

REVIEW

Advanced paternal age and reproductive outcome

Zofnat Wiener-Megnazi, Ron Auslender and Martha Dirnfeld

Women have been increasingly delaying the start of motherhood in recent decades. The same trend is seen also for men. The influence of maternal age on fertility, chromosomal anomalies, pregnancy complications, and impaired perinatal and post-natal outcome of offspring, has been thoroughly investigated, and these aspects are clinically applied during fertility and pregestational counseling. Male aging and reproductive outcome has gained relatively less attention. The purpose of this review is to evaluate updated and relevant literature on the effect of paternal age on reproductive outcome.

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INTRODUCTION

The rise in life expectancy, women's entry into the labor market and the popular use of contraception, have contributed to the social phenomena of delaying family planning and parenthood to the mid or late thirties.

Assisted reproductive technologies have become more accessible to the general population, enabling older couples to hope that they can materialize their aspirations for healthy offspring later in life. It is well established that the main limiting factor to fertility and good reproductive outcome has been female's age.^{1,2}

The trend of older parenthood is true also for males. In Germany, the median age of married fathers has increased from 31.3 years in 1991 to 33.1 years in 1999.³ The same trend has also been observed in England. In 1993, fathers aged 35–54 years accounted for 25% of live births. Ten years later, these percentages grew to 40%⁴ (Figure 1). Therefore, attention should also be paid to the influence of advanced paternal age on reproductive outcome. In this review, we have extensively investigated the present data on male age-related effects on sexuality, testicular function, sperm parameters, risk of chromosomal disorders, specific genetic mutations, epigenetic changes, multifactorial disorders and perinatal outcome.

Testicular function and spermatogenesis

In contrast to menopause, which marks the cessation of ovarian function due to the inevitable loss of female gametes, spermatogenesis continues throughout life.⁵ Nevertheless, male aging does exert a certain detrimental effect on reproductive organs and tissues. These reported changes develop gradually without a sudden age threshold. Testicular volume decreases only towards the eighth decade⁶ and in healthy men, it is 31% lower than in the 18- to 40-year age group. This change is accompanied by alterations in testicular histomorphology observed in older men.^{7,8} In a multivariate analysis, follicle-stimulating hormone (FSH), inhibin B and testosterone were independently associated with bilateral testicular volume in the elderly. This moderately decreased bilateral testicular volume observed in elderly men was strongly

associated with a decrease in the inhibin B/FSH ratio, and was consistent with a reduced Sertoli cell mass, compensated by increased FSH stimulation resulting in only limited decrease of Sertoli cell function.⁹ FSH increases slightly in men between 40 and 70 years.^{10,11} This is concomitant with a decrease in inhibin B and an increase in activin A.¹⁰ In addition, the decrease in Leydig cell function yields in an age-related reduction in testosterone levels,^{8,10} starting at the age of 30 years.¹² The manifestations include decreased bone and muscle mass, sexual body hair and beard growth, and increased abdominal obesity, as well as higher incidence of erectile dysfunction, which increases to >30%–40% in the eighth decade.^{7,12,13} Age-related erectile dysfunction has also been associated with endothelial dysfunction due to atherosclerosis of the penile arteries and a reduction in the production of nitric oxide.⁷ In a retrospective study of 3698 infertile patients, the incidence of infection in the accessory glands increased from 6.1% among males aged <25 years to 13.6% of patients aged >40 years, and total sperm count was lower ($P<0.0001$).¹⁴ Numerous studies have examined the age-related effect on semen characteristics.³ There is a trend of age-related decline in daily sperm production per testis.⁶ Semen volume and motility (evaluated both qualitatively and by computer-assisted sperm analysis method), tend to decrease with age.^{15,16}

In some studies, a paternal age-related decline in semen characteristics was not observed, but this is probably due to relative small numbers of older-aged men in the study population. One of the proposed mechanisms for the age-related decrease in testicular function is a change in the production of reactive oxygen species by Leydig cells mitochondria.¹² Oxidative potential in seminal plasma (measured by the thermochemiluminescence assay) from 148 men undergoing semen analysis exhibited¹⁷ was inversely correlated with patients' age (Lissak *et al*, 2004, unpublished data).

