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Research Article

EFFECT OF VARIOUS BIOMOLECULES FOR NORMAL FUNCTIONING OF HUMAN SPERM FOR FERTILIZATION: A REVIEW

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ABSTRACT

Semen is a mixture of sperm and secretions of seminal vesicle, prostate gland and Cowper's gland. The molecules present in the semen includes Sperm, amino acids, proteins, lipids, Anti-sperm antibodies and trace elements like include zinc, megnesium, sodium and potasium. Each of these molecules is having specific function. Trace elements play a major impact on the quality of the semen and inturn, these trace elements helps in motility, metabolism, and acrosome reaction. The cumulative function of all these molecules determines the quality of semen to accomplish the task of fertilization.

Due to increased industralization and environmental pollution over the past decades, numerous studies have reported that a decline in semen quality in men in various parts of the world has been observed. Besides, the semen quality and acticity is reported to be declined 50% between 1930 and 2011. There are several proteins identified in the semen that are used as a biomarker, acting as an identification marker for fertility of an individual. The seminal plasma proteins are identified to help in transport and elimination in female reproductive tract. Reactive oxygen species (ROS) are identifide to be present even in the normal semen (15%), and it is found to be increasing in the infertile samples. So, in this review paper we have dissused about the effects and functions of the various micro and macromolecules in the semen to make the future generation alert on the male infertility.

INTRODUCTION

World Health Organization (W.H.O) [1] defines infertility as biological inability of a person to contribute to conception. Approximately 10–15 of every 100 couples reported to be incapable to produce offspring. Among these, it is estimated that in about 30–40% of these cases, defects were identified among the males. Male contributes semen for the conception. Semen is a complex biological fluid, consists of sperm (male gamete) and seminal fluid. Seminal fluid is secretions from several glands, comprises of several organic and inorganic compounds includes free amino acids, proteins, lipids and its derivatives, zinc and other scavenging elements that includes Mg^{2+}, Ca^{2+}, $K^{+,}$ Na. Therefore, in view of the development of novel approaches to male contraception, overall understanding of the biochemical and molecular composition and its role in regulation of sperm quality and enable to be potential human spermatozoa is highly desirable[2].

FREE AMINO ACIDS OF SEMINAL FLUID

In human seminal plasma, almost all the amino acids are present free form. Those amino acids are produced/arises due to proteolysis subsequent to semen ejaculation. Comparison of seminal plasma, amino acids of fertile and infertile pateints reveals that the amino acids are needed for sperm activity. It has been assumed that full episode behind the infertility problems seems to arise from this aminoacids quality and quantity. The presence of all free amino acids and its concentration in the seminal plasma indicates that their presence is not simply the result of diffusion from extracellular fluids [3, 4]. The functions of these free amino acids are largely unknown[5]. However, available literature reveals that, the seminal plasma proline and threonine is negatively correlated with the sperm motility[6]. Where as in bull semen there is a positive correlation between the concentration of amino acid present in seminal fluid and the fertilizing capacity in the bull semen[7]. Most of the free amino acids presented in the seminal plasma originate from the testis or epididymis[8]. The amino acids are considered to be oxidizable substrate for energy yielding reactions in the semen which increases the quality of the semen[9]. Arginine is an essential amino acid that plays important role in determining the semen quality that inturn decides the fertility. L-carnitine plays an important role in the process of sperm formation, sperm maturation and the maintenance of sperm quality during the time of ejaculation. L- Carnitine is also important in the vital development of the sperm membrane, maturation of the sperm cells in the testes, and also in the metabolic process and enzymatic reaction followed by ejaculation. This overall contribution is only associated with proper sperm motility and deplition in this process will reduce the semen quality. When there is depletion of L-carnitine, then there is poor or no sperm motility which led to the male infertility. Tyrosine acts as an important contributor to the antioxidant capacity of seminal plasma[10]. Tyrosine scavange the free radicals and enhance the motility. Lack of tyrosine amino acid in the semen decreases the motility of the sperm and consequently male infertility.

