

## REVIEW

# Long-term effects of delayed parenthood

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**The present study aims to define, characterize and compare the long-term effects on offspring of delayed parenthood. Data published so far on this topic show that maternal and paternal ageing may affect offspring by different mechanisms. Delayed motherhood is characterized by increased probability of obstetric complications and/or fetal and perinatal problems which, in turn, may increase the risks of mortality and morbidity in newborns and later life. Furthermore, maternal ageing is distinguished by a decreased ratio of male to female infants and higher odds of conceiving a trisomic child and/or an individual suffering from mitochondrial DNA disorders. In contrast, delayed fatherhood is associated with higher risks of conceiving an individual suffering from new inheritable-mutation disorders. The different pattern of disease in offspring associated with maternal and paternal ageing may be explained, among other factors, by the fact that (i) oocytes of middle-aged women may suffer oxidative stress because their mitochondria produce higher amounts of reactive oxygen species; (ii) diplotene oocytes and to a lesser extent metaphase I and II oocytes have an efficient DNA repair system which is essentially independent of maternal age; and (iii) mitochondria are transmitted to the next generation along the matrilineal line. Moreover, (i) the activities of antioxidant enzymes within the seminal plasma and spermatozoa from older men may be reduced and so spermatozoa may be more vulnerable to mutational changes than spermatozoa from younger men; and (ii) late spermatids, and immature and mature spermatozoa do not have a DNA repair system.**

*Key words:* maternal age/mitochondrial diseases/mutation rate/oocytes/paternal age

## Introduction

The *modus vivendi* (prolonged education, difficulties in finding stable employment, etc.) of the present-day occidental society is forcing women increasingly to delay the moment of the first pregnancy until their mid-thirties or even beyond, i.e. the latter part of the woman's childbearing years. Although some authors

claim that the risks of delayed parenting are overstated (Ales *et al.*, 1990; Berkowitz *et al.*, 1990; Antinori *et al.*, 1995; Bowman and Saunders, 1995), overwhelming evidence shows that delayed motherhood is not only associated with infertility and obstetrical problems, such as hypertension and/or toxemia, gestational diabetes mellitus, premature rupture of membranes, late pregnancy bleeding, delivery by Caesarean section and maternal mortality (Vercellini *et al.*, 1993; Dildy *et al.*, 1996; for reviews, see Hansen, 1986; Lansac, 1995), but also with fetal and perinatal problems (see Table I).

It has been suggested that delayed motherhood is associated with an increased proportion of premature (delivery before 37 weeks) and dysmature (infants born small for gestational age) babies with a birthweight less than 2500 g as well as with higher perinatal morbidity and mortality rates (Vercellini *et al.*, 1993; Dildy *et al.*, 1996; for reviews, see Hansen, 1986; Lansac, 1995). Nonetheless, when data are adjusted for other low-birthweight risk factors, such as parity, education, early prenatal care, pre-pregnancy weight, weight gain and smoking status, the differences in birthweight and mortality between younger and older women are no longer significant (for review and references, see Kramer, 1987; Cogswell and Yip, 1995, respectively). Early studies suggested also that older maternal age, which may be associated with higher pregnancy oestrogen levels, is associated with increased risks of breast and testicular cancer of offspring. However, recent studies have found this association to be weak or non-existent (for references, see Moller and Skakkebaek, 1996; Weis *et al.*, 1997).

