Age-related alterations in the genetics and genomics of the male germ line

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Paternal aging is associated with increased risk of genetic disease transmission to the offspring. The changes associated with aging arise predominantly through formation of single nucleotide variation through DNA replication errors, as well as possibly chronic exposure to environmental toxins and reactive oxygen species exposure. Several age-related reproductive factors are also contributory, including the systemic hormonal milieu, accumulation of environmental toxin exposure, aging germ cells, and accumulation of de novo genetic and genomic abnormalities in germ cells. In this article we review the age-related genetic and genomic changes that occur in the male germ line. (Fertil Steril® 2017;107:319–23. ©2017 by American Society for Reproductive Medicine.)
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Over the last 6 decades, couples have delayed marriage and reproduction because of various socioeconomic factors and shifting gender roles in the work force. During this time the median age of fathers at the time of first marriage increased by 25% to the current median age of 29 years (1). Similarly, from 1980 to 2010 the number of men fathering children between the age groups of 35–39 and 40–44 years rose by 48% and 51%, respectively (2). Although the reproductive effects of advancing maternal age are well known, the effects of advancing paternal age are less well studied. However, as increasingly more babies are born to older fathers, interest in studying the implications of advancing paternal age on reproductive outcomes and offspring health has similarly risen.

Multiple studies investigating the association between paternal age and reproductive outcomes concluded that increasing age is associated with impaired semen parameters; fivefold longer time to pregnancy; and reduced fertilization rates, embryo quality, implantation rates, pregnancy rate, and live-born deliveries (3–9). Furthermore, increased paternal age is linked to a broad range of developmental abnormalities (Table 1), such as congenital birth defects and neurologic disorders, and a statistically significant increase in 5-year offspring mortality related to the severity of the congenital malformations, malignancies, and other external causes (11, 12). Several age-related reproductive changes drive these impaired outcomes, including changes to the systemic hormonal milieu, accumulation of environmental toxin exposure, aging germ cells, and accumulation of de novo genetic and genomic abnormalities in germ cells. In this article we review the age-related genetic and genomic changes that occur in the male germ line.

REACTIVE OXYGEN SPECIES AND DNA FRAGMENTATION

For fertilization to take place, spermatozoa require a certain amount of reactive oxygen species (ROS), which are the byproducts of oxygen metabolism and consist of reduced oxygen molecules with chemically reactive unpaired electrons, to undergo biologic functions such as capacitation, hyperactivation, acrosome reaction, and oocyte fusion (13). In addition, ROS modulate nuclear maturation and facilitate nuclear condensation in spermatozoa by oxidizing nuclear proteins (14). Although a certain amount of ROS is

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TABLE 1

Offspring genetic conditions associated with advanced paternal age.

Condition	Paternal age (y)	Relative risk	Population risk	Adjusted risk
Achondroplasia	>50	7.8	1/15,000	1/1,923
Apert syndrome	>50	9.5	1/50,000	1/5,263
Pfeiffer syndrome	>50	6	1/100,000	1/16,666
Crouzon syndrome	>50	8	1/50,000	1/6,250
Neurofibromatosis I	>50	3.7	1/3,000–1/4,000	1/810–1/1,080
Retinoblastoma	>45	3	1/15,000–1/20,000	1/5,000–1/6,667
Down syndrome	40–44	1.37	1/1,200[a]	1/876[a]
Klinefelter syndrome	>50	1.6	1/500 men	1/312 men
Epilepsy	40–45	1.3	1/100	1/77.0
Breast cancer	>40	1.6	1/8.5	1/5.3
Childhood leukemia	>40	1.14	1/25,000	1/21,930
Childhood central nervous system tumor	>40	1.69	1/36,000	1/21,302

Note: Adapted with permission from Ramasamy et al. (10).
[a] Maternal age 20–29 years.

