Effect of Sperm Molecular Factors, Oxidative Damage and Transcripts in Childhood Disorders

Vidhu Dhawan, Manoj Kumar and Rima Dada

Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India

Disruptions in the spermatozoal genetic integrity play a major role in determining the subsequent embryonic development trajectory. Sperm contributes an important role in post fertilization induction of normal development but also processes extending beyond the fertilization. Damaged or defective spermatozoa affect the outcome of pregnancy but also the health of the offspring, resulting not only in paternally mediated increase in miscarriages but also dominant genetic disorders in the progeny, including neuropsychiatric disorders like autism and schizophrenia, and even childhood carcinomas [1-3]. Sperm, a highly polarized cell is unique in its morphology, chromatin structure, and function, is characterized by a myriad of changes which occur during spermatogenesis and spermatogenesis. The haploid sperm chromatin undergoes chromatin packaging into a volume that is typically 10% or less than that of other somatic cell nucleus. This remarkable level of compaction is achieved by significant changes of ordered histone replacement by transition proteins, followed protamine's [4,5]. 85% of the normal human sperm chromatin remains packaged into toroid's by protamine's and 5–15% remains associated with histones, as compared to <5% being retained by other mammalian species, e.g. bulls, stallions, hamsters, and mice making it less compact tan other mammalian species [6,7]. Transcriptional and translational machinery of the spermatozoa are temporarily disengaged during the post-meiotic stage of spermatogenesis. The retained histones in the peripheral histone bound nucleosome complex remains transcriptionally active and have been explored for its epigenetic role (particularly histones carrying post-translational modifications) and contain telomeric DNA and promoters of genes of developmental importance [8].

The male reproductive functions are seen to witness gradual decline with age over a period of years and fathers are seen to bequeath more mutations with advanced age and the germline mutation rate is 6 times higher than in females, because of many more germ-cell divisions [9-11]. With age-dependent clonal expansion, mutant spermatogonial stem cells having a proliferative advantage over nonmutated cells. This germ line selection model explains the origin of autosomal dominant genetic disorders such as achondroplasia, complex polygenic conditions (schizophrenia, autism, epilepsy, bipolar disorder), cardiovascular malformations, diaphragmatic hernia, cleft palate, lower intelligence in children born to older fathers (higher sperm DNA damage [2,3,9,12-14].

Corresponding author:
Dr. Rima Dada

✉ rima_dada@rediffmail.com

Professor, Department of Anatomy, Laboratory of Molecular Reproduction and Genetics, All India Institute of Medical Sciences, New Delhi, India.

Tel: +91-11-2658 8500/3517, 26593517
Fax: +91-11-26588663

The more vulnerability of DNA damage in sperm as compared to somatic cells may be due to its susceptibility to damage at various stages of spermatogenesis, function and transport, however majority of damage occurs post spermiogenesis It is multifactorial and may be due to both intrinsic as well as extrinsic factors. Intrinsic factors can be the result of protamine deficiency, abortive apoptosis, and excessive Reactive Oxygen Species (ROS) levels, presence of morphologically abnormal and immature germ cells. Various extrinsic factors include the following: paternal age, environmental exposures, radiotherapy, chemotherapy, electromagnetic radiation and possibly lifestyle factors such as nicotine and alcohol users, sedentary lifestyle, psychological stress/ depression consumption of fatty foods [1-3,15-20].

Oxidative Stress (OS) is one of the major causes of defective sperm function. It is mediated by a variety of Reactive Oxygen Species (ROS) which are highly reactive oxidizing agents and includes superoxide anion ($O_2 \cdot$), nitric oxide (NO •), peroxyl (ROO.), or the hydroxyl (OH.) radicals (OH •) as well as powerful oxidants such as hydrogen peroxide (H_2O_2) or peroxynitrite

(ONOO-). Sperm as a professional generator of ROS, has its vast majority of these free radicals generated as a consequence of mitochondrial electron leakage during intrinsic apoptotic cascade and is also contributed by activated leukocytes in the seminal plasma [20]. Spermatozoa are more prone oxidative stress as they have limited antioxidant capacity because their cytoplasm is extruded outside during the process of spermatogenesis with a concomitant reduction in cytoplasmic antioxidants such as catalase and superoxide dismutase. Mild OS is required for driving the tyrosine phosphorylation event associated with sperm capacitation but supraphysiological ROS levels impede sperm membrane fluidity and permeability [21]. Moderate levels are beneficial for maintenance of telomere length and thus play vital role in maintenance of genomic integrity. OS disrupts the sperm DNA integrity and limit the fertilizing potential as a result of collateral damage to proteins and lipids in the sperm plasma membrane. Sperm cells are vulnerable attack by ROS as they contain high concentrations of unsaturated fatty acids, particularly docosahexaenoic acid with six double bonds per molecule [12]. The lipid peroxidation chain reaction culminates in the generation of small molecular mass electrophilic lipid aldehydes such as 4-hydroxynonenal (4HNE), acrolein, and malondialdehyde [22,23]. OS is also witnessed to modulate the epigenome by altered methylation patterns; it may have a significant impact on sperm epigenome and thus have adverse effects on developing embryo. Sperm OS also targets the telomeres, which are tandemly repeating hexameric units (5'TTAGGG3') that cap chromosomal ends and are vital for genomic integrity and chromosomal stability. These are histone bound, located in periphery of sperm nucleus and are rich in guanine, the nucleotide with lowest oxidative potential. Both its location and guanine content make telomeres highly susceptible to oxidative damage [24-26].