Evidence of possible associations between maternal ageing and the incidence of specific non-cytogenetic congenital malformations, such as spina bifida, cleft palate, syndactyly, limb deficiencies, male genital tract malformations, etc., is controversial. On the one hand, there are studies suggesting that middle-aged women have higher risks of giving birth to a child with non-cytogenetic congenital malformations (Hay and Barbano, 1972; el-Shafei *et al.*, 1986; Swerdlow and Melzer, 1988; Chaturvedi and Banerjee, 1993; Halliday *et al.*, 1993; Castilla *et al.*, 1995). In contrast, several studies (Abudu *et al.*, 1988; Baird *et al.*, 1991; 1994; Hsieh *et al.*, 1995), some of them analysing very large samples (Baird *et al.*, 1991), show no effect at all. Maternal ageing may indirectly increase the risks of non-cytogenetic congenital malformations by increasing the frequency of diabetes mellitus among older pregnant women (Schaefer *et al.*, 1997) and/or extending the follicular phase (pre-ovulatory oocyte overripeness) of the

**Table I.** Long-term effects of maternal ageing on offspring

**Fetal and perinatal problems**

Complete hydatidiform mole and choriocarcinoma<sup>a, 1-2</sup>

Dizygotic twin pregnancy<sup>b, 3-6</sup>

Spontaneous abortion<sup>7-8</sup>

Newborns with a birthweight more than 4000 g<sup>c, 9</sup>

**Decreased ratio of male to female infants<sup>d, 10-12</sup>**

**Trisomy<sup>e, 13</sup>**

**Mitochondrial DNA diseases<sup>f</sup>**

Congenital sensorial hearing loss<sup>14-15</sup>

Cerebellar ataxia<sup>16</sup>

Type I (insulin-dependent) diabetes mellitus<sup>17-18</sup>

Alzheimer's disease<sup>19-21</sup>

<sup>a</sup>This anomaly is likely to be due to ovulation of an increased proportion of anuclear oocytes by middle-aged women (Yamaguchi, 1987).

<sup>b</sup>The probability of having dizygotic twins increases with maternal age, which is likely to be due to a quantitative decrease of an inhibitor factor of follicular maturation and/or increase of follicle stimulating hormone secretion leading to double ovulation. However, after 37 years of age, dizygotic twin rate decreases, which is likely to be due to the reduced fertility in older women (Bulmer, 1970; Lazar, 1996; for review, see Bomsel-Helmreich and Al Mufti, 1995).

<sup>c</sup>This outcome is consistent with the higher frequency of diabetes mellitus among older pregnant women (for review, see Hansen, 1996). Gestational diabetes, in turn, may increase the risks of mortality and morbidity in newborns including macrosomia, hypoglycaemia, hyperbilirubinaemia, hypocalcaemia and polycythaemia, or even cause delayed chronic morbidity to the offspring by inducing obesity and diabetes (Hod *et al.*, 1991).

<sup>d</sup>Although factors such as parental hormone levels at the time of conception (James, 1996) and coital frequency (Martin, 1997) should be taken into account when analysing the causes of decreased male:female ratios at advanced maternal age, it appears that human endometria from older women select against fast-cleaving concepti (males) at implantation (Tarín *et al.*, 1995) or post-implantation (Juntunen *et al.*, 1997) stages.

<sup>e</sup>The incidence of the majority of trisomies among all clinically recognized pregnancies is highly correlated with increasing maternal age. In contrast, there is no significant effect of maternal age on the frequency of sex chromosome monosomy or polyploidy (Hassold and Chiu, 1985). Maternal-age associated trisomy may have a multifactorial origin. Nonetheless, oocytes of middle-aged women may suffer oxidative stress because their mitochondria produce higher amounts of reactive oxygen species. The intracellular oxidative stress of oocytes may, in turn, affect the dynamic equilibrium of microtubules and microfilaments and so induce mistakes in chromosome segregation (Tarín, 1995, 1996).

<sup>f</sup>We should bear in mind that sensorial hearing loss in both children and adults and ataxia are typical neurological manifestations of mitochondrial disorders (for review, see Gold and Rapin, 1994; Zeviani and Antozzi, 1997). Moreover, clinical evidence exists showing an involvement of mitochondrial DNA and/or oxidative phosphorylation defects in the development of some cases of diabetes (for review, see Luft, 1994; Zeviani and Antozzi, 1997) and Alzheimer's disease (for review, see Wallace, 1992). The biochemical and genetic heterogeneity of Alzheimer's disease may explain, however, the absence of maternal-age effect on Alzheimer's disease reported in some studies (Prince *et al.*, 1994; for references, see Farrer *et al.*, 1991; Rocca *et al.*, 1991; van Duijn and Hofman, 1992).

