Review Article

The role of genotoxicity in infertility and cancer development

Kanad Dev Nayar[1], Sabina Sanan[1], Ankita Sethi[1], Sakshi Miglani[1], Gaurav Kant[1], Kapil Dev Nayar[1]

[1]Akanksha IVF Centre, Mata Chanan Devi Hospital, New Delhi, India

Abstract According to recent literature, genotoxicity is one of the important causes of infertility and the rising incidence of various cancers worldwide. DNA damage and its effect on DNA segregation are the mechanisms by which genotoxicity causes infertility or carcinogenesis. In this article, we discuss about genotoxicity and the various chemicals and environmental pollutants that cause genetic damage and their mechanisms of action. Hazardous effects of chemicals and pollutants can be evaluated by various genotoxicity and mutagenicity tests. These are important and initial steps in industrial development and the regulation of their effect on health. The detailed knowledge of the effects of genotoxins on fertility at the molecular, subcellular, cellular and tissue or organ system levels is crucial for a better understanding of occupational and environmental hazards and the need to find safe alternatives. In addition, new biomarkers using OMICS can render genotoxicity evaluation to decrease the infertility and cancer risk.

Keywords: Carcinogenesis, genotoxicity, infertility, mutagen

Address for correspondence: Kanad Dev Nayar, Akanksha IVF Centre, Room Number 711, Mata Chanan Devi Hospital, C-1 Block, Janakpuri, New Delhi 110058, India.
E-mail: kdnayar@usa.net
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INTRODUCTION

Genotoxicity is defined by geneticists as substances that destroy the cellular genetic material (DNA, RNA), therefore hampering the cell's integrity.[1,2] Genotoxins are the agents that possess genotoxicity properties.[3,4] The study of DNA and chromosomal damage in the cell due to potential agents or substances is called genetic toxicology.[5] People confuse genotoxicity with mutagenicity.[6] But all genotoxic substances do not possess mutagenic properties; nevertheless, all mutagens can cause genotoxicity, for example, ionizing radiation and chemical genotoxins.

Humans are affected mainly by following three kinds of agents:

(1) cancer-causing agents (carcinogens).
(2) mutation-causing agents (mutagens).
(3) birth defect-causing agents (teratogens).[6,7]

The genetic damage to somatic cells leads to cancer development, whereas genetic damage to germ cells results in heritable mutations. Mutational effects can lead to chromosomal abnormalities, like duplication, insertion, or deletion.[2,8] The cell has DNA repair mechanism, which prevents and regulates DNA mutations. Cell repairs itself by the following pathways[9-12]:

(a) Single/double-strand break repair.
(b) Mismatch repair.
(c) Direct repair.

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(d) Base excision repair.
(e) Nucleotide excision repair (NER) cell repair.

Ionizing radiation, chemical substances and environmental pollutants can have hazardous effects, which are identified by genotoxicity and mutagenicity tests. Genotoxicity testing is a crucial step in the evaluation of the safety of substances for regulatory approval. The management of real and functional genetic toxicology problems depends profoundly on the awareness of DNA damage mechanisms at the molecular, subcellular, cellular, tissue, organ system and organism levels. The knowledge of the impact of environment on the causation of infertility and cancer development is always intriguing for the reproductive medicine specialists.

There have been advances in the past decade to identify causes of infertility, with a recent surge in the number of patients with unexplained infertility. The incidence of infertility has increased many fold over the past decade. Similar is the rise in diseases arising from lifestyle changes. It can be attributed to carcinogens and other substances in the environment leading to subtle changes in germ cells that are not evident on various tests. Over the past few decades, the effects of man-made chemicals have shown to impact fertility in a rather subtle way, which is realized long after the agent enters the system.

Genotoxic agents exert their effect by interacting and sometimes integrating with the genetic material within the cells. The dependence of man on various man-made chemicals has led to the use of a large variety of chemicals and impurities. Sometimes these impurities interact with each other to synergise their effects. Such an effect is difficult to anticipate and scrutinize. One such example is the reaction between sulfonic acid and alcohol to form sulfonate esters.

Occupational exposure may be responsible for genotoxic and reprotoxic effects and unfortunately, the toxic agents are not well recognized and the effects are subtle. The toxic substances include certain solvents, pesticides, infectious agents, etc.

