, these defects appear not to lead to the failure of an oocyte to be fertilised, to form an embryo, to implant or to give rise to an offspring, otherwise there would be no maternally inheritable mtDNA disease. Indeed, it is argued that mutated mtDNA is eliminated or fixed in the female germline and that this relates directly to the severity of the mutation (Fan et al. 2008; Stewart et al. 2008). Consequently, a mutation that is lethal will not be transmitted with the likelihood that these oocytes do not mature.

Nevertheless, mitochondrial rearrangements do seem to play a role in the aging process of the oocyte. The most commonly observed rearrangement is the 4977-bp deletion. This "common" deletion is inherited in maternal fashion but can also arise as part of the aging process. Its presence is, however, variable in oocytes with some studies suggesting a clearly identifiable link with an increase in age (Chan et al. 2005; Keefe et al. 1995), whereas others have reported no discernible link (Barritt et al. 1999; Brenner et al. 1998; Chen et al. 1995; Hsieh et al. 2002). Although the deletion might be present in a number of oocytes from patients seeking in vitro fertilisation (IVF; Barritt et al. 1999), it needs to be in present at extremely high levels for it to have a discernible affect. Furthermore, if one common deletion is present, then a number of other large-scale deletions might also be present. Interestingly though, there appears to be a significant increase in the common deletion in oocytes and embryos following the administration of gonadotrophins as part of superovulation programmes (Gibson et al. 2005). Furthermore, the levels of the 4977-bp deletion have been shown to be higher in the cumulus cells of older women (aged 34 years or older) undergoing IVF treatment than in younger women and this is correlated negatively with pregnancy rates (Tsai et al. 2010). Nevertheless, oocytes do not seem to accumulate the large number of mtDNA deletions that have been observed in sperm (Reynier et al. 1998) suggesting that male gametes are predisposed to carrying deleterious mutations as a means of limiting the effectiveness of their mitochondrial genomes to a functional basis only (Innocenti et al. 2011).

Previously, the mitochondrial genome was assumed to reside in the mitochondrion in a "naked" fashion, unprotected by protein-binding complexes and exposed to free radicals that would mediate a number of mutations or large-scale deletions (Ozawa 1995). Now, significant evidence exists to suggest that mtDNA is packaged by mitochondrial transcription factor A (TFAM; Kaufman et al. 2007) and perhaps other members of the mitochondrial nucleoid (discussed below). Therefore, the high mutation rate in mtDNA is probably linked to its increased propensity for replication, which is independent of the cell cycle (Clayton 1992) and to the proofreading efficiency of the mitochondrial-specific polymerase gamma (POLG).

As is the case for sperm mtDNA, there is a close association between the oocyte mtDNA copy number and fertilisation outcome. Several studies in mammals have shown that, at ovulation, metaphase II fertilisable oocytes possess between 1.9×10^5 to 3×10^5 mtDNA copies of mDNA (Chen et al. 1995; Santos et al. 2006; Reynier et al. 2001; Steuerwald et al. 2000; May-Panloup et al. 2005a; Spikings et al. 2007) and that each mitochondrion consists in one mitochondrial genome (Satoh and Kuroiwa 1991). Observations in the pig (El Shourbagy et al. 2006) and human (Santos et al. 2006; Reynier et al. 2001; May-Panloup et al. 2005a) indicate a significant difference in the mtDNA copy number between oocytes that successfully fertilise and those that fail. Furthermore, significantly more mitochondria are present in fully grown porcine oocytes when compared with those that are not (Spikings et al. 2007). Similarly, ovarian insufficiency is associated with lower mtDNA content (May-Panloup et al. 2005b), as are the cumulus cells surrounding the oocytes of women of increasing age, both of which have impacts on pregnancy rates (Tsai et al. 2010) .

MtDNA replication

Replication of mtDNA follows transcription of the mitochondrial genome (Clayton 1992). MtDNA replication is

initiated by TFAM, which generates a primer that is used by the catalytic subunit of POLG, namely POLGA, to copy mtDNA (Shadel and Clayton 1997). Replication is supported by three other factors: POLGB, an accessory subunit to POLGA; the mitochondrial single-stranded DNA-binding protein; and the helicase Twinkle (Kucej and Butow 2007). These factors are bound to the mitochondrial genome and form the mitochondrial nucleiod (Kucej and Butow 2007), which consists in a central core and a peripheral region. The proteins within the central core are involved in mtDNA replication and transcription, whereas translation and protein assembly are mediated by the proteins in the peripheral region (Bogenhagen et al. 2008). Furthermore, evidence suggests that TFAM has an anchoring and packaging role, since it is a member of the high mobility group and is located in distinct regions of the mitochondrial genome (Kaufman et al. 2007).

Nevertheless, the expression of mtDNA and the generation of ATP through OXPHOS can occur only when mtDNA is continuously replicated (Trounce 2000). This is essential for high-energy-requiring cells, such as nerve, heart and muscle cells, which rely extensively on OXPHOS to mediate their complex and energy-demanding cellular functions (Moyes et al. 1998). For these cells, OXPHOS is the most appropriate process by which to produce energy as it generates 32 molecules of ATP, compared with every two molecules generated by its anaerobic counterpart, glycolysis (Pfeiffer et al. 2001). Consequently, the mtDNA copy number is an effective indicator of the competency of OXPHOS to supply the cell with ATP.

MtDNA replication during development

MtDNA replication events can be divided into four key phases during differentiation and/or development, phases that are likely to be the key regulators of mtDNA transmission in a range of large mammals, including cattle, pig, sheep, monkeys and human (Fig. 4). These are the periods during oogenesis, preimplantation development, pregastrulation and post-implantation development.

Phase I—during oogenesis Primordial germ cells possess ~200 copies of mtDNA (Cao et al. 2007; Cree et al. 2008; Wai et al. 2008) and these copies are clonally expanded during oogenesis (Fig. 4). Observations in the human (Santos et al. 2006; Reynier et al. 2001; May-Panloup et al. 2005b), pig (El Shourbagy et al. 2006) and other species (May-Panloup et al. 2005a; Thundathil et al. 2005) have demonstrated the importance of the mtDNA copy number to fertilisation outcome. Developmentally competent and incompetent oocytes can be selected based on their ability to reduce the dye, brilliant cresyl blue (BCB). Consequently, at the beginning of in vitro culture (0 h; equivalent to the 1st oocyte in the red box in Fig. 5), competent oocytes suppress the activity of the enzyme glucose-6-phosphate dehydrogenase and cannot reduce BCB (i.e. are BCB^+), whereas incompetent oocytes continue to express active glucose-6-phosphate dehydrogenase and thus reduce BCB (i.e. are BCB^-). BCB^+ oocytes have >200,000 copies of mtDNA at 0 h of in vitro maturation, progress to metaphase II (42–44 h) and develop as embryos once fertilised (Spikings et al. 2007; El Shourbagy et al. 2006), whereas BCB^- oocytes have significantly fewer copies (<100,000) at 0 h and either fail to fertilise or arrest during preimplantation development (Spikings et al. 2007; El Shourbagy et al. 2006). By 42–44 h, BCB^+ oocytes also exhibit decreased expression of the mtDNA replication factors (Spikings et al. 2007) suggesting that mtDNA replication is complete and the population of mtDNA in the oocyte is fixed for transmission. Nevertheless, during 42 h of in vitro culture, a notable biphasic change occurs in the mtDNA copy number where there is a significant decrease in the mtDNA copy number in BCB^+ oocytes by 24 h but numbers are reinstated by 42 h. These changes in the mtDNA copy number are key events in oocyte maturation and are essential to determining the population of mtDNA that is transmitted to the offspring.

