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REVIEW

Sperm Mitochondria in Reproduction: Good or Bad and Where Do They Go?

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ABSTRACT

The mitochondrion is the major energy provider to power sperm motility. In mammals, aside from the nuclear genome, mitochondrial DNA (mtDNA) also contributes to oxidative phosphorylation to impact production of ATP by coding 13 polypeptides. However, the role of sperm mitochondria in fertilization and its final fate after fertilization are still controversial. The viewpoints that sperm bearing more mtDNA will have a better fertilizing capability and that sperm mtDNA is actively eliminated during early embryogenesis are widely accepted. However, this may be not true for several mammalian species, including mice and humans. Here, we review the sperm mitochondria and their mtDNA in sperm functions, and the mechanisms of maternal mitochondrial inheritance in mammals.

KEYWORDS: Mitochondrial DNA; Maternal inheritance; Paternal inheritance; Autophagy; Oxidative phosphorylation; Ubiquitination

INTRODUCTION

Mitochondria are among the critically important yet intriguing organelles with multiple cellular functions. Besides their primary function to produce ATP, mitochondria also contribute to many physiological processes, such as calcium homeostasis, apoptosis, lipid and amino acid metabolism, and others (Logan, 2007; Suen et al., 2008; Tait and Green, 2012). Mitochondria have their own genome, and in mammalian species, the mitochondrial genome contains 15−17 kb circular double-stranded DNA, and includes 37 genes encoding for 13 peptides, 22 tRNA and 2 rRNA (Park and Larsson, 2011). When mutations or/and deletions occur in mitochondrial DNA (mtDNA), individuals may suffer from mitochondrial disorders (Park and Larsson, 2011); furthermore, when even only two different normal mtDNAs (NZB and 129S6) are mixed, some physiological defects including reduced activity and food intake, attenuated stress response, and cognitive impairment have been observed in mice (Sharpley et al., 2012). However, due to the many copies of mtDNA in one cell, mitochondrial disorders may not emerge except for cases in which damaged mtDNA is accumulated to a certain degree (Park and Larsson, 2011; Sharpley et al., 2012). Therefore, keeping mtDNA homogeneity is very important for an organism (Yan et al., 2011).

In most mammalian species, along with the sperm being incorporated into the oocyte during fertilization, mitochondria are also incorporated into the zygote (Ankel-Simons and Cummins, 1996). However, because sperm mtDNA is less condensed, it is likely to be easily damaged by reactive oxygen species (ROS) during the fertilization process (Donnelly et al., 2000; Aitken et al., 2012). Hence, the viewpoint that sperm mitochondria and their mtDNA should be eliminated at the early embryo stages to protect embryos and offspring from being affected by damaged paternal mtDNA is widely accepted (Marchetti et al., 2002; Ford, 2004). However, very recently, by utilizing a specific transgenic mouse which

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bearded RFP-labeled mitochondria, we found that sperm mitochondria were not eliminated through the early embryonic degradation system, i.e., ubiquitin−proteasome and autophagy; instead, they could persist evenly in several cells until the morula stage. Moreover, by using a highly sensitive allele-specific nested PCR and restriction analysis, we confirmed that most of the active motile sperm cells eliminated their mtDNA during spermatogenesis, but if mtDNA occasionally remained and entered the zygote, it could be transmitted to offspring (Luo et al., 2013). Thus, the view that sperm mtDNA is eliminated at early embryo stages to maintain maternal mitochondrial inheritance may not be true in mice. Instead, a possible mechanism of maternal mitochondrial inheritance in mice is the pre-elimination of sperm mtDNA before fertilization. Moreover, by examining relevant references, it appears that similar phenomena may also be true for humans (May-Panloup et al., 2003). In the present review, we will mainly address the roles of mitochondria in sperm and the mechanisms of maternal mitochondrial inheritance in mammals.

CAN MITOCHONDRIA BE INDICATIVE OF SPERM QUALITY?

Currently, density gradient centrifugation and swim-up, as standard preparation techniques to obtain good-quality sperm samples, are widely used in assisted reproduction (Sousa et al., 2011); however, these two techniques are mechanical methods and cannot reflect sperm DNA integrity. Thus, other methods that can be used to evaluate sperm quality at biochemical levels are needed. Mitochondria are thought to be the most important organelles for the evaluation of sperm quality, and this may be due to the fact that mitochondria contain their own DNA and membrane potential which can easily be examined *in vitro* to reflect sperm DNA integrity and motility, respectively (Carra et al., 2004; Amaral and Ramalho-Santos, 2010). However, when mtDNA and mitochondrial membrane potential are used to evaluate sperm quality, two issues should be addressed. First, besides mitochondrial oxidative phosphorylation, another energy production system, glycolysis, is also functional in sperm. Can oxidative phosphorylation be used to evaluate sperm motility and quality? Second, can the copy number and quality of mtDNA be used to indicate the integrity of the nuclear genome?

The role of oxidative phosphorylation in sperm function

The spermatozoon is a terminally differentiated cell, and most of its components are removed after meiosis II (Smith and Alcivar, 1993), with only 22−28 mitochondria ultimately remaining, as studied in mice (Otani et al., 1988). However, sperm mitochondria morphology and biochemistry are different from those in somatic cells. Firstly, mitochondria in spermatozoa are tandem and tightly wrapped by disulphide bridges in the midpiece (Otani et al., 1988). But, in somatic cells, mitochondria form a dynamic network with distribution throughout the entire cell, and each mitochondrion is subjected to fusion and fission with other mitochondria (Chen and Chan, 2005). Secondly, many proteins and enzymes such as subunit VIb of the cytochrome *c* oxidase (Huttemann et al., 2003), E1-pyruvate decarboxylase and creatine kinase (CK) (Gerez de Burgos et al., 1994; Huszar et al., 2000), are specific isoforms in sperm mitochondria (Yang et al., 2011), and these isoforms may allow sperm cells to modulate mitochondrial function at different fertilization stages. Besides mitochondria, there are many glycolytic enzymes distributed in the sperm tail, and some of these enzymes are also isoforms which contain appropriate modifications at their N-terminus to aid proteins being anchored at the fibrous sheath (Bunch et al., 1998; Vemuganti et al., 2007). Similar to other cells, there are two energy production systems within sperm cells, i.e., mitochondrial oxidative phosphorylation and glycolysis. However, due to the small number and unique structure of mitochondria in sperm cells, whether mitochondria are the main energy resource for sperm motility has been debated for several decades up to the present time (Piomboni et al., 2012).

