Advancing Paternal Age: The Ticking Biological Clock

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Abstract

Since spermatogenesis is a continuous process that occurs throughout life, paternal age often gets less consideration for childbearing. However, a number of studies have reported a significant impact of advanced paternal age on the time to pregnancy, adverse pregnancy outcomes and the birth of children with congenital deformities. This chapter provides an overview of the impact of advanced paternal age on the loss of fertility and increased likelihood of passing birth defects and genomic changes that can have significant impact on the coming generations.

Keywords

Paternal age • Age and infertility • Age and congenital abnormalities

Key Points

- Testosterone level declines by 0.4–2% per year, resulting in a decrease in libido and spermatogenesis.
- There is evidence of epigenetic changes with ageing that may affect the quality of gametes.
- DNA accumulates defects with ageing that are more likely to pass on to the next generation.
- Advanced paternal age is more likely to contribute to congenital defects.

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19.1 Introduction

Over the last few decade, the trend for advanced parental age has increased in couples due to professional and social commitments. There is a precipitous decline in female fecundity after 30–35 years of age with an increase in various adverse reproductive events from infertility, pregnancy complications to perinatal morbidity and mortality. The 'ticking biological clock' for women is well understood, but this clock ticks for men too and appears to tick faster (Crow 2000; Thacker 2004). The anecdotal reports about older fathers have always sounded fascinating, and the oldest paternity has been noted scientifically as 94 years (Seymour et al. 1935). However, the advanced paternal age adversely affects testicular functions, semen parameters, sperm DNA integrity and sperm telomere length and increases de novo mutation rate and chromosomal and epigenetic alterations. Accumulated chromosomal aberrations and mutations in male germ cells may lead to the increased risk of reduced fertility, poor implantation and pregnancy rates and an increased risk of birth defects and childhood disease burden.

A steep increase in the age of childbearing by men tends to invite a host of negative reproductive outcomes. According to CDC birth statistics, the birth rate for men 25–44 years is increasing with a decline seen in men <25 years (Hamilton et al. 2003). There is a strong association between paternal age and extension of sperm DNA strand breaks. With increased age, there is a concomitant increase in DNA damage with increase in the incidence of semen abnormalities (Singh et al. 2003; Moskovtsev et al. 2006, 2009). With age, the incidence of mutations in spermatozoa rises due to repeated premeiotic cell divisions, decreased antioxidant capacity and other diseases which are more likely to appear with ageing. This adversely affects embryogenesis because advanced paternal age is also associated with advanced maternal age and suboptimal quality of oocyte. This may lead to incomplete, inefficient aberrant repair of sperm DNA damage by oocyte postfertilization. The advanced age at the planning of the first child not only decreases the chances of conception but also increases the risk of DNA damage, gene deletions and chromosomal aneuploidies. A host of these factors are associated with pregnancy loss or the birth of children with congenital abnormalities. This chapter highlights the impact of advanced paternal age on fertility, its possible odd outcomes and the need to counsel couples for timely planning of family.

19.2 Testicular Morphology and Semen Parameters

Age-related morphological changes in testes affect spermatogenic efficiency, characterized by a predominance of multinucleated spermatogonia, megalospermatocytes, giant spermatids along with seminiferous tubular diverticula and thickening of the basal membrane (Johnson et al. 1988; Kuhnert and Nieschlag 2004; Dakouane et al. 2005). Testicular sclerosis occurs as a result of defective vascularization in senile testis and systemic arteriosclerosis (Sasano and Ichijo 1969). A decrease in

testicular volume is attributed to a decrease in both Sertoli cells and Leydig cells. The Sertoli cells are seen to accumulate cytoplasmic lipid droplets, and Leydig cells may also be multinucleated (Johnson 1986; Holstein 1989). A pioneering study by Auger et al. (1995) found a 2.6% decline in sperm concentration, a 0.3% decline in motile sperm number and a 0.7% decline in the percentage of normal sperm morphology, with increased paternal age. There is a decrease in seminal volume and seminal fructose concentration with age, whereas zinc and α-glucosidase, secreted by prostate and epididymis, remain constant (Rolf et al. 1996).