REPRODUCTIVE OUTCOME IN *IN VITRO* FERTILIZATION (IVF)

Age-related decline in testicular function could be overcome by the use of IVF–intracytoplasmic sperm injection techniques in terms of

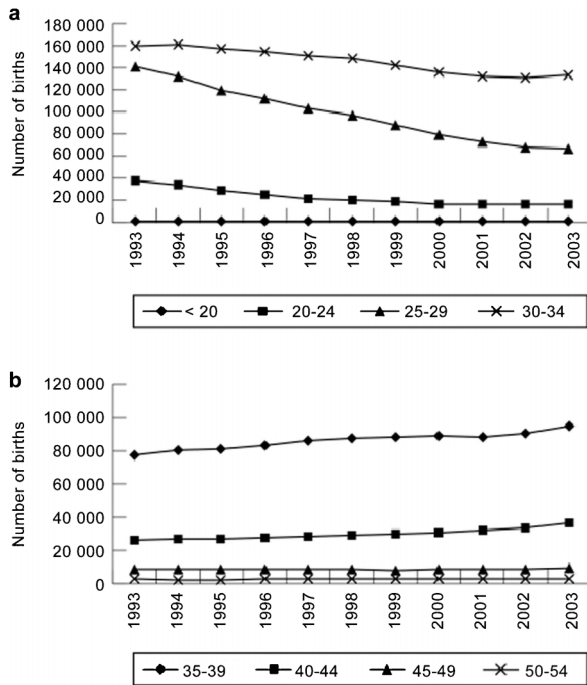


Figure 1 Trends in paternal age for live births within marriage in England and Wales, 1993–2003: (a) decreasing trends (<35 years); (b) increasing trends (35–54 years). Source: Birth Statistics, England and Wales, Series FM1 no. 32 (Office for National Statistics, 2003). (Births to fathers over 54 years account for less than 0.5% of live births within marriages and are not shown.) Reproduced with permission from Ref. 4.

fertilization and pregnancy rates. Dain *et al.*¹⁸ found in their review that except for an age-dependent decrease in semen volume, other parameters such as concentration, motility and morphology, were not found to decrease with advancing age.

Moreover, they have found no correlation between advanced paternal age and rates of fertilization, implantation, pregnancy, miscarriage and live birth in IVF (Table 1).^{18–28} Although embryo quality at the cleavage stage (days 2–3) was not affected by increasing males' age, it was associated with a significant decrease in blastocyst embryo formation, probably reflecting male genomic activation within the embryo.

CHROMOSOMAL ABNORMALITIES

Aneuploidy accounts for 30%–50% of all pregnancies. Most are lethal and found in fetal material from miscarriage. Aneuploidy arises mostly from non-disjunction during meiosis (MI+MII).^{29,30} The 45,XO monosomy and trisomies of 16, 21 and 22 chromosomes comprise 60% of aneuploidy in pregnancy loss. Trisomies of chromosomes 13, 18 and 21, and sex chromosome can reach birth (0.3% of all births). Most autosomal trisomies arise from maternal origin.³¹ The influence of paternal age on the incidence of Down syndrome (DS) is controversial. Most studies have not found paternal effect on the incidence of trisomy 21 and several other aneuploidies. Analysis of 352 cases of DS³¹ has found that only 9.1% of cases derived from paternal origin. The risk of DS among offspring of aged fathers is low. No clear association between fathers' age (>50 years) and the occurrence of DS or several other chromosomal aneuploidies was found.³² In contrast, very young fathers (age <20 years) were found to be at increase risks for the occurrence of DS and trisomy 22 (relative risk=4), oral clefts, neural tube disease, cystic kidney and hypospadias, when compared to 25- to 29-year-old fathers.³³ This study was limited by a small number of cases and difficulties separating maternal and paternal age effects. Sex chromosome aneuploidies are the most common abnormalities in live birth. Around 55% of the cases derive from paternal origin (80% of monosomy 45,XO, 6% of 47,XXX, 100% of 47,XYY and 50% of Klinefelter syndrome (47, XXY)).³⁴ No clear association was found between older fathers' age (>50 years) and XY chromosomes aneuploidy. There is no information regarding males' age and 47,XXX/47,XYY. This is due to a paucity of data available on affected offspring (most abnormal embryos are lost) and difficulties in separating the maternal and paternal contributions.

Sperm chromosomal anomalies

The genetic material in the mature sperm nucleus is highly condensed. First chromosomal information derived from the human sperm/hamster egg method, came from zona pellucida-denuded hamster eggs inseminated by human sperm.³⁵ Method limitations included high price, time consumption, the need for intensive labor, paucity of spermatozoa available for analysis, the need for fertile men only (only ejaculated sperm) and the inability to distinguish between diploid sperm and dispermy. In a study which included seven men aged 59–74 years and five men aged 23–39 years, Sartorelli *et al.*³⁶ have found that the frequency of numerical and structural chromosomal aberrations (acentric fragments and complex radial figures) was significantly

Table 1 The effect of paternal age on assisted reproduction outcome (reproduced with permission from Ref. 18)

