Invited review

New perspectives on the genetics of male infertility

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Abstract

Infertility is a genetically heterogenous disorder with a multifactorial etiology. In a large number of male infertility cases the etiology is not known. About 35-40% men in the reproductive age group have either a qualitative or a quantitative defect in sperm production. The gene-gene and gene environment interactions in regulation of male fertility are poorly understood. With the advent of ART there is a strong and urgent need to understand the genetic basis of infertility to prevent the iatrogenic transmission of these genetic abnormalities to the offspring and prevent the actual doubling of the infertile population. In the past decade tremendous progress has been made in field of genetics and male reproductive health. The completion of human genome project has aided in better understanding of the genetic aspects of several factors regulating male fertility. This article focuses on our understanding of genetic aspects of infertility and the development of newer technologies and methodologies which will aid in a better understanding of molecular etiopathogenesis of male infertility.

Key words: genetics, male infertility, azoospermia, oligozoospermia.

Introduction

Infertility affects 15 to 20% of couples in the reproductive age group. It is a genetically heterogeneous disorder with multifactorial etiology. Changes in lifestyle, exposure to environmental pollutants and delayed marriage and childbearing have resulted in an increased number of couples experiencing difficulty in conception. About 30 to 40% of men of reproductive age have either a qualitative or quantitative defect in sperm production; however, in about 40% of cases, the etiology of infertility cannot be identified and are classified as idiopathic cases.

The causes of male infertility may be classified as secretory, which affect spermatogenesis, or excretory, which affect sperm outflow. Although several factors affect spermatogenesis, some of the important factors that cause irreversible, complete or partial spermatogenic arrest are genetic in origin. These factors may be chromosomal and involve chromosomal structural or numerical abnormalities or may involve autosomal, sex-chromosomal genes [1]. Several studies have reported an increased incidence of genetic abnormalities in cases with low sperm count. About 20 to 25% of azoospermic and 14-16% of oligozoospermic men harbor genetic abnormalities, and 9.8%

of men with severe oligozoospermia and azoospermia harbor Yq microdeletions in blood and 11.3% in sperm DNA [1, 2]. In addition a large number of infertile asthenozoospermic men show nucleotide substitutions in mitochondrial genes regulating oxidative phosphorylation [3, 4]. Thus, genetic studies in infertility help to define an etiology in a large number of idiopathic cases, but are also of prognostic value as they help to decide the future management of couples, especially those opting for assisted reproduction.

By offering assisted reproductive technology/intracytoplasmic sperm injection (ART/ICSI) to infertile men with genetic abnormalities, we actually may double the population of infertile men. Babies fathered by these men and born as a result of these techniques may harbor secondary larger deletions and structural alterations that may predispose them to an increased risk of both major and minor congenital malformations, epigenetic alterations and cancer.

Unlike other diseases, little is known of the role of gene and environment interactions in regulation of spermatogenesis and infertility. Subfertility is a multifactorial trait in which environmental toxins (environmental hormones), radiation, heavy metals like lead and electromagnetic radiation may disrupt the genes involved in gametogenesis and increase susceptibility to certain diseases. More than 500 environmentally responsive genes have been discovered, including several that regulate testicular function or the hypothalamo-pituitary gonadal axis. These genes play a critical role not only in germ cell development and differentiation but also in sexual differentiation and development of internal and external genitalia. Thus, many cases of male infertility are genetic in origin. However, the study of genetic diseases in humans is limited by small family size and diploidy of the genome, and the most conclusive evidence of the genetic basis of infertility is from genetic knockout studies and its direct link with sperm disorders.

An estimated more than 2000 genes are involved in spermatogenesis, spermiogenesis and sperm function. Although a number of genes regulate the differentiation of diploid to haploid spermatozoa, the diversity of gene products regulating their functions is larger. About 35 to 65% genes undergo alternative splicing, increasing the complexity of regulatory and functional mechanisms.

About 3000 m-RNA are expressed in mammalian testis. Non-protein-coding RNA's may be essential for normal cellular function. These micro RNA's (over 800 human micro RNA genes have been identified) are essential regulators of gene expression and are very relevant to sperm function. Thus Miller et al. [5] reported that about 10% genes in the human genome may be related to spermatogenesis and fertility.

