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The maternal age effect: a hypothesis based on oxidative phosphorylation

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Summary

The 'maternal age effect' in human reproduction, characterized by a negative relationship between maternal age and reproductive efficiency, remains a poorly understood phenomenon. Current data suggest that oocyte physiology determines this relationship. In this review, we present a hypothesis of a mitochondrial role in the physiology of ageing in human oocytes. We suggest that the efficiency of oxidative phosphorylation in the ageing human oocyte is degraded by free radical attack on the primordial oocytes residing in the ovary. Although deficiencies in oxidative phosphorylation can be accounted for in the short term by anaerobic respiration, we suggest that, in the long term, the level of oxidative phosphorylation strongly influences oocyte quality.

Keywords: Ageing, Human oocyte, Human reproduction, Mitochondria, Oxidative phosphorylation, Respiration

Introduction

Europe and the United States are currently affected by an ageing demographic population due both to a fall in the birth rate and to an increase in the mean age at which couples choose to have children. Reasons for this include the pressure of work on the couple and the woman's desire to advance her career before taking time out to have children. The increase in the demographic age of these populations not only causes problems for the economy of the countries involved but also increases pressure on the health services due to the increase in numbers of patients seeking treatment for age-related conditions including infertility. The success rate of both natural conception and assisted reproduction in couples in which the maternal age is over 37 years is significantly lower than the level reported for younger couples (Cummins *et al*., 1994; Janny & Menezo, 1996; Keefe, 1997). This is also correlated with an increase in the incidence of genetic diseases such as Down's syndrome (trisomy 21; Hassold & Jacobs, 1984; Antonarakis *et al*., 1992; Mikkelsen *et al*., 1995; Yoon *et al*., 1996), suggesting that the physiology of ageing causes negative effects on the reproductive system. Furthermore, although it is early to test the effect of assisted conception on the health of adults born with this technique, births after both natural and assisted conception to mothers of advanced age are often characterized by indicators of poor health, such as low birth weight, high infant mortality and infertility (Tarin *et al*., 2000, 2001, 2002; Tough *et al*., 2000, 2002; Zuppa *et al*., 2001; Kandstaal *et al*., 2001). These data are in agreement with hypotheses from the human population which suggest that the health of the potential mother can adversely influence the health of the fetus and neonate (Godfrey & Barker, 1995; Barker, 1997).

The maternal age effect thus not only leads to a lower probability of a successful pregnancy, but increases the probability of the birth of an infant affected by poor health. Several hypotheses have been proposed to explain the maternal age effect. These are based on receptivity of the uterus, ovarian reserve, oocyte energy

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levels and oocyte aneuploidy (Navot *et al*., 1994; Cano *et al*., 1995; Faddy *et al*., 1992; Faddy & Gosden, 1996; Keefe *et al*., 1995; Zheng & Byers, 1992; Munne *et al*., 1995; Van Blerkom *et al*., 1995). Although these diverse hypotheses are certainly valid, and the regression of fertility in relation to maternal age does depend on a wide range of factors, experimental and clinical evidence supports the hypothesis that the decline in oocyte quality is the major factor that determines the maternal age effect in human reproduction.

What happens to the oocyte that is so decisive in underscoring oocyte quality? In this review, we will present evidence suggesting that oocyte quality is determined primarily by the level of metabolism and in particular the physiology of mitochondria. We will then present data that suggest the regression of oocyte quality is due to the loss of mitochondrial metabolic efficiency in relation to maternal age.

What underlies the 'maternal age effect' in oocytes?

The relationship between the decrease in oocyte quality and maternal age suggests that some factor or factors within human oocytes decrease in relation to the age of the potential mother, thus negatively affecting oocyte quality. Basically, such factors could include a decrease in protein synthesis, mistakes in DNA synthesis or the inability to correctly form an operational meiotic apparatus, leading to the production of oocytes with low development potential. All these processes require an input of energy. This suggests that the efficiency of energy production (i.e. respiration) is the process that underlies the 'maternal age effect'. Energy in mammalian cells and oocytes is available in the form of adenosine trisphosphate (ATP). ATP is produced in the mammalian cell by both aerobic respiration and anaerobic respiration. Aerobic respiration occurs within mitochondria and involves the complete oxidation of pyruvate to carbon dioxide and water. Anaerobic respiration is cytoplasmic and involves the breakdown of pyruvate to lactic acid. Mammalian oocytes are no different to other mammalian cells in this respect in that these specialized cells possess the enzymes for both respiratory pathways. The current body of data suggests that mitochondrial respiration appears to be strongly subject to ageing (Harman, 1956, 1972). Mitochondria contain DNA coding for proteins that include many elements of the respiratory chain (Anderson *et al*., 1981; Saraste, 2000). However, mitochondrial DNA (mtDNA) does not contain protective histones and has little in the way of DNA repair mechanisms (Linnane *et al*., 1989, 1992). Because oxidative phosphorylation causes the production of free radicals, mitochondria and therefore mtDNA are particularly sensitive to free-radical-induced damage (Wallace, 1987; Tarin, 1995). This brings about an age-related effect on mitochondrial physiology including the ATP production capacity. Oocytes are subject to this effect because oocyte mitochondria originate from the replication of a small pool in the primordial oocyte to form the terminal complement in the mature oocyte (Hauswirth & Laipis, 1982). Primordial oocytes are formed during fetal development and therefore may reside within the ovaries for as long as 40 years before transformation into mature oocytes. This suggests that a low level of free radical production, when extended over a 40–50 year lifetime, could damage the oocyte mtDNA sufficiently to diminish the oocytes' capacity for oxidative phosphorylation. Therefore, in the reproductive system, the effect of mitochondrial damage on primordial follicles may help to explain the 'maternal age effect'.