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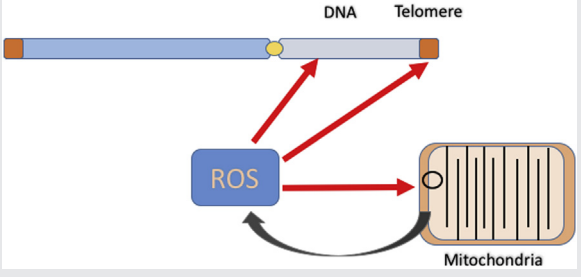
necessary for normal sperm function, excess ROS can place a detrimental oxidative stress on spermatogenesis and fertilization via damage to sperm DNA and proteins and are associated with poor semen quality and function. Studies comparing ROS levels of healthy fertile men aged <40 years vs. those aged >40 years found significantly lower ROS levels in the seminal ejaculate (15). Reactive oxygen species harm sperm by entering the nucleus, binding to DNA, and inducing double-strand breaks (16). The relationship between age and DNA fragmentation is well established, with higher levels of DNA damage more often found in older men. Even when matched comparisons are made between young and old subjects who are normozoospermic, a statistically significant correlation ($P<.001$) remains between age and percent sperm DNA fragmentation (17). Using the sperm chromatin structure assay, Das et al. (17) compared 107 normozoospermic young men (<40 years of age) with 41 normozoospermic older men (≥41 years of age) and found higher DNA damage levels in the older cohort (17% ± 13% vs. 12% ± 18%). Moskovtsev et al. (18) also used sperm chromatin structure assay to compare DNA fragmentation values in a cohort of men ≥45 years of age with a cohort of men <30 years of age and found that damage levels were twice as high in the ≥45 years cohort (32.0% ± 17.1% vs. 15.2% ± 8.4%). These findings were corroborated by a large meta-analysis of 10,220 patients by Johnson et al. (19), who identified a statistically significant age-dependent increase in DNA fragmentation.

Although the mechanism for elevated DNA fragmentation in older men remains unclear, several factors may contribute, including a higher incidence and prevalence of varicoceles (the incidence of varicoceles increases by 10% with each decade of life [20]), environmental exposure to pollutants, and comorbidities such as obesity, diabetes, infections, and other lifestyle issues (reviewed by Sabeti et al. [21]). Coupled with age-related reduction in antioxidant enzymatic activity (reviewed by Aitken and De Iullis [22]), higher ROS levels in the semen of older men increase the oxidative stress, resulting in impaired sperm DNA integrity via creation of double-strand DNA breaks, mutations of genomic and mitochondrial DNA, perturbation of DNA repair enzymes, and the accumulation of single nucleotide variants with each mitotic and meiotic replication (Fig. 1). Defective DNA repair in turn may result in greater numerical and structural chromosomal abnormalities, increasing the risk of aneuploidy by twofold among fathers aged >50 years compared with fathers aged 25–29 years in one study by McIntosh et al. (23). This oxidative stress–mediated DNA damage has been linked with IVF/intracytoplasmic sperm injection failure and abnormal offspring development, particularly with learning disorders and impaired cognition (24–26), although some studies were not performed with the rigor required for evidenced-based medicine.

STRUCTURAL AND NUMERICAL CHROMOSOMAL ABNORMALITIES, DNA MUTATIONS, AND PATERNAL AGE

Genomic instability is characterized as a higher frequency of spontaneous genetic and genomic mutations. Genomic instability associated with aging is a multifactorial, complex process that represents the cumulative effects of chronic ROS

FIGURE 1



Reactive oxygen species generated from the mitochondrial electron transport chain (*black arrow*) damage DNA and mitochondrial DNA and disrupt telomerase activity, causing germ cell senescence.

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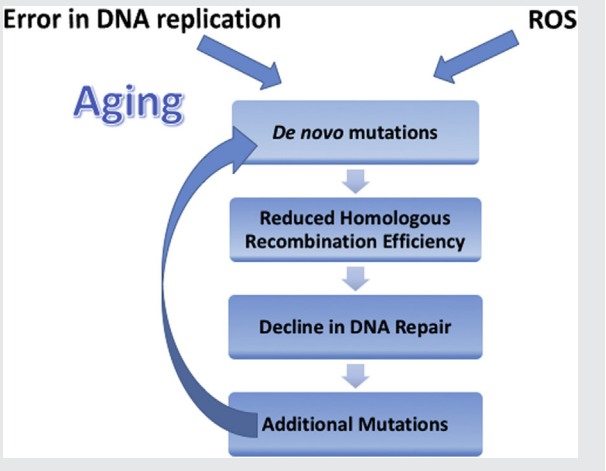
exposure, telomere shortening, and DNA replication errors, inducing a vicious cycle of chromosome recombination errors, reduced DNA repair efficiency, and further de novo mutations (Fig. 2). Recombination errors can result in chromosomally balanced and unbalanced gametes that ultimately result in aneuploid and often nonviable offspring. Studies assessing the type of chromosomal aneuploidy associated with paternal aging have reported mixed relative risk results for chromosomes 13, 18, 21, and X (reviewed by Sharma et al. [27] and Sloter et al. [28]). However, a statistically significant positive association between centromeric deletions of chromosome 1 and paternal age was reported by McInnes et al. (29), who performed fluorescent in situ hybridization (FISH) on sperm from 18 men belonging to one of six age groups (20–24, 25–29, 30–34, 35–39, 40–44, and ≥50 years) using probes for chromosomes 13, 21, 1 and a unique probe to detect duplications and deletions of 1p. Historically, reports from the 1950s suggested an association between Down syndrome and advanced paternal age; however, several studies have since emerged, refuting this association (28).