Reference	Fertilization	Implantation	Embryo quality	Pregnancy rate	Pregnancy loss rate	Liveborn deliveries
Ferreira <i>et al.</i> ¹⁹	—	Decrease only in oligozoospermic	Days 2–3 (NS)	Decrease only in oligozoospermic	NS	—
Aboulghar <i>et al.</i> ²⁰	Decrease	—	Days 2–3 (NS)	NS	—	—
Paulson <i>et al.</i> ²¹	NS	—	—	NS	—	NS
Bellver <i>et al.</i> ²²	NS	NS	Days 2–3 (NS)	NS	NS	—
Spandorfer <i>et al.</i> ²³	NS	NS	Increase in digynity	NS	NS	NS
Frattarelli <i>et al.</i> ²⁴	NS	NS	Days 2–3 NS, decrease at day 5	NS	Increase	Decrease
de la Rochebrochard <i>et al.</i> ²⁵	—	—	—	Decrease	—	—
Luna <i>et al.</i> ²⁶	Decline in IVF >60 years	Decline in men >60 years	Decrease at days 3, 5 and 6	NS	NS	—
Klonoff-Cohen and Natarajan ²⁷	NS	—	—	Decrease	NS	Decrease
Gallardo <i>et al.</i> ²⁸	NS	NS	NS	NS	NS	—

Abbreviations: IVF, *in vitro* fertilization; NS, non-significant.

greater ($P=0.02$) in chromosomes of older donors when compared with those of the younger group. There is no consensus on the relationship between males' age and aneuploidy in spermatozoa. Buwe *et al.*³⁷ used human fluorescence *in situ* hybridization (FISH) karyotyping, which allows simultaneous analysis of several chromosomes in a shorter time. In addition, analysis can be made using a very low number of sperm cells, including samples retrieved from the genital tract or directly from the testicular tissue.^{38,39} Limitations of the method are probe specificity and the inability to distinguish between absence of signal due to hybridization failure and nullisomy. Age studies have yielded no evidence for an age-related increase in sperm disomy frequencies. These methods (FISH and sperm/hamster egg) demonstrated an increased rate of aneuploid sperm among infertile men, compared to fertile population,⁴⁰ even when somatic karyotype is normal. Meiotic studies demonstrated that infertile men have impaired chromosome synapsis, decreased frequency of recombination and increased frequency of chromosomes completely lacking a recombination site, errors rendering cells to meiotic arrest and production of aneuploid gametes.⁴⁰

Sex and autosomal chromosomal aneuploidy. Y chromosome is unique as it does not have an equal partner for pairing in male meiosis. Sex chromosome non-disjunction in meiosis I results in XY sperm whereas in meiosis II it forms XX/YY sperm. Evidence was found for an age-related increase in XY sperm disomy with advanced donor age (36–60 years compared with 18–35 years).^{32,37} In addition, there is a more pronounced age-related effect on XX/YY disomy rate, a two- to three-fold higher frequency being seen when sperm originate from men of over 50 years. Dakouane *et al.*⁴¹ have examined testicular biopsies from 36 older men (61–102 years old) and 10 younger men (29–40 years old) by histomorphometry and FISH (X, Y and 18 chromosomes). The rate of aneuploidy in older subjects (61–95 years old) with preserved spermatogenesis was not different from that found in the control group and was increased in older subjects with altered or impaired spermatogenesis. Triploidy is one of the most frequent chromosomal aberrations (~1%). It is usually associated with an early abortion. Diandric (paternal) triploids, originate from dispermy, whereas digynic (maternal) triploids originate from errors in meiosis II.⁴² However, data are lacking in regard to the effect of paternal age on the incidence of triploidy.

Structural chromosomal anomalies in sperm analysis

Structural chromosomal anomalies comprise 0.25% of births, 0.4% of stillbirths and 2% of abortions. Population-based studies did not find evidence for an increased rate with advancing paternal age (fetuses and abortions).⁴² Around 80% of the *de novo* cases (partial deletions) originate in male germ line. Their frequency in men with normal sperm varies from 7% to 14%. Cytogenetic studies are rare. Despite of predominance of paternal origin, a significant influence of paternal age has not been demonstrated. Using sperm FISH analysis to demonstrate centromeric and subtelomeric regions, a significant association with donors' age was detected for the frequency of acentric fragments of chromosome 1 ($P<0.05$).⁴³ Slotter *et al.*⁴⁴ have found that frequency of sperm with segmental duplications and deletions of chromosome 1 was twice among older men, and frequency of sperm carrying breaks within the 1q12 fragile site region nearly doubled.