19.3 Testicular Functions and Reproductive Hormones

Alterations in testicular functions develop gradually, which has been considered as a rough indicator of spermatogenesis. The testicular volume remains constant over quite a long time and is documented to decrease only in the eighth decade of life (Kuhnert and Nieschlag 2004). The changes in testicular volume are seen to be associated with the levels of follicle-stimulating hormone (FSH), inhibin B and testosterone. The resultant increased FSH levels associate with a decrease in the inhibin B/FSH ratio as well as a decrease in Sertoli cell mass and testosterone levels in the testis (Weiner-Megnazi et al. 2012). Testosterone levels peak around 20 years of age (Bhasin and Buckwalter 2001) and continue to decline by about 0.4–2% every year after 30 years of age (Feldman et al. 2002; Abram McBride et al. 2016). Decreasing testosterone levels were quantified by Massachusetts Male Ageing Study (MMAS), as a cross-sectional decline of 0.8%/year of age and a longitudinal decline of 1.6%/year, over a 10-year follow-up data (Morley et al. 1997).

The decrease in testosterone levels with age has led to the development of 'late-onset' hypogonadism (LOH) in contrast to hypogonadism, which is a more general term referring to the state with decreased testicular volume, impaired sperm production and low testosterone levels. Age-related below-normal testosterone levels and associated symptoms have also been addressed as 'andropause', 'symptomatic androgen deficiency', 'age-related hypogonadism' and 'testosterone deficiency (TD)'. This hypogonadism in ageing men is characterized by poor libido, fatigue and loss of cognitive functions (Sharma et al. 2015; Abram McBride et al. 2016). Testosterone production is regulated by the hypothalamic–pituitary–gonadal (HPG) axis via the production of luteinizing hormone (LH) (Abram McBride et al. 2016). Failure in this delicate balance can result in primary, secondary or mixed hypogonadism. The predominant form of testosterone deficiency in ageing men is mixed with primary and secondary hypogonadism components. The levels of luteinizing hormone may vary with age due to the decrease in Leydig cell number and subsequent decrease in sensitivity of the HPG axis to feedback inhibition and/or decreased LH pulse amplitude despite normal pulse frequency. The decrease in LH pulse amplitude may subsequently be related to decreased neuronal cell secretion of gonadotrophin-releasing hormone (Kaufman and Vermeulen 2005).

19.4 Spermatozoa Have Limited Repair Capacity

The aetiology of DNA damage in the spermatozoa is complex and is chiefly induced by oxidative stress and thus making it vital to understand the nature and extent of DNA damage. Oxidative stress is one of the major causes of defective sperm function, which disrupts the sperm DNA integrity, induces single double-strand breaks, shortens telomeres, alters sperm methylome, oxidizes DNA bases and interstand and intrastand crosslinking and also causes fragmentation of mt and nuclear DNA also (Shamsi et al. 2008; Mishra et al. 2014). It thus limits the fertilizing potential because of parallel damage to lipids and proteins in the sperm plasma membrane. Spermatozoa are particularly vulnerable to lipid peroxidation because they have high concentrations of unsaturated fatty acids, which further triggers the mitochondria for the generation of high levels of superoxide anion as a prelude to entering the intrinsic apoptotic cascade (Aitken and De Iullis 2010; Aitken et al. 2012, 2013).

Unfortunately, spermatozoa have very little capacity to respond to such an attack because they have a highly truncated base excision repair mechanism as they only possess the first enzyme in the base excision repair (BER) pathway, 8-oxoguanineglycosylase 1 (OGG1). The latter successfully creates an abasic site, but the spermatozoa cannot process the oxidative lesion further because of the lack of downstream proteins (APE1, XRCC1) needed to complete the repair process. These are repaired only by oocyte at the time of fertilization. However, ageing oocyte and the presence of extensive sperm DNA damage in ageing sperm may overwhelm the oocyte repair mechanism postfertilization with persistence of these mutagenic bases in the child, and that may be an alternative explanation for paternal age effects (Smith et al. 2013).

19.5 Paternal Age and Mutations in Sperm DNA

The female fertility reaches a natural limit marked by the occurrence of menopause, which leads to cessation of ovarian function due to inevitable loss of female gametes. The male reproductive functions have been observed to decline gradually with age and spermatogenesis throughout life. Each oocyte produced by the female undergoes 22 germ line divisions and two meiotic divisions, and regardless of her age, female oocytes do not undergo any further divisions after that. In males, as spermatogenesis is continuous throughout life, ageing increases the number of cell divisions, hence increased number of chromosomal replications with advanced age reaching a number of 840 replications by the age of 50 years. This increase in the number of cycles of DNA replication with advanced paternal age brings more copy-error mutations as per the Penrose's *copy-error hypothesis* (Penrose 1955).