Hyaluronan, an important protein derivative found in reproductive fluids is reported to be involved in sperm penetration[11]. Hyaluronan is known to play an important role in sperm motility and is used as sperm-select in medium for the isolation of motile sperms for IVF[12]. A 34 kDa glycoprotein is found to regulate the sperm motility[13]. Absence of this protein will lead to low sperm motility and the quality of the semen.

SEMINAL PLASMA PROTEINS

It has been demonstrated in human sperm surface that property the ejaculated spermatozoa surface is coated with a number of seminal plasma proteins. These surface proteins have various biochemical activities such as haemagglutination, heparin-binding or zona pellucida-binding[14]. Majority of these proteins are 12–16 kDa Glycoproteins that bind to the sperm surface which helps in acrosome reaction [15]. Human seminal plasma (HSP) contains a family of major proteins designated HSP-A1/A2 and HSP-A3 with molecular masses ranging from 15 to 16.5 kDa and HSP 30 kDa with an approximate molecular mass of 28–30 kDa. These proteins, collectively called Human seminal plasma proteins (HSP proteins), bind to the sperm surface and modulate sperm functions. These proteins are important in proper management of sperm-oocyte binding and motility. In recent years, various proteins from the seminal plasma have been identified, isolated and characterized[16]. The protein composition of the seminal plasma is found to be different in different species and is associated with the fertility and thought to increase the semen quality.

There are number of glycoproteins responsible for the sperm-egg interaction. The cascade of reactions starts with the cell-specific binding site at the surface of the zona pellucida (ZP) and the complementry receptor on the sperm plasma membrane should recognise. This results in the recognition of the sperm and egg which ultimately result in the acrosomal reaction folllowing the penetration of the sperm into the oozyte.

The molecular structure reveled that ZP consists of three major extacellular glycoprotein complexes which iniates the binding of the sperm to the egg and even finally enhancing the meshwork after acrosomal reaction. It has been reported that all the three proteins are having the N-terminal sequences that are cleaved from mature protein. [17, 18]

MEMBRANE PROTEINS OF SPERM AND MALE INFERTILITY

Membrane cofactor protein (MCP) CD46 is considered to be a complement regulatory protein. It functions in the IgG mediated cleavage of C3b and C4b, acts as a cofactor and in turn (cannot understand), regulates the complement cell cascade at the sperm cell surface[19]. Its expressions are strongly found in the human reproductive tracts the acrosomal region of condensing spermatids and spermatozoa, germinal epithelium of the testis and glandular epithelium of the prostate [20]. It has been suggested that this protein (membrane bound) could have a role in human reproduction by protecting spermatozoa from complement-mediated lysis in the female reproductive tract[21]. It also has the function in human sperm-oocyte interaction [22, 23]. Soluble forms of these proteins are found in the human seminal fluid. Absence of this particular protein in the seminal plasma led to the low motility which reduces the semen quality. CD46 is found to have an exclusive function in the sperm and egg interaction. It is exclusively thought to originate in the inner membrane of the acrosome.

GROWTH FACTOR

Neurotrophins, (a group of protein) are considered to be the growth factor found in the nervous system and plays very important in the neuronal survival and in its differentiation. This is also found in the expressed state in the non-neuronal tissues like the cardivasculor, and immune system. In addition it is also found in the reproductive system[24, 25]. The expression of neurotrophins in the reproductive system as a valid function in the spermatogenesis and in the post ejaculatory functions of the sperm. Recently the expression of the neurotrophins and their receptors has been detected in the prenatal and in the adult human testis. This indicates the potential role of neurotrophin in the morphogenesis of the testis and even in the mature testis[26].