Telomeres and paternal age

Telomeres are repetitive linear DNA sequences, 3–17 kb long, located at the ends of chromosomes, protecting them from deterioration,

clumping, fusion with neighboring chromosomes and DNA breaks. During cell division, enzymes that duplicate DNA cannot continue their duplication all the way to the end of the chromosome. Therefore, telomere regions deter degradation of genes near the ends of chromosomes by allowing for the shortening of chromosome ends, thereby acting as disposable buffers that blocking chromosomal ends. During each cell division, telomeres shorten and are replenished by the enzyme telomerase reverse transcriptase. After a critical number of cell divisions when telomere length (TL) shortens considerably, this marks the termination of proliferation stops.⁴⁵ Cells may enter apoptosis.^{46,47} Males lose telomere sequence relatively faster than females (31 bp year⁻¹ vs. 14 bp year⁻¹) and the rate of telomere loss slows throughout lifespan. TL is linked to age-associated diseases, when shorter TL increases the risk of mortality due to infection or heart disease, and has recently been regarded as a biological marker for aging.^{48,49} Evidence was found for father-offspring heritage of TL.^{50,51} This correlation diminishes with increasing age, suggesting that non-inheritable factors have an impact on TL during life.⁵² Increasing TL was found in offspring of advanced paternal age (~17 bp year⁻¹).⁴⁷ This implies that TL is not completely reset in the zygote. Implications of TL on health are unknown. For every additional year of paternal age, TL in offspring increased at a magnitude ranging for half to more than twice of the annual attrition in TL with age.⁵³ Sperm TL analyses were compatible with the emergence in older men of a subset of sperm with elongated telomeres.⁵³ One of the hypotheses for this phenomenon is that this paternal effect is an adaptive signal of the expected age of male reproduction in the environment where offspring are born into. The offspring of lineage of older fathers will tend to have longer telomeres better preparing them for an environment with higher expected age at reproduction.⁴⁷

Spontaneous abortions

A case-control study of 13 865 women found that increasing paternal age is significantly associated with spontaneous abortion, independent of maternal age and multiple other factors.⁵⁴ Slama *et al.*⁵⁵ observed that the risk of spontaneous abortion was almost twice higher among men aged >45 years than among those aged <25 years. Paternal age of >40 years becomes relevant concerning miscarriages when the woman was >30 years old.⁵⁶

AUTOSOMAL DOMINANT (AD) DISEASES

Mutation at fibroblast growth factor receptor 3 (FGFR3)

Until 1975, four AD diseases were related to pure paternal age effect: achondroplasia (ACH), Apert syndrome, myositis ossificans and Marfan syndrome.⁵⁷ The average age of fathers at the time of birth of an affected child was 6.1 years older than that of fathers of normal children in the same population, whereas maternal age and birth order showed no significant effect. In 1976, Francke *et al.*⁵⁸ studied five families in which the Lesch-Nyhan mutation appeared first in a heterozygous mother. The mean age of maternal grandfathers at the birth of a daughter was about 7.3 years older than the population mean, and though numbers were small, they could suggest an age-related paternal effect on X-linked recessive disorders.⁵⁸ ACH was the first genetic disorder that hypothesized to have paternal age influence.⁵⁹ ACH is the most common form of skeletal dysplasia characterized by disproportionately short stature, lumbar lordosis and relative macrocephaly with incidence of 1:25 000 live births.⁶⁰ It is caused by mutations in *FGFR3* gene. A predominant *FGFR3* mutation, G to A transition at nucleotide 1138, has been found in more than 98% of the affected

individuals from different ethnic groups. A second mutation described in about 2% of ACH patients is G to C transversion at the same codon. The mutation causes an arrest of chondrocyte terminal differentiation and a blockade of bone elongation and growth. Affected individuals have shorter life expectancy due to accidental, neurological and heart disease-related deaths. Heart disease-related mortality between ages of 25 and 35 years, was more than 10 times higher than the general population. Overall survival and the average life expectancy for this ACH population were decreased by 10 years.⁵⁰ Dakouane Giudicelli *et al.*⁶¹ examined sperm cells from ejaculate and testicular biopsies. They have found that the rate of *G1138A* mutation did not significantly vary with age in sperm, whereas in testis biopsies, it increased markedly past the age of 70 years. They have also identified a mosaic for this mutation which was detected in the testis of three subjects who were >80 years of age. The association between paternal age and incidence of ACH has been also reported by Orioli *et al.*⁶² Mean paternal age was highly elevated in ACH. Approximately 50% of ACH patients were born to fathers above the age of 35 years. All sporadic ACH cases have been found to inherit the *G1138A* mutations from their fathers. This association was also found by Wyrobek *et al.*⁶³ The paternal age effect has been attributed to spermatogonial replication errors that accumulate with increased age. Mutations in *FGFR3* gene cause other different disorders of skeletal dysplasias, including hypochondroplasia and thanatophoric dysplasia (which is lethal). The pattern of increase of these mutations with paternal age is exponential.⁶¹ Tiemann-Boege *et al.*⁶⁴ have determined the frequency of the nucleotide substitution in the fibroblast growth factor *FGFR3* gene that causes ACH. They have found that the age-related increase of the mutation for ACH in sperm is lower than expected and does not explain the exponential increase of the disease with paternal age. They have proposed a number of alternative mechanisms which may explain this discrepancy, including selection for sperm that carry the mutation or an age-dependent increase in premutagenic lesions that remain unrepaired in sperm and are inefficiently detected by the PCR assay.

