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Environmental Stressors: Is the Sperm Cell Vulnerable or Resilient?

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Abstract

The sperm is a unique cell, differing from other cells in physiology and function. It survives an arduous journey through male and female reproductive tracts, to ensure that an intact male genome reaches the site of fertilization. In addition, it has also been recognized to wield epigenetic control over early embryonic development.

Environmental degradation over the past years has exposed the human sperm to myriads of toxicants which have adversely affected its structure and function. In correlation, there are reports of decline in sperm counts and fertility, although there is still no concrete evidence to substantiate these claims. Moreover population statistics in most developing nations do not conform to data of declining fertility. In addition there are certain researches, in agreement with our data of normal, fertile volunteers, which also suggest no change in semen characteristics or sperm numbers over the past three decades.

These findings indicate that despite the barrage from environmental agents, spermatozoa have withstood the test of time and have emerged resilient. Hence, in addition to the manifold machinery that operate to protect the spermatozoa including toll-like receptors, anti-oxidants, heat shock proteins etc., there possibly exists an in-built, genetically programmed, population specific mechanism that reinforces its nuclear integrity to protect this cell from the onslaught of toxic influences. Research in this direction is underway at our laboratory and other research centers to delve into the enigma of sperm endurance.

Keywords: Human spermatozoa; Environmental toxicants; Fertility decline; Chromatin; Genetic regulation; Resilience

Introduction

The sperm cell has proven to be most enigmatic, the most intriguing of all the varied cells in the living world. It is one of the most unique in morphology, metabolism and function and is perhaps the most investigated (after neoplastic cells). Being endowed with distinctive organelles, spermatozoa are highly specialized cells that do not grow, divide, replicate their DNA nor synthesize protein, but have the dual function of:

(1) Ensuring that its haploid genome reaches the site of fertilization intact, not withstanding its long and arduous journey through variable terrain, and

(2) The sperm cell is known to exert an epigenetic control over early steps in implantation and embryonic development.

Despite their high degree of specialization, Aitken [1] has stated that human spermatozoa are extremely inadequate and are major contributory factors to poor fertility. He has further attributed the increasing incidence of defective sperm to both genetic and environmental impacts. Earlier, Aitken and Sawyer [2] termed poor DNA integrity and Reactive Oxygen Species (ROS) the key attributes to faulty spermatozoa and called for identification of the underlying environmental factors and mechanisms that lead to erosion of sperm quality. Correlating semen quality parameters with couple fecundity, Louis et al. [3] have confirmed that persistent environmental pollutants work as reproductive toxicants. Monitoring the deterioration in semen quality in recent years has therefore taken a prime locus in research and several techniques have evolved to evaluate sperm function.

Despite the early warning from Rachael Carson in her book 'The Silent Spring' and not withstanding endless discussions on the degrading quality of the environment, we still continue to generate toxic emissions beyond rescue [4]. The radical environmental deterioration has proven damaging to the human sperm both morphologically and functionally. Toxins, effluents, chemicals, drugs, metal ions, radiation, have all been reported by several researchers to cause a decline in sperm concentration, motility, viability and nuclear integrity. Global warming and climate change have subjected this cell to varied effects of hyperthermia. As Aitken has observed in his article the causes of defective function are complex and involve both genetic and environmental impacts [1]. Moreover, Rocco et al. [5] have demonstrated that active pharmacological agents discharged into waters have led to a significant increase in sperm DNA fragmentation. Earlier, Klassen et al. [6] have provided an exhaustive list of various agents that affect the sperm, lowering male reproductive potential. With each passing decade, an exponential rise occurs in the number of environmental toxicants and xenobiotics as fallout of the escalating industrial, chemical and technological advances.

Effect of Environmental Agents on the Sperm

Research at our laboratories over the past few decades, have also reflected the damaging effects of radiation [7], heavy metal ions [8], aluminum [9], fluoride [10], aflatoxins [11], pesticides [12], organic solvents [13] and other agents on the sperm. Environmental degradation has therefore been intrinsically implicated with a direct

impact on the sperm cell, possibly leading to a loss of sperm fertilizing ability.