Where in the developmental pathway is oxidative phosphorylation most critical?

The precise metabolic needs of mammalian embryos during preimplantation development have been notoriously difficult to define. This is because the mammalian embryo is highly flexible in its culture requirements, and can develop normally in a wide range of culture conditions (Gardner & Leese, 1988; Leese, 1995). However, differences between the carbohydrate supply and demand can alter the quality of mammalian embryos (Lane & Gardner, 1998), suggesting that although the embryo can respond to alterations in the carbohydrate supply, this does affect the embryos' developmental potential. The current literature suggests that the pathways of respiration of mammalian gametes may be slightly different between oogenesis, preimplantation embryo development and fetal development. During oogenesis, the evidence suggests that mitochondrial metabolism predominates, although it must be stated here that the metabolism referred to originates mainly in the follicle cells surrounding the oocyte and the oocyte itself may reside in a relatively hypoxic environment (Gosden & Byatt-Smith, 1986). For the purposes of the maternal age effect, this is probably not important as the contribution to the oocyte from the follicular environment determines oocyte quality. In fact, correlations between the state of perifollicular vascularity of developing follicles, the dissolved oxygen content of follicular fluid, and the quality of oocytes retrieved from these follicles suggests that oxidative phosphorylation plays an important role in oogenesis (Van Blerkom *et al*., 1997; Van Blerkom, 2000). Furthermore, mitochondrial mutations in follicle cells have been correlated with age (Seifer *et al*.,

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2002), suggesting that oxidative phosphorylation in the follicle is compromised in advancing age. The available evidence also suggests that after the LH surge mitochondrial metabolism plays a fundamental role in the maturation of the formed oocyte. The rate of oxygen consumption, a measure of mitochondrial metabolism, increases dramatically in the isolated oocyte after the LH surge (Magnusson *et al*., 1977, 1981). Furthermore, although an increase in lactic acid is observed after the LH surge, suggesting anaerobic respiration (Nilsson, 1974; Hillensjo, 1976; Tsafriri *et al*., 1976), the use of iodoacetate to block anaerobic respiration does not inhibit oocyte maturation, suggesting that anaerobic respiration is not necessary for oocyte maturation (Tsafriri *et al*., 1976). In contrast, oocyte maturation is blocked when aerobic respiration is suppressed (Zeilmaker & Verhamme, 1974; Gwatkin & Haidri, 1974; Hu *et al*., 2001).

After fertilization in mammalian embryos, measurements of the relative levels of aerobic and anaerobic respiration suggest that both pathways are active during preimplantation embryo development. Mammalian oocytes and preimplantation embryos prefer pyruvate as an energy source (Brinster, 1969, 1971; Cross & Brinster, 1973; Rushmer & Brinster, 1973; Leese & Barton, 1984; Gardner *et al*., 1993; Leese, 1995; Butcher *et al*., 1998; Mehta, 2001; Biggers, 2002). The preference for pyruvate, however, does not eliminate either aerobic or anaerobic respiration as a possible route to ATP production. Lactic acid is present in fluid sampled from human tubules, and is also produced during mammalian embryo development, suggesting a component of anaerobic respiration in the ATP-generating mechanism. Mitochondria within the preimplantation embryo do not replicate, and are characterized by poorly formed cristae, suggesting that the level of mitochondrial respiration is low during this stage (Piko & Taylor, 1987; Ebert *et al*., 1988; Mereilles & Smith, 1998). Evidence from mice also suggests that embryos can develop and even implant adequately in the absence of mitochondrial respiration, although development does not continue until birth (Piko & Chase, 1973; Larsson *et al*., 1998; Li *et al*., 2000). These data suggest that anaerobic respiration can support preimplantation embryo development, at least in the mouse. Aerobic respiration appears to be upregulated at blastocyst development because the mitochondrial cristae become more compact, mitochondrial replication is initiated and the utilization of glucose as a carbohydrate substrate increases at this stage (Leese & Barton, 1984; Leese *et al*., 1993; Leese, 1995; Martin Leese, 1995; Gardner *et al*., 1993; Barnett & Bavister, 1996). These data suggest that the intracellular energy supply during both oocyte maturation and embryo implantation is controlled mainly through aerobic respiration, but anaerobic respiration predominates during mammalian preimplantation development.