Previously, the most widely used methods to investigate the role of mitochondria in sperm motility and fertilization were adding diverse oxidative phosphorylation substrates or inhibitors to the incubation systems and then monitoring the oxygen consumption (Piomboni et al., 2012). However, because several factors, such as substrates (pyruvate, malate, fumarate, citrate or 2-oxoglutarate), spermatozoa status (intact or ruptured membrane), and sample origin (human, mice, rat or other animals), can impact the results, a definite conclusion of the roles of mitochondria in sperm motility and fertilization is still lacking (Piomboni et al., 2012). A general and reasonable concept is that sperm cells can modulate glycolysis and oxidative phosphorylation functions to satisfy their energy need based on different conditions and fertilization stages (John and John, 2011). Glycolysis, a vital, even exclusive resource for ATP production in human spermatozoa was confirmed in some studies (Peterson and Freund, 1970). However, most of these studies were conducted on intact sperm samples, and whether the added exogenous substrates were effectively delivered to mitochondria for oxidation awaited validation (Peterson and Freund, 1970; Piomboni et al., 2012). Indeed, when sperm samples were exposed to hypotonic buffer before the oxygen consumption assay to selectively allow the sperm cell membrane but not the mitochondrial membrane to be disrupted, sperm mitochondria were able to undergo respiration very efficiently by adding pyruvate, malate and lactate (Ferramosca et al., 2008). Besides, it was discovered that, when oxidative phosphorylation inhibitors were added into a 2 mmol/L glucose environment, similar to the female reproductive tract (Quinn et al., 1985), the motility of human spermatozoa was significantly inhibited; but these inhibitory effects disappeared when glucose was modified to 5 mmol/L (St John et al., 2005). Moreover, in some asthenozoospermia sperm samples, abnormalities in mitochondrial number and morphology were also found (Wilton et al., 1992; Rawe et al., 2002). Thus, although oxidative phosphorylation and glycolysis may have different roles in spermatozoa from diverse species, oxidative

phosphorylation is not dispensable for the sperm normal functions, and any defect of it may affect the sperm cell's fertilization ability.

Mitochondrial membrane potential as an indicator used to evaluate sperm quality

Because maintenance of positively charged membrane potential is a prerequisite for mitochondria to produce ATP, many commercial fluorescent dyes are widely used, such as JC-1 and MitoSOX Red, to examine mitochondrial membrane potential and mitochondria superoxide to evaluate mitochondrial health and cell quality (Liesa et al., 2009). In addition, flow cytometry, which is used to separate cells of different quality, can also be used to evaluate and obtain good-quality sperm cells and examine the health of mitochondria (Marchetti et al., 2002). By utilizing MitoTracker Green FM (MT-G) to separate and examine human sperm cells of different quality, Sousa et al. (2011) confirmed that MT-G could be utilized to efficiently obtain good-quality sperm cells, and samples obtained with this method contained more capacitated and acrosome-intact sperm cells, lower chromatin damage and better capabilities to decondense and participate in early development after chemical induction or injection into mature bovine oocytes. However, several problems may be noted when using mitochondrial fluorescence dyes to examine sperm quality. By comparing three dyes, MT-G, MitoTracker Red CMH2XRos (MT-R) and JC-1, Amaral and Ramalho-Santos (2010) found that JC-1 could stain sperm cells displaying orange, green or non-staining depending on the mitochondrial membrane potential; but when MT-G or MT-R was used, sperm samples comprised only two subpopulations: stained or unstained spermatozoa. Also, they found that Mitotrackers were able to respond to mitochondrial membrane potential alterations in sperm cells, and this was contradictory to the results obtained in somatic cells (Amaral and Ramalho-Santos, 2010). In addition, although JC-1, MT-G and MT-R could not be retained in human sperm cells after fixation (Amaral and Ramalho-Santos, 2010), MT-G staining remained after fixation, as observed in bovine, mouse and primate sperm samples (Sutovsky et al., 1996, 2000). Therefore, mitochondrial membrane potential can only indicate sperm quality to some extent; whereas many other factors that may also affect the results obtained with mitochondrial probes should be taken into account (Table 1).

mtDNA as an indicator used to evaluate sperm quality

Due to the loosely packaged structure and the nearby localization to ROS production, mtDNA is more easily

Table 1
The features of different mitochondrial indicators used for sperm quality evaluation

	Characteristics	Reference
MitoTracker Green FM (MT-G)	Staining sperm cells with green or non-staining depending on the MMP	Amaral and Ramalho-Santos, 2010
	Fractionizing a subpopulation of human sperm with better fertilization potential	Sousa et al., 2011
MitoTracker Red CMH2XRos (MT-R)	Staining sperm cells with red or non-staining depending on the MMP	Amaral and Ramalho-Santos, 2010
JC-1	Staining sperm cells with orange, green or non-staining depending on the MMP	Amaral and Ramalho-Santos, 2010; Wang et al., 2010
	Revealing a positive correlation between the motility and MMP in human sperm cells	Paoli et al., 2011
mtDNA point mutation	mtDNA point mutations always being associated with bad sperm quality	Folgero et al., 1993; Holyoake et al., 2001; Spiropoulos et al., 2002; Thangaraj et al., 2003; Selvi Rani et al., 2006; Pereira et al., 2007; Mossman et al., 2012
	Some mtDNA point mutations having no effect on sperm quality	Pereira et al., 2007; Pereira et al., 2008; Mossman et al., 2012
mtDNA rearrangement	Some deletions being associated with sperm dysfunctions	Kao et al., 1995, 1998; Lestienne et al., 1997; St John et al., 2001; Thangaraj et al., 2003; Ieremiadou and Rodakis, 2009
	Most of the mtDNA polymorphisms significantly correlated with infertility in general populations	Palanichamy and Zhang, 2011
mtDNA copy number	Low copy number as good-quality indicator for sperm cells	Diez-Sanchez et al., 2003; May-Panloup et al., 2003; Song and Lewis, 2008; Luo et al., 2013
	HIV-positive men displayed large scale deletion of mtDNA and azoospermy after administration of NRTIs	White et al., 2001
	A decrease in sperm mtDNA content being detected in patients with asthenospermia or with poor sperm motility	Kao et al., 2004