Genetic causes of male infertility

Sperm dysfunction is the single most defined cause of infertility. Although the population of subfertile men has increased significantly, diagnosis is still poor and ART is still the treatment of choice due to our poor understanding of sperm cellular, biochemical, and molecular functioning. To address this in our laboratory, we provide a thorough workup of infertile men with idiopathic non-obstructive azoospermia or oligozoospermia. Following traditional semen analysis as described by World Health Organization (WHO) guidelines in 1999 [6], all samples are assessed for reactive oxygen species (ROS) and antioxidant levels. The next step is a comprehensive cytogenetic analysis. In cytogenetically normal men, Yq microdeletion screening is performed from genomic DNA isolated from blood and sperm. Since Yq microdeletions occur with greater frequency in sperm than in blood and encompass different *loci*, we postulated that sperm Yq microdeletion screening should be done in all men opting for ART [1, 2].

In all asthenozoospermic cytogenetically normal men, we perform mitochondrial mutation analysis to understand the etiology of the sperm motility defect. This is further correlated with the testicular phenotype and sperm ultrastructure [4]. In infertile men harboring a high number of nucleotide substitutions after whole genome amplification of mitochondrial genes, we found a partially formed and poorly organized microtubular apparatus in sperm axoneme. This was accompanied by dysplasia of the fibrous sheath and totally dysmorphic mitochondria with few mitochondrial cristae, which were loosely arranged around the sperm midpiece [4]. We also are analyzing cases with severe oligozoospermia for epimutations and imprinting defects. In all cases that are cytogenetically normal and have no Yq microdeletion, sperm DNA integrity analysis is done by comet assay [7].

This detailed, stepwise workup for infertile men not only provides a diagnosis of infertility but also has prognostic significance. Constitutional chromosomal abnormalities are found in 2 to 14% of infertile men. Sex chromosomal numerical abnormalities are predominant in azoospermic cases (97%) while autosomal structural abnormalities are predominant in oligozoospermic cases (63%). Results of a detailed diagnostic analysis of more than 1400 cases over the last 12 years has shown that about 16 to 18% of infertile men harbor structural or numerical chromosomal abnormalities [8]. About 9.83% men harbor a Yq microdeletion in blood DNA and 11.3% in sperm DNA [2]. Asthenozoospermic showed a high number of nucleotide substitutions in mtDNA [3] and ultrastructural defects [4]. Shamsi et al. [4] also reported the loss of DNA integrity and increased DNA damage in the sperm of infertile men. The maximum

DNA damage was observed in cases with oligoasthenoteratozoospermia (OAT) as compared to cases with oligozoospermia [7]. Thus we propose that ICSI, in which sperms with very high DNA damage can be used, may result in pre-post implantation failure, early fetal loss or an increased risk of major and minor congenital malformation and certain cancers.

Paternal DNA damage also may be one of the causes of poor success rate for ART/ICSI. We also found elevated levels of ROS in infertile men [9]. Varicocele is one of the most common, surgically reversible causes of infertility. Varicocele was believed to be associated with spermatogenic arrest due to testicular hyperthermia and varicocelectomy improved semen parameters. However, we found that many varicocele cases did not show any improvement in semen parameters and harbor Yq microdeletions [2]. Recently, we also found that these cases had very high ROS levels and DNA damage. We also observed significant decline in ROS levels one month post-varicocelectomy, but sperm DNA still showed increased damage with no significant change (unpublished data). However, these are immediate postoperative changes and are being followed up over one year. Although these men show marked improvement in semen parameters following varicocelectomy, sperm may still harbor DNA damage that may be transmitted to the offspring.

Fertility is a function of an individual's genetic makeup and his/her interaction with the environment. Several environmental agents/pollutants (persistent organic pollutants, phthalates, glycol esters, pesticides) disrupt genes involved in gametogenesis or interact with multiple alleles that increase susceptibility to environmental modulation [10]. Several studies have reported that infertility is a part of monogenic syndromes, but the majority of idiopathic cases are non-syndromic.

Mouse knockout studies have shown the most conclusive evidence of a genetic basis for infertility. In large, multi-generation human families, genetic studies have focused on linkage association between markers and have helped in identification of several genes in monogenic disorders. However, since infertility is a complex disorder, genome-wide associations studies are more useful. Several genes like *SYPC3*, *PRM1*, *PRM2*, *TNP1* and *DAZL* have shown an association with infertility. Several thousand nuclear genes may need to be tested for an association with male infertility.