BCB^- oocytes only initiate mtDNA replication at 42 h suggesting that nuclear and cytoplasmic maturation are not synchronised in these oocytes (Spikings et al. 2007; El Shourbagy et al. 2006). The importance of the mtDNA copy number has been verified by supplementing BCB^- oocytes at 0 h of in vitro maturation with mitochondria from BCB^+ oocytes; this results in fertilisation rates comparable with non-supplemented BCB^+ oocytes (El Shourbagy et al. 2006). As embryos have been been hypothesised to undergo embryonic developmental arrest because their oocytes possess too few or less functional mitochondria (Cohen et al. 1997), the supplementation of oocytes with autologous sources of mtDNA might prove an effective approach and circumvent the problems associated with heteroplasmy that has arisen from cytoplasmic transfer (Cohen et al. 1997; St John et al. 2010; Barritt et al. 2001).

Phase II—preimplantation development In mouse and pig embryos, an mtDNA replication event occurs between fertilisation and the two-cell stage (Spikings et al. 2007). In larger mammals, a highly significant reduction then takes place in mtDNA content between the four- and 16-cell stages (May-Panloup et al. 2005a; Spikings et al. 2007) with each newly divided blastomere having fewer copies of mtDNA (El Shourbagy et al. 2006). This reduction in the mtDNA copy number during early development coincides with the generation of ATP by glycolysis rather than OXPHOS (Van Blerkom 2004) and is matched by low levels of expression of the mtDNA-encoded genes (Pikó and Taylor 1987) and nuclear-encoded mtDNA replication factors (Spikings et al. 2007; Bowles et al. 2007). At the

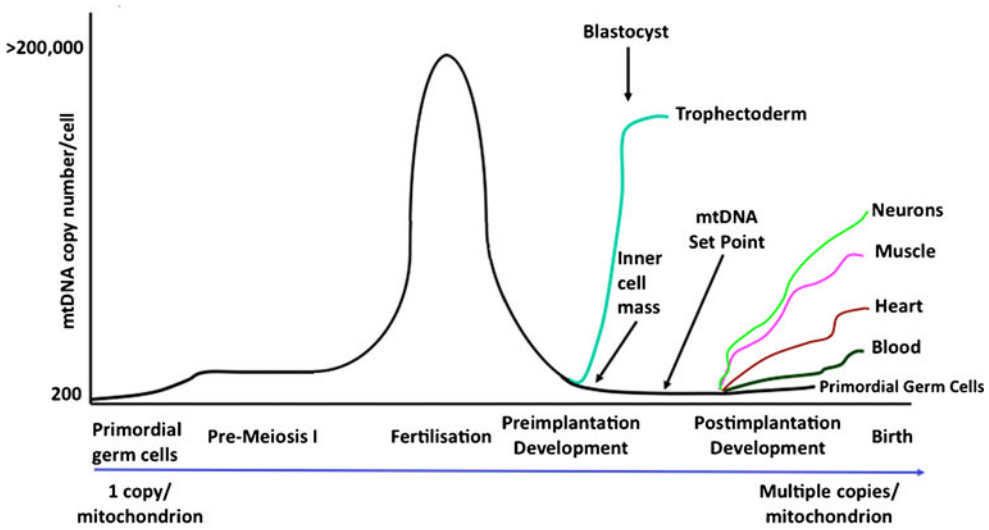

Fig. 4 Regulation of the mtDNA copy number during development. The mtDNA copy number increases during oogenesis but decreases during preimplantation development. At the blastocyst stage, replication is initiated in the trophectoderm. The inner cell mass cells continue to reduce the copy number and establish the "mtDNA set point". When cells differentiate into specialised cell types, they increase the copy number to match their needs for ATP

blastocyst stage, the mtDNA copy number significantly increases in all mammals so far investigated (May-Panloup et al. 2005a; Spikings et al. 2007; Thundathil et al. 2005; Bowles et al. 2007) matched by the increased expression of POLGA (Spikings et al. 2007; Bowles et al. 2007) and the embryo's increased requirement for OXPHOS-derived ATP (Houghton 2006). These increases are restricted to the trophectoderm (Spikings et al. 2007; Houghton 2006), which gives rise to the placenta. The inner cell mass cells, which are pluripotent and give rise to the embryo and fetus, have considerably reduced expression of the mtDNA replication factors (Spikings et al. 2007), maintain undifferentiated mitochondria and continue to generate ATP through glycolysis (Houghton 2006). Whereas in the porcine (Spikings et al. 2007) and bovine (May-Panloup et al. 2005a) embryo, the mtDNA copy number decreases significantly, in the mouse, it remains constant during preimplantation development (Thundathil et al. 2005).

Phase III—pre-gastrulation As distinct mtDNA replication events occur during preimplantation development, it is perhaps most useful to analyse pre-gastrulation events by using embryonic stem cells. They originate from the inner cell mass of the blastocyst-stage embryo (Evans and Kaufman 1981). The analysis of three murine embryonic stem cell lines, which express the key members of the pluripotency gene network, Oct4, Sox2 and Nanog (Facucho-Oliveira et al. 2007; Facucho-Oliveira and St John 2009), suggests that the inner cell mass cells, which contain >1000 mtDNA (Cao et al. 2007) continue to dilute their mtDNA copy number as they give rise to embryonic stem cells. Embryonic stem cells have 30–50 copies of mtDNA/cell and low levels of expression of the mtDNA replication factors and depend on glycolysis rather than OXPHOS for energy production (Facucho-Oliveira et al. 2007; Facucho-Oliveira and St John 2009). This suggests that any mtDNA replication is associated with the repopulation of each newly divided cell and that a minimum number of mtDNA copies is retained.

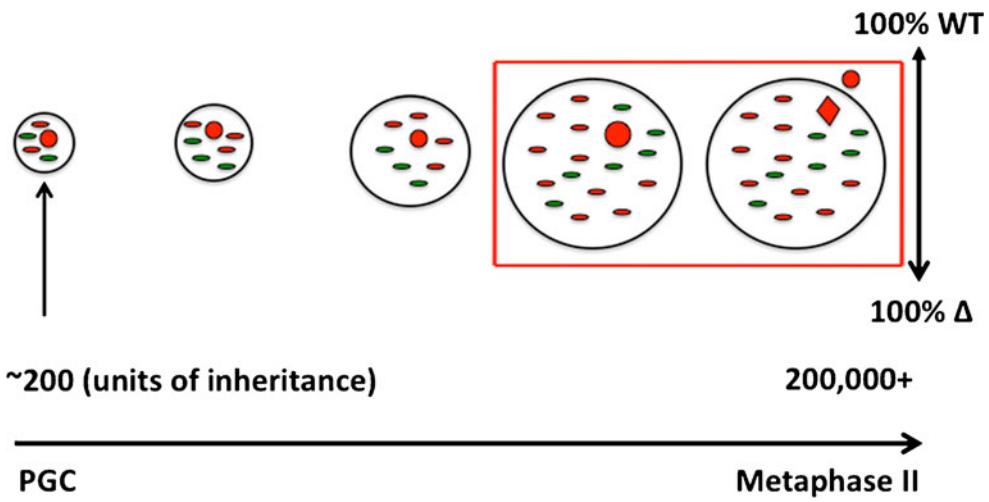

Fig. 5 MtDNA expansion during oogenesis. MtDNA is randomly segregated to the primordial germ cells (*PGC*). As the mtDNA copy number is expanded during development, the degree of heteroplasmy in a cohort of mature metaphase II oocytes cannot be predicted. During later stages of oogenesis, wild type (*WT*) or mutant (*Δ*) molecules might be preferentially replicated (*red box* in vitro maturation, *red diamond* spindle)

Indeed, the relationship between pluripotency and the mtDNA copy number is likely to be finely balanced, as the inhibition of *PolgA* expression results in the loss of pluripotency and the onset of cellular differentiation (Facucho-Oliveira et al. 2007).