MMP, mitochondrial membrane potential; NRTIs, nucleoside reverse transcriptase inhibitors.

damaged than the nuclear genome (Yakes and Van Houten, 1997). And damaged mtDNA may affect the sperm cell's motility, which can be easily examined by PCR (Spiropoulos et al., 2002). Therefore, besides mitochondrial membrane potential, mtDNA is another candidate that could be used to evaluate sperm quality. As there is a lack of animal models whose mtDNA abnormalities can be controlled *in vivo*, most of the results related to sperm mtDNA and its functions are derived from human clinical diagnosis. Based on its characteristics, mtDNA abnormalities mainly include mutation, rearrangement, and copy number alteration (Table 1).

In the past decade, many human mtDNA point mutations have been detected, among which 622 point mutations have been proved to be associated with somatic diseases (Dimauro and Davidzon, 2005; Park and Larsson, 2011; MITOMAP, 2013). In human sperm cells, it has also been reported that mutations of 3243 (A > G), 8821 (T > C) and 11994 (T > C) could be associated with sperm qualities (Folgero et al., 1993; Holyoake et al., 2001; Spiropoulos et al., 2002; Selvi Rani et al., 2006). Except for 3243 (A > G) (Lorenc et al., 2003; Mayr et al., 2008), the other two mutations are sperm cell-specific. Aside from these single point mutations, multiple point mutations also appeared in oligoasthenoteratozoospermia (Thangaraj et al., 2003). However, some controversial results were reported. For example, the 11994 (T > C) mutation was not correlated with motility in a Portuguese population study (Pereira et al., 2008); mitochondrial haplotype did not influence sperm motility in a UK population (Mossman et al., 2012); and the mtDNA haplogroup did not affect sperm motility as confirmed by complete sequencing of asthenozoospermic males (Pereira et al., 2007).

Rearrangement is another major mtDNA abnormality; it includes mtDNA deletions, mtDNA inversions and mtDNA insertions, for which, 131, 1 and 6 cases have been detected in humans, respectively (MITOMAP, 2013). A 4977-bp deletion occurred between two 13-bp direct repeats (8470−8482 and 13447−13459) which is associated with many pathological phenotypes, such as hearing loss (Bai and Seidman, 2001), end stage renal disease (ESRD) (Rao et al., 2009), and Kearns−Sayre syndrome (Mohri et al., 1998). Besides this common deletion (Kao et al., 1995; Ieremiadou and Rodakis, 2009), several other deletions including 7345-bp and 7599-bp deletions (Kao et al., 1998), 2-bp deletions (Thangaraj et al., 2003), an average 9-kb deletions (Lestienne et al., 1997), and 7.4-kb and 15-bp deletions associated with sperm dysfunction have been reported (St John et al., 2001). Moreover, through density centrifugation, a standard preparation technique to obtain good-quality sperm samples has been widely used in assisted reproduction. O'Connell et al. (2003) found that the high-density fraction displayed significantly more wild-type mtDNA than the low-density fraction. Therefore, examining mtDNA deletions may be another method to evaluate sperm quality. However, when PCR is utilized to detect mtDNA deletions, one may use caution when interpreting the results, because most of the mtDNA polymorphisms reported as significantly correlated with infertility were commonly found in general populations (Palanichamy and Zhang, 2011).

Although there is no direct evidence indicating that the abnormality of mtDNA copy number can be a single cause for sperm dysfunction, many studies indicate that a low mtDNA copy number may be an indicator for good-quality sperm samples. For example, May-Panloup et al. (2003) found that increased sperm mtDNA content appeared in male infertility and the 40% layer of density centrifugation samples rather than the normal and 100% layer of density centrifugation samples. Diez-Sanchez et al. (2003) found the absolute content of mtDNA was 700 and 1200 mtDNA copies per cell in progressive and non-progressive human spermatozoa, respectively. A significant increase in mtDNA copy number and a decrease in mtDNA integrity were also observed in spermatozoa samples from patients with abnormal semen parameters (Song and Lewis, 2008). In addition, we showed that good-quality sperm samples and bad-quality sperm samples from mice contained 1.29 and 45.93 copies mtDNA, respectively (Luo et al., 2013). Males could tolerate at least a three-fold reduction in mtDNA copy number in their sperm without impaired fertility, and preferentially transmitted a deleted *Tfam* allele that may have less mtDNA in the sperm cell which was also observed in mice (Wai et al., 2010). Thus, it seems that a lesser mtDNA copy number may be an indicator for good-quality spermatozoa. However, both the mtDNA replication system defect and the mtDNA elimination system can result in a low copy number of sperm mtDNA, and the former may induce sperm dysfunction but the latter may help sperm function effectively. It should be noted which case is at work when interpreting the relationship between sperm mtDNA copy number and sperm quality. For instance, when HIV-positive men were administered NRTIs (nucleoside reverse transcriptase inhibitors) for treatment, polymerase gamma (POLG) activity was inhibited and large scale deletion of mtDNA and azoospermy were induced (White et al., 2001); whereas, in normal cases, good-quality sperm samples exhibited high POLG activity and low copy number of mtDNA in synchrony (Amaral et al., 2007).