Although several nuclear genes (sex chromosome and autosomal) regulate fertility, the role of mitochondrial genes in sperm function cannot be ignored. Unlike nuclear DNA, mitochondrial DNA is not protected by histones. It is closely associated with the inner mitochondrial membrane, where highly mutagenic oxygen radicals are generated [11]. Reactive oxygen species are highly reactive biomolecules that can damage mtDNA [12]. In addition, lack of proofreading and DNA repair mechanism [13], self-replication and lack of introns makes them more vulnerable to mutation.

Mitochondria and the integrity of its DNA have been recognized as important factors for normal spermatogenesis and sperm motility [14, 15]. The energy for germ cell development, differentiation and optimal sperm function is supplied by mitochondria in the form of ATP through oxidative phosphorylation (OXPHOS). Number and DNA content of mitochondria vary in different tissues according to their energy demand. Tissues with high energy demand such as neurons, muscle and oocytes have high mitochondria content and DNA copies to manage their efficient function. However, alteration in mitochondria function and its genome may severely disturb tissue physiology.

Mitochondria and its DNA are important in male fertility because the development of mature spermatozoa from primordial germ cells involves morphological changes and mitochondrial rearrangement. During such processes, energy supplied from the mitochondria is used to complete spermatogenesis and spermiogenesis. Moreover, motility in mature spermatozoa is highly dependent on energy supplied by the mitochondria arranged around the sperm midpiece. However, any genomic alteration in mtDNA genes that code for the OXPHOS or alteration in the nuclear genome that codes for mitochondrial proteins may result in decreased ATP production and impairment of spermatogenesis and sperm motility. The impact of such genetic alterations and its consequences with respect to the impairment of male fertility has been the focus of recent studies.

Point mutations, small and large deletions in mitochondrial genes, have been found to impair sperm motility [3, 16, 17]. Alterations in the mitochondria genome results in malfunction of proteins that result in formation of an abnormal OXPHOS system. Consequently, ATP production decreases and free radical leakage increases. As a high amount of energy is required for spermatogenesis, insufficient ATP would result in spermatogenic arrest. Low ATP levels result in production of sperm with abnormal morphology like retained cytoplasmic residues, improper arrangement of mito-chondria around sperm midpiece and impaired microtubular arrangement.

Male infertility with severely impaired sperm motility is one of the most frequently observed disorders among the male infertile population. Nine missense and 27 silent mutations were found in sperm mtDNA but not in blood DNA in an infertile Indian man with oligoasthenoteratozoospermia (OAT) [17]. Another study [18] revealed homoplasmic mutant mtDNA with a novel missense mutation

(C11994T) in the ND4 gene in an oligoasthenozoospermic man. Kumar R et al. [3] reported high frequency of nucleotide changes in the mitochondrial gene (ATPase 6, ATPase 8, ND2, ND3, ND4 and ND 5) in the semen of infertile men. As the number of mtDNA is few per mitochondria in spermatozoa, any mutation results in a phenotypic defect. mtDNA mutation or deletions affect respiratory chain function and result in oligozoospermia, asthenozoospermia and teratozoospermia leading to infertility in male mice [19]. Large-scale deletions of 4977bp, 7345bp and 7599 bp in mtDNA have also been reported in infertile oligoasthenozoospermic patients [15, 16]. Alternatively, mitochondria have been reported to contain both wild- type DNA and mutated DNA in a condition called heteroplasmy; the presence of either wild-type or mutated DNA is called homoplasmy. Studies have reported a threshold value exists above which the onset of disease occurs [20-22].

In most of the mitochondrial diseases, the expression of abnormal phenotype occurs only when the ratio of mutated or deleted mtDNA to wild mtDNA exceeds a critical threshold value. For example, greater than 60% of mutant mtDNA are expected to express the phenotype in LHON patients and greater than 85% for myoclonic epilepsy and ragged red fibres [23]. However, sperm mitochondria with few mtDNA show phenotypic defects even when there are low levels of mutant mtDNA. Organs like brain, muscle and heart with high energy demands are mostly affected by heteroplasmy [24]. Shamsi et al. [4] reported increased DNA damage in infertile men with increased number of mtDNA mutation and oxidative stress. More than 100 mitochondrial proteins are encoded by nuclear genes to control OXPHOS. A defect in any of these nuclear genes may also affect the production of ATP. One of the important nuclear gene products, transcription factor A mitochondrial (TFAM), regulates mtDNA transcripts [25]. TFAM, which is expressed in the early spermatid stage and down-regulated in late spermatogenesis, accounts for the reduction of mtDNA copies in mature sperm [25]. Hence, increased mtDNA copies can be explained as a marker for spermatogenic dysfunction. Mutation in nuclear-encoded polymerase-gamma (POLG) is also believed to affect mitochondrial function and is associated with secondary mitochondrial defects [26]. Studies also have reported a higher percentage of sperm cells with nuclear DNA fragmentation and low mitochondrial activity in varicocele patients [27]. Reduced mitochondria activity in this group might be due to fragmentation of the nuclear gene that encodes for mitochondrial protein. Therefore, screening for nuclear mutations associated with mitochondria dysfunction is important.