LIPID AND MALE INFERTILITY

It is assumed that fatty acid is essential for the high fluidity of the cell membrane of the spermatozoa, its ability to become potentially fusogenic and therefore, for the quality of the semen. Several reports indicates that a close correlation between the fatty acid composition of spermatozoa and sperm motility as one of the main determinants of quality of the semen. Human semen consists of unique fatty acid composition. It consists of high amounts of docosahexaenoic acid (22:6) in phospholipids [27]. The concentration of fatty acids is varying among individuals and ranges between 20% and 40%[28, 29]. The presence of these fatty acids gives high fluidity and fusogenic to the cell membrane of the sperm cells and is associated to the fertility inturn increases the semen quality[30]. There is a close association between the concentration of the fatty acids in the semen and sperm motility after ejaculation into the female reproductive tract. The relation between the presence of lipids and their influence on semen malfunction or male infertility are not yet understood well. The only reason so far identified is the motility of the sperm, associated with the phospholipids. The efflux of the cholesterol from the plasma membrane of the sperm and the decrease in cholesterol-phospholipids ratio is found to be important in the quality of the semen, capacitating process[31]. An alteration in the efflux of cholesterol and cholesterol-phospholipid may show some problems in the sperm of the infertile men and the capacitating process[32]. The acrosomal response to the P4 (progesterone) is regulated by the unesterified cholesterol content of the sperm in the humans[33]. The composition, dynamics, and the amount of the lipids in the plasma membrane of the seminal fluid is useful in the determinants of the physical post ejaculatory functions such as capacitation, acrosomal exocytosis, and motility of the sperm inside the vagina[34]. Membrane lipid like docosahexaenoic acid (DHA) is found to decrease in the process of epidydimis. The lipid and their composition in the plasma membrane plays major role in microenvironments encountered by the sperm in the female genital tract[35]. When the semen is ejaculated it requires some metabolic efficiency in processing fats and sugars into energy and this energy, in turn, will help in enhancing the sperm motility. Cholesterol is secreted into the seminal plasma of the semen through the prostrate gland. It is very important in protecting the sperm membrane integrity by various environmental shocks through the chemicals or various pollutants present in the environment.

The ejaculated semen sample will travel inside the female genital tract by loosing the cholesterol on its plasma membrane for capacitation which takes several hours. Cholesterol also plays a major role in the fusion of the plasma membrane and the acrosome which plays a quite role in the male fertility. An alteration in the cholesterol and phospholipid ratio plays an important role in the human male fertility. Any change in this ratio will show the underlining problem in the capacitation and the acrosomal reaction rates. Changes in the lipid metabolism will lead to lower motility and low acrosomal rates. No correlation has been shown between triglycerides and the phospholipid concentrations in normal and abnormal semen samples and even some reports suggestt that there is no connection between the ratio in the capacitation and the fertilizing capacity in the human beings. The lipid concentration is found to be high in the azoospermic patients and also increase in the triglycerides led to decrease the semen quality, through affecting the spermatogenesis.

ZINC AND OTHER TRACE ELEMENTS LEVEL IN SEMEN AND MALE INFERTILITY

Human seminal plasma contains several trace elements including zinc and they play an important role in the normal function of the sperm. Zinc has antioxidative properties and plays an important role in scavenging reactive oxygen species. Zinc has an important role in the testis development, sperm motility, sperm count, and sperm physiological functions. Decrease in the zinc level in the semen results in hypogonadism, atropy of seminiferous tubules, inadequate semen volume while ejaculation, improper development of testes, and low motility of the sperm inside the female reproductive tract. The concentration of the zinc in the human seminal plasma is higher than other tissues.[36]

Zinc is acting as a cofactor for DNA-binding proteins that contain the zinc finger motif. Recent studies hypothesized that insufficient intake of Zn can impair antioxidant defence and may be an important risk factor in oxidant release, compromising the mechanism of DNA repair, and making the sperm cell highly susceptible to oxidative damage [37, 38]. Intracellular calcium (Ca) is essential for the sperm motility[39], metabolism and acrosomal reaction. Magnesium is necessary for proper ejaculation. Magnesium is found in higher concentrations in the prostrate and is released into the seminal fluid. Decrease in magnesium concentration led to decrease the semen quality. Potassium and sodium are also present in high concentrations in the seminal plasma that have the great role in acrosomal reactions.