Therefore, since not only mitochondrial membrane potential and mtDNA but many other factors can impact sperm function, utilizing sperm mitochondrial membrane potential or mtDNA as single indicator to evaluate sperm quality is not adequate. When combined with other technologies such as density gradient centrifugation or swim-up, examining mitochondrial membrane potential or mtDNA can provide more accurate interpretations for diagnosis of male infertility and improve the assisted reproduction results (Lewis, 2007).

MATERNAL MITOCHONDRIAL INHERITANCE

After Hutchison et al. (1974) discovered that the mtDNA in mule was inherited from the female parent, it has been determined that mtDNA is inherited only from one parent in almost all organisms (Basse, 2010; St John et al., 2010). In mammals, the mtDNA is always transmitted only through the female which is termed as maternal mitochondrial inheritance (St John et al., 2010). Indeed, at the beginning of life, maternal mitochondrial inheritance is very important for keeping

mtDNA homogeneity and an individual's health, because even if two normal mtDNAs are mixed, they may produce several adverse physiological disorders, as studied in mice (Sharpley et al., 2012). Although the exact mechanism of maternal mitochondrial inheritance is not yet completely clear, existing studies indicate that distinct mechanisms are involved in different species to guarantee paternal mtDNA elimination in the offspring (Sato and Sato, 2013).

Diverse mechanisms involved in maternal mitochondrial inheritance

Due to different methods and different animal models used in different studies, the reports on the number of mtDNA copy in oocyte and sperm cells vary greatly. However, the fact that the oocyte contains more mtDNA copy numbers than the sperm cell has been confirmed by a series of studies (Steuerwald et al., 2000; Reynier et al., 2001; May-Panloup et al., 2003; Wai et al., 2010; Luo et al., 2013). Thereby, simple dilution, i.e., sperm mtDNA being too diluted to be detected after fertilization, is suggested as one of the mechanisms of maternal mitochondrial inheritance (Gyllensten et al., 1991). Indeed, considering that heterogenous mtDNA can be recombined and replicated in one cell (Rokas et al., 2003; Sharpley et al., 2012), the simple dilution explaining maternal mitochondrial inheritance may be true in some organisms; however, solid evidence is still lacking. Besides dilution, several other mechanisms were suggested in different organisms. In *Medaka*, by using optical tweezer technology, it has been observed that, after fertilization, sperm mtDNA was selectively eliminated before degradation of mitochondria through unknown mechanisms, leaving vacuolar mitochondria in early embryos (Nishimura et al., 2006). In *Caenorhabditis elegans*, post-fertilization degradation of sperm mitochondria by autophagy was confirmed. Sperm mitochondria disappeared before the 16-cell stage, whereas, when autophagy-related genes such as *Atg-7* and *Atg-18* were knocked down by RNAi or when *lgg-1* was mutated, paternal mitochondria and their DNA could persist in the embryo even through the first larval stage (Al Rawi et al., 2011; Sato and Sato, 2011; Zhou et al., 2011). Furthermore, it has also been reported that in *Drosophila*, maternal mitochondrial inheritance was due to the elimination of sperm mtDNA before fertilization. During spermatogenesis, mtDNA was eliminated by endonuclease G and removed by a cellular remodeling process, just leaving vacuolar mitochondria in sperm to complete fertilization (DeLuca and O'Farrell, 2012).

Maternal mitochondrial inheritance in mammals

In most mammals, along with the sperm genome, the sperm tail and midpiece are also incorporated into the oocyte during fertilization (Ankel-Simons and Cummins, 1996); however, it was also reported that due to the giant volume of the sperm midpiece and tail in Chinese hamster (*Cricetulus griseus*), its mitochondria remained outside the zygote after fertilization (Pickworth and Change, 1969; Yanagimachi et al., 1983). Although in some cases such as in mouse interspecies crossing (Gyllensten et al., 1991; Kaneda et al., 1995), and human abnormal embryos, paternal mtDNA was detected (St John et al., 2000), maternal mitochondrial inheritance is accepted as the most prevalent phenomenon in mammals. By utilizing immunofluorescence methods, sperm mitochondria ubiquitination during spermatogenesis was found in rhesus monkeys and cows, which suggested that the ubiquitin−proteasome-dependent proteolytic machinery might be involved in the degradation of sperm mitochondria after fertilization (Sutovsky et al., 1999). Indeed, subsequent studies indicated that the degradation of sperm mitochondria could be inhibited by microinjection of anti-ubiquitin antibodies into zygotes or treatment of zygotes with the lysosomotropic agent ammonium chloride (Sutovsky et al., 2000), and the target of ubiquitin was a mitochondrial membrane protein, prohibitin (Thompson et al., 2003). Thereby, the mechanism of maternal mitochondrial inheritance was suggested to be divided into two steps: firstly, sperm mitochondria are pre-labeled with ubiquitin during spermatogenesis, so they can be targeted by the zygote degradation system; secondly, upon fertilization, ubiquitin-labeled sperm mitochondria were selectively recognized by the ubiquitin−proteasome-dependent proteolytic machinery and eliminated by lysosomes (Sutovsky, 1999, 2000, 2003; Thompson et al., 2003). Now, the molecular events between the ubiquitin−proteasome and lysosomes are still not understood. By utilizing immunofluorescence methods, it was found that P62 and microtubule-associated protein 1 light chain 3 (LC3) proteins were co-localized to the sperm tail after fertilization, so the idea that autophagy might link these two aspects was proposed (Al Rawi et al., 2011). However, these results were only derived from immunofluorescence studies and solid evidence is still lacking.