Mutations in the *FGFR2* gene

Crouzon syndrome, Apert syndrome and Pfeiffer syndrome are all AD craniosynostotic disorders that can be caused by mutations in the *FGFR2* gene.⁶⁵ The frequency of new mutations, responsible for craniosynostosis syndromes is estimated as 1:65 000 live births. Most Apert syndrome cases result from one of two site (C755G and C758G) mutations in the fibroblast *FGFR2* occurring in a normal father's germ line. Eleven different mutations that are responsible for sporadic cases of either Crouzon syndrome or Pfeiffer syndrome also arise in the paternal germ line. All mutations were associated with increased paternal age and molecularly proved to be a paternal origin of mutation.^{66,67} Glaser *et al.*⁶⁸ have found that two nucleotide substitutions in the human *FGFR2* gene (C755G or C758G) are responsible for virtually all sporadic cases of Apert syndrome. This condition is 100–1000 times more common than that genomic mutation frequency data predict. Choi *et al.*⁶⁹ have performed testis analysis of the *FGFR2* mutation of Apert syndrome in four individual testes from three older individuals (45, 54 and 62 years) and two testes from younger donors (23 and 19 years). Each testis was dissected into 192 pieces. They demonstrated that spontaneous mutations are not uniformly distributed throughout normal testes. Instead, they have found foci where mutation frequencies were 3–4 orders of magnitude greater than the remaining tissue. They concluded that the nucleotide site was not a mutation hot spot. An alternative explanation for such foci involves positive selection

acting on adult self-renewing spermatogonia experiencing the rare mutation which could not be rejected. Further, the two youngest individuals studied (19 and 23 years old) had lower mutation frequencies and smaller foci at both mutation sites compared with the older individuals. This implies that the mutation frequency of foci increases with age, and thus, selection could explain the paternal age effect for Apert syndrome and other genetic conditions. Based on their genetic analysis of human sperm, Goriely *et al.*⁷⁰ proposed that specific *FGFR2* mutations are positively selected in sperm because they encode proteins with gain-of-function properties, favoring clonal expansion of mutant spermatogonial cells. Among *FGFR2* mutations, those causing Apert syndrome may be especially prevalent because they enhance signaling by fibroblast growth factor ligands specific for each of the major expressed isoforms. More than 10 genetic disorders are associated with advanced paternal age; half of them (Apert syndrome, ACH, multiple endocrine neoplasia type 2B,⁷¹ Crouzon syndrome and Pfeiffer syndrome) have definitively been shown to be the result of mutations that are paternal in origin. The mean paternal age at birth of 80 mutant cases of dominant osteogenesis imperfecta was significantly higher than that of population controls and remained so after adjusting for maternal age.⁷² The weak paternal age effect in neurofibromatosis may be due to the fact that new mutations are not base substitutions. The overall risk calculation for AD diseases (0.3%–0.5% among offspring of fathers aged >40 years)⁷³ may have been overestimated. The calculation was based on the assumption that the paternal age effect found in ACH is typical of all AD diseases, an assumption which was not accurate. Prenatal screening for paternally transmitted AD disease is not yet recommended routinely. Penrose⁵⁹ has proposed the concept of 'copy error hypothesis': in males, germ cells (spermatogonia) divide continuously and undergo many more mitotic replications than oogonia. The higher number of germ cell replications among males compared with females (oogonia-21 replications until the stage of oocyte; spermatogonia-30 replications until puberty and then 23 replications every year) may serve as an explanation for the increased rate of paternally-inherited point mutations compared with maternal inheritance.⁷⁴ One explanation for this could be that the efficiency of the proofreading of the replication machinery (DNA repair mechanism) in spermatogenesis declines with age⁷⁵ due to endogenous (oxidative stress) or exogenous mutagenic damage. This is due to the absence of DNA repair mechanism in late spermatids, immature and mature spermatozoa,^{76,77} and the reduced levels of antioxidant enzymes in seminal plasma of older men,⁷⁸ a finding which is in concordance with the age-dependent decrease in oxidative potential of seminal plasma¹⁷ (Lissak *et al*, 2004, unpublished data).