Phase IV—post-implantation development The ongoing dilution of mtDNA prior to gastrulation establishes the "mtDNA set point" (Facucho-Oliveira et al. 2007; Facucho-Oliveira and St John 2009). The mtDNA set point provides a small founder population of mtDNA that allows any undifferentiated cell progressing to organogenesis to accumulate the appropriate numbers of mtDNA to meet its newly specialised function (St John et al. 2010; see Fig. 4). For example, human skeletal muscle and cardiac cells have ~6800 and 3650 copies/cell, respectively (Miller et al. 2003), which differ significantly from the ~10 copies in mature human sperm (Amaral et al. 2007). These key mtDNA replication and restriction events will affect the distribution and transmission of heteroplasmic molecules to the offspring and could thus be a main determiner of the way in which the offspring acquires mtDNA, especially when levels have been sufficiently low in previous generations to prevent any observable phenotypic symptoms.

The fate of sperm mtDNA

In the vast majority of cases, mammals inherit their mtDNA from the population of mtDNA present in the oocyte at fertilisation (Birky 1995; Giles et al. 1980). Sperm mtDNA was previously thought to be simply eliminated through dilution during preimplantation and post-implantation development or not even to enter the oocyte at fertilisation (Ankel-Simons and Cummins 1996). Nevertheless, sperm contributes a few copies of the mitochondrial genome (Otani et al. 1988; Amaral et al. 2007; Song and Lewis 2008; Pavili et al. 2010) but these account for less than 0.005 % of the zygote's mtDNA content following fertilisation (Amaral et al. 2007). They are packaged within mitochondria arranged end-to-end in a helical fashion in the midpiece. The elimination of sperm mtDNA is a targeted process of destruction in which it is actively eliminated by the process of ubiquitination mediated by the cytoplasm of the oocyte (Sutovsky et al. 1999).

Another proposal for the elimination of sperm mitochondria is a two-step process in which a gradual loss of the mitochondrial-specific nucleoids occurs during spermatogenesis followed by the fairly imminent digestion of sperm mtDNA just after fertilisation (Nishimura et al. 2006). Both the digestion and ubiquitination mechanisms are supported by the evidence of the loss of sperm mtDNA content during preimplantation development (Cummins et al. 1997). In this instance, sperm mtDNA is present up to the pronucleus stage following the intraspecific crossing of mice. Studies of other species also suggest that the elimination process takes place prior to embryonic genome activation and is thus an oocyte-only mediated event (Sutovsky et al. 1999; St John and Schatten 2004). However, those mice generated through interspecific crossings are able to transmit sperm mtDNA through to the neonate (Kaneda et al. 1995). Although sperm mtDNA is detectable in the offspring, it is not transmitted to subsequent generations (Gyllensten et al. 1991).

The transmission of sperm mtDNA has now been detected in a wide-range of species following interspecific crossing, including fruit flies (Kondo et al. 1990), honeybees (Meusel and Moritz 1993), birds (Kvist et al. 2003), sheep (Zhao et al. 2004) and non-human primates (St John and Schatten 2004) and following intra-specific crossing of mussels (Zouros et al. 1992) and fruitflies (Kondo et al. 1990). Consequently, the mechanism of elimination is likely to be highly specific and targeted to sperm mitochondria, as somatic cell mitochondria that have been introduced into the oocyte are transmitted and are thus not targeted for destruction (Shitara et al. 2000). Nevertheless, in humans, sperm mtDNA has been detected in a few polyploid IVF-derived blastocysts (St John et al. 2000). This is especially worrying as a male patient has been diagnosed as having a mitochondrial myopathy with 90% of his muscle tissue mtDNA being inherited from his father's mtDNA together with a de novo 2-bp deletion that gave rise to his myopathy (Schwartz and Vissing 2002). Whereas there is considerable speculation as to how the 2-bp deletion arose (Kraytsberg et al. 2004), sperm mtDNA is evidently highly susceptible to mtDNA mutation (Folgerø et al. 1993; Spiropoulos et al. 2002) and deletion (St John et al. 2001; Lestienne et al. 1997; Reynier et al. 1997).

Maternal inheritance, mtDNA segregation and the bottleneck

During the course of evolution, the oocyte, embryo and fetus have developed processes for regulating the transmission of mtDNA. This ensures that the offspring can generate ATP at maximum efficiency (Innocenti et al. 2011). Primarily, mammalian mtDNA is maternally inherited and, in most cases, is uniform in its transmission to those offspring derived from the same mother (Birky 1995). Each primordial oocyte has approximately 200 copies of the mitochondrial genome (Cao et al. 2007; Cree et al. 2008; Wai et al. 2008; Fig. 5) and only one copy is assumed to be present per mitochondrion (Pikó and Taylor 1987). These are clonally amplified during oogenesis yielding a 45-fold increase in the mature oocyte (Smith and Alcivar 1993; see Fig. 5). Furthermore, these molecules are often identical and the resultant offspring are thus homoplasmic.

Metaphase II oocytes most likely possess a high mtDNA copy number as an investment in the future development of the embryo, fetus and the offspring. This is evidenced by the early death of homozygous knockout mice for TFAM (Larsson et al. 1998) and PolgA (Hance et al. 2005); such mice have low numbers of mtDNA copy in the oocytes from which they arise and die in utero. Although mouse preimplantation embryos maintain similar levels of copy number throughout development (Thundathil et al. 2005), they reduce their copy number later as they progress to gastrulation. This occurs similarly but at an earlier stage in porcine and bovine development (May-Panloup et al. 2005a; Spikings et al. 2007). These are most likely the mitochondrial bottlenecks that restrict the transmission of mtDNA to the fetus and offspring. However, they are not effective filters as mutant mtDNA transits through and is subsequently randomly segregated to some or all of the tissues. It is evident from the cleaving embryo that the anomalies in mtDNA segregation could also arise from the variable distribution of mitochondria to each newly divided blastomere, thereby skewing the segregation of mutant mtDNA (Van Blerkom et al. 2000). Indeed, there is also an unequal distribution of mtDNA in blastomeres of preimplantation embryos (El Shourbagy et al. 2006).

Interest in the segregation and transmission of mutant mtDNA through the oocyte first came to our attention following the observations that variants in Holstein cattle became rapidly fixed within a few generations (Hauswirth and Laipis 1982). This led to the mitochondrial bottleneck hypothesis being proposed and being used by others to account for the considerable variations in mutant mtDNA transmitted to the next generation from the small "founder" population of mitochondrial genomes present in the primordial germ cells (Marchington et al. 1997). Others have argued that the "bottleneck" acts a purification process for mtDNA (Jenuth et al. 1997; Bergstrom and Pritchard 1998) allowing any variant to be tested by Darwinian natural selection processes. This would entail failing to complete maturation in the most severe cases (Fan et al. 2008; Stewart et al. 2008; Wallace 2007) or allowing these molecules to pass through, resulting in the almost immediate death of the offspring once it has to survive without the mother's endometrial support post-parturition. This would thus explain why we observe older offspring with severely debilitating but not lethal phenotypes. The latter outcome is probably the most likely considering the wide-ranging levels of mutant mtDNA that have been observed in the cohort of oocytes of a carrier of a mitochondrial disease (Blok et al. 1997).

Potential assisted reproductive techniques to prevent the transmission of mutant mtDNA

For women who are carriers of mtDNA disease or for those women who are unable to achieve a viable ongoing pregnancy, some of the assisted reproductive technology options are very similar. These technologies rely on the transfer of chromosomes from one oocyte to another and are effectively an exploitation of the cloning technology that led to "Dolly the Sheep" (Wilmut et al. 1997).