MITOCHONDRIA AND MALE INFERTILITY

After ejaculation of the semen into the vagina of the female reproductive tract, it will travel through the mucus filled cervix for the fertilization. The sperm cell mitochondria supply the energy for the sperm to reach the ovum. It is estimated that roughly 72-75 mitochondria are present in a single spermatozoon for this purpose[40]. Defect in the mitochondria leads to decrease in motility and improper binding of the sperm to the oocyte. A study on mouse reported that, male could tolerate at least a threefold reduction in mtDNA copy number in their sperm without impairing the fertility [41]. Mitochondrial proteins are essential in the capacitation process for the proper management of the fertilization. Sperm capacitation includes a series of steps that including the acrosomal reaction and the tyrosine phosphorylation[42]. Mitochondrial DNA plays a major role in the spermatogenesis and their copy number plays a dual role in the sperm motility and the sperm count. Oxidative phosphorylation is found to be the determinant for the sperm motility. But, when the copy number is low the significance of the mitochondrial DNA is

unclear and uncertain related to sperm. The down-regulation of mitochondrial DNA during spermatogenesis results in a decrease in mtDNA copy number in sperm[43]. It is estimated that crictical thershold is needed for sperm function. Oligozoospermic and asthenozoospermic males are found to be with elevated levels of the mitochondrial DNA[44]. Tyrosine phosphorylation occurs in numerous mitochondrial proteins present in the sperm[45]. But all the mitochondrial proteins are not localized to the mitochondria. The enzymes of the Electron Transport Chain such as voltage-dependent anion channel, phospholipid hydroperoxide glutathione peroxidise (PHGPx), are localised in the mitochondrion (sperm head and tail) while enzymes like dihydrolipoamide dehydrogenase (DLD), pyruvate dehydrogenase α-2 and Glycerol-3-phosphate dehydrogenase 2 (GPD2) are extra-mitochondrial in localization [45]. The human spermatozoa are identified to undergo capacitation dependent tyrosine phosphorylation[46]. The mitochondrial protein namely Akinase anchoring protein (AAP), a major structural protein found in the fibrous sheath of sperm, has been identified as a capacitating dependant phosphorylated protein[47]. This protein plays a key role in the spermatogenesis and in capacitation process of the sperm in the female reproductive tract.

Chaperones

Chaperones play a major role in the sperm motility, and it also influences the capacitation and fertilization in humans[48]. Sperm-surface chaperones are tyrosine phosporylated and the induced conformational changes are identified to be required for sperm-zona pellucida interaction[49]. It has been identified that Reactive Oxygen Speceis (ROS) is associated with the mitochondria and excessive of ROS lead to the male idiopathic infertility. ROS has been shown essential in the pre-step of sperm to reach the ovum for the proper fertilitzation. Nicotinamide-adenine dinucleotide phosphate oxidase and Nicotinamide-adenine dinucleotide oxidase-dependent oxidoreductase (diaphorase) are integrated and found in the mitochondria (respiratory system) [50].

In the somatic cells, the copy number of the mitochondria is high and therefore will be able to manage the polymorphisms within limit. But, in the small cells like sperm, the copy number of mitochondria is very less, and when mutations will result in great impact[51]. Human oocyte consists of nearly 200000 mitochondria and this is correlated with the fertility[52]. Mitochondrion is first identified and considered to the factor for male infertility in the year of 1990[53]. Sperm will be survived by the glycolysis, and sperm motility is dependent on the oxidative metabolism[54]. There exists structural and functional relationship between the mitochondrial halotype and the respiratory chain of the sperm cell for the asthenozoospermia patients[55]. There is an association between the mitochondrial mutation, polymorphic variant in the CAG microsatellite of the mitochondria and the male infertility. In mitochondria, the frequency of the mutation/deletion is less than 1% and the probability increases slowly with increase in age[56]. Mitochondrial DNA is 100- 2000 times more susceptible for oxidative insult than the nuclear DNA because the lack of the histone proteins in the mitochondria [57].