Garcia-Ferreyra et al. (30) recently showed a significantly higher embryo aneuploidy rate associated with fathers aged ≥50 years (73.9% aneuploidy) compared with fathers aged 40–49 (61.1% aneuploidy) and ≤39 years (59.1% aneuploidy). Interestingly, no statistically significant difference was detected in fertilization rates, percentages of zygotes that underwent cleavage, and the quality of embryos at day 3. Although the authors reported that all subjects had normal karyotype, additional cytogenetic testing (such as FISH) was not performed before intracytoplasmic sperm injection, and embryo FISH results were not provided to determine which chromosome(s) was most often affected. The effects of age on chromatin integrity, gene mutations, and aneuploidy in a group of 97 men with an age range of 22–80 years were assessed by Wyrobek et al. (31), who showed no association between X-Y-21 aneuploid sperm per 10,000 sperm vs. age. Because most sperm aneuploidy arises from nondisjunction during meiosis I and meiosis II, the lack of an association between sperm aneuploidy and paternal age but higher rate of embryo aneuploidy implies an error in sperm-related centrosomal function during the embryo cleavage stage. Further studies are necessary to determine the mechanism of aneuploidy transmission from sperm to embryo in men with advanced paternal age.

Advanced father's age is also associated with increased incidence of clinical disorders in offspring, ranging from congenital malformations to neurodegenerative disorders. Similarly, paternal age is associated with an increased incidence of autosomal dominant disorders such as Apert Syndrome, achrondroplasia, osteogenesis imperfecta, progeria, Marfan syndrome, and Waardenburg syndrome. These disorders are caused by single nucleotide variants, which are more likely to arise in male gametes rather than female gametes owing to the continuing mitotic divisions of spermatogonial stem cells throughout a male's adult life, which create more opportunities for random mutation and mismatch repair failure. In a study of 78 parent–child trios in Iceland, Kong et al. (32) found that the rate of de novo mutation strongly correlated with paternal age and generated a model predicting that paternal germ-line mutations double every 16.5 years. Francioli et al. (33) analyzed 11,020 de novo mutations from whole genomes of 250 families and found after accounting for random Poisson distribution that 95% of variation in the global human mutation rate could be attributed to paternal age. They confirmed Kong et al.'s findings that de novo mutations are more common in the children of older fathers and found that, relative to 20-year-old fathers, 40 year olds had twice the rate of mutation and that those mutations were more likely to have functional consequences.

A number of single gene mutations are enriched in the setting of advanced paternal age. The fibroblast growth factor receptor 3 (*FGFR3*) gene codes for a member of the fibroblast growth factor receptor family and is responsible for initiating cell signaling cascades that ultimately result in mitogenesis and differentiation. Mutations of this gene cause skeletal malformations, such as craniosynostosis and achondroplasia (1138G>A). Achondroplasia is inherited in an autosomal dominant pattern and leads among the causes of dwarfism (reviewed by Ramasamy et al. [10]). An increased risk of achondroplasia associated with advanced paternal age, however, has long been described, with original reports by Penrose in 1955 (34). Corroborating data were provided by Wyrobek et al. (31), who reported a statistically significant and positive correlation between age and the incidence of *FGFR3* mutation. The decade-specific incidence of achondroplasia causing *FGFR3* mutation increased from 0.55 per 10,000 genomes in subjects aged 20–29 years to 1.85 for men over the age of 60 years or a 3.3% increase per year in the frequency of the mutation. Mutations of other FGFR family members, such as *FGFR2*, have also been associated with advanced paternal age (35). Mutations of *FGFR2* result in Apert syndrome, which is characterized by acrocephalosyndactyly.

FIGURE 2



Development of genomic instability with aging.