Preimplantation genetic diagnosis, which is available to potentially all in vitro fertilisation patients, assesses whether an embryo is carrying a specific genetic defect that would be harmful. For the carriers of mitochondrial disease, it has limitations for determining the potential transmission of mutant mtDNA (St John et al. 2010). Preimplantation genetic diagnosis often relies on analysing a single blastomere that is representative of the embryo (Chiaratti et al. 2011; Gigarel et al. 2011). It is however only likely to be successfully used for carriers of the NARP mutation as the segregation of the NARP mutation tends to result in high or low mutant loading, which is not the case for the other mtDNA disorders (Schaefer et al. 2008). Consequently, the embryos from many women will fall into a "non-determinable" zone and potentially pose a risk if they are transferred. As a result, these women would have to undergo later-stage genetic diagnosis, such as amniocentesis when they could choose to terminate the pregnancy (St John and Campbell 2010).

The recent advances in assisted reproductive technologies provide opportunities to generate offspring free of their mother's transmissible mutant mtDNA. The two proposed approaches are metaphase II spindle transfer (MII-ST) and pronuclear transfer (PNT), which are described in Fig. 6. MII-ST has been successfully used to generate cattle (Bao et al. 2003), mouse (Wang et al. 2001) and monkey offspring (Tachibana et al. 2009). Although the cattle and mouse studies did not attempt to determine the mtDNA content in their offspring, the monkey studies suggested that the resultant offspring were homoplasmic for recipient oocyte mtDNA. These findings should be treated with caution as the assays were not sensitive enough to detect low levels of mtDNA and only a few tissues were analysed. There was also no evidence to support the conclusion that no mtDNA accompanied the spindle as it was transferred (St John and Campbell 2010). This latter aspect is especially unconvincing since mitochondria cluster around the metaphase II spindle of oocytes prior to fertilisation (Dumollard et al. 2008) clearly indicating that it would be extremely difficult to transfer the metaphase II spindle without carrying over some of this mitochondrial shroud. Furthermore, the metaphase II spindle is susceptible to damage, as the nuclear envelope will have been broken down at the germinal vesicle stage (Jones 2007).

PNT has produced live mice. Donor karyoplast mtDNA was transmitted and ranged from 0% to 69% of the offspring's total mtDNA content (Meirelles and Smith 1997). More recently, two human pronuclei from clinically discarded three-pronuclei zygotes were transferred into enucleated zygotes, which resulted in blastocyst-stage embryos (Craven et al. 2010).

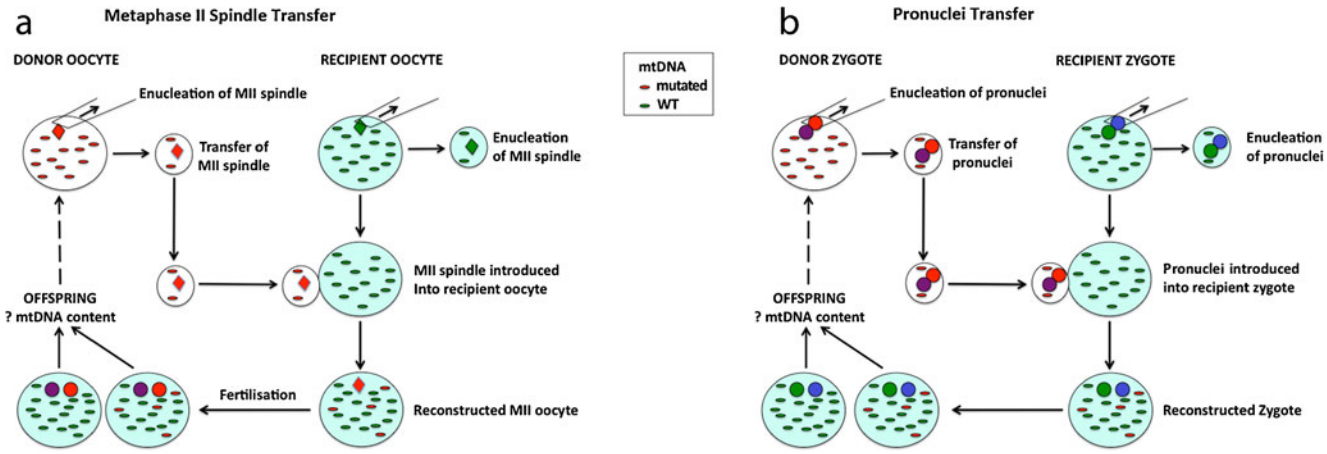

Fig. 6 Proposed assisted reproductive technologies to prevent the transmission of mutant mtDNA from one generation to the next. **a** Metaphase II spindle transfer (MII-ST). The metaphase II spindle containing the mother's chromosomes is removed from the mature oocyte of a carrier of mtDNA disease. It is then transferred into an enucleated oocyte from an oocyte donated by a non-carrier of mtDNA disease (recipient). The reconstructed oocyte is fertilised and cultured in vitro and transferred to the uterus of the "chromosomal" mother. **b** Pronuclear transfer. The male and female pronuclei of a fertilised oocyte are transferred to a "non-affected" recipient zygote, which is then cultured in vitro as for MII-ST. In each case, mtDNA accompanying the karyoplast can be transferred and transmitted. Modified from St John and Campbell (2010)

However, the amount of accompanying mtDNA influenced the segregation of mtDNA during the early stages with levels varying between <0.5% and 11.4%. This technique appears to be equally susceptible to the transfer of mitochondria, as these organelles will still surround the pronuclei to be transferred.

Lessons to be learnt from embryonic and somatic cell nuclear transfer

Embryonic cell and somatic cell nuclear transfer are similar to MII-ST and PNT, except that a cell from a preimplantation embryo or a somatic cell is introduced into an enucleated oocyte. The outcomes provide significant insights into the potential problems arising from MII-ST and PNT. Nuclear transfer poses two major problems in respect of mtDNA transmission. First, both embryos and offspring can harbour mtDNA from the donor cell. Reported contributions of donor mtDNA to the total mtDNA content range from 0% to 63% for embryos (Meirelles et al. 2001) and 0% to 59% for offspring (Takeda et al. 2003). However, the persistence of donor mtDNA appears to be random and independent of whether intra- or inter-specific or cross-species nuclear transfer is performed (St John et al. 2004). For example, donor mtDNA has been detected in bovine embryos derived by both intra-specific (Steinborn et al. 1998) and inter-specific (Meirelles et al. 2001) nuclear transfer, although not in all cases (Meirelles et al. 2001; Takeda et al. 2003). Donor mtDNA has also been detected in porcine offspring (Takeda et al. 2006) derived by inter-specific nuclear transfer. Following cross-species nuclear transfer, donor mtDNA can survive up to the 16-cell stage in human-bovine crosses (Chang et al. 2003) and persists in macaque-rabbit blastocysts (Yang et al. 2003). Second, mtDNA is not transmitted through the maternally related oocyte but rather through an undetermined surrogate oocyte. This results in the "clone" lacking true genetic identity.

Why does the donor cell mtDNA persist? The mtDNA present in the donor cell accompanies the chromosomes as it is introduced into the recipient oocyte and persists. This is caused by the failure of the embryonic genome to silence the expression of the mtDNA-specific nuclear-encoded replication factors during early preimplantation development (Bowles et al. 2007), unlike fertilised-derived embryos (Spikings et al. 2007; Bowles et al. 2007). When donor cell mtDNA is depleted to 0.002% of its original content, it also persists in hatched blastocysts (Lloyd et al. 2006). Consequently, these molecules are present just before gastrulation and are available for segregation to some or all of the cells of the fetus and for replication during organogenesis. Serial nuclear transfer, which is a combination of somatic cell nuclear transfer followed by PNT, results in the transfer of mtDNA from the recipient oocyte and that of the zygote to the offspring (St John et al. 2005). For somatic cell nuclear transfer, this has been overcome by completely depleting the donor cell of its mtDNA prior to transfer into an enucleated recipient oocyte (Bowles et al. 2007; Lloyd et al. 2006). The resultant sheep were homoplasmic for the recipient oocyte's mtDNA only (Lee et al. 2010).