CANCER AND MALE INFERTILITY

There is an increasing in the percentage of men survived with malignant disease and they are affected by infertility. The main reason for this is the improved prognosis for many childhood cancers and malignancies of young adulthood, like testicular germ cell cancer (TGCC) and lymphoma. 1 in 650 children develop cancer at the age of 15 years and 50-60% has been reported to be curable. It should be noted that there is a close relationship between the male fertility and the malignancies. In testicular cancer, there is a specific link between TGCC and the gonadal functions (fertility). Sperm output in men with TGCC is only 25-30% that of a control group of normal men or of patients with newly diagnosed lymphoma [58].

Y-CHROMOSOME MICRO-DELETIONS

The average Y chromosome microdeletions for infertile males were 8.2% and the majority of deletions (84.3%) were associated with azoospermia[59]. These deletions in fertile controls have been <1% and no deletion has been reported in men with normal semen analysis. The human Y chromosome consists of euchromatic and heterochromatic regions and the overall length of the Y chromosome is 60MB. Y chromosome consists of specific regions called pseudo-autosomal regions. These specific regions are homologous with the X chromosome and it is paired with X region during meiosis. All euchromatic regions fall into three classes, namely the ampliconic, X-degenerate, and X-transposed regions. These regions are said to be male specific Y (MSY) and it corresponds to the X region. This MSY is helpful in sex differentiation in human beings and accounts for 95% of the total Y chromosome. The MSY was previously known as NRY (non-recombination region of the human Y chromosome) because it is believed that no recombining event occurred between the X and Y chromosomes during meiosis in this region [60]. It is discovered that one subset of gene rearrangements on the Y chromosome, "micro-deletions", is a major cause of male infertility in some populations. However, controversies exist about different Y chromosome haplotypes. Six AZFs of the Y chromosome have been discovered including AZFa, AZFb, AZFc, and their combinations AZFbc, AZFabc, and partial AZFc called AZFc/gr/gr. Different deletions in AZF lead to different content spermatogenesis loss from teratozoospermia to infertility in different populations depending on their Y haplotypes. The causes of micro-deletions in human Y chromosome and their relationship with male infertility from the view of chromosome evolution has been reviewed recently[61].

Micro-deletions in the long arm of the Y chromosome lead to the spermatogenic and the ejaculatory failure. The paternal lineages of the Y chromosome are associated with the low sperm count and even with the motility. The regions underlining the heterochromatin in the Y chromosome deletions lead to the morphological abnormalities in the sperm cell. The shape of the sperm cells is abnormal in the acrosomal or neck regions resulting in the lack of binding capacity with the Oocyte. The capacitation process is affected when the heterochromatic region of the Y chromosome has been deleted. There must be a genetically functional azoospermia factor (AZF) on the long arm of the human Y chromosome. The genes required for spermatogenesis are located in the AZF region of the Y chromosome. The cases with azoosperimia are associated with the deletions in the six AZF regions. The oligospermia cases are not associated with the deletions inside the AZF region and it remains outside the deletion region[63].

The percentage of non-obstructive azoospermia with deletions in AZFa, AZFb, AZFc, AZFbc, and AZFabc are 4.9%, 15.8%, 59.6%, 13.6%, and <1%, respectively, and about of severe oligozoospermia cases occur with deletions outside the AZFs[64]. Primary spermatogenic failure (SgF, also termed as idiopathic infertility) accounts for more than one half of the cases[65].

CHROMOSOMAL ABNORMALITIES

Chromosomal abnormalities are found higher in infertile men. Those who are affected by these abnormalities are inversely related to the sperm count. Based on the largest published series it could be estimated that the overall incidence of a chromosomal factor in infertile males ranges between 2% and 8%, with a mean value of 5%[66]. The common type of genetic abnormality found in male infertile human population is Klinfelter's Syndrome and Y-chromosome long arm deletions is not associated with chromosomal alteration[67]. The frequency of the Klinefelter syndrome among the infertile men is very high and is found to be upto 8% in severe oligozoospermia and up to 10% in azoospermia. This is associated with the testicular failure and low sperm count in the ejaculation.