Birth defects

Non-Mendelian disease characters may depend on two, three or more genetic loci. In addition, contributions from environmental factors, nutrition during organogenesis and exposure to chemicals may be involved. These multifactorial conditions include the common birth defects (cleft palate, congenital dislocation of the hip, congenital heart disease, *etc.*), behavioral traits such as IQ test performance or mental diseases such as schizophrenia. Yang *et al.*⁷⁹ have performed a population-based retrospective cohort study of 5 213 248 subjects from the 1999–2000 birth registration data of the United States of America. They included 22 categories of birth defects and divided parental age into eight standard 5-year-old age groups: I, <20 years; II, 20–24 years; III, 25–29 years; IV, 30–34 years; V, 35–39 years; VI, 40–44 years; VII, 45–49 years; and VIII,

>50 years. The overall prevalence of ascertained serious birth defects in the United States of America during 1999–2000 reported was 1.5%. A progressively elevated risk of birth defects was observed with a 15% increase risk in the age group of 50 years old. Advanced maternal age was associated with a progressively elevated risk of birth defects with a 2.2-fold higher risk in the age group of 45 years old. The adjusted odds ratios (ORs) were 1.04, 1.08, 1.08 and 1.15, respectively, for infants born to fathers 30–35, 40–44, 45–49 and over 50 years (test for trend, $P=0.0155$), when compared with those infants born to fathers aged 25–29 years for any birth defect. Advanced paternal age was associated with increased risks of heart defects, tracheo-oesophageal fistula, esophageal atresia, other musculoskeletal/integumental anomalies, DS and other chromosomal anomalies. Fathers under 25 years of age were also at increased risks of spina bifida/meningocele, microcephalus, omphalocele/gastroschisis and other musculoskeletal/integumental anomalies. Younger fathers were at increased risks of spina bifida/meningocele, microcephalus omphalocele/gastroschisis. However, results could be explained by associated maternal risk factors: smoking, alcohol and folic acid. Kazaura *et al.*⁸⁰ investigated 1 869 388 births from The Medical Birth Registry of Norway. There was little evidence of increased risks by high paternal age for any category of defects, except for a category of 'other central nervous system' where risk estimates were 2.5-fold (95% confidence interval: 1.2–5.5) for fathers aged between 45 and 49 years compared with the reference age group (25–29 years). The risk of neural tube defects was 1.3-fold (95% confidence interval: 1.1–1.5) when the father's age was between 20 and 24 years relative to the reference. A pattern of moderately higher risks for younger fathers was consistent for anencephaly and spina bifida. An increased risk of heart defects was also estimated among children of young fathers. Zhu *et al.*⁸¹ investigated the Danish fertility database ($n=71\ 937$) between 1980 and 1996. Advanced paternal age (>40 years) may be associated with an excess occurrence of some specific malformations (syndromes of multiple systems and malformations of extremities). Green *et al.*,⁸² using the 1997–2004 data from the National Birth Defects Prevention Study, have found elevated ORs for each year increase in paternal age for cleft palate (OR=1.02), diaphragmatic hernia (OR=1.04), right ventricular outflow tract obstruction (OR=1.03) and pulmonary valve stenosis (OR=1.02). At younger paternal ages, each year increase in paternal age correlated with increased odds of having offspring with encephalocele, cataract, esophageal atresia, anomalous pulmonary venous return and coarctation of the aorta, but these increased odds were not observed at older paternal ages. The effect of paternal age was modified by maternal age for gastroschisis, omphalocele, spina bifida, all oro-facial clefts and septal heart defects.

Perinatal outcome. Nybo Andersen *et al.*⁸³ examined 23 821 pregnant women recruited consecutively to the Danish National Birth Cohort from 1997 to 1999 to assess the association between paternal age and fetal death. Pregnancies fathered by men aged 50 or more years ($n=124$) had almost twice the risk of ending in a fetal loss compared with pregnancies with younger fathers (hazard ratio=1.88; 95% confidence interval: 0.93–3.82), after adjustment for maternal age, reproductive history and maternal lifestyle during pregnancy. Chen *et al.*⁸⁴ have performed a retrospective cohort study of 2 614 966 live singletons born to women aged 20–29 years and estimated the independent effect of paternal age on adverse birth outcome. Teenagers (<20 years old) had an increased risk of low birth weight (OR=1.13) preterm birth (OR=1.15), small-for-gestational-age births (OR=1.17),

low Apgar score (OR=1.13), neonatal mortality (OR=1.22) and post-neonatal mortality (OR=1.41). In contrast, advanced paternal age (>40 years) was not associated with the risk of adverse birth outcomes.