The relationship between the donor cell's and the recipient oocyte's mtDNA Compatibility between the karyoplast's and the enucleated recipient oocyte's mtDNA is essential for

successful development. First, the donor karyoplast and the enucleated recipient oocyte need to be from the same stage of development (Liu et al. 1999). Second, enhanced developmental outcome has been demonstrated when the mtDNA content of the donor cell and the recipient oocyte is from an unrelated source, i.e. another haplotype (Bowles et al. 2007, 2008). Indeed, mtDNA haplotypes can confer both positive and negative traits on offspring. For example, they can affect milk quality in Holstein cows (Brown et al. 1989), growth and physical performance in mice (Nagao et al. 1998), fertility in beef cattle (Sutarno et al. 2002) and pigs (El Shourbagy et al. 2006) and sperm motility in men (Ruiz-Pesini et al. 2000). Experimental investigations have shown that interactions between the chromosomal genome and a different mitochondrial haplotype can affect developmental outcomes. For example, certain cow mtDNA haplotypes tend to result in improved outcomes when oocytes are fertilised, whereas others favour somatic cell nuclear transfer (Bruggerhoff et al. 2002; Tamassia et al. 2004).

Nevertheless, the importance of donor cell and recipient oocyte mtDNA compatibility is demonstrated by the observations of the subtle anomalies occurring at the functional level in cross-species somatic cell hybrids. In respect of mouse-rat fusions, rat mtDNA is efficiently replicated, transcribed and translated by murine nuclear-encoded transcription and replication factors. However, OXPHOS function, unlike that in murine-murine fusions, is compromised (McKenzie et al. 2003; McKenzie and Trounce 2000). Furthermore, recent observations in interspecific bovine and porcine NT offspring have indicated the possibility of a similar occurrence for these species. Here, the transmission of both donor cell and recipient oocyte mtDNA results in sequence variation at specific sites, which will encode for different amino acids within one offspring (St John et al. 2005; Steinborn et al. 2002). Consequently, we predict that: (1) the mixing would occur of nuclear-encoded proteins from one species with an mtDNA genome from another and (2) the mixing of diversely encoding mtDNA genomes would result in a dysfunctional electron-transfer chain and decreased levels of ATP. This would explain the developmental arrest in caprine-sheep embryos at the 20+ cell stage (Bowles et al. 2007).

Epigenetic modifications Somatic cell nuclear transfer is associated with a range of developmental abnormalities (Cibelli et al. 2002). These include incomplete or inappropriate epigenetic reprogramming of the donor chromosomes (Morgan et al. 2005) resulting in abnormal gene expression (Meirelles and Smith 1997). Consequently, studies of karyoplast transfer need to clearly address any epigenetic modifications that may occur during development, otherwise we may be eliminating mtDNA disease but generating epigenetic disorders, as have been reported following other human assisted reproductive technologies (Cox et al. 2002; DeBaun et al. 2003). This would explain the continued expression of TFAM and PolgA in cloned embryos.

Consequently, the transfer of chromosomes from one oocyte or zygote to another is fraught with many problems. These problems are initiated from the moment that the chromosomes are transferred, as there is a substantial risk that mitochondria will accompany the chromosomes. Not only can this lead to the mixing of mitochondrial genomes but other potential deleterious outcomes might occur; whether such aspects are detrimental to the offspring's health and survival remains to be determined. Nevertheless, it may be possible to deplete the oocyte of its mtDNA content (Spikings et al. 2007) and then transfer the chromosomes from this oocyte into an enucleated oocyte, as demonstrated in interspecies somatic cell nuclear transfer (Jiang et al. 2011).

Concluding remarks

It is clearly evident that mtDNA transmission, inheritance and replication are strictly regulated during development. In terms of reproductive and development biology, these events are extremely important for the genetic integrity of the offspring, gamete quality and the offspring's ability to function effectively as an individual. As reproductive technologies advance, they might lead to considerable progress that would allow us to develop specific genetic traits or to rescue endangered species or perhaps even to prevent the transmission of mutant mtDNA. However, these technologies might also result in the mixing of mitochondrial genotypes, so that they may equally be deleterious. Consequently, extreme caution is required if these techniques, which modulate mitochondrial genotypes, are to be effectively used.

Acknowledgments Professor Justin St John would like to acknowledge the support of the Victorian Government's Operational Infrastructure Support Program.

References

Amaral A, Ramalho-Santos J, St John JC (2007) The expression of polymerase gamma and mitochondrial transcription factor A and the regulation of mitochondrial DNA content in mature human sperm. Hum Reprod 22:1585–1596

Anderson S, Bankier AT, Barrell BG, Bruijn MH de, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Ankel-Simons F, Cummins JM (1996) Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. Proc Natl Acad Sci USA 93:13859–13863

Bao S, Ushijima H, Hirose A, Aono F, Ono Y, Kono T (2003) Development of bovine oocytes reconstructed with a nucleus from growing stage oocytes after fertilization in vitro. Theriogenology 59:1231–1239

Barritt JA, Brenner CA, Cohen J, Matt DW (1999) Mitochondrial DNA rearrangements in human oocytes and embryos. Mol Hum Reprod 5:927–933

Barritt JA, Brenner CA, Malter HE, Cohen J (2001) Rebuttal: interooplasmic transfers in humans. Reprod Biomed Online 3:47–48

Bergstrom CT, Pritchard J (1998) Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. Genetics 149:2135–2146

Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA (1981) Sequence and gene organization of mouse mitochondrial DNA. Cell 26:167–180

Birky CW Jr (1995) Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc Natl Acad Sci USA 92:11331–11338

Blok RB, Gook DA, Thorburn DR, Dahl HH (1997) Skewed segregation of the mtDNA nt 8993 (T→G) mutation in human oocytes. Am J Hum Genet 60:1495–1501

Bogenhagen DF, Rousseau D, Burke S (2008) The layered structure of human mitochondrial DNA nucleoids. J Biol Chem 283:3665–3675

Boulet L, Karpati G, Shoubridge EA (1992) Distribution and threshold expression of the tRNA(Lys) mutation in skeletal muscle of patients with myoclonic epilepsy and ragged-red fibers (MERRF). Am J Hum Genet 51:1187–1200

Bowles EJ, Lee JH, Alberio R, Lloyd RE, Stekel D, Campbell KH, St John JC (2007) Contrasting effects of in vitro fertilization and nuclear transfer on the expression of mtDNA replication factors. Genetics 176:1511–1526

Bowles EJ, Tecirlioglu RT, French AJ, Holland MK, St John JC (2008) Mitochondrial DNA transmission and transcription after somatic cell fusion to one or more cytoplasts. Stem Cells 26:775–782

Brenner CA, Wolny YM, Barritt JA, Matt DW, Munné S, Cohen J (1998) Mitochondrial DNA deletion in human oocytes and embryos. Mol Hum Reprod 4:887–892

Brown DR, Koehler CM, Lindberg GL, Freeman AE, Mayfield JE, Myers AM, Schutz MM, Beitz DC (1989) Molecular analysis of cytoplasmic genetic variation in Holstein cows. J Anim Sci 67:1926–1932