<sup>1</sup>Parazzini *et al.*, 1986; <sup>2</sup>for review, see Palmer, 1994; <sup>3</sup>Bonnelykke, 1990; <sup>4</sup>Fretts *et al.*, 1995; <sup>5</sup>Campana and Roubicek, 1996; <sup>6</sup>Lazar, 1996; <sup>7</sup>Coste *et al.*, 1991; <sup>8</sup>Smith and Buyalos, 1996; <sup>9</sup>for review, see Hansen, 1996; <sup>10</sup>Juntunen *et al.*, 1997; <sup>11</sup>Tarín *et al.*, 1995; <sup>12</sup>for review, see James, 1987; <sup>13</sup>Hassold and Chiu, 1985; <sup>14</sup>Liu *et al.*, 1994; <sup>15</sup>Sutton and Rowe, 1997; <sup>16</sup>Skre, 1970; <sup>17</sup>Flood *et al.*, 1982; <sup>18</sup>Wagener *et al.*, 1983; <sup>19</sup>for references, see Farrer *et al.*, 1991; <sup>20</sup>for references, see Rocca *et al.*, 1991; <sup>21</sup>for references, see van Duijn and Hofman, 1992.

conceptional cycle because of the endocrine irregularities associated with the later pre-menopausal years (Troya *et al.*, 1985). Nonetheless, the relatively low frequency of gestational diabetes among middle-aged women (less than 3%) together with the unpredicted nature of variations in length of the menstrual cycles during the pre-menopausal years may conceal any existing effect of maternal ageing on the incidence of non-cytogenetic congenital malformations. Furthermore, the multifactorial origin of non-cytogenetic congenital malformations may contribute further to obscure any direct or indirect effect of maternal ageing on offspring. Indeed, non-cytogenetic congenital malformations may be caused by age-independent factors, including maternal exposure during pregnancy to teratogenic agents, fetal alcohol syndrome and maternal virus infections such as rubella, toxoplasmosis, cytomegalovirus infection and, possibly, varicella.

Other inconsistencies in the literature are found when analysing the effect of maternal ageing on neurological disorders of offspring. Childbearing at advanced maternal age has been associated with cerebral palsy (Durkin *et al.*, 1976), epilepsy (Degen, 1978; Monetti *et al.*, 1995), autism (Gillberg, 1980), dyslexia (Jayasekara and Street, 1978), psychotic dis-

orders (Gillberg, 1982; Kinnell, 1983) and minor neurodevelopmental disorders including fine-motor problems, visual-perceptual dysfunction and attentional deficit signs (Gillberg *et al.*, 1982). However, most of these studies are limited by the fact that a case-control method was not used. Recent case-control studies fail to show any effect of maternal ageing on autism (Cryan *et al.*, 1996) and psychotic disorders (Bertranpetit and Fañanás, 1993). It has been also suggested that the association between cerebral palsy or psychotic disorders and increased maternal age may be secondary to a paternal age effect on fresh dominant genetic mutation (Crow, 1987; Fletcher and Foley, 1993). Moreover, maternal-age-associated epilepsy may be caused by prenatal and perinatal exposure to risk factors such as toxæmia of pregnancy and low or heavy weight at birth rather than maternal ageing *per se* (Degen, 1978; Fiaschi *et al.*, 1986).