NEUROCOGNITIVE DISORDERS

Autism

Autism spectrum disorder (ASD) refers to a syndrome of social communication deficits and repetitive behaviors or restrictive interests.⁸⁵ In an historical population-based cohort study on 132 271 males, Reichenberg *et al.*⁸⁶ have found a significant monotonic association between advanced paternal age and the risk of ASD. Offspring of men aged 40 years or older were 5.75 times ($P<0.001$) more likely to have ASD compared with offspring of men younger than 30 years. Buizer-Voskamp *et al.*⁸⁷ examined the Dutch registry using 14 231 patients and 56 924 matched controls. ASD was significantly associated with increased paternal age. Older fathers (>40 years of age) have 3.3 times increased odds of having a child with ASD compared to young fathers (<20 years of age). In sporadic cases of ASD, mutations in transcription factors which regulate the expression of genes associated with neurodevelopmental disorders may play a causal role. Alter *et al.*⁸⁸ have used micro-arrays to measure the expression levels of $\geq 47\ 000$ transcripts including 38 500 well-characterized human genes in RNA from lymphocytes from each of 82 children with autism and 64 control subjects. A decrease in the variance in the distribution of gene expression levels in lymphocytes was associated with the diagnosis of autism and increased paternal age. Gene expression pathways involved in transcriptional regulation were found to be downregulated in the blood of children with autism and children of older fathers. These results may suggest a possible mechanism for decreased variance in gene expression in the etiology of paternal age-dependent autism.

Schizophrenia

Sipos *et al.*⁸⁹ have conducted a population-based cohort study in Sweden. They have found that advanced paternal age was associated with schizophrenia, and the results were independent from sociodemographic or socioeconomic parameters, family history or early parental death. Meta-analysis of paternal age and schizophrenia risk in male vs. female offspring made by Miller *et al.*⁹⁰ revealed that relative risk in the oldest fathers (≥ 50 years) was 1.66 compared with younger fathers. The population attributable risk percentage was 10% for paternal age of >30 years and 5% for paternal age of <25 years. Buizer-Voskamp *et al.*⁸⁷ have found that schizophrenia had significant associations for fathers aged >35 years (OR=1.27). Lee *et al.*⁹¹ have identified distinct characteristics of paternal age-related schizophrenia patients in regard to other schizophrenia patients (a discrepancy between verbal and performance intelligence and an early age of onset among females). The results of these studies support the theory that accumulating *de novo* mutations in the germ cells of older fathers might contribute to an increased risk of schizophrenia in their offspring. Point mutations are well known to increase with advancing paternal age^{92,93} while other errors such as an increased allele length mutation rate⁹⁴ and chromosome breakage⁹⁵ have in some cases also been associated with advanced paternal age.

Bipolar disorder

Offspring of men aged 55 years and older were 1.37 times more likely to be diagnosed as having bipolar disorder than the offspring of men aged 20–24 years.⁹⁶ In contrast, Buizer-Voskamp *et al.*⁸⁷ have not found any association between paternal age and bipolar disorder, but have found that for major depressive disorder, both younger and older fathers had increased odds.

Neurocognitive development

Saha *et al.*⁹⁷ have examined a sample of singleton children ($n=33\ 437$) from the US Collaborative Perinatal Project. Outcome measures were assessed at 8 months, 4 years and 7 years (Bayley scales, Stanford Binet Intelligence Scale, Graham–Ernhart Block Sort Test, Wechsler Intelligence Scale for Children and Wide Range Achievement Test). Advanced paternal age showed significant associations with poorer scores on all of the neurocognitive measures apart from the Bayley Motor score. The findings were broadly consistent in direction and effect size at all three ages. Malaspina *et al.*⁹⁸ examined the association of paternal age and IQ scores, using the Israeli Army Board data on 44 175 individuals from a richly described birth cohort, along with maternal age and other potential modifiers. A significant inverted U-shaped relationship was observed between paternal age and IQ scores, which was independent from a similar association of IQ scores with maternal age. These relationships were not significantly attenuated by controlling for multiple possible confounding factors. Overall, parental age accounted for approximately 2% of the total variance in IQ scores, with later paternal age lowering non-verbal IQ scores more than verbal IQ scores. Vestergaard *et al.*,⁹⁹ in a population-based cohort study of 96 654 children, found that fathers aged 35 years or older were slightly more likely to have a child diagnosed with epilepsy compared to fathers aged 25–29 years.