As early as 1988, Overstreet et al. [14] recognized that the human sperm is more vulnerable to environmental and occupational exposures and this cell in particular, although a completely differentiated cell, is relatively sensitive to exogenous attack. Comparing the effects of xenotoxins on DNA of somatic cells to that of the human sperm, Anderson et al. [15] have observed that spermatozoa are much more susceptible to environmental toxicants and this susceptibility has been attributed to the fact that mature sperm do not carry the enzymatic machinery to repair the damage to their DNA. Recently, Sakkas and Alvarez [16] have suggested that induced DNA aberrations could result in a protamination deficiency that would subsequently render the sperm DNA increasingly vulnerable to a variety of environmental stressors. In addition, these researchers have observed that a wide range of such agents could activate caspases and endonucleases triggering DNA fragmentation, which has been indexed as the first sign of a cell in distress.

It has been noted by Delbes [17] that exogenous factors such as radiation, heat, drug, alkylating chemotherapeutic drugs, pesticides as well as altered lifestyle, habits and addictions can affect the sperm nuclear integrity. Aitken et al. [18] have recognized that the origin of sperm DNA damage involves complex mechanisms at both testicular and post-testicular sites. As Delbes et al. [17] have aptly indicated, the mechanisms by which such damage is triggered are still largely unresolved and the susceptibility will depend on the genetic background, lifestyle and the form of exposure to various insults

Among the myriad of environmental factors that have been linked to sperm assault, are hyperthermia and electromagnetic radiation. Levine et al. [19] have convincingly demonstrated that semen quality effectively deteriorates in summer. In a separate study these researchers also highlighted the differences in semen quality in outdoor workers as compared to controls [20]. Ionizing radiation, ultraviolet and microwave radiation are known bio-hazards. Research from our laboratory has shown conclusively that even infrared radiation (IR) at low doses affects male reproductive function [21]. The data obtained as shown in Figure 1, indicates that key enzymes of the steroidogenic pathway are altered on exposure to short duration infrared radiation (900 nm), leading to a fall in testosterone levels, which in turn lowers the fertility of the animals. The findings reveal the influence of infrared radiation exposure on reproductive function (Figure 1).

Kumar et al. [22] have shown the presence of apoptotic bodies, micronuclei and DNA strand breaks on exposure to 10 GHz electromagnetic radiation. Cell phones work in the frequency range 400 MHz to 2000 MHz and emit radiofrequency radiations which have shown as to decrease sperm count by Agarwal et al. [23]. In an earlier study Fejes et al. [24] have also reported that cell phone emissions adversely affect sperm motility.

Mortazavi et al. [25] have cautioned that while our changing lifestyle has increased our dependency on electricity and gadgets, it leads to exposure of varied levels of EMFs through mobile phones, laptops, wireless-internet servers, which in turn decrease human semen quality. These researchers have further warned that male reproductive health is under threat, since short term emissions from mobile jammers could significantly inhibit sperm motility. Research by La Vignera et al. [26] has confirmed that Rf-EMF causes a decline in sperm count and motility through formation of ROS. However, there are conflicting reports regarding the reproductive toxicity of radiofrequency radiation and scientists [27,28] have stressed the need for well-designed, structured studies in this direction.

Although there has been a flood of publications reflecting the repercussions of environmental changes on spermatozoa, very few sporadic researches have been aimed at identifying factors that actually protect the sperm from damage and conserve the integrity of its nuclear DNA.