**Transmission of maternal-age-associated mitochondrial DNA diseases**

It has been reported that oocytes/ovaries from older and/or post-menopausal women are more likely to contain mitochon-

drial DNA with the so-called 'common deletion', which removes 4977 bp between the *ATPase8* and *ND5* genes, than oocytes from younger women (Kitagawa *et al.*, 1993; Keefe *et al.*, 1995). Moreover, pre-ovulatory oocytes from middle-aged women show increased mitochondrial numerical density, mitochondrial volume ratio and mitochondrial profile area suggesting, therefore, subtle but generalized changes in the oxidative phosphorylation capacity (Muller-Hocker *et al.*, 1996). Such generalized damage to mitochondria may increase the probability of defective mitochondria being transmitted to the next generation and so cause patent phenotypical expression of mitochondrial DNA diseases (see Table I), reduce the reproductive potential (biological fitness) of offspring, or even decrease the longevity in otherwise normal-looking individuals. No paternally inherited mutations of mitochondrial DNA have been reported so far owing to the 1000:1 ratio of maternal to paternal mitochondrial DNA present in zygotes and/or the total or partial failure of sperm mitochondria to survive and replicate throughout conceptus development (for reviews, see Cummins *et al.*, 1993; Smith and Alcivar, 1993).

Inheritance of excess loads of maternal defective mitochondria allocated to ovarian/testis-forming tissues may cause infertility in offspring (Cummins *et al.*, 1994). Although it remains to be ascertained whether maternal ageing is associated with increased levels of mitochondrial DNA mutations in spermatozoa of offspring, it appears that point mutations in the mitochondrial tRNA leucine (*UUR*) gene (Folgerø *et al.*, 1993) or large-scale rearrangements, such as the mitochondrial 'common deletion', the 7436-bp deletion and the 260-bp tandem duplication in the D-loop of mitochondrial DNA, are associated with diminished fertility and motility of human spermatozoa (Kao *et al.*, 1995). Late-conceiving women may also produce infertile offspring by two different but interconnected events. First, mitochondrial DNA from oocytes of older mothers may be exposed to endogenous and/or exogenous mutagen metabolites during the long period of arrested prophase that oocytes spend in the ovaries before the conception cycle. Secondly, oocytes from middle-aged women may suffer from pre-ovulatory overripeness because of endocrine irregularities during the last pre-menopausal years. Each one of these events acting either separately or together may induce mitochondrial DNA and/or oxidative phosphorylation defects in oocytes (Tarín, 1995; 1996), and so decrease the biological fitness of offspring. Interestingly, preliminary data (sample size  $n = 118$ ) show that women over 40 years of age have a significantly greater chance of their sons being infertile, due to asthenozoospermia, than women under the age of 20 (St. John *et al.*, 1997). Advanced maternal age at conception ( $\geq 40$  years of age) is also associated with increased risk of menstrual disorders, and so subfecundity of the daughter (Smits *et al.*, 1997b). In addition, Smits *et al.* (1997a) have reported that fecundability, i.e. the risk of conception per month or menstrual cycle, is associated with the month of birth of the conceiving women. This result agrees with the hypothesis of 'seasonal pre-ovulatory overripeness of the oocyte' and with the trans-generational concept of familial subfecundability along matrilineal lines (Jongbloet, 1993).

Inheritance of excess loads of maternal defective mitochon-

**Table II.** Long-term effects of paternal ageing on offspring

**Dominant disorders<sup>1-4</sup>**

Wilms' tumour,<sup>5</sup> thanatophoric dysplasia,<sup>6</sup> retinitis pigmentosa,<sup>7</sup> osteogenesis imperfecta type IIA,<sup>8</sup> acrodysostosis, achondroplasia, Apert's disease, fibrodysplasia ossificans progressiva, aniridia, bilateral retinoblastoma, multiple exostoses, Marfan's, Lesch-Nyhan's, Pfeiffer's, Waardenburg's, Treacher-Collin's, Soto's and Crouzon's syndromes, basal cell nevus, cleidocranial dysostosis, polyposis coli, oculodentodigital syndrome, Costello syndrome, progeria, Recklinghausen's neurofibromatosis, tuberous sclerosis and renal polycystic kidney disease