Robertsonian translocations are the predominant sex chromosomal abnormality associated with the infertility in the humans. It affects the structure and sperm count in the semen and various degrees of alterations in the sperm. This occurs when two acronetic chromosome fuse together to form a single chromosome. This results in the formation of abnormal dicentric chromosome that modulates the sperm count, morphology, and the motility after ejaculation. Reciprocal translocations are also identified as a

problem related male infertility in humans. There is mutual exchange of information between the two chromosomes. In couples experiencing repeated pregnancy losses, the incidence of chromosomal translocations is higher than the incidence present in newborn series[68]. The reciprocal translocation leads to the unbalanced sperm and their morphology. This results in severe oligozoospermia and azoosperimia in males. 47, XYY is found to be second frequent sex chromosome aneuploidy in humans. 46, XX chromosomal abnormality is observed mainly in azoospermic males, with frequency of 0.9%[69].

ANTI-SPERM ANTIBODIES (ASA)

Anti-Sperm Antibodies (ASA) are small proteins that are identified to impair the sperm fertilizing capacity. These types of proteins were commonly found in the infertile patients and in the men after vasectomy. ASA are thought to impair the fertility and the quality of the semen in men through inhibiting acrosomal reaction [70], invoking the complement cascade that will result in the sperm lysis [71, 72], or inhibition of sperm motility[73] and sperm penetration in the cervical mucus, capacitation[74]. Significant levels of anti-sperm antibodies are found to be present in the infertile conditions like azoospermia and oligozoospermia patients, and inturn this is associated with the genital tract problems in the males. Anti-sperm antibodies will act in the infertile pateints either directly or indirectly. It directly interacts with the sperm surface and suppresses the motility power of the sperm. In some cases cytokines are secreted that result in some adverse effects on the sperm motility and on the sperm and egg interactions and it is thought to be the indirect effect of ASA[75, 76].

ENVIRONMENT AND MALE INFERTILITY

After the World War II, there as been large number of the chemicals and their bi-products are released into the environment and these in turn affect the normal reproduction of the various species including the human beings. Continuous exposure to these endocrine disrupting chemicals results in permanent or irreversible damage to the reproductive system[77]. Recent studies showing that the male infertility develops upto 15-20 % in the industrialized countries just because of increase and continuous exposure of the endocrine disrupting chemicals alone and it is compared in the early 1960's and it was found to be 7-8%[78]. Some studies revealed that about 2-2.5% of the sperm count is decreased to the human males considered between1940-1990 (Carlsen *et al*., 1992). Clinical and epidemiological evidence supports that androgens protect more male than female subjects from the development of immune inflammatory diseases[79]. Recent studies show that there is a direct relation between the testicular cancer and decrease in the sperm count. The sperm count is directly proportional to the male reproduction. Epidemiological research shows the genuine connection of the sperm motility and the continuous exposure to the endocrine disrupting chemicals[80]. The occupational pesticide workers are affected by the male infertility[81]. The sperm DNA damage due to the environmental toxicants has been detected by using the sperm chromatin structure assay (SCSA) [82]. The reproductive toxicants are found to integrate with the nuclear double strandard DNA and break them which can be evaluated by using the flow cytometry[83]. Studies suggest that the multiple mechanisms were disrupting the male reproduction by continuous exposure to the heavy metals. These toxicants affect germ cell, spermatogenesis, acrosomal reaction, sperm motility, sperm-egg interaction and in all the post ejaculatory functions of the sperm in the female genital tract[84].