Very recently, by utilizing two transgenic mouse strains, one bearing GFP-labeled autophagosomes and the other bearing RFP-labeled mitochondria, we confirmed that autophagy was not involved in the sperm mitochondrial degradation (Luo et al., 2013). Furthermore, by utilizing allele-specific-PCR and restriction enzyme analysis, we found that most motile spermatozoa that could reach the oviduct to complete fertilization, had eliminated their mtDNA, just leaving vacuolar mitochondria to supply energy for fertilization; however, if sperm bearing mtDNA occasionally were able to fertilize, mtDNA could be detected in newborn mice (Luo et al., 2013). Thus, as in *Drosophila* (DeLuca and O'Farrell, 2012), pre-elimination of mtDNA during spermatogenesis also contributes to maternal mitochondrial inheritance in mice (Fig. 1A). Because sperm mitochondria are structurally highly packaged, upon fertilization, they are disaggregated and always limited to one single blastomere during the 1- to 4-cell stages. Considering the fact that only two blastomeres of a 4-cell embryo can finally contribute to an animal body (Tarkowski et al., 2001; Piotrowska-Nitsche et al., 2005), despite mtDNA remaining, there is a one half (50%) opportunity for individuals to inherit paternal mtDNA (Kaneda et al., 1995; Shitara et al., 1998). Furthermore, the uneven distribution of sperm mitochondria during early

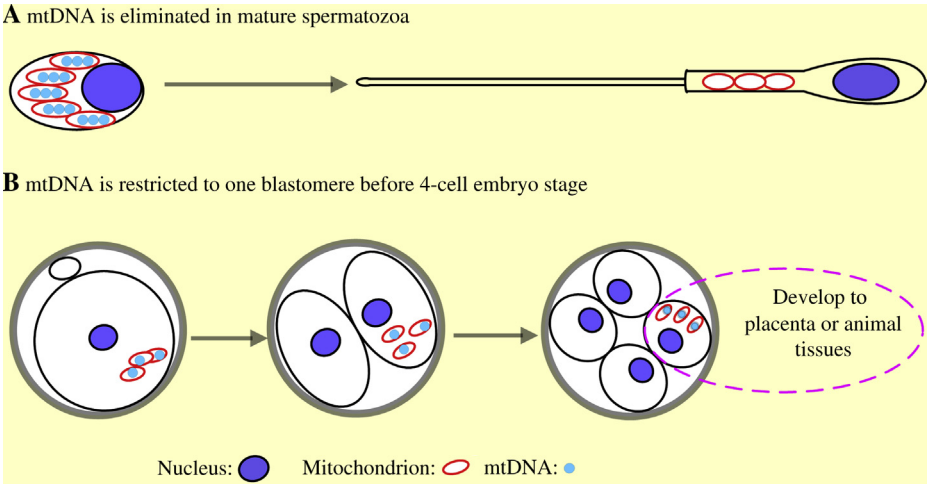

Fig. 1. Two mechanisms are involved in maternal mitochondrial inheritance in mice.
A: mtDNA is eliminated in mature sperm cells. Most of the sperm cells have eliminated their mtDNA, just leaving vacuolar mitochondria to supply energy for completing fertilization; **B:** mtDNA is restricted to one blastomere before the 4-cell embryo stage. The sperm mitochondria are highly concentrated, and they are always restricted to one blastomere before the 4-cell embryo stage. In rare cases, if a sperm bearing mtDNA is able to fertilize an egg, paternal mtDNA will be distributed to either placenta or to some tissues.

development may also result in the phenomenon that only some cells and tissues rather than the whole body could inherit paternal mtDNA (Shitara et al., 1998; Schwartz and Vissing, 2002; Luo et al., 2013). Thereby, the uneven distribution of sperm mitochondria is another mechanism of maternal mitochondria inheritance in mice (Fig. 1B).

In humans, it was reported that motile sperm contained only 1.4 mtDNA molecules on average, which means that the majority of sperm probably do not contain any mtDNA (May-Panloup et al., 2003). This is similar to our results obtained in mice: the motile sperm contained only 1.29 mtDNA on average (Luo et al., 2013). Furthermore, in a myopathy patient only his muscle rather than all tissues inheriting a 2-bp deletion mtDNA from his paternal parent was reported (Schwartz and Vissing, 2002); due to the helical structures (Sutovsky et al., 1997), the uneven distribution of sperm mitochondria during early embryo stages may also be true in humans. Therefore, it seems that the two mechanisms, i.e., pre-elimination of sperm mtDNA before fertilization and uneven distribution of sperm mitochondria in early embryos, may be involved in maternal mitochondrial inheritance in humans. In this case, when assisted reproductive technologies are performed, two points should be noted. First, due to the concentrated effects from uneven distribution of mtDNA, the impact of paternal mtDNA cannot be ignored; it has been reported that in the aforementioned myopathy patient, the paternal mtDNA contributed 90% of the muscle mtDNA (Schwartz and Vissing, 2002). Second, due to the natural selection of mtDNA-free sperm during the process of sperm swimming to the oviduct being absent, the rate of paternal mtDNA transmitted to the embryo may be increased (St John et al., 2000).

CONCLUSIONS AND PERSPECTIVES

Diverse mechanisms of maternal mitochondria inheritance may function in different organisms; however, the main mechanism for *Drosophila*, mice and humans, even other mammals may be pre-elimination of mtDNA. Although endonuclease G and cell remodeling have been shown to be involved in the elimination of mtDNA in *Drosophila* (DeLuca and O'Farrell, 2012), whether this also holds true for mammals is unknown. Future studies are needed to answer the following questions: how and when is sperm mtDNA eliminated in mammals and does mtDNA-free sperm have a better fertilization ability? By answering these questions, it will not only be helpful to interpret the mechanisms of maternal mitochondrial inheritance and prevent mitochondrial disorders due to biparental mitochondrial inheritance, but also be helpful to diagnose and treat cases of relevant male infertility.

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REFERENCES

Aitken, R.J., Jones, K.T., Robertson, S.A., 2012. Reactive oxygen species and sperm function − in sickness and in health. J. Androl. 33, 1096−1106.
Al Rawi, S., Louvet-Vallee, S., Djeddi, A., Sachse, M., Culetto, E., Hajjar, C., Boyd, L., Legouis, R., Galy, V., 2011. Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. Science 334, 1144−1147.
Amaral, A., Ramalho-Santos, J., 2010. Assessment of mitochondrial potential: implications for the correct monitoring of human sperm function. Int. J. Androl. 33, e180−e186.
Amaral, A., Ramalho-Santos, J., St John, J.C., 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.
Ankel-Simons, F., Cummins, J.M., 1996. Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. Proc. Natl. Acad. Sci. USA 93, 13859−13863.