Environmental toxic chemicals are endocrine disrupting chemicals (EDC), which are not produced by the human body and that disrupt the functioning of our natural hormone system by acting on the hypothalamic–pituitary-gonadal axis, leading to adverse health effects.

These include pesticides, heavy metals, plastics and chemicals from industrial waste and electronic waste.

Table 1: How people are exposed to endocrine disrupting chemicals (EDCs)

Category/use	Examples EDCs
Pesticides	DDT, chlorpyrifos, atrazine, 2.4-D
Children's products	Lead, phthalates, cadmium
Food contact material	BPA, phthalates, phenol
Electronics, building materials	Brominated flame retardants
Personal care products, tubing	Phthalates
Antibacterials	Triclosan
Textiles and clothing	Perfluorochemicals

2,4D, 2,4-dichlorophenoxyacetic acid; BPA, bisphenol A; DDT, dichlorodiphenyltrichloroethane.

They are present everywhere in the air, soil, food, toys, plastic containers and wrappings, furniture, clothing, digital receipts, household dust, electronic waste, water sources and even in personal care products. Table 1 mentions the endocrine disrupting substances present in day-to-day used items.

Air pollutants include particulate matter (PM) 2.5 and 10 μm in diameter (PM2.5, PM10, respectively), poly-aromatic hydrocarbons, heavy metals, volatile organochlorines, CO_2 and SO_2 (sulphur dioxide).

Some of these pollutants are in cigarette smoke, major components of exhausts from cars, trucks and airplanes, industrial pollution and from the burning of fossil fuels (coal, oil and gas).

EXPOSURE TO CHEMICAL AGENTS AND ITS TOXIC EFFECT ON REPRODUCTION

Occupational exposures to agents like organic solvents, pesticides, metals and pharmacological agents have their impact on fertility. There are several fields of industries that are associated with adverse reproductive outcomes like dry cleaning, printing, dyeing, painting, electronics and petrochemical industry.

Glycol ethers that are frequently used in varnishing agents, inks, paints and cleaning agents were found to be associated with low sperm quality and thus the most toxic ethers were withdrawn in 1990s but the effects of newer agents of same category have not been thoroughly evaluated.[13]

Reproductive outcomes of women working in laundry and dry cleaning industry were studied in a retrospective study involving 7305 women. Exposure to perchloroethylene was deemed responsible for sub fertility and spontaneous abortions.[14]

Sulphur mustard (SM): A chemical warfare

The effect of SM on male fertility has recently been studied. Sulphur mustard works by various mechanisms, like DNA methylation and the generation of free radicals and reactive oxygen species, thereby causing oxidative stress and inflammatory responses. It exerts its effect on male fertility by decreasing spermatogenesis and impairing sperm quality. Though the exact mechanism of action at the molecular level is not known, its effects are also observed in the next generation as hormonal disturbance, testicular atrophy, reduced sperm count and impaired sperm quality and male infertility. Chronic effects may include congenital defects in children; thus, the chronic genotoxic and reprotoxic effects need to be considered and studied further.[15]

Use of pesticides

Organophosphorus pesticides are frequently used in the agricultural industry. Acephate is a toxic and unfortunately extensively used pesticide and insecticide used in agriculture and domestic purpose.[16] It causes alterations in sperm structure, integrity, viability and motility. Oraganophosphates are suspected of causing infertility by reducing the activity of acetylcholinesterase in the brain affecting pituitary gonadotrophin secretion. Acephate not only acts through this mechanism but also by acting as a delayed neurotoxic agent. This requires the need for strict regulation of pesticides, which is currently based mainly on animal models and the effects on humans are studied less.