Hypothesis

The above data suggest, therefore, that oxidative phosphorylation is critical for mammalian oogenesis and postimplantation development, and may programme the developmental competence of the embryo. However, considering the presence of an active anaerobic respiratory pathway, it is difficult to understand the influence of repressed mitochondrial respiration, such as may be the case in advanced maternal age, in mammalian preimplantation embryo development. The present data suggest both that mitochondrial respiration underlies the 'maternal age effect' and that it is not essential for mammalian embryo development. This seemingly contradictory set of data becomes clearer when presented as a hypothesis of mammalian embryo development where aerobic and anaerobic respiratory pathways can be considered complementary (Fig. 1*a*). In this sense, we suggest that the oocytes' requirement for ATP is met through both aerobic and anaerobic respiration. In the absence of sufficient ATP from the aerobic pathway, anaerobic respiration steps in, and vice versa. Aerobic respiration does not lead to side products as the pyruvate precursor is completely degenerated to carbon dioxide and water. Anaerobic respiration causes the production of lactic acid. Lactic acid is eliminated from the cell, but is also a substrate for the cytoplasmic enzyme lactate dehydrogenase, and so a proportion of lactic acid is reconverted to pyruvate (Fig. 1*a*). The conversion of lactate to pyruvate through lactate dehydrogenase requires NAD^+ (Fig. 1*a*). NAD^+ is mainly produced during oxidative phosphorylation (Newsholme & Leech, 1983 (Fig. 1*a*)). These data suggest that aerobic and anaerobic respiration are interlinked. In normal circumstances (in our case oocytes derived from young women), the supply of NAD^+ is sufficient to enable the complete catabolism of the lactate built up during anaerobic respiration (Fig. 1*a*). However, when mitochondrial metabolism is insufficient, two situations occur. First, the level of anaerobic respiration increases to high levels, causing high levels of lactate production, and consequently depleting exogenous carbohydrate sources without creating an adequate supply of ATP (Fig. 1*b*). Second, the high level of lactate forces the lactate-dehydrogenase-catalysed reaction towards the conversion of lactate to pyruvate, depleting the NAD^+ pool (Fig. 1*b*). Because this cannot be replaced by sufficient oxidative phosphorylation, the rundown of both aerobic and anaerobic respiration results, leading to insufficient energy production, and causing the degeneration in embryo development.

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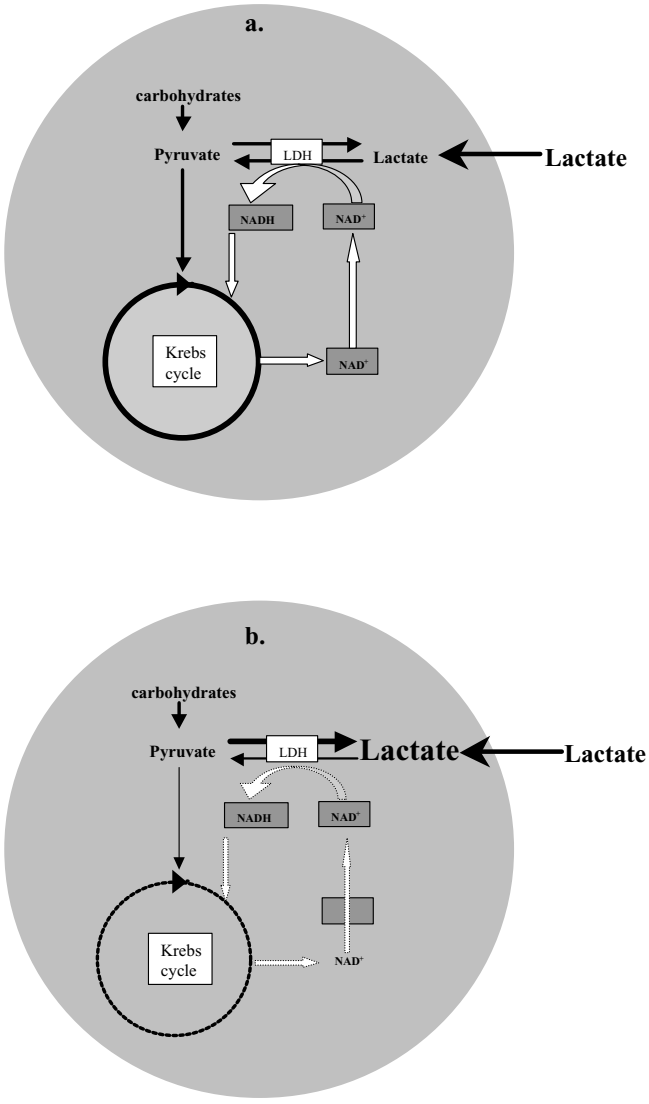