**X-linked recessive diseases**

Haemophilia A and Duchenne's muscular dystrophy<sup>2</sup>

**Non-cytogenetic congenital defects**

Congenital cataracts, reduction defects of the upper limb, nasal aplasia, pulmonic and urethral stenosis, preauricular cyst, cleft palate, neural tube defects<sup>4,9</sup>

**Athetoid/dystonic cerebral palsy and congenital hemiplegia<sup>10</sup>**

**Psychotic disorders<sup>11-14</sup>**

**Decreased learning capacity and/or mental retardation of unknown aetiology<sup>2,4</sup>**

<sup>1</sup>For review, see Risch *et al.*, 1987; <sup>2</sup>for review, see Auroux, 1995; <sup>3</sup>for review, see Crow, 1997; <sup>4</sup>for review, see Gavrilo and Gavrilo, 1997; <sup>5</sup>Olson *et al.*, 1993; <sup>6</sup>Martínez-Frís *et al.*, 1988; <sup>7</sup>Kaplan *et al.*, 1990; <sup>8</sup>Young *et al.*, 1987; <sup>9</sup>McIntosh *et al.*, 1995; <sup>10</sup>Fletcher and Foley, 1993; <sup>11</sup>Hare and Morgan, 1979; <sup>12</sup>Gillberg, 1982; <sup>13</sup>Kinnell, 1983; <sup>14</sup>Axelsson and Lagerkvist-Briggs, 1992.

dria may also induce daughters of middle-aged women to have increased risks of conceiving a trisomic child. First-generation women may inherit defective mitochondria from their mothers and so their oocytes/ovarian cells produce, irrespectively of women's age, increased levels of reactive oxygen species. As result of this intrinsic higher rate of reactive oxygen species production, oocytes may have higher risks of making mistakes in chromosome segregation during the first and/or the second meiotic division of oocytes (Tarín, 1995, 1996). Data supporting this hypothesis come from the study of Aagesen *et al.* (1984) in which a significant advanced grandmaternal age at birth of mother was found in cases of Down's syndrome when compared with that of the control groups. Previously, Papp *et al.* (1977) found a higher maternal and paternal grandmaternal age in Down's syndrome children born to mothers under 30 years of age. However, no association of grandmaternal age and Down's syndrome of children born to mothers aged  $\geq 30$  years was observed in that study (Papp *et al.*, 1977). This result may be explained by the independent effect on chromosomal segregation of both maternal and grandmaternal ageing at the time of conception (Aagesen *et al.*, 1984) together with the so-called 'bottleneck', whereby a small amount of mitochondrial DNA populates the organism (for reviews, see Poulton and Marchington, 1996; Boore, 1997). Such a genetic filter may decrease the relative frequency of grandmaternal-induced versus maternal-induced trisomy, and so conceal any potential association between grandmaternal age at birth of mother and Down's syndrome in children born to women aged  $\geq 30$  years.

A role of extranuclear factors in the lifespan of individuals has been demonstrated in *Drosophila melanogaster*. In this species it appears that, in addition to a few nuclear genes, lifespan is influenced by extranuclear, presumably mitochondrial, factors (Yonemura *et al.*, 1991). In humans, preliminary data show that both maternal and paternal ageing does not

affect the lifespan of sons, although they may decrease the lifespan of daughters (Gavrilov *et al.*, 1997a,b). These authors analysed the lifespan of offspring from European aristocratic families with well-known genealogy. They found that (i) daughters born to fathers of 50 to 59 years of age lose about 4.4 years of their life when compared with daughters of 20 – 29 year old fathers (Gavrilov *et al.*, 1997a); and (ii) daughters born to mothers over 40 years old show a lifespan about 3.6 years lower than that of daughters born to mothers younger than 40 years. This difference was increased to 4.5 years when data on ‘long-lived’ mothers (those who lived 70 years or longer) were analysed (Gavrilov *et al.*, 1997b). Although multiple regression analysis is needed to discriminate between the maternal- and paternal-age effect, it is possible that longevity of offspring is affected independently by ageing of both parents before the conception cycle. According to Gavrilov and his colleagues, the paternal effect may be explained by accumulation of gene mutations in ‘housekeeping’ genes on the paternally transmitted X chromosome. They explain the absence of the maternal-age effect on lifespan of sons by a stronger ‘rejection’ (higher mortality) of males during embryonic/fetal stages and early childhood.