The backbone of DNA helix is usual cleaved in the sperm and results either in single- and double-break strands (SSBs and DSBs), and oxidative attack occurs primarily at the guanine bases and causes the formation of base adducts, particularly 8-hydroxy-2'-deoxyguanosine (8OHdG) and 8–oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) [18,23,27]. SSB are repaired by the Base Excision Repair (BER) and Nucleotide Excision Repair (NER) pathways, while DSB are repaired by Non-Homologous End Join (NHEJ) and Homologous Recombination (HR). Sperm has a very limited capacity for repair as they only possess the first enzyme in the BER pathway, 8-oxoguanine glycosylase 1 (OGG1), but the repair cannot be proceeded further because they lack the downstream enzymes (APE1, XRCC1). Sperm is dependent on the oocyte to resume the repair of basic site created by OGG1 to continue the BER pathway prior to initiation of S-phase of the first mitotic division. Sperm being transcriptionally and translationally inert, lack cytosolic antioxidants and as they have a very basic repair mechanism is unable to repair DNA damage. They may overwhelm oocyte repair mechanism if damage is extensive and thus unresolved damage may not only limit fertilization potential, but may persist post fertilization. In era of ART/ICSI use of sperm of suboptimal quality and aged oocytes (with inefficient and aberrant DNA repair mechanisms) further compounds the problem and may actually double the population in need for this technology in future generations.

Unresolved DNA damage post fertilization has potential to disrupt the integrity of both its DNA and RNA, create mutations/epimutations in the offspring that can have and also have profound impact on the development potential of embryo through dysregulation of sperm transcripts [28-31]. The non-genomic paternal delivery of selective transcripts by the transcriptionally inert spermatozoa has been seen to contribute to the transcriptome of embryo prior to activation of embryonic genome. Various mRNA transcripts like *FOXG1, WNT5A, SOX3, and STAT4* have a critical role in cell fate determination, primary embryonal axis, and development of embryonic forebrain, hypothalamic pituitary axis and morphogenesis in the developing embryo. Dysregulation in sperm transcripts along with ODD may alter sperm methylation pattern and affect the sperm epigenome, lifelong health of the offspring and also have transgenerational effects. This may be underlying cause of embryo implantation failures, recurrent pregnancy losses, congenital malformations and even childhood cancers [32,33].

This explains the reason that why it becomes pertinent to undertake the relevant investigations and assays for assessment of male reproductive parameters not only because they reveal the status of the damage on the sperm but also reflect the underlying quality of spermatogenesis. And more importantly they may aid in revealing the detrimental effects of the said derangements on the developmental normality of the embryo and health of the future progeny.

As OS is caused by a host of modifiable factors especially unhealthy lifestyle and social habits (smoking, excessive alcohol intake, sedentary lifestyle, psychological stress, intake of nutritionally depleted food), lifestyle modifications and simple practices like incorporation of yoga and meditation can have a significant positive impact on reduction of OS and reduce ODD [3,14]. This may not only improve health of the individual but also reduce the incidence of genetic and epigenetic disorders in the offspring and thus reduce disease burden in future generations.

References

1. Aitken RJ, Baker MA, Sawyer D (2003) Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. Reprod Biomed Online 7: 65-70.

2. Kumar SB, Chawla B, Bisht S, Yadav RK, Dada R (2015) Tobacco use increases oxidative DNA damage in sperm - possible etiology of childhood cancer. Asian Pac J Cancer Prev 16: 6967-6972.

3. Rima D, Shiv BK, Bhawna CH, Shilpa B, Saima KH (2016) Oxidative stress induced damage to paternal genome and impact of meditation and yoga- can it reduce incidence of childhood cancer? Asian Pac J Cancer Prev 17: 4517-4525.

4. Li Y, Lalancette C, Miller D, Krawetz SA (2008) Characterization of nucleohistone and nucleoprotamine components in the mature human sperm nucleus. Asian J Androl 10: 535-541.

5. Miller D, BrinkworthM, Iles D (2010) Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. Reproduction 139: 287-301.

6. Erenpreiss J, Spano M, Erenpreisa J, Bumgum M, Giwercman A (2006) Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J Androl 8: 11-29.

7. Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT (2009) Distinctive chromatin in human sperm packages genes for embryo development. Nature 460: 473-478.

8. Ioannou D, Miller D, Griffin DK, Tempest HG (2016) Impact of sperm DNA chromatin in the clinic. J Assist Reprod Genet 33: 157-166.

9. Crow JF (2000) The origins, patterns and implications of human spontaneous mutation. Nature reviews. Genetics 1: 40-47.

10. Taylor J, Tyekucheva S, Zody M (2006) Strong and weak male mutation bias at different sites in primate genomes: insights from the human-chimpanzee comparison. Mol Biol Evol 23: 565-573.

11. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, et al. (2012) Rate of de novo mutations, father's age, and disease risk. Nature 488: 471-475.

12. Aitken RJ, Smith TB, Mathew SJ, Baker MA, De Iuliis GN (2014) Oxidative stress and male reproductive health.

13. Dhawan V, Kumar M, Malhotra N, Singh N, Dada R (2016) Paternal influence of sperm transcripts and DNA damage in recurrent implantation failure. Hum Reprod 31: i190.

14. Tolahunase M, Sagar R, Dada R (2017) Impact of Yoga and Meditation on Cellular Aging in Apparently Healthy Individuals: A Prospective, Open-Label Single-Arm Exploratory Study. Oxid Med Cell Longev.

15. Cocuzza M, Sikka SC, Athayde KS, Agarwal A (2007) Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: An evidence based analysis. International Braz J Urol 33: 603-621.

16. Shamsi MB, Kumar R, Dada R (2008) Evaluation of nuclear DNA damage in human spermatozoa in men opting for assisted reproduction. Indian J Med Res pp: 115-123.

17. Venkatesh S, Riyaz AM, Shamsi MB, Kumar R, Gupta NP, et al. (2009) Clinical significance of reactive oxygen species in semen of infertile Indian men. Andrologia 41: 251-256.

18. Aitken RJ, De Iuliis GN (2010) On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod 16: 3-13.

19. Venkatesh S, Singh A, Shamsi MB, Thilagavathi J, Kumar R, et al. (2011) Clinical significance of sperm DNA damage threshold value in the assessment of male infertility. Reprod Sci 18: 1005-1013.

20. Aitken RJ (2013) Human spermatozoa: revelations on the road to conception. F1000 Prime Rep 5: 39.

21. Aitken RJ, Gibb Z, Mitchell LA, Lambourne SR, Connaughton HS, et al. (2012) Sperm motility is lost *in vitro* as a consequence of mitochondrial free radical production and the generation of electrophilic aldehydes but can be significantly rescued by the presence of nucleophilic thiols. Biol Reprod 87: 110.

22. De Lamirande E, Gagnon C (1992) Reactive oxygen species and human spermatozoa: I. Effects on the motility of intact spermatozoa and on sperm axonemes. J Androl 13: 368-368.

23. Aitken RJ, De Iuliis GN, McLachlan RI (2009) Biological and clinical significance of DNA damage in the male germ line. Int J Androl 32: 46-56.

24. Thilagavathi J, Kumar M, Mishra SS (2013a) Analysis of sperm telomere length in men with idiopathic infertility. Arch Gynecol Obstet 287: 803-807.

25. Thilagavathi J, Mishra SS, Kumar M (2013b) Analysis of telomere length in couples experiencing idiopathic recurrent pregnancy loss. J Assist Reprod Genet 30: 793-798.

26. Mishra S, Kumar R, Malhotra N, Singh N, Dada R (2016) Mild oxidative stress is beneficial for sperm telomere length maintenance. World J Methodol 6: 163-170.

27. Aitken RJ, Baker MA (2013) Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development. Int J Dev Biol 57: 265-272.

28. Kumar K, Deka D, Singh A, Chattopadhyay P, Dada R (2012) Expression pattern of PRM2, HSP90 and WNT5A in male partners of couples experiencing idiopathic recurrent miscarriages. J Genet 91: 363-366.

29. Kumar M, Kumar K, Jain S, Hassan T, Dada R (2013) Novel insights into genetic and epigenetic paternal contribution to the human embryo. Clinics 68: 5-14.

30. Kumar M, Dhawan V, Kranthi V, Dada R (2016) Paternal factors- Role in idiopathic recurrent pregnancy losses. Int J Reprod Fertil Sex Health pp: 1-6.

31. Dhawan V, Misra S, Biswas VK, Malhotra N, Dada R (2016) Impact of paternal aging on the seminal oxidative stress and sperm DNA damage. JISNAH 3: 1266.

32. Dhawan V, Kumar M, Deka D, Dadhwal V, Dada R (2016) Expression pattern of RPS6, RBM9 and RPL10A in recurrent pregnancy loss. Andrology 4: 110.

33. Kumar M, Dhawan V, Deka, D, Dhadhwal V, Dada R (2016) Role of paternal factors in early embryonic development. Andrology 4: 84.