Figure 1 Association between aerobic and anaerobic respiration. The figure is representative of the metabolic state of a mammalian oocyte in two metabolic situations: the upper figure represents normal metabolism including both aerobic and anaerobic components and the lower figure represents the case in which aerobic respiration is severely repressed. As demonstrated in the figures, lactic acid, present in the external medium, enters the oocyte and is also produced by anaerobic respiration in the oocyte cytoplasm. Conversion of lactate to pyruvate requires the cofactor NAD^+, which is reduced to NADH and then reoxidized to NAD^+ during the Krebs (tricarboxylic acid) cycle (Newsholme & Leech, 1983). In the normal situation (*a*), NADH is adequately reoxidized and both aerobic and anaerobic respiration can continue. When aerobic respiration is repressed (*b*; which may represent oocytes from older women), two imbalances occur: first, excess lactic acid is produced, and leads to build-up of this product in the oocyte cytoplasm; second, insufficient reoxidization of NADH occurs. We hypothesize that this leads in the long term to the rundown of NAD^+ and consequently causes ATP insufficiency.

Evidence supporting the hypothesis

It is well established that ageing causes negative effects on human cells, such as slow growth and senescence (Dice, 1993; Fossel, 2002). However, the evidence of a direct association between oxidative phosphorylation and ageing is more recent. In the human, one of the measurable effects of maternal age on cell physiology is a decrease in the ATP production capacity of mitochondria (Shigenaga *et al*., 1994). Human preimplantation embryos are probably particularly prone to the influence of this factor, as the embryo implantation potential is correlated with the ATP content of the embryo (Van Blerkom *et al*., 1995) and may show a relationship with the mitochondrial membrane potential (Wilding *et al*., 2001, 2002). In particular, embryos with chaotic mosaicism, thought to indicate a zero implantation potential, were shown to be characterized by a significantly lower mitochondrial membrane potential than other embryos (Wilding *et al*., 2003). The expression of chaotic mosaicism in the embryo was suggested to be derived from the relationship between low mitochondrial activity in the oocyte and a poorly formed meiotic apparatus (Wilding *et al*., 2003). Furthermore, a relationship between maternal age and meiotic aberrations has been suggested in both mice and humans (Tarin, 1995, 1996; Liu & Keefe, 2002; Wilding *et al*., 2003) and antioxidant therapy appears to counteract this (Tarin *et al*., 1998). Embryos affected by chaotic mosaicism tend to divide at slower rates than normoploid embryos and often block during preimplantation development (Delhanty *et al*., 1997; Bielanska *et al*., 2002; Wilding *et al*., 2003). The rate of blastocyst formation is also negatively correlated with age (Janny & Menezo, 1996). These data suggest that oogenesis programmes the developmental fate of the future embryo, and further defines the role of mitochondria in the expression of the maternal age effect.

Conclusions

Although there is a large body of evidence on the clinical effects of advanced maternal age on human reproduction, the underlying causes of these effects are still not well understood. Current clinical data suggest, however, that the physiological effects of advanced maternal age reside within the oocyte, and not with uterine receptivity. Our discussion of what is known about ageing suggests that the physiological effect on the human oocyte of advanced maternal age is determined by the damaging effects of free radical production during oxidative phosphorylation on the mitochondrial genome. We further suggest that,

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although anaerobic respiration can account for the excess requirement for ATP of the oocyte and preimplantation embryo, the reduction in the respiratory capacity of mitochondria can also negatively affect anaerobic respiration. This hypothesis accounts for the observation that embryos can develop *in vitro* in the absence of oxidative phosphorylation, and further suggests that mitochondrial metabolism can influence the ATP production capacity of the human preimplantation embryo in the long term, even when ATP production is accounted for in the short term. From what is known about oogenesis in mammalian systems, the current data suggest that oxidative phosphorylation is critical during this stage, and is an integral part of the foundation of what is currently termed 'oocyte quality' in that the evidence supports oxidative phosphorylation being required for the establishment of a morphologically normal meiotic apparatus capable of forming an embryo without chromosomal defects. These hypotheses suggest that an area of future development for the field of *in vitro* fertilization should be in the improvement of protocols for controlled ovarian hyperstimulation concentrating on the level of ovarian oxidative phosphorylation.

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