Oxidative stress plays a key role in inducing mitochondrial mutation and is an independent marker of male infertility [9]. Since mitochondria DNA are related to the inner mitochondrial membrane containing ETC, leaked free radicals may further damage mitochondria DNA and further increase free radical production. Since lipids, proteins, DNA and other biomolecules are vulnerable to free radical attack, increased ROS would increase the overall damage to the mitochondrial genome. Increased free radicals also overwhelm antioxidant defenses, resulting in oxidative stress. Increased malondialdehyde (MDA) levels as a measure of indirect oxidative stress have been found in infertile men with a high frequency of mitochondria DNA mutation (unpublished data). Although free radical-induced mtDNA accumulation is a well known phenomenon in human aging [28], studies have reported that leukocytes, as a contaminant in the semen, also produce ROS a hundred times greater than sperm itself. Reactive oxygen species induce a variety of DNA lesions by oxidation of bases as 8-oxo guanosine (8-oxo G), 8-OH guanosine, etc. 8-oxo G, one of the most abundant products of nitrogenous base oxidation [29] has mutagenic tendency and also blocks transcription.

Oxidative stress and DNA damage

Germ cells produce high levels of free radicals during development, particularly ROS [30], which can induce DNA lesions and oxidative stress. The antioxidant mechanism comprising enzymatic (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase etc.) and non-enzymatic components (glutathione, taurine, vitamin E etc.) scavenges ROS to maintain them within their physiological limits.

Apart from the antioxidant molecules and proteins involved in intrinsic and extrinsic apoptotic pathways, more than 130 genes are involved in protecting genome integrity in humans. These genes are expressed in testis, and their products are involved in mismatch repair (repairs small nucleotides or loops), nucleotide excision repair (principally associated with repair of UV-induced lesions and also against some bulky and oxidative DNA lesion), base excision repair (replaces aberrant bases as oxidized bases in DNA), single- and double-strand break repair. Histone variant H2AX recruits DNA repair factors to DNA damage sites, where it is rapidly phosphorylated [31].

Maintenance of genomic integrity

Maintenance of genomic integrity is of key importance in gametogenesis. Cell cycle checkpoints at the G_1/S, S and G_2/M phases control proliferation and allow the cell to proceed to the next stage of cell cycle only if the conditions are favorable, otherwise the cell cycle is paused for DNA repair, however if the damage cannot be repaired

then the apoptosis of cell is initiated [32]. A number of DNA repair mechanisms have evolved that recognize and repair DNA damage and maintain fidelity of DNA sequence. It is of prime importance that DNA integrity is maintained in germ cells so that damaged DNA is not transmitted to the offspring as this not only affects embryogenesis and placentation but has lifelong health implications. The damage response is cell-type specific, and the amount of damage required for initiation of apoptosis varies between cell types. During the recombination phase of crossing over, ds DNA breaks are initiated in early prophase I [33] which is mediated by topoisomerase II [34, 35].

Several genes affect somatic and germ cell integrity in the testis. These include growth factors, their receptors, genes in hormone biosynthesis, cell function maintenance and signal transduction pathways, cell remodeling chromatin packaging and nuclear condensation. Thus, genes controlling genetic fidelity are of prime importance in spermatogenesis.

In the post-meiotic phase of spermiogenesis, histones are replaced, first by transition proteins and subsequently by protamines, to remodel the germ cells and form the characteristic condensed protamine-DNA toroid structure [36]. During the transition from the round spermatid phase to the elongation phase, transient DNA breaks are induced along with the histone hyperacetylation. Hyperacetylation helps in the removal of DNA supercoils before binding of transition proteins [37].

Since chromatin remodeling does not occur in somatic cells, the occurrence of breaks in sperm DNA is comparatively higher, although most of the breaks induced are transient and are repaired by ligation [38].