Bai, U., Seidman, M.D., 2001. A specific mitochondrial DNA deletion (mtDNA4977) is identified in a pedigree of a family with hearing loss. Hear. Res. 154, 73−80.

Basse, C.W., 2010. Mitochondrial inheritance in fungi. Curr. Opin. Microbiol. 13, 712−719.

Bunch, D.O., Welch, J.E., Magyar, P.L., Eddy, E.M., O'Brien, D.A., 1998. Glyceraldehyde 3-phosphate dehydrogenase-S protein distribution during mouse spermatogenesis. Biol. Reprod. 58, 834−841.

Carra, E., Sangiorgi, D., Gattuccio, F., Rinaldi, A.M., 2004. Male infertility and mitochondrial DNA. Biochem. Biophys. Res. Commun. 322, 333−339.

Chen, H., Chan, D.C., 2005. Emerging functions of mammalian mitochondrial fusion and fission. Hum. Mol. Genet. 14 (Suppl. 2), R283−R289.

DeLuca, S.Z., O'Farrell, P.H., 2012. Barriers to male transmission of mitochondrial DNA in sperm development. Dev. Cell 22, 660−668.

Diez-Sanchez, C., Ruiz-Pesini, E., Lapena, A.C., Montoya, J., Perez-Martos, A., Enriquez, J.A., Lopez-Perez, M.J., 2003. Mitochondrial DNA content of human spermatozoa. Biol. Reprod. 68, 180−185.

Dimauro, S., Davidzon, G., 2005. Mitochondrial DNA and disease. Ann. Med. 37, 222−232.

Donnelly, E.T., O'Connell, M., McClure, N., Lewis, S.E., 2000. Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. Hum. Reprod. 15, 1552−1561.

Ferramosca, A., Focarelli, R., Piomboni, P., Coppola, L., Zara, V., 2008. Oxygen uptake by mitochondria in demembranated human spermatozoa: a reliable tool for the evaluation of sperm respiratory efficiency. Int. J. Androl. 31, 337−345.

Folgero, T., Bertheussen, K., Lindal, S., Torbergsen, T., Oian, P., 1993. Mitochondrial disease and reduced sperm motility. Hum. Reprod. 8, 1863−1868.

Ford, W.C., 2004. Regulation of sperm function by reactive oxygen species. Hum. Reprod. Update 10, 387−399.

Gerez de Burgos, N.M., Gallina, F., Burgos, C., Blanco, A., 1994. Effect of L-malate on pyruvate dehydrogenase activity of spermatozoa. Arch. Biochem. Biophys. 308, 520−524.

Gyllensten, U., Wharton, D., Josefsson, A., Wilson, A.C., 1991. Paternal inheritance of mitochondrial DNA in mice. Nature 352, 255−257.

Holyoake, A.J., McHugh, P., Wu, M., O'Carroll, S., Benny, P., Sin, I.L., Sin, F.Y., 2001. High incidence of single nucleotide substitutions in the mitochondrial genome is associated with poor semen parameters in men. Int. J. Androl. 24, 175−182.

Huszar, G., Stone, K., Dix, D., Vigue, L., 2000. Putative creatine kinase M-isoform in human sperm is identified as the 70-kilodalton heat shock protein HspA2. Biol. Reprod. 63, 925−932.

Hutchison 3rd, C.A., Newbold, J.E., Potter, S.S., Edgell, M.H., 1974. Maternal inheritance of mammalian mitochondrial DNA. Nature 251, 536−538.

Huttemann, M., Jaradat, S., Grossman, L.I., 2003. Cytochrome *c* oxidase of mammals contains a testes-specific isoform of subunit VIb − the counterpart to testes-specific cytochrome *c*? Mol. Reprod. Dev. 66, 8−16.

Ieremiadou, F., Rodakis, G.C., 2009. Correlation of the 4977 bp mitochondrial DNA deletion with human sperm dysfunction. BMC Res. Notes 2, 18.

John, J.S., John, B., 2011. Sperm mitochondrial DNA. In: Zini, A., Agarwal, A. (Eds.), Sperm Chromatin. Springer, New York, pp. 81−94.

Kaneda, H., Hayashi, J., Takahama, S., Taya, C., Lindahl, K.F., 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, H.T., Wei, Y.H., 1995. Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm. Biol. Reprod. 52, 729−736.

Kao, S.H., Chao, H.T., Liu, H.W., Liao, T.L., Wei, Y.H., 2004. Sperm mitochondrial DNA depletion in men with asthenospermia. Fertil. Steril. 82, 66−73.

Kao, S.H., Chao, H.T., Wei, Y.H., 1998. Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. Mol. Hum. Reprod. 4, 657−666.

Lestienne, P., Reynier, P., Chretien, M.F., Penisson-Besnier, I., Malthiery, Y., Rohmer, V., 1997. Oligoasthenospermia associated with multiple mitochondrial DNA rearrangements. Mol. Hum. Reprod. 3, 811−814.

Lewis, S.E., 2007. Is sperm evaluation useful in predicting human fertility? Reproduction 134, 31−40.

Liesa, M., Palacin, M., Zorzano, A., 2009. Mitochondrial dynamics in mammalian health and disease. Physiol. Rev. 89, 799−845.

Logan, D.C., 2007. The mitochondrial compartment. J. Exp. Bot. 58, 1225−1243.

Lorenc, A., Bryk, J., Golik, P., Kupryjanczyk, J., Ostrowski, J., Pronicki, M., Semczuk, A., Szolkowska, M., Bartnik, E., 2003. Homoplasmic MELAS A3243G mtDNA mutation in a colon cancer sample. Mitochondrion 3, 119−124.