Fathers bequeath more mutations with advanced age, and the germ line mutation rate is higher than in females, mainly because of many more germ-cell divisions. As compared to females, the number of cell divisions in males is seven times higher at the age of 20 and 25 times higher at the age of 40 (Crow 2000; Taylor et al. 2006). The mutation rate tends to further increase due to the decrease in sperm DNA integrity with ageing and accumulation of highly mutagenic oxidized DNA adducts like

8-hydroxy 2-deoxy guanosine. Oxidative DNA damage gets accumulated with advanced paternal age and also by the exposure to various exogenous and lifestyle factors like smoking, excess alcohol intake, psychological stress, increased BMI and exposure to xenobiotics and infections (Kumar et al. 2015). In a genome-wide association study by Kong et al. (2012), the average de novo mutation rate was found to be 1.20×10^{-8} per nucleotide per generation with an average father's age of 29.7 years. There is a striking effect of over two additional mutations every year or an exponential effect of doubling of paternal mutations every 16.5 years (Kong et al. 2012). The effect of hazardous environmental conditions and various demographic characteristics driven by forces of genetic drift, gene flow and natural selection cannot be negated.

The mutation rate for base substitutions is much higher in the ageing male. The critical phase for induction of de novo mutations is the post-meiotic events during spermiogenesis (Wyrobek et al. 2006; Crow 2006). NR5A1 nuclear receptor, also known as steroidogenic factor 1 mutations, has been reported in 46,XY disorders of sex development in 4% men with unexplained severe spermatogenic failure (Bashamboo et al. 2010). Men with non-obstructive azoospermia were reported to have de novo point mutations in Y-chromosomal gene USP9Y (Sun et al. 1999). On the contrary, small deletions or rearrangements do not show the paternal age effect. This has been observed in larger genes encoding for neurofibromatosis, Duchenne muscular dystrophy, Wilms' tumour or retinoblastoma (Crow 2000). Ongoing studies in our lab (Kumar et al. 2015) in fathers of children with nonfamilial sporadic heritable retinoblastoma (RB) showed higher levels of oxidative DNA adducts in blood of children with RB who were born to fathers who smoked or who were above 35 years of age. Advanced age in fathers and limited detection of DNA damage and repair in sperm and its dependence on oocyte to repair DNA damage may result in incomplete removal of DNA lesions due to suboptimal quality of oocyte (associated with advanced maternal age) and extensive DNA damage thus persists.

A variable incidence of different dominant mutations due to varied base substitutions and deletions has been observed in children as the age of the father increased. Advanced maternal age has been reported as the only well-documented non-genetic risk factor for trisomies in humans, but recent studies have found that trisomy 21 is primarily associated with advanced paternal age when the female partner is >35 years of age (Sartorius and Nieschlag 2010). Advanced paternal age has not been associated with trisomy 18 and even less likely with trisomy 13. No relationship of advanced paternal age has been observed with the birth of an offspring with anencephaly or encephalocele. No significant relationship between either maternal or paternal age has been observed in Klinefelter's syndrome as well. Nevertheless, an increase in the likelihood of these disorders with advancing paternal age cannot be denied.

19.6 Paternal Age Effect (PAE) Disorders

The first remarkable statement about the association of paternal age with birth disorders was given by Wilhelm Weinberg in 1912 when he noticed the sporadic cases of achondroplasia in the last-born children of sibship. This was further strengthened 40 years later by Penrose who gave the 'copy-error hypothesis' owing to more

number of germ line mutations in men. Paternal age effect (PAE) disorders are small group of such type of rare disorders with an increased risk for spontaneous congenital disorders and common complex diseases (some cancers, schizophrenia, autism, bipolar disorder) (Goriely and Wilkie 2012; Goriely et al. 2013). Replication error is not the only underlying mechanism in such disorders. The common factor in these disorders lies in dysregulation of spermatogonial cell behaviour with an effect mediated by specific mutations in genes encoding components of the tyrosine kinase receptor/RAS/MAPK signalling pathways (Maher et al. 2014). These are random mutations occurring during mitotic divisions of spermatogonial stem cells (SSCs) that confer a selective/growth advantage on mutant SSCs, leading to a clonal expansion of mutant cells significantly above the background mutation rate. The clonal expansion takes place in the testes of all men, leading to the relative enrichment of mutant sperm over time. This phenomenon is known as *Selfish Spermatogonial Selection* and skews the mutational profile of sperm as men age, enriching the de novo mutations in offsprings of older fathers (Goriely et al. 2013).