Heavy metals are identified to be the major cause for the male infertility. Lead is an important heavy metal and exposure to lead is directly proportional to the depression in the male reproduction. From the history it is shown to be the 'reproductive toxin' and plays the role both directly and indirectly in the sperm quality as well as in post- ejaculatory functions [85]. High levels of prenatal intake ie, exposure to lead > 40 µg/l for a year will affect the male semen quality[86]. Lead affects the pituitary gland membrane and its receptors that arrest the secretions of the gland which are found to be the important for the gonadal functions. Alterations in this gland will automatically affect the male sterility and semen quality. Cadmium is also found to be the highly toxic heavy metal that causes male infertility. It accumulates in the human body in the prenatal periods and affects the reproduction in the adult life. Both the cadmium and lead are thought to reduce the gonadotrophin binding which reduces the hormone secretions that thought to reduce the semen quality. Smokers accumulate more cadmium than non-smokers. Mercury is found to have some adverse effect on the human reproduction. Mercury is commonly used in the dental procedures and in the thermometers. The population in India is commonly exposed to the mercury through diet and in the dental amalgam[1]. According to the studies, mercury exposure causes various malfunctions in the reproduction including abortion, stillbirths, abstruction in the vas deferens and totally leading to the male infertility.

BPA (Bisphenol A) is identified to be the toxicant; that is released into the environment during the period of industrializatin. It is inhaled by the human population through the contaminated food and water.[87] BPA is found to be the most relevant endocrine disrupter in the world. It affects the spermatogenesis by altering the gene expression which is predient to the sperm formation. It also affects the steroidogenesis by altering the epigenetic effects, which affect the male reproduction up to four generation. Exposure to some pthalates will reverse the reproductive functions. It results in the irreversible changes in the reproductive tract. Exposure and inhalation of this chemical will bind to the androgen receptor and altering the signaling pathway and its affiliated pathways which alters the reproductive tract and its functions in male reproduction. Cancer is the third main type of HIV induced pathological manifestations[88]. Studies show that there is a gradual decline in the human male fertility, due to changes in the sperm count, sperm motility, and capacitation process in the industrialized countries[89]. These chemicals possess anti-oestrogenic, antigestagenic and antiandrogenic, through endogeneous effects by entering into the cell, binding to the receptor, alter the transcription and translational process of the cells and alter the normal functions of the androgen action.

Sperm DNA damage is found to occur in the infertile patients, and result in spermatogenesis. However contradictory results have been reported in literature that some fertile samples also consist of detectable damaged DNA[90]. The damaged DNA is found to be multi-factorial in nature, it cannot be clearly understood. But, this result in excess reactive oxygen species (ROS), protamine deficiency, and problems in the testis and post ejaculatory functions of the sperm in the reproductive tract[10].

Cigarette smoking will directly affect the every part included in the male reproductive system. Smokers are found to be with the reduced sperm count, low motility, and morphological defects in the sperm. Smoking will affect the fertilizing capacity of the egg [18]. It will create high negative mood variability in the youths. Studies suggest that smoking has strong impact on the male infertility by changing the semen parameters. Smoke creates the DNA damage and therefore might prevent the fertilization of the egg. The chromosomal damage was observed to be 1.15% in smokers and 0.8% in non-smokers. Significantly higher level or ratio of single strand/ double stranded DNA spermatozoa is found in the smokers rather than non-smokers. The percentage of the fragmented DNA in the spermatozoa in smokers is higher than the non-smokers. Sperm mutagenecity seems to be debated[91]. The parantal smoking will led to the spermatogenesis disturbance in reproductive adult age and childhood cancer in the prenatal age. There is no clear view about the alcohol consumption and the semen quality, but some suggests that it will directly interrupt with its own toxic effects and interrupt the spermatogenesis and the motility of the sperm, but does not have adverse effect on the epidemological functions related to fertility[64]. There is no report available on the combined effect of the alcohol consumption and smoking. Reactive oxygen species is produced through cigarette is due to the harmful substances or chemicals present including alkaloids, nitrosamines, nicotine, cotinine and hydroxycotinine. It involves in the modification of chromatin structure, damaging the double strands, attacking of the DNA integrity[89]. The sperm DNA is compact and it is structurally designed well which will keep the nuclear chromatin highly stable and even compact. This type of

nuclear chromatin when breaks, affects the normal fertilization[92]. The oxidants present in the cigarette increase the Reactive Oxygen Species (ROS) that might interact with the semen quality and decrease the integrity of the DNA. Increased level of leukocyte infiltration is found in the semen of the smokers, due to the integrity of DNA[91].

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