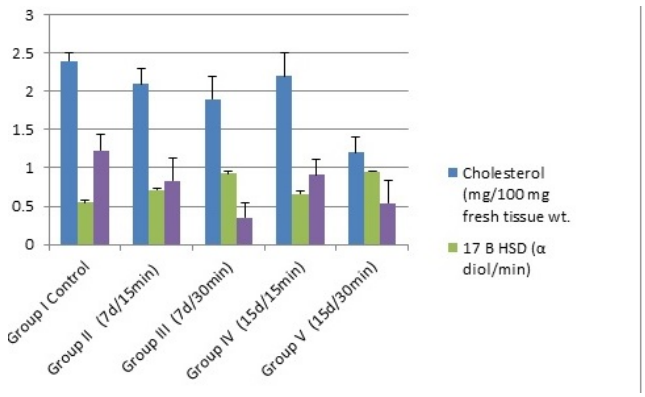

Figure 1: Figure showing cholesterol, 17-β, 3-β hydroxysteroid dehydrogenases and testosterone levels in testis of Control and IR-radiation exposed animals.

Fertility Decline- Loss of Sperm Function?

A spurt of research papers recording a decline in male fertility emerged the world over in the early nineties, with evidence of falling sperm counts and deterioration of semen quality [29,30]. These publications met with sharp criticism regarding study design and statistical validation; conversely, the mounting birth rates and simultaneous population explosion appear incongruous to reports of decline in sperm count or male fertility. It is clear that there is no consistent evidence of a worldwide effect on human male fertility.

Reports from various centers all over the globe have statistically confirmed the increase in sperm anomalies with concomitant decline in sperm numbers. Mendiola et al. [31] have asserted, through research focused on young University students of Southern Spain, that sperm counts have declined in populations of that region. Several studies have investigated temporal trends in semen quality in Northern Europe, but none has examined this question in Southern Europe where a prior study conducted in Almeria Province (Southern Spain) reported higher sperm. The global fertility decline has been reinforced by projection analyses that predict a drastic fall in fertility rates by 2050 (Figure 2).

Although some recent studies have corroborated this decline in semen quality [31-33], others have found no such change [34,35]. The ambiguity on the issue of sperm count decline has been discussed in an interesting review article by Pacey [36].

Fertility Decline - The Indian Scenario

Even in our country India, there is no consensus regarding the trends of fertility decline. Certain Scientists have recorded a fall in sperm numbers in the Indian population [37-39]. Earlier, Gopalkrishnan [40] and Mukhopadhyay et al. [41] had noted a

significant decline in many semen parameters in men from Indian populations. On the other hand, there were certain researchers who did not support the contention of a decrease in the semen quality in Indian men [42,43] and reported no change in the semen quality among Indian subjects. With both pros and cons providing data, there appears to be no clinching evidence to conclude whether sperm count or fertility is on the decline in this country. When equated with figures related to population dynamics, any reference to a decline in fertility seems inconsistent.

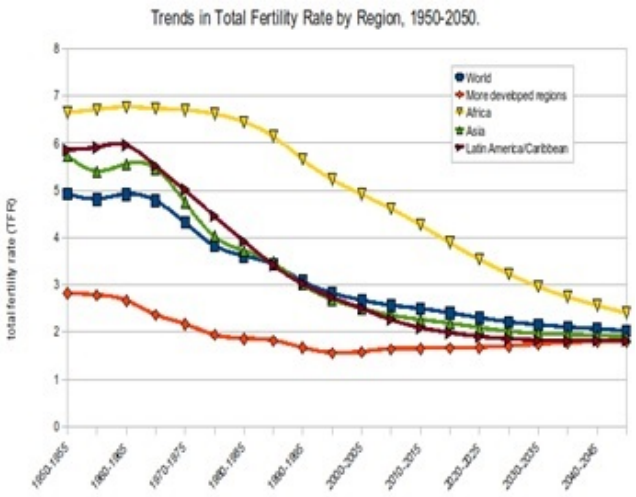

Figure 2: Prediction analysis for global fertility rate.

YEAR	SPERM DENSITY (million/ml)	MOTILITY (%)
1990-1995 (n=410)	116 ± 24.0	79.5 ± 14.0
1995-2000 (n=390)	118 ± 13.0	75.8 ± 11.5
2000-2005 (n= 370)	121 ± 14.2	81.5 ± 7.4
2005-2010 (n=364)	125 ± 11.7	74.4 ± 17.3
2010-2015 (n=330)	122 ± 14.5	78.3 ± 11.2
*Values are MEAN ± S.E.		