Post-meiotic condensation of chromatin causes transcriptional and translational inactivity of the genome. This means that post-meiotic spermatids have minimal capacity for DNA repair [39, 40], and therefore their DNA could be damaged in a cumulative manner. The sperm cannot respond by inducing either apoptosis or DNA repair as they are transcriptionally silent. Studies also have reported that infections in the female reproductive tract (e.g. by *Chlamydia*, *Ureaplasma urealyticum*) cause premature decondensation of sperm chromatin and sperm DNA fragmentation associated with adverse pregnancy outcome [41].

Although the testis contain an active DNA repair machinery, backed by the antioxidants and elimination of germ cells with damaged DNA *via* apoptosis, lesions in sperm DNA still are detected in ejaculated sperm. These DNA lesions cannot be predicted by conventional semen parameters of motility, morphology and count. Several genes are involved in DNA repair, recombination and replication. This indicates the importance of DNA repair genes in maintaining genomic integrity and fidelity and optimal spermatogenesis. A significant percentage of infertile men harbor genetic defects in these functionally important genes.

Epigenetic alterations in infertility

Histone modification such as acetylation, phosphorylation and methylation play an important role in fundamental cellular processes, including epigenetic regulation of gene expression, organization of chromatin structure, chromosome segregation and DNA replication and repair [42]. Developmental processes are regulated largely by the epigenome because different cell types maintain their fate during cell division even though their DNA sequences are essentially the same. Epigenetic changes encompass an array of molecular modifications to both DNA and chromatin, the most extensively investigated of which are DNA methylation, which takes place at the carbon-5 position of cytosine in CpG dinucleotide, and changes to the chromatin packaging of DNA by post-translational histone modifications. In general, DNA methylation is associated with gene silencing and the formation of heterochromatin.

Both male and female gametes are highly specialized and are, in fact, terminally differentiated cells. An important question about the mechanism, timing and consequences of epigenetic disruption remains unexplained. Germ cell development is regulated by both genetic and epigenetic mechanism. Epigenetics and its role in infertility has been poorly investigated. Epigenetic changes occur most commonly during gestation, neonatal development, puberty and old age. On fertilization, the oocyte and sperm cell fuse to form the zygote. The zygote is totipotent, acquired through extensive epigenetic reprogramming [43, 44]. At fertilization, sperm and oocytes represent different epigenetic organizations in that: i) differentially methylated regions have been established at various imprinted loci; ii) the sperm genome is more methylated than the oocyte genome; and iii) chromatin has been compacted with protamines in sperm and with histones in oocytes.

After fertilization, decondensation of the male pronucleus is followed by a rapid and active demethylation, as both male and female-specific epigenetic programs are reset to allow the gametes to give rise to a zygote that is totipotent and thus able to develop into a new organism [45, 46]. Methylation marks the imprinted genes differently in egg and sperm, and inheritance of these epigenetic marks is known to lead differential gene expression. Normal embryonic development in mammals requires differential imprinting of both male and female genomes. During development,

the epigenetic profile of the germ cells changes dynamically. In primodial germ cells, inherited imprinted genes erase their methylation pattern, and *de novo* monoallelic sex-specific methylation then takes place during gametogenesis [47, 48].

After the oocyte is activated by sperm and completes meiosis, haploid oocyte chromosomes transform into a maternal pronucleus, and the haploid sperm nucleus transforms into a paternal pronucleus. When sperm chromatin protamines are replaced with oocyte cytoplasmic histones, extensive demethylation of paternal, but not maternal, DNA occurs [45, 46]. In human male germ cells, re-establishment of paternal imprints starts prenatally and is completed postnatally at the pachytene stage. The male germ cell pattern of DNA methylation remains hypomethylated as compared with the somatic cell DNA. Epigenetically disrupted development can occur in various biological pathways or systems. Defects in genomic imprinting and in other epigenetic process results in aberrant development of the embryo and may have serious lethal consequences.

Imprinted genes are susceptibility loci for disease since their normal function can be altered by a single genetic or epigenetic event. The genome-wide DNA methylation pattern changes little during spermiogenesis [49]. Studies have demonstrated that sperm DNA global methylation is essential for normal embryo development and pregnancy outcome in mice and humans. Sperm from infertile patients, especially those with oligozoospermia, carry a higher risk of incorrect primary imprints and contain imprinting/DNA methylation defects [50, 51].