Nine autosomal-dominant disorders (Apert, Crouzon, Pfeiffer and Muenke syndromes, achondroplasia, Costello and Noonan syndromes and multiple endocrine neoplasia types 2A and 2B), corresponding to specific point mutations within five genes (FGFR2, FGFR3, HRAS, PTPN11and RET), have been ascribed to the PAE disorders. 99% of individuals with Apert syndrome carry either of the two transversions (c.755C>G or c.758C>G), encoding substitutions in two adjacent amino acids (p. Ser252Trp or p. Pro253Arg, respectively) located within the extracellular region of the receptor tyrosine kinase protein fibroblast growth factor receptor-2 (FGFR2) (Wilkie et al. 1995). It is characterized by craniosynostosis (premature fusion of the cranial sutures) and severe syndactyly of both hands and feet. Single nucleotide substitution mutation (encoding a p. Gly380Arg mutant protein) in FGFR3 causes more than 95% of achondroplasia cases (Rousseau et al. 1994), which is the most common cause of short-limbed dwarfism.

Crouzon and Pfeiffer syndromes are witnessed to overlap clinically and are caused by any of more than 50 specific activating point mutations in fibroblast growth factor receptor 2 (FGFR2) gene. Craniosynostosis is seen to occur in Apert syndrome, but limb abnormalities are milder (Kan et al. 2002). Muenke's syndrome develops because of a single c.749C>G transversion in FGFR3 (resulting in a point substitution Pro250Arg equivalent to the FGFR2 Apert-causing Pro253Arg) and is the most common genetic cause of coronal craniosynostosis (Vajo et al. 2000). Costello and Noonan syndromes are a part of neuro–cardio–facial cutaneous syndromes or RASopathies and present with variable combinations of distinctive craniofacial features, short stature, failure to thrive, developmental delay and skin, cardiac and skeletal abnormalities (Aoki et al. 2008). 90% of Costello syndrome patients have the c.34G>A transition in HRAS (Gly12Ser) at a well-known mutation hotspot in tumorigenesis, while ~50% of Noonan syndrome mutations are detected within the PTPN11 gene (encoding SHP2-containing tyrosine phosphatase). The last two PAE disorders, multiple endocrine neoplasia types 2A (Men2A) and 2B (Men2B), are caused by allelic mutations within the RET receptor tyrosine kinase (Aoki et al. 2008; Tartaglia et al. 2010).

A noticeable phenotypic overlap is observed between different PAE syndromes though they are clearly distinct and have well-defined complex pathological entity. These features highlight the pleiotropic role played by the PAE genes during development, whereas the clinical overlaps of these features point out to the fact that these genes are required in common cellular contexts in shared molecular pathways.

19.7 Paternal Age, Sperm DNA Integrity and Reproductive Outcomes

Moskovtsev et al. (2006) reported that there was twice an increase in DNA fragmentation index (DFI) from less than 30 years of age (15.2%) to ≥45 years of age (32.0). In an ongoing study in our department, we observed that an increase in DNA damage is associated with decreased probability of conception with increase in time to pregnancy. Decreased sperm DNA integrity is associated with an increased risk of recurrent miscarriages, congenital birth defects and childhood carcinomas. Advanced paternal age has been seen to affect the rates of fertilization, implantation, pregnancy and miscarriage. The impact of paternal age on the seminal oxidative stress and DNA integrity in our laboratory showed an increase in seminal ROS from 58.3 to 115.7 relative light units (RLU)/s/million sperm and an increase in DFI from 32.6 to 42.3% from 2 to 40 years of age. Damage to the germ cells entering meiosis will precipitate an increase in apoptosis, thus making the sperm cell susceptible to accumulate damage to its genome and epigenome right from the time they are formed till conception (Tremellon 2008; Dada et al. 2012; Aitken et al. 2012, 2013).