Table 1: Sperm density and motility in semen samples from men of normal proven fertility over the 25 year period.

Research at our laboratory since 1990, has been directed towards the investigation of infertility, spontaneous fetal loss and congenital anomalies, in association with which semen parameters of the male counterpart have been systematically evaluated. The scrutiny revealed that there was no significant change in semen profile, among the males studied from the local population, over the given period [44]. Semen parameters from men of proven fertility in the age range of 20 to 40 years were evaluated along with males with referral diagnosis of infertility. The data accrued over the period from 1990 to 2015, was statistically analyzed (SPSS, version 16) and no significant change was found in the sperm count and motility of sperm from samples of the Normal, control volunteers (n=1864; Table 1). Similarly as shown in Figure 3, there is an insignificant alteration in the sperm viability and morphology as obtained on analysis of our data over the twenty-five year period.

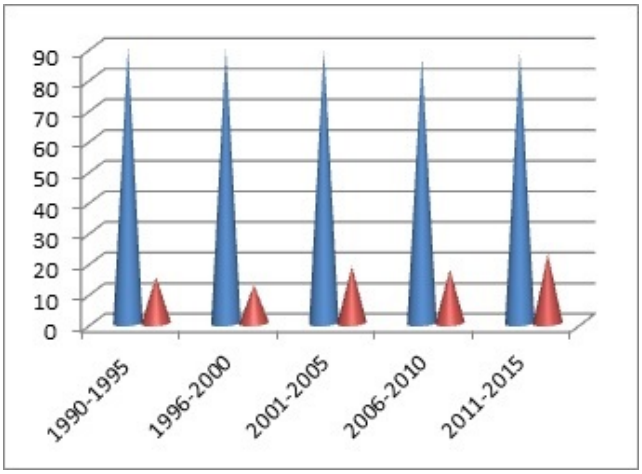

Figure 3: Sperm viability and morphology in semen samples from men of normal proven fertility over the 25 year period.

Our results therefore, fail to agree with reports of declining sperm count or motility despite the increasing barrage from environmental mutagens and disruptors. Could it be then, that the sperm cell, once termed extremely sensitive, is instead (in certain populations) genetically resilient?

Does the Sperm Have In-Built Protection Mechanisms?

Pierce et al. [45] have conjectured that 'a general fitness factor' reflects an overall mutation load, where key metabolic pathways which are targets for mutation, are perhaps protected. The revelation of the presence of Toll-like receptors (TLR) and the expression of *TLR* genes in the sperm [46] that play a critical role in the protection of these relatively fragile cells against microbial attack, lends support to the theory of protective mechanisms operating around this cell. Could there be a similar defense mechanism against the pressure of environmental factors? Or else - the volley of fuel exhaust, industrial emissions, effluents, bio-medical and electronic waste, pesticides, food contaminants, plastics, endocrine disruptors, heat, toxic metal ions would have corroded the vitality of even this most specialized cell.

The most reported natural defense mechanism is the innate anti-oxidant defense that exists in the seminal plasma and plays a vital role in protection of the spermatozoa from free radical attack [47]. There are volumes related to research in this direction.

Heat shock proteins (HSP) have been described as natural protectors of the sperm cell against physical stress factors such as temperature elevations. Heat shock proteins (HSP) are essential mammalian and bacterial stress proteins. At the cellular level, they act as chaperones, have important regulatory functions. HSP have been identified as a critical component of a very complex and highly conserved cellular defense mechanism to preserve cell survival under adverse environmental conditions. These molecules are preferentially expressed in response to an array of insults, including hyperthermia, free oxygen radicals, heavy metals, ethanol, amino acid analogues, inflammation and infection. HSP interact with intracellular polypeptides and prevent their denaturation or incorrect assembly.