Brüggerhoff K, Zakhartchenko V, Wenigerkind H, Reichenbach HD, Prelle K, Schernthaner W, Alberio R, Küchenhoff H, Stojkovic M, Brem G, Hiendleder S, Wolf E (2002) Bovine somatic cell nuclear transfer using recipient oocytes recovered by ovum pick-up: effect of maternal lineage of oocyte donors. Biol Reprod 66:367–373

Cao L, Shitara H, Horii T, Nagao Y, Imai H, Abe K, Hara T, Hayashi J, Yonekawa H (2007) The mitochondrial bottleneck occurs without reduction of mtDNA content in female mouse germ cells. Nat Genet 39:386–390

Chan CC, Liu VW, Lau EY, Yeung WS, Ng EH, Ho PC (2005) Mitochondrial DNA content and 4977 bp deletion in unfertilized oocytes. Mol Hum Reprod 11:843–846

Chang KH, Lim JM, Kang SK, Lee BC, Moon SY, Hwang WS (2003) Blastocyst formation, karyotype, and mitochondrial DNA of interspecies embryos derived from nuclear transfer of human cord fibroblasts into enucleated bovine oocytes. Fertil Steril 80:1380–1387

Chen X, Prosser R, Simonetti S, Sadlock J, Jagiello G, Schon EA (1995) Rearranged mitochondrial genomes are present in human oocytes. Am J Hum Genet 57:239–247

Chiaratti MR, Meirelles FV, Wells D, Poulton J (2011) Therapeutic treatments of mtDNA diseases at the earliest stages of human development. Mitochondrion 11:820–828

Cibelli JB, Campbell KH, Seidel GE, West MD, Lanza RP (2002) The health profile of cloned animals. Nat Biotechnol 20:13–14

Clayton DA (1992) Transcription and replication of animal mitochondrial DNAs. Int Rev Cytol 141:217–232

Cohen J, Scott R, Schimmel T, Levron J, Willadsen S (1997) Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. Lancet 350:186–187

Cox GF, Burger J, Lip V, Mau UA, Sperling K, Wu BL, Horsthemke B (2002) Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet 71:162–164

Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, Murdoch AP, Chinnery PF, Taylor RW, Lightowlers RN, Herbert M, Turnbull DM (2010) Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. Nature 465:82–85

Cree LM, Samuels DC, Sousa Lopes SC de, Rajasimha HK, Wonnapinij P, Mann JR, Dahl HH, Chinnery PF (2008) A reduction of mitochondrial DNA molecules during embryogenesis explains the rapid segregation of genotypes. Nat Genet 40:249–254

Cummins JM, Wakayama T, Yanagimachi R (1997) Fate of microinjected sperm components in the mouse oocyte and embryo. Zygote 5:301–308

Cummins JM, Jequier AM, Martin R, Mehmet D, Goldblatt J (1998) Semen levels of mitochondrial DNA deletions in men attending an infertility clinic do not correlate with phenotype. Int J Androl 21:47–52

DeBaun MR, Niemitz EL, Feinberg AP (2003) Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet 72:156–160

Dumollard R, Campbell K, Halet G, Carroll J, Swann K (2008) Regulation of cytosolic and mitochondrial ATP levels in mouse eggs and zygotes. Dev Biol 316:431–440

El Shourbagy SH, Spikings EC, Freitas M, St John JC (2006) Mitochondria directly influence fertilisation outcome in the pig. Reproduction 131:233–245

Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF (2008) Pathogenic mitochondrial DNA mutations are common in the general population. Am J Hum Genet 83:254–260

Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154–156

Facucho-Oliveira JM, St John JC (2009) The relationship between pluripotency and mitochondrial DNA proliferation during early embryo development and embryonic stem cell differentiation. Stem Cell Rev 5:140–158

Facucho-Oliveira JM, Alderson J, Spikings EC, Egginton S, St John JC (2007) Mitochondrial DNA replication during differentiation of murine embryonic stem cells. J Cell Sci 120:4025–4034

Fan W, Waymire KG, Narula N, Li P, Rocher C, Coskun PE, Vannan MA, Narula J, Macgregor GR, Wallace DC (2008) A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. Science 319:958–962

Folgerø T, Bertheussen K, Lindal S, Torbergsen T, Oian P (1993) Mitochondrial disease and reduced sperm motility. Hum Reprod 8:1863–1868

Gibson TC, Kubisch HM, Brenner CA (2005) Mitochondrial DNA deletions in rhesus macaque oocytes and embryos. Mol Hum Reprod 11:785–789

Gigarel N, Hesters L, Samuels DC, Monnot S, Burlet P, Kerbrat V, Lamazou F, Benachi A, Frydman R, Feingold J, Rotig A, Munnich A, Bonnefont JP, Frydman N, Steffann J (2011) Poor correlations in the levels of pathogenic mitochondrial DNA mutations in polar bodies versus oocytes and blastomeres in humans. Am J Hum Genet 88:494–498

Giles RE, Blanc H, Cann HM, Wallace DC (1980) Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci USA 77:6715–6719

Gyllensten U, Wharton D, Josefsson A, Wilson AC (1991) Paternal inheritance of mitochondrial DNA in mice. Nature 352:255–257

Hance N, Ekstrand MI, Trifunovic A (2005) Mitochondrial DNA polymerase gamma is essential for mammalian embryogenesis. Hum Mol Genet 14:1775–1783

Hauswirth WW, Laipis PJ (1982) Mitochondrial DNA polymorphism in a maternal lineage of Holstein cows. Proc Natl Acad Sci USA 79:4686–4690

Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA (1990) A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 46:428–433

Houghton FD (2006) Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst. Differentiation 74:11–18

Hsieh RH, Tsai NM, Au HK, Chang SJ, Wei YH, Tzeng CR (2002) Multiple rearrangements of mitochondrial DNA in unfertilized human oocytes. Fertil Steril 77:1012–1017

Innocenti P, Morrow EH, Dowling DK (2011) Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. Science 332:845–848

Jenuth JP, Peterson AC, Shoubridge EA (1997) Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. Nat Genet 16:93–95

Jiang Y, Kelly R, Peters A, Fulka H, Dickinson A, Mitchell DA, St John JC (2011) Interspecies somatic cell nuclear transfer is dependent on compatible mitochondrial DNA and reprogramming factors. PLoS One 6:e14805

Jones KT (2007) Intracellular calcium in the fertilization and development of mammalian eggs. Clin Exp Pharmacol Physiol 34:1084–1089

Kaneda H, Hayashi J, Takahama S, Taya C, Lindahl KF, Yonekawa H (1995) Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. Proc Natl Acad Sci USA 92:4542–4546

Kao S, Chao HT, Wei YH (1995) Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm. Biol Reprod 52:729–736

Kaufman BA, Durisic N, Mativetsky JM, Costantino S, Hancock MA, Grutter P, Shoubridge EA (2007) The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. Mol Biol Cell 18:3225–3236

Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S (1995) Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril 64:577–583

Kirby DM, Boneh A, Chow CW, Ohtake A, Ryan MT, Thyagarajan D, Thorburn DR (2003) Low mutant load of mitochondrial DNA G13513A mutation can cause Leigh's disease. Ann Neurol 54:473–478

Kobayashi Y, Momoi MY, Tominaga K, Momoi T, Nihei K, Yanagisawa M, Kagawa Y, Ohta S (1990) A point mutation in the mitochondrial tRNA(Leu)(UUR) gene in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). Biochem Biophys Res Commun 173:816–822

Kondo R, Satta Y, Matsuura ET, Ishiwa H, Takahata N, Chigusa SI (1990) Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. Genetics 126:657–663

Kraytsberg Y, Schwartz M, Brown TA, Ebralidse K, Kunz WS, Clayton DA, Vissing J, Khrapko K (2004) Recombination of human mitochondrial DNA. Science 304:981