### Spontaneous mutation and paternal age

If we compare the long-term effects on offspring of maternal ageing with those induced by ageing of fathers, we find that, in contrast to the female counterpart, delayed paternity is associated with increased risks of conceiving a child suffering from a new inheritable-mutation disorder (see Table II).

It appears that, for approximately two-thirds of syndromes, the mutation rate increases with paternal age at a rate much faster than linear. In particular, the increase in number of mutations with paternal age fits an exponential model with a cubic term. In the remaining syndromes, mutation rate shows a low rate of increase with paternal age (Risch *et al.*, 1987; Crow, 1997). According to Crow (1997), the low rate-of-increase syndromes would be induced by a minority of base substitutions or point mutations with a strong paternal effect and by a majority of deletions/duplications coming from both parents. However, syndromes associated with an exponential increase in mutation rate with paternal age would be induced mainly, if not entirely, by point mutations. Crow (1997) invokes the greater number of cell divisions in the male germ line when compared with the female germ line as the major explanation of the high ratio of male-to-female mutation rate found in human beings. This assumption is based on the ‘copy-error’ model proposed by Penrose (1955) whereby oogonia/spermatogonia would accumulate mutations with each replication cycle. In fact, whereas in the female, an oogonium divides approximately 21 times before becoming an oocyte, in the male, spermatogonia divide approximately 30 times before puberty, and thereafter it is assumed they keep a constant rate of 23 divisions per year (Vogel and Rathenberg, 1975). Furthermore, Crow (1997) has suggested that age-associated deterioration of the fidelity of replication, efficiency of editing and error correction machinery explains the exponential increase of point mutations with paternal age. However, other

factors such as the intrinsic differences between spermatozoa and oocytes in their potential for repairing endogenous (e.g. age-associated oxidative stress) or exogenous (e.g. parental exposure to ionizing and non-ionizing radiation or to other mutagenic agents) damage to DNA should also be taken into account. Indeed, it is known that (i) the activities of antioxidant enzymes within the seminal plasma and spermatozoa from older men may be reduced (Kelso *et al.*, 1996, 1997), and so spermatozoa may be more vulnerable to mutational changes than spermatozoa from younger men; (ii) late spermatids and immature and mature spermatozoa do not have a DNA repair system (Matsuda *et al.*, 1989; Inoue *et al.*, 1993); and (iii) diplotene oocytes, and to a lesser extent metaphase I and II oocytes, have an efficient DNA repair system which is essentially independent of maternal age (for review, see Ashwood-Smith and Edwards, 1996).

In conclusion, we have outlined the potential long-term effects on offspring of both maternal and paternal ageing at the moment of conception. It should be borne in mind, however, that it is not possible to infer an unequivocal correspondence between parental ageing and increased morbidity and mortality in offspring from the existence of epidemiological associations between these variables. It appears that delayed motherhood is associated with few obstetric, fetal and neonatal risks within the context of ‘modern obstetric care’ (Antinori *et al.*, 1995; Bowman and Saunders, 1995). In addition, middle-aged mothers may transmit to their offspring different amounts of mutated mitochondrial DNA due to the ‘bottleneck’ that mitochondria pass from one generation to another (for reviews, see Poulton and Marchington, 1996; Boore, 1997). Trisomy incidence can be controlled by monitoring chromosomal disorders using appropriate preimplantation and/or prenatal diagnosis tests. Finally, the mutational effect on nuclear DNA of paternal ageing is clinically relevant only at very advanced ages, i.e. in the fifties and beyond (for reviews, see Auroux, 1995; Crow, 1997; Gavrilov and Gavrilova, 1997).

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