In infertile men it has been demonstrated that the number of HSP60-expressing spermatogonia paralleled the loss of spermatogenic function [48]. These observations suggest that a low level of HSP60 expression in spermatogonia might lead to a decreased level of protection, which in turn could be involved in low spermatogenic efficiency. In a recent study, Dix [49] has shown in a mouse model that the disruption of the *HSP70-2* gene results in failed meiosis, germ cell apoptosis and male infertility.

While these natural gladiators maybe contributing to the in-born protection system of the sperm cell, there is no doubt that it is the genetic constitution of the cell that governs its ability to combat stressors.

A Possible Genetic Link to Resilience

Storgaard et al. [50] carried out a twin-based study and suggested a substantial hereditary component in sperm cell chromatin stability. Confirming these observations, Enciso et al. [51] have described a differential resistance of mammalian sperm chromatin to oxidative stress, based on the differences in protamine structure and have demonstrated that the sperm DNA of certain species is less susceptible to attack, due to extensive disulfide cross-linking. These authors further suggest that the oxidation of thiols to disulfides for chromatin condensation during epididymal transit in mammals is likely to provide nuclear stability and protect these cells from the genotoxic effects of adverse environments. Gonsalvez et al. [52] have substantiated these findings, proving that normal human spermatozoa showed greater chromatin stability due to specific protamine 1-to-protamine 2 ratio, which defines the integrity of the sperm nucleus. In addition, resistance to iatrogenic damage was also ascribed to genomic design.

Differential gene expression is produced by variability in the DNA-associated protein and its modifications (methylation, demethylation, acetylation, and deacetylation). These modifications are responsible for variable gene expression and constitute an integral component of epigenetics, which has a crucial role in sperm development and function, fertilization, and post-fertilization events. Hence, among different individuals of various populations, there possibly lies a differential pattern of gene expression based on the associations of the DNA with its nucleoprotein that perhaps causes some spermatozoa to withstand stress while others are more vulnerable.

Specific genes *PRM1* and *PRM2* encode for the protamines, while genes *TNP 1* and *TNP2* for the transition proteins that collectively comprise the compact chromatin assembly which in turn determines the nuclear integrity. Any change in these genes or their expression, leads to protamine anomalies that lead to aberration in chromatin packaging in spermatozoa. A deviation from the normal P1/P2 ratio or any change in these proteins would ultimately mean a disrupted toroid structure. Consequently, when the DNA is not correctly packaged, it is vulnerable to attack. Hence poor protamination and impaired chromatin compaction are indicative of increased susceptibility to DNA damage and poor semen quality [53,54]. Alternately, abnormalities in protamine packaging of DNA cause aberrant gene expression resulting in either hypertranscription or transcriptional arrest, leading to failure in spermatogenesis [55]. Thus, there is huge genetic link to the differential susceptibility of spermatozoa to external stress.

If the mechanisms that confer such innate resilience in the sperm cell could be proven and elucidated the findings would generate immense information in understanding the basis of sperm survival in certain human races, despite tremendous environmental degradation. These findings would eventually have important clinical implications in current times where there is a heavy dependence on Intracytoplasmic Sperm Insemination (ICSI) and related IVF technologies to manage conditions of infertility.

Conclusion

The deleterious impact of environmental toxicants on the structure and function of the sperm cell has been extensively researched and reported. Despite these harsh effects of a wide range of toxic agents on spermatozoa, these cells appear relatively resilient, as reflected by evidence of insignificant change in sperm density over the years. In addition to the micro-environmental protection system that safeguards the sperm, there appears to be a strong genetic influence that may work to shield this highly specialized cell from the plethora of environmental stressors that are increasing exponentially with time. These genetic factors may be population specific, related to compaction of the sperm nuclear chromatin and susceptibility of its DNA.

However, it will require time and in-depth research to conclusively state whether the sperm cell is genetically programmed to be vulnerable or resilient to the insult and injury from an ever changing, stressful environment.

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