Kucej M, Butow RA (2007) Evolutionary tinkering with mitochondrial nucleoids. Trends Cell Biol 17:586–592

Kvist L, Martens J, Nazarenko AA, Orell M (2003) Paternal leakage of mitochondrial DNA in the great tit (*Parus major*). Mol Biol Evol 20:243–247

Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS, Clayton DA (1998) Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat Genet 18:231–236

Lee JH, Peters A, Fisher P, Bowles EJ, St John JC, Campbell KH (2010) Generation of mtDNA homoplasmic cloned lambs. Cell Reprogram 12:347–355

Lee S, Kim S, Sun X, Lee JH, Cho H (2007) Cell cycle-dependent mitochondrial biogenesis and dynamics in mammalian cells. Biochem Biophys Res Commun 357:111–117

Lestienne P, Reynier P, Chrétien MF, Penisson-Besnier I, Malthièry Y, Rohmer V (1997) Oligoasthenospermia associated with multiple mitochondrial DNA rearrangements. Mol Hum Reprod 3:811–814

Liu H, Wang CW, Grifo JA, Krey LC, Zhang J (1999) Reconstruction of mouse oocytes by germinal vesicle transfer: maturity of host oocyte cytoplasm determines meiosis. Hum Reprod 14:2357–2361

Lloyd RE, Lee JH, Alberio R, Bowles EJ, Ramalho-Santos J, Campbell KH, St John JC (2006) Aberrant nucleo-cytoplasmic cross-talk results in donor cell mtDNA persistence in cloned embryos. Genetics 172:2515–2527

Manwaring N, Jones MM, Wang JJ, Rochtchina E, Howard C, Mitchell P, Sue CM (2007) Population prevalence of the MELAS A3243G mutation. Mitochondrion 7:230–233

Marchington DR, Hartshorne GM, Barlow D, Poulton J (1997) Homopolymeric tract heteroplasmy in mtDNA from tissues and single oocytes: support for a genetic bottleneck. Am J Hum Genet 60:408–416

May-Panloup P, Vignon X, Chrétien MF, Heyman Y, Tamassia M, Malthièry Y, Reynier P (2005a) Increase of mitochondrial DNA content and transcripts in early bovine embryogenesis associated with upregulation of mtTFA and NRF1 transcription factors. Reprod Biol Endocrinol 3:65

May-Panloup P, Chrétien MF, Jacques C, Vasseur C, Malthièry Y, Reynier P (2005b) Low oocyte mitochondrial DNA content in ovarian insufficiency. Hum Reprod 20:593–597

McKenzie M, Trounce I (2000) Expression of *Rattus norvegicus* mtDNA in *Mus musculus* cells results in multiple respiratory chain defects. J Biol Chem 275:31514–31519

McKenzie M, Chiotis M, Pinkert CA, Trounce IA (2003) Functional respiratory chain analyses in murid xenomitochondrial cybrids expose coevolutionary constraints of cytochrome b and nuclear subunits of complex III. Mol Biol Evol 20:1117–1124

Meirelles FV, Smith LC (1997) Mitochondrial genotype segregation in a mouse heteroplasmic lineage produced by embryonic karyoplast transplantation. Genetics 145:445–451

Meirelles FV, Bordignon V, Watanabe Y, Watanabe M, Dayan A, Lôbo RB, Garcia JM, Smith LC (2001) Complete replacement of the mitochondrial genotype in a *Bos indicus* calf reconstructed by nuclear transfer to a *Bos taurus* oocyte. Genetics 158:351–356

Meusel MS, Moritz RF (1993) Transfer of paternal mitochondrial DNA during fertilization of honeybee (*Apis mellifera* L.) eggs. Curr Genet 24:539–543

Miller FJ, Rosenfeldt FL, Zhang C, Linnane AW, Nagley P (2003) Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: lack of change of copy number with age. Nucleic Acids Res 31:e61

Morgan HD, Santos F, Green K, Dean W, Reik W (2005) Epigenetic reprogramming in mammals. Hum Mol Genet 14 (Spec No 1): R47–R58

Moyes CD, Battersby BJ, Leary SC (1998) Regulation of muscle mitochondrial design. J Exp Biol 201:299–307

Nagao Y, Totsuka Y, Atomi Y, Kaneda H, Lindahl KF, Imai H, Yonekawa H (1998) Decreased physical performance of congenic mice with mismatch between the nuclear and the mitochondrial genome. Genes Genet Syst 73:21–27

Nishimura Y, Yoshinari T, Naruse K, Yamada T, Sumi K, Mitani H, Higashiyama T, Kuroiwa T (2006) Active digestion of sperm mitochondrial DNA in single living sperm revealed by optical tweezers. Proc Natl Acad Sci USA 103:1382–1387

Otani H, Tanaka O, Kasai K, Yoshioka T (1988) Development of mitochondrial helical sheath in the middle piece of the mouse spermatid tail: regular dispositions and synchronized changes. Anat Rec 222:26–33

Ozawa T (1995) Mechanism of somatic mitochondrial DNA mutations associated with age and diseases. Biochim Biophys Acta 1271:177–189

Pavili L, Daudin M, Moinard N, Walschaerts M, Cuzin L, Massip P, Pasquier C, Bujan L (2010) Decrease of mitochondrial DNA level in sperm from patients infected with human immunodeficiency virus-1 linked to nucleoside analogue reverse transcriptase inhibitors. Fertil Steril 94:2151–2156

Pfeiffer T, Schuster S, Bonhoeffer S (2001) Cooperation and competition in the evolution of ATP-producing pathways. Science 292:504–507

Pikó L, Taylor KD (1987) Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. Dev Biol 123:364–374

Reynier P, Chrétien MF, Penisson-Besnier I, Malthièry Y, Rohmer V, Lestienne P (1997) Male infertility associated with multiple mitochondrial DNA rearrangements. C R Acad Sci III 320:629–636

Reynier P, Chrétien MF, Savagner F, Larcher G, Rohmer V, Barrière P, Malthièry Y (1998) Long PCR analysis of human gamete mtDNA suggests defective mitochondrial maintenance in spermatozoa and supports the bottleneck theory for oocytes. Biochem Biophys Res Commun 252:373–377

Reynier P, May-Panloup P, Chrétien MF, Morgan CJ, Jean M, Savagner F, Barrière P, Malthièry Y (2001) Mitochondrial DNA content affects the fertilizability of human oocytes. Mol Hum Reprod 7:425–429

Ruiz-Pesini E, Lapeña AC, Díez-Sánchez C, Pérez-Martos A, Montoya J, Alvarez E, Díaz M, Urriés A, Montoro L, López-Pérez MJ, Enríquez JA (2000) Human mtDNA haplogroups associated with high or reduced spermatozoa motility. Am J Hum Genet 67:682–696

Sacconi S, Salviati L, Nishigaki Y, Walker WF, Hernandez-Rosa E, Trevisson E, Delplace S, Desnuelle C, Shanske S, Hirano M, Schon EA, Bonilla E, De Vivo DC, DiMauro S, Davidson MM (2008) A functionally dominant mitochondrial DNA mutation. Hum Mol Genet 17:1814–1820

Santos TA, El Shourbagy S, St John JC (2006) Mitochondrial content reflects oocyte variability and fertilization outcome. Fertil Steril 85:584–591

Satoh M, Kuroiwa T (1991) Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. Exp Cell Res 196:137–140

Schaefer AM, McFarland R, Blakely EL, He L, Whittaker RG, Taylor RW, Chinnery PF, Turnbull DM (2008) Prevalence of mitochondrial DNA disease in adults. Ann Neurol 63:35–39

Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, DiMauro S (1989) A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. Science 244:346–349