OTHER CONDITIONS

Yip *et al.*¹⁰⁰ have examined a population of 4.3 million children (≤ 14 years old) with their parents, born between 1961 and 2000. Paternal age was significantly associated with leukaemia (oldest age group's incidence rate ratio=1.31). For central nervous system cancer, the effect of paternal age was found to be significant (oldest age group's relative risk=1.69) when maternal age was included in the analysis. Weiss-Salz *et al.*¹⁰¹ have found an association of paternal age at birth and the risk of breast cancer under the age of 40 years in offspring. Choi *et al.*¹⁰² have found that the risk of breast cancer significantly increased as the paternal age increased (P (for trend)=0.025). The association was stronger after controlling for maternal age. Women whose fathers were aged ≥ 40 years at their birth had a 1.6-fold increased risk of breast cancer compared with fathers aged < 30 years. This association was profound in breast cancer cases in premenopausal women (OR=1.9 for paternal aged ≥ 40 years vs. < 30 years) (P (for trend)=0.031). In a retrospective analysis using the Swedish Inpatient Register, Montgomery *et al.*¹⁰³ identified 4443 patients with a diagnosis of multiple sclerosis. Twenty-four thousand, one hundred and ninety-four controls were selected with similar characteristics for year, county of birth and survival until at least the age at diagnosis of the matched cases. The risk of multiple sclerosis increased steadily with fathers' age but not mothers' age, up to 2.00 (1.35–2.96) for 51- to 55-year-old fathers (compared with 21- to 25-year-old fathers).

Epigenetics

The non-genomic pathway of inheritance, otherwise known as epigenetics (which involves the methylation or acetylation of DNA or histones as a mechanism modulating gene transcription and expression), has recently gained intensive scientific attention due to its possible role in experience-dependent mother–infant influence. Since epigenetic patterns are only metastable and may be changed by environmental as well as endogenous factors, this may serve as an alternative mechanism by which age-related changes in parental epigenetic 'footprints' may affect the phenotype of their offspring, regarding the susceptibility to certain diseases. Adkins *et al.*¹⁰⁴ have used a genome-wide survey of 27 578 cytosine guanine dinucleotides in a cohort of 168 newborns. They

examined the association between DNA methylation in newborns and parents, and newborn traits. They have found that the methylation level of 144 cytosine guanines in 142 genes was correlated with maternal age. A weaker correlation was observed with paternal age. The end products of these genes involved glucose/carbohydrate metabolism, neurological regulation, processes related to cancer, nucleocytoplasmic transport and transcriptional regulation. They concluded that congenital differences in cytosine guanine methylation are associated with parental age and may contribute to future risk of certain diseases. Perrin *et al.*¹⁰⁵ and Sipos *et al.*⁸⁹ have raised the question whether epigenetic changes that occur as paternal age advances, may be casually related to the susceptibility of schizophrenia. Errors in epigenetic processes, such as parental imprinting may possibly have serious effects on offspring both pre- and post-natally and into adulthood. Interestingly, paternal exposure to toxicant and nutritional state as well as age, have been found to influence not only offspring but sometimes grand-offspring.¹⁰⁶

SUMMARY

In Western societies, advanced paternal age is a phenomenon that parallels advanced maternal age and is associated with various reproductive hazards. Testicular function and sperm parameters deteriorate rather gradually with advanced age. However, unlike in the female, assisted reproductive techniques such as IVF–intracytoplasmic sperm injection, may circumvent these obstacles and enable fertilization in cases with severe male factor infertility, otherwise unlikely to conceive.

In view of the rise in late parenthood combined with these powerful techniques, it is inevitable that other aspects may emerge regarding late parenthood and be expressed in the offspring.

There is an increased age-associated risk of sperm germ line mutations and specific genetic disorders, specifically AD type.

Epigenetic imprinting which alters levels of gene transcription, may contribute to the susceptibility of offspring of old men to diseases of multifactorial origin, including neurocognitive and psychiatric disorders, and even malignancy. Recent micro-array techniques reported a reduced variance in the distribution of gene expression levels, which was associated with autism and advanced paternal age.

Possible mechanisms may be prolonged periods of exposure to nutritional and environmental toxicants, an age-dependent decrease in anti-oxidant mechanisms and a positive selection of mutated spermatozoa, vulnerable to the accumulation of mutations due to their high rate of divisions.

Interestingly, the offspring of a lineage of older fathers will tend to have longer telomeres, which may imply better preparation for an environment with a higher expected age at reproduction.

Different evolutionary aspects are involved in the aged male father in humans. This may dictate a different approach to the impact of males' age on reproduction. Therefore, in view of the growing number of older patients attending fertility and assisted reproductive facilities, proper counseling about the implications of age effects on gamete quality should be provided to both partners prior to family planning and infertility treatment.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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