Luo, S.M., Ge, Z.J., Wang, Z.W., Jiang, Z.Z., Wang, Z.B., Ouyang, Y.C., Hou, Y., Schatten, H., Sun, Q.Y., 2013. Unique insights into maternal mitochondrial inheritance in mice. Proc. Natl. Acad. Sci. USA 110, 13038−13043.

Marchetti, C., Obert, G., Deffosez, A., Formstecher, P., Marchetti, P., 2002. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum. Reprod. 17, 1257−1265.

May-Panloup, P., Chretien, M.F., Savagner, F., Vasseur, C., Jean, M., Malthiery, Y., Reynier, P., 2003. Increased sperm mitochondrial DNA content in male infertility. Hum. Reprod. 18, 550−556.

Mayr, J.A., Meierhofer, D., Zimmermann, F., Feichtinger, R., Kogler, C., Ratschek, M., Schmeller, N., Sperl, W., Kofler, B., 2008. Loss of complex I due to mitochondrial DNA mutations in renal oncocytoma. Clin. Cancer Res. 14, 2270−2275.

MITOMAP, 2013. MITOMAP: A Human Mitochondrial Genome Database. http://www.mitomap.org.

Mohri, I., Taniike, M., Fujimura, H., Matsuoka, T., Inui, K., Nagai, T., Okada, S., 1998. A case of Kearns−Sayre syndrome showing a constant proportion of deleted mitochondrial DNA in blood cells during 6 years of follow-up. J. Neurol. Sci. 158, 106−109.

Mossman, J.A., Slate, J., Birkhead, T.R., Moore, H.D., Pacey, A.A., 2012. Mitochondrial haplotype does not influence sperm motility in a UK population of men. Hum. Reprod. 27, 641−651.

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.

O'Connell, M., McClure, N., Powell, L.A., Steele, E.K., Lewis, S.E., 2003. Differences in mitochondrial and nuclear DNA status of high-density and low-density sperm fractions after density centrifugation preparation. Fertil. Steril. 79 (Suppl. 1), 754−762.

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.

Palanichamy, M.G., Zhang, Y.P., 2011. Identifying potential pitfalls in interpreting mitochondrial DNA mutations of male infertility cases. Indian J. Med. Res. 134, 447−451.

Paoli, D., Gallo, M., Rizzo, F., Baldi, E., Francavilla, S., Lenzi, A., Lombardo, F., Gandini, L., 2011. Mitochondrial membrane potential profile and its correlation with increasing sperm motility. Fertil. Steril. 95, 2315−2319.

Park, C.B., Larsson, N.G., 2011. Mitochondrial DNA mutations in disease and aging. J. Cell Biol. 193, 809−818.

Pereira, L., Goncalves, J., Bandelt, H.J., 2008. Mutation C11994T in the mitochondrial *ND4* gene is not a cause of low sperm motility in Portugal. Fertil. Steril. 89, 738−741.

Pereira, L., Goncalves, J., Franco-Duarte, R., Silva, J., Rocha, T., Arnold, C., Richards, M., Macaulay, V., 2007. No evidence for an mtDNA role in sperm motility: data from complete sequencing of asthenozoospermic males. Mol. Biol. Evol. 24, 868−874.

Peterson, R.N., Freund, M., 1970. ATP synthesis and oxidative metabolism in human spermatozoa. Biol. Reprod. 3, 47−54.

Pickworth, S., Change, M.C., 1969. Fertilization of Chinese hamster eggs *in vitro*. J. Reprod. Fertil. 19, 371−374.

Piomboni, P., Focarelli, R., Stendardi, A., Ferramosca, A., Zara, V., 2012. The role of mitochondria in energy production for human sperm motility. Int. J. Androl. 35, 109−124.

Piotrowska-Nitsche, K., Perea-Gomez, A., Haraguchi, S., Zernicka-Goetz, M., 2005. Four-cell stage mouse blastomeres have different developmental properties. Development 132, 479−490.

Quinn, P., Kerin, J.F., Warnes, G.M., 1985. Improved pregnancy rate in human in vitro fertilization with the use of a medium based on the composition of human tubal fluid. Fertil. Steril. 44, 493−498.

Rao, M., Li, L., Demello, C., Guo, D., Jaber, B.L., Pereira, B.J., Balakrishnan, V.S., 2009. Mitochondrial DNA injury and mortality in hemodialysis patients. J. Am. Soc. Nephrol. 20, 189−196.

Rawe, V.Y., Olmedo, S.B., Benmusa, A., Shiigi, S.M., Chemes, H.E., Sutovsky, P., 2002. Sperm ubiquitination in patients with dysplasia of the fibrous sheath. Hum. Reprod. 17, 2119−2127.

Reynier, P., May-Panloup, P., Chretien, M.F., Morgan, C.J., Jean, M., Savagner, F., Barriere, P., Malthiery, Y., 2001. Mitochondrial DNA content affects the fertilizability of human oocytes. Mol. Hum. Reprod. 7, 425−429.

Rokas, A., Ladoukakis, E., Zouros, E., 2003. Animal mitochondrial DNA recombination revisited. Trends Ecol. Evol. 18, 411−417.

Sato, M., Sato, K., 2011. Degradation of paternal mitochondria by fertilization-triggered autophagy in *C. elegans* embryos. Science 334, 1141−1144.

Sato, M., Sato, K., 2013. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. Biochim. Biophys. Acta 1833, 1979−1984.

Schwartz, M., Vissing, J., 2002. Paternal inheritance of mitochondrial DNA. N. Engl. J. Med. 347, 576−580.

Selvi Rani, D., Vanniarajan, A., Gupta, N.J., Chakravarty, B., Singh, L., Thangaraj, K., 2006. A novel missense mutation C11994T in the mitochondrial *ND4* gene as a cause of low sperm motility in the Indian subcontinent. Fertil. Steril. 86, 1783−1785.