A germ line abnormal epigenetic mechanism is proposed as a possible mechanism compromising fertility in some men. A recent study described a relationship between sperm methylation promoter sequence and gene function. It also indicates that methylation of the sperm genome is an important factor in sperm maturation and also in embryogenesis. There is increasing evidence that genetic as well as environmental factors (hormones and culture media) in infertile couples can have adverse effects on epigenetic processes controlling implantation, placentation, organ formation and fetal growth. Environmental toxins such as heavy metals disrupt DNA methylation and chromatin organization. Estrogenic and anti-androgenic toxins that disrupt the hypothalamo-pituitary-gonadal axis alter DNA methylation, and these changes are inherited by subsequent generations [52, 53]. Dietary modifications also can have a profound effect on DNA methylation and genomic imprinting.

It has been suggested that *in vitro* culture of gametes, zygotes and embryos, a common feature of all ART techniques, results in an accumulation of epigenetic alterations leading to an enhancement in fetal growth, known as large offspring syndrome [54]. In addition, loss of epigenetic control may expose hidden genetic variation. ART is a surprising environmental modulator of the epigenome is and has been shown to be the method of conception at higher than expected frequency in Beckwith-Wiedemann syndrome and Angelman syndrome [55]. Kobayashi et al. [51] reported abnormal DNA methylation in sperm of oligozoospermic men. Defects were present in paternally and maternally imprinted genes.

Increased knowledge of epigenetic regulation will affect a wider biomedical area, and knowledge of genetic and epigenetic mechanism of germ cells is necessary for the production of functional gametes and overcoming infertility. Whether common diseases have an epigenetic basis is still open to speculation, but if they do, this holds great promise for medicine. Given that epigenetics is at the heart of phenotypic variation in health and disease, it seems likely that understanding and manipulating the epigenome holds enormous promise for preventing and treating common human illness. Epigenetics also offers an important window to understanding the role of the environment's interactions with the genome in causing disease, and in modulating those interactions to improve human health. Our increasing knowledge over the last 10 years is beginning to be translated into new approaches to molecular diagnosis and targeted treatments across the clinical spectrum.

Role of telomere length and telomerase in infertility

Telomeres are nucleoprotein structure that protects the ends of chromosomes from DNA damage and degradation [56]. Most adult cells progressively lose telomeres during cell division and tissue renewal. In humans telomere length is maintained from spermatogonia to spermatozoon (i.e. spermatogenesis).

Telomerase maintains the length of the telomere. Many studies have addressed the issue of telomere length and telomerase activity in infertile couples. Telomerase has two important components: telomerase reverse transcriptase (TERT) and telomerase RNA. Schrader et al. [57] showed that expression of telomerase subunits and telomerase activity in testicular tissue are highly sensitive and specific markers of gametogenesis in infertile patients. Low-level expression of TERT and telomere RNA in the testis of infertile males is one of the factors for germ cell maturation arrest. Loss of telomerase appears to alter the end structure, rendering the telomere more susceptible to nucleolytic attack and end-to-end fusion. Loss of telomerase RNA leads to defects in cellular differentiation and an increase in genetic instability

and infertility. Eloisa et al. [58] showed that infertility, aging, immune system defects and other phenotypes appear as a consequence of telomere loss. Telomere shortening with age is associated with loss of organismal viability.

Telomerase is found only in the germ line, with some activity in bone marrow and peripheral blood leukocytes. It is present during early development, ensuring that replicative telomere shortening is suppressed to keep telomere length constant. Telomeres were observed to shorten in human tissues, including peripheral blood cells, liver, kidney, spleen, dermal fibroblasts and mucosal keratinocytes [59, 60]. However, in most somatic human cells telomerase is not expressed, which results in shortening of chromosome ends every time the cell divides [61]. Ultimately this leads to generation of very short telomeres. Telomere shortening limits the natural replicative life span of somatic human cells. In most tissues, therefore, telomere length decreases over time with replication.

Increasing evidence suggests an important role for histone and DNA methylation in regulating mammalian telomere length and integrity [62]. One study suggests that the telomeric restriction fragments isolated from DNA from human sperm cells are significantly longer than such fragments isolated from corresponding replicating cells *in vivo* [63]. Heterogeneity in telomere length leads to heterogeneity in the loss of telomere function.

In conclusion, ART has offered hope to millions of infertile couples, but the safety of these procedures is questionable in cases where parents harbor genetic abnormalities. Genomic and proteomic analysis offer great hope for understanding the etiology of infertility and further advance our knowledge of the complex processes that regulate fertility.

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