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PATERNAL AGE AND EPIGENETICS

Alternate routes exist that allow a father's age to influence offspring. Epigenetics refers to the stable and heritable mechanism of gene expression regulation that does not involve DNA sequence. The major groups of epigenetic changes are DNA methylation, histone modifications, and microRNA expression. An individual can acquire many epigenetic changes throughout his or her life, depending on external stimuli. Although these changes can be stably propagated in somatic cells and regulate cell fate, they do not alter the genetic code. During normal embryonic development the cells undergo a reset of the epigenetic marks, to allow for proper cell fate differentiation (reviewed in references [36, 37]). Because of these events, it was believed that epigenetic mutations could not be passed down through the germ line. However, this view is being challenged by increasing evidence linking maternal epigenetic states to effects on progeny (38). Of particular interest are the analyses linking advanced age to an increased likelihood of diseases such as autism spectrum disorder, schizophrenia, or Down syndrome in the progeny (39). These diseases are associated with epigenetic changes, and it is important to investigate whether the paternal epigenetic state affected these children. There is increasing literature suggesting that some marks can be passed down through maternal nongenetic means to the new generation (38); however, a precise mechanism has not been established, and the role of the paternal epigenetic state needs further investigation.

Several studies have associated advanced paternal age with a greater burden of DNA methylation changes. Oakes et al. (40) showed increased ribosomal DNA methylation in the sperm of older rats, suggesting a mechanism for age-related DNA methylation changes. Restriction landmark genomic scanning, a method used to determine specific methylation patterns of CpG island sequences, allowed Oakes et al. to find a region of the ribosomal DNA locus that is preferentially hypermethylated with age in both spermatozoa and liver. These findings suggest that the maintenance of normal DNA methylation levels in spermatozoa over the course of a lifetime changes and may explain age-related abnormalities in the offspring (40). Another study, by Jenkins et al. (41), evaluated the global methylation levels in sperm of fertile men. They discovered that there is a statistically significant increase of global sperm 5-mC and 5-hmC levels by 1.76% and 5%, respectively, per year. These findings underscore the importance of epigenetic aberrations and their contribution to the offspring risks associated with advanced paternal age (reviewed in reference [10]).

Although the evidence arguing that epigenetic changes occur with age and that they are associated with poor outcomes in the progeny is increasing, the factors that lead to transgenerational epigenetic inheritance need further investigation. One such factor is diet. A study in mice by Terashima et al. (42) found that hepatic messenger RNA levels in seven imprinted genes were significantly altered in the offspring of male mice given a high-fat diet (HFD). Although they did not detect DNA methylation changes in the fathers' spermatozoa, they found differential histone H3 occupancy at genes involved in the regulation of embryogenesis and differential H3K4me1 enrichment at transcription regulatory genes in HFD fathers. Another study, by Barbosa et al. (43), evaluated HFD effects on rat offspring. They found altered expression of the microRNA let-7c in the sperm of F0 rats and their F1 offspring, thus showing a transgenerational epigenetic inheritance that alters the metabolic tissues in the offspring. An earlier study by Anderson et al. (44) suggested that paternal food deprivation results in a consistent decrease in average serum glucose in male and female offspring in mice, which was also accompanied by significant changes in corticosterone and insulin-like growth factor-1.

Human studies also suggest a link between parental obesity and epigenetic changes in the offspring. Soubry et al. (45) showed in 79 newborns that differential changes in DNA methylation in multiple human imprinted genes (such as *IGF2*) associate with both paternal and maternal obesity. A father's obesity was significantly correlated with hypomethylation of the differentially methylated regions of the *IGF2* gene. Another study, by Pembrey et al. (46), investigated paternal ancestors' food supply and effects of smoking on the progeny's health. They found that the paternal grandfather's food supply was linked to the mortality risk ratios only of the grandsons, suggesting a sex-linked inheritance of these traits. All of these studies provide compelling evidence that the paternal contribution to the development of the progeny goes beyond the DNA sequence. The influence of a father's poor nutrition habits, such as overeating, may extend across multiple generations. A large-scale epidemiologic study by Kaati et al. (47) showed the transgenerational metabolic effects of a father's overfeeding, with elevated cardiovascular disease risk and diabetes in his grandchildren. Further research is needed to elucidate how the paternal epigenetic state is transferred to the progeny, what factors affect the epigenetic state, and why some marks are inherited whereas others are not. Most importantly, researchers need to address the question of what external factors, such as parental age and lifestyle choices, affect future generations the most.

In conclusion, paternal aging is associated with increased risk of genetic disease transmission to the offspring. The changes associated with aging arise predominantly through DNA replication errors, as well as possibly chronic exposure to environmental exposure to toxins and ROS exposure. More importantly, age-related reproductive problems are an inevitable complication of an aging population. Recognition and thorough understanding of male reproductive disorders associated with aging will continue to gain in importance because of the medical and societal burden of developmental disorders in offspring. Guidelines on proper evaluation and clinical counseling are lacking.

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