Sharpley, M.S., Marciniak, C., Eckel-Mahan, K., McManus, M., Crimi, M., Waymire, K., Lin, C.S., Masubuchi, S., Friend, N., Koike, M., Chalkia, D., MacGregor, G., Sassone-Corsi, P., Wallace, D.C., 2012. Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. Cell 151, 333−343.

Shitara, H., Hayashi, J.I., Takahama, S., Kaneda, H., Yonekawa, H., 1998. Maternal inheritance of mouse mtDNA in interspecific hybrids: segregation of the leaked paternal mtDNA followed by the prevention of subsequent paternal leakage. Genetics 148, 851−857.

Smith, L.C., Alcivar, A.A., 1993. Cytoplasmic inheritance and its effects on development and performance. J. Reprod. Fertil. 48 (Suppl.), 31−43.

Song, G.J., Lewis, V., 2008. Mitochondrial DNA integrity and copy number in sperm from infertile men. Fertil. Steril. 90, 2238−2244.

Sousa, A.P., Amaral, A., Baptista, M., Tavares, R., Caballero Campo, P., Caballero Peregrin, P., Freitas, A., Paiva, A., Almeida-Santos, T., Ramalho-Santos, J., 2011. Not all sperm are equal: functional mitochondria characterize a subpopulation of human sperm with better fertilization potential. PLoS ONE 6, e18112.

Spiropoulos, J., Turnbull, D.M., Chinnery, P.F., 2002. Can mitochondrial DNA mutations cause sperm dysfunction? Mol. Hum. Reprod. 8, 719−721.

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, J.C., 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.

St John, J.C., Jokhi, R.P., Barratt, C.L., 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, J.C., Jokhi, R.P., Barratt, C.L., 2005. The impact of mitochondrial genetics on male infertility. Int. J. Androl. 28, 65−73.

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

Suen, D.F., Norris, K.L., Youle, R.J., 2008. Mitochondrial dynamics and apoptosis. Genes. Dev. 22, 1577−1590.

Sutovsky, P., 2003. Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: killing three birds with one stone. Microsc. Res. Tech. 61, 88−102.

Sutovsky, P., Moreno, R.D., Ramalho-Santos, J., Dominko, T., Simerly, C., Schatten, G., 1999. Ubiquitin tag for sperm mitochondria. Nature 402, 371−372.

Sutovsky, P., Moreno, R.D., Ramalho-Santos, J., Dominko, T., Simerly, C., Schatten, G., 2000. Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos. Biol. Reprod. 63, 582−590.

Sutovsky, P., Navara, C.S., Schatten, G., 1996. Fate of the sperm mitochondria, and the incorporation, conversion, and disassembly of the sperm tail structures during bovine fertilization. Biol. Reprod. 55, 1195−1205.

Sutovsky, P., Tengowski, M.W., Navara, C.S., Zoran, S.S., Schatten, G., 1997. Mitochondrial sheath movement and detachment in mammalian, but not nonmammalian, sperm induced by disulfide bond reduction. Mol. Reprod. Dev. 47, 79−86.

Tait, S.W., Green, D.R., 2012. Mitochondria and cell signalling. J. Cell Sci. 125, 807−815.

Tarkowski, A.K., Ozdzenski, W., Czolowska, R., 2001. How many blastomeres of the 4-cell embryo contribute cells to the mouse body? Int. J. Dev. Biol. 45, 811−816.

Thangaraj, K., Joshi, M.B., Reddy, A.G., Rasalkar, A.A., Singh, L., 2003. Sperm mitochondrial mutations as a cause of low sperm motility. J. Androl. 24, 388−392.

Thompson, W.E., Ramalho-Santos, J., Sutovsky, P., 2003. Ubiquitination of prohibitin in mammalian sperm mitochondria: possible roles in the regulation of mitochondrial inheritance and sperm quality control. Biol. Reprod. 69, 254−260.

Vemuganti, S.A., Bell, T.A., Scarlett, C.O., Parker, C.E., de Villena, F.P., O'Brien, D.A., 2007. Three male germline-specific aldolase A isozymes are generated by alternative splicing and retrotransposition. Dev. Biol. 309, 18−31.

Wai, T., Ao, A., Zhang, X., Cyr, D., Dufort, D., Shoubridge, E.A., 2010. The role of mitochondrial DNA copy number in mammalian fertility. Biol. Reprod. 83, 52−62.

Wang, H., Chen, X.X., Wang, L.R., Mao, Y.D., Zhou, Z.M., Sha, J.H., 2010. AF-2364 is a prospective spermicide candidate. Asian J. Androl. 12, 322−335.

White, D.J., Mital, D., Taylor, S., St John, J.C., 2001. Sperm mitochondrial DNA deletions as a consequence of long term highly active antiretroviral therapy. Aids 15, 1061−1062.

Wilton, L.J., Temple-Smith, P.D., de Kretser, D.M., 1992. Quantitative ultrastructural analysis of sperm tails reveals flagellar defects associated with persistent asthenozoospermia. Hum. Reprod. 7, 510−516.

Yakes, F.M., Van Houten, B., 1997. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc. Natl. Acad. Sci. USA 94, 514−519.

Yan, H., Yan, Z., Ma, Q., Jiao, F., Huang, S., Zeng, F., Zeng, Y., 2011. Association between mitochondrial DNA haplotype compatibility and increased efficiency of bovine intersubspecies cloning. J. Genet. Genomics 38, 21−28.

Yanagimachi, R., Kamiguchi, Y., Sugawara, S., Mikamo, K., 1983. Gametes and fertilization in the Chinese hamster. Gamete Res. 8, 97−117.

Yang, M., Ge, Y., Wu, J., Xiao, J., Yu, J., 2011. Coevolution study of mitochondria respiratory chain proteins: toward the understanding of protein−protein interaction. J. Genet. Genomics 38, 201−207.

Zhou, Q., Li, H., Xue, D., 2011. Elimination of paternal mitochondria through the lysosomal degradation pathway in *C. elegans*. Cell Res. 21, 1662−1669.