19.8 Increased Telomere Length in Offsprings of Old Fathers

The response of the germ cells to an increase in stress is up-regulation of telomerase activity and increase in telomere length of the spermatozoa. Milder level of oxidative stress may thus compensate, and this is one of the effects which may favour the survival of the offspring by increasing the telomere length. The telomere length is a paternally inherited trait so the offsprings of ageing fathers will have longer telomeres and this can be explained as a biological resistance to ageing process (Unryn et al. 2005). A strong and positive correlation has been showed between increasing paternal age and telomere length (Aston et al. 2012). By contrast if the germ cells are exposed to oxidative stress post-meiotically as in cases of infertility patients undergoing ART, the telomerase can no longer increase and the telomere length will be abnormally short, posing serious health hazards for the offspring.

With age the telomeres of leukocytes tend to decrease, while that of sperm tend to increase in length (Aston et al. 2012). Consistent with increase in sperm telomere length, a correlation between paternal age at birth and leukocyte telomere length of the individuals has been reported (Prescott et al. 2012). The paternal age

contribution to offspring leukocyte telomere length is stronger than the maternal contribution (Broer et al. 2013), and the paternal age shows cumulative effect across generations (Eisenberg et al. 2012). A recent study analysed leukocyte and sperm telomere length in the same individual in relation to spermatogenic activity and parents' age at birth by recruiting 18–19 years old high school students. Sperm and leukocyte telomere length showed correlation, but sperm telomere length was significantly longer. Also, a positive correlation between sperm telomere length and total sperm number was observed. This increase telomere length may have health implications, though they do not seem to affect the risk of cancer (Chang 2012).

19.9 Age-Related Changes in Sperm Epigenome

Epigenetics is a stable heritable modification on histone tails but not the DNA sequence that leads to altered gene expression. The sperm cell has a highly differentiated and specialized morphology, and the epigenome of human sperm matters for embryogenesis. Epigenetic factors suggest that sperm play diverse and critical roles in embryonic development. Methylation of cytosine residues, typically found at cytosine phosphate guanine dinucleotides (CpGs), in the DNA by DNMTs (DMA methyl transferases) is the most important mechanism regulating the process of gene expression and capable of regulatory control over gene activation or silencing. DNA hypomethylation is associated with gene transcriptional activity, whereas hypermethylation is associated with gene silencing activity (Carrell and Hammoud 2010; Carrell 2012). Epigenetic patterns are shown to be silenced/disrupted by various environmental and endogenous factors such as age, diet and lifestyle factors, including smoking or drug intake (Sharma et al. 2015). These epigenetic events may impair or inhibit key steps of fertilization, implantation and/or embryo development.

Epigenetic modifications have been shown to not only affect normal cellular function but also to be involved in ageing and cancer and as a mechanism where environmental influences come into play. Jenkins et al. studied the impact of ageing on DNA methylation in 17 fertile donors by methylation array approach. They identified 139 regions in sperm DNA that were hypomethylated and eight regions that were significantly hypermethylated with age. They reported that 117 genes were associated with these regions and a portion of age-related changes in sperm DNA methylation were located at genes associated with schizophrenia and bipolar disorder (Jenkins et al. 2014). Epigenetic marks within sperm are specifically associated with genes that regulate transcription and developmental processes. As the embryo grows, these imprints are maintained in somatic tissues, but erased in primordial germ cells so that imprints can be re-established during gametogenesis. The various methylation changes during development make the epigenome vulnerable to interference from environmental exposure (Kumar et al. 2015). Epigenetic programming plays an important role in an organism's response to environmental stress during critical developmental periods. Understanding the epigenetics of sperm can be a

potential mean to decipher the mechanisms of pluripotency, which has broad implications for potential therapies.

Conclusion

In the last decade, an alarming increase in delayed marriages and delayed parenthood has been observed. This is due to several reasons like increased contraceptive use and professional pressures. Though the effect of advanced maternal age on fertility and health of the offspring is well documented, the impact of advanced paternal age on fertility is less well investigated. Hence, there is little awareness about the impact of father's age on fertility and the health of offspring. The use of sperm from old men for ART may also lead to pre- and post-implantation losses and congenital malformations. In a number of cases of miscarriage, the role of paternal age may be significant; however, investigation of this requires well-planned studies on aborted foetuses. Thus, there is a need to increase awareness to not delay childbearing as ageing affects the quality of gametes, which is usually associated with adverse pregnancy outcomes. Nevertheless, the rate of testicular ageing can be slowed by adopting a healthy lifestyle and practice of meditation and yoga.

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