Schwartz M, Vissing J (2002) Paternal inheritance of mitochondrial DNA. N Engl J Med 347:576–580

Shadel GS, Clayton DA (1997) Mitochondrial DNA maintenance in vertebrates. Annu Rev Biochem 66:409–435

Shitara H, Kaneda H, Sato A, Inoue K, Ogura A, Yonekawa H, Hayashi JI (2000) Selective and continuous elimination of mitochondria microinjected into mouse eggs from spermatids, but not from liver cells, occurs throughout embryogenesis. Genetics 156:1277–1284

Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. Cell 61:931–937

Smith LC, Alcivar AA (1993) Cytoplasmic inheritance and its effects on development and performance. J Reprod Fertil Suppl 48:31–43

Song GJ, Lewis V (2008) Mitochondrial DNA integrity and copy number in sperm from infertile men. Fertil Steril 90:2238–2244

Sousa Lopes SM de, Roelen BA (2010) An overview on the diversity of cellular organelles during the germ cell cycle. Histol Histopathol 25:267–276

Spikings EC, Alderson J, St John JC (2007) Regulated mitochondrial DNA replication during oocyte maturation is essential for successful porcine embryonic development. Biol Reprod 76:327–335

Spiropoulos J, Turnbull DM, Chinnery PF (2002) Can mitochondrial DNA mutations cause sperm dysfunction? Mol Hum Reprod 8:719–721

St John JC, Campbell KH (2010) The battle to prevent the transmission of mitochondrial DNA disease: is karyoplast transfer the answer? Gene Ther 17:147–149

St John JC, Schatten G (2004) Paternal mitochondrial DNA transmission during nonhuman primate nuclear transfer. Genetics 167:897–905

St John J, Sakkas D, Dimitriadi K, Barnes A, Maclin V, Ramey J, Barratt C, De Jonge C (2000) Failure of elimination of paternal mitochondrial DNA in abnormal embryos. Lancet 355:200

St John JC, Jokhi RP, Barratt CL (2001) Men with oligoasthenoteratozoospermia harbour higher numbers of multiple mitochondrial DNA deletions in their spermatozoa, but individual deletions are not indicative of overall aetiology. Mol Hum Reprod 7:103–111

St John JC, Lloyd RE, Bowles EJ, Thomas EC, El Shourbagy S (2004) The consequences of nuclear transfer for mammalian foetal development and offspring survival. A mitochondrial DNA perspective. Reproduction 127:631–641

St John JC, Moffatt O, D'Souza N (2005) Aberrant heteroplasmic transmission of mtDNA in cloned pigs arising from double nuclear transfer. Mol Reprod Dev 72:450–460

St John JC, Facucho-Oliveira J, Jiang Y, Kelly R, Salah R (2010) Mitochondrial DNA transmission, replication and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. Hum Reprod Update 16:488–509

Steinborn R, Zakhartchenko V, Wolf E, Müller M, Brem G (1998) Non-balanced mix of mitochondrial DNA in cloned cattle produced by cytoplast-blastomere fusion. FEBS Lett 426:357–361

Steinborn R, Schinogl P, Wells DN, Bergthaler A, Müller M, Brem G (2002) Coexistence of *Bos taurus* and *B. indicus* mitochondrial DNAs in nuclear transfer-derived somatic cattle clones. Genetics 162:823–829

Steuerwald N, Barritt JA, Adler R, Malter H, Schimmel T, Cohen J, Brenner CA (2000) Quantification of mtDNA in single oocytes, polar bodies and subcellular components by real-time rapid cycle fluorescence monitored PCR. Zygote 8:209–215

Stewart JB, Freyer C, Elson JL, Wredenberg A, Cansu Z, Trifunovic A, Larsson NG (2008) Strong purifying selection in transmission of mammalian mitochondrial DNA. PLoS Biol 6:e10

Sutarno, Cummins JM, Greeff J, Lymbery AJ (2002) Mitochondrial DNA polymorphisms and fertility in beef cattle. Theriogenology 57:1603–1610

Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G (1999) Ubiquitin tag for sperm mitochondria. Nature 402:371–372

Tachibana M, Sparman M, Sritanaudomchai H, Ma H, Clepper L, Woodward J, Li Y, Ramsey C, Kolotushkina O, Mitalipov S (2009) Mitochondrial gene replacement in primate offspring and embryonic stem cells. Nature 461:367–372

Takeda K, Akagi S, Kaneyama K, Kojima T, Takahashi S, Imai H, Yamanaka M, Onishi A, Hanada H (2003) Proliferation of donor mitochondrial DNA in nuclear transfer calves (*Bos taurus*) derived from cumulus cells. Mol Reprod Dev 64:429–437

Takeda K, Tasai M, Iwamoto M, Akita T, Tagami T, Nirasawa K, Hanada H, Onishi A (2006) Transmission of mitochondrial DNA in pigs and progeny derived from nuclear transfer of Meishan pig fibroblast cells. Mol Reprod Dev 73:306–312

Tamassia M, Nuttinck F, May-Panloup P, Reynier P, Heyman Y, Charpigny G, Stojkovic M, Hiendleder S, Renard JP, Chastant-Maillard S (2004) In vitro embryo production efficiency in cattle and its association with oocyte adenosine triphosphate content, quantity of mitochondrial DNA, and mitochondrial DNA haplogroup. Biol Reprod 71:697–704

Thundathil J, Filion F, Smith LC (2005) Molecular control of mitochondrial function in preimplantation mouse embryos. Mol Reprod Dev 71:405–413

Trounce I (2000) Genetic control of oxidative phosphorylation and experimental models of defects. Hum Reprod 15 (Suppl 2):18–27

Tsai HD, Hsieh YY, Hsieh JN, Chang CC, Yang CY, Yang JG, Cheng WL, Tsai FJ, Liu CS (2010) Mitochondria DNA deletion and copy numbers of cumulus cells associated with in vitro fertilization outcomes. J Reprod Med 55:491–497

Ursing BM, Arnason U (1998) The complete mitochondrial DNA sequence of the pig (*Sus scrofa*). J Mol Evol 47:302–306

Van Blerkom J (2004) Mitochondria in human oogenesis and preimplantation embryogenesis: engines of metabolism, ionic regulation and developmental competence. Reproduction 128:269–280

Van Blerkom J, Davis P, Alexander S (2000) Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. Hum Reprod 15:2621–2633

Vandebona H, Mitchell P, Manwaring N, Griffiths K, Gopinath B, Wang JJ, Sue CM (2009) Prevalence of mitochondrial 1555A→G mutation in adults of European descent. N Engl J Med 360:642–644

Wai T, Teoli D, Shoubridge EA (2008) The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. Nat Genet 40:1484–1488

Wallace DC (2007) Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. Annu Rev Biochem 76:781–821

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ 2nd, Nikoskelainen EK (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427–1430

Wang MK, Chen DY, Liu JL, Li GP, Sun QY (2001) In vitro fertilisation of mouse oocytes reconstructed by transfer of metaphase II chromosomes results in live births. Zygote 9:9–14

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997) Viable offspring derived from fetal and adult mammalian cells. Nature 385:810–813

Yang CX, Han ZM, Wen DC, Sun QY, Zhang KY, Zhang LS, Wu YQ, Kou ZH, Chen DY (2003) In vitro development and mitochondrial fate of macaca-rabbit cloned embryos. Mol Reprod Dev 65:396–401

Zhao X, Li N, Guo W, Hu X, Liu Z, Gong G, Wang A, Feng J, Wu C (2004) Further evidence for paternal inheritance of mitochondrial DNA in the sheep (*Ovis aries*). Heredity (Edinb) 93:399–403

Zouros E, Freeman KR, Ball AO, Pogson GH (1992) Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. Nature 359:412–414