The EARTH (Environment and Reproductive Health) study was published in 2018 to bring attention to the increasing rates of female infertility and the role of diet and pesticides in its causation. They also segregated the response to high-pesticide containing and low-pesticide containing foods and it was concluded that women who ate more than two servings of high-pesticide fruits or vegetables per day compared to one or less were 18% less likely to become pregnant and 26% less likely to have a live birth.[17]

Bisphenol A

Bisphenol A is incorporated in many products used that are for daily use that utilize polycarbonate plastics and epoxy resins. BPA has been demonstrated to be a reproductive toxicant and it was detected in reproductive tissues like ovarian follicular fluid, placenta and breast milk. It has been associated with decreased methylation in the TSP50 gene promoter. BPA was also seen to be associated with increased implantation failure.[18]

Exposure to diethyl stilbestrol (DES)

DES, which has estrogenic property, was widely used from the 1940s onwards in early pregnancy. A randomized control trial published in 1953 showed that DES was not effective in the prevention of miscarriage, and its clinical use was banned in 1971 when the hazardous effects were identified. Girls exposed to DES in utero were shown to have reproductive tract abnormalities, reduced fertility, increased spontaneous abortions and preterm births. There was also an increased risk of clear cell adenocarcinoma and breast cancer.[19]

Impact of air pollution on fertility

Air pollutants can be categorized in following four forms:

(1) Gaseous pollutants (sulphur dioxide, nitrate oxide, carbon monoxide).
(2) Organic compounds (organic solvents and dioxins).
(3) Heavy metals (lead, mercury, arsenic and cadmium).
(4) Particulate matter (PM).

Exposure to NO_2 and O_3 was shown to be associated with reduced live births. The effect of air pollutants on spermatogenesis has been studied with a significant association between PM10 and PM2.5 and sperm chromosomal abnormalities (disomy Y and disomy chromosome 21),[20] DNA fragmentation was seen with elevated levels of air pollution. Moreover, DNA methylation is also affected by air pollution.[21,22]

Heavy metals tend to accumulate in the food chain and cause damaging effects even at very low concentrations. Lead disrupts the ovarian steroidogenesis pathway, thereby affecting female fertility. Evidence suggests that exposure to lead, mercury, cadmium, or chromium could adversely affect male fertility.[23]

Radiation exposure and infertility

Radiation exposure to the ovaries, as a part of cancer treatment, causes disruption to the ovarian activity. Some chemotherapeutic drugs like cyclophosphamide, busulfan and melphalan have the potential to cause infertility. Radiation exposure to the pelvis, abdomen, spine and/or whole body can damage eggs and sperms.[24,25] This advocates the utilisation of oocyte and sperm freezing before scheduling the patient for chemoradiation. Ovarian transposition is a surgical option for fertility cryopreservation.[26] Not only the patients, but medical care givers like doctors, nurses and staff involved in procedures like endoscopic retrograde

cholangiopancreatography (ERCP) and endoscopic ultrasonography (EUS) are also at increased risk.[27] Radiation protective curtains and lead aprons are claimed to have some protective effect.

GENOTOXICITY IN CANCER DEVELOPMENT

Cancer is recognized as a genetic disease, and carcinogens are agents causing genetic damage or changes in gene expression.[28] In the context of cancer development, the terms genotoxin and mutagen cannot be used interchangeably. A mutagen is an agent that can cause DNA damage in such a way that it can be processed by the cell to cause mutation, whereas a genotoxin causes DNA damage that may or may not be processed into mutation; therefore, genotoxin is a rather general term. This is important because some assays like p32 post labelling, the comet assay detect DNA damage and few other assays like the *Salmonella typhimurium* reverse mutation test, the Hprt gene mutation assay in Chinese hamster ovary cells and transgenic mouse mutation assays detect the mutagenic potential.[29] Many in vivo and in vitro tests have been developed for genotoxicity to detect DNA damage and its potential biological effects. The mechanism of action of carcinogens is by binding covalently to DNA and forming DNA adducts. The DNA adducts can be detected and the potential carcinogens can be identified.

International Agency of Research on Cancer (IARC) has identified more than 100 carcinogens. The classification of carcinogens by IARC is as follows[30]:
Group 1 Carcinogenic to humans.
Group 2A Probably carcinogenic to humans.
Group 2B Possibly carcinogenic to humans.
Group 3 Not classifiable as to its carcinogenicity.
Group 4 Probably not carcinogenic to humans.

A comprehensive analysis showed that more than 90% of Group 1 IARC chemical carcinogens are genotoxic. It is estimated that 5% of cancers are caused by viruses, 5% by radiation and the remaining 90% by chemicals.[30] Up to 8% of all human cancers are related to occupational chemical exposure, hence the importance of chemical products in carcinogenesis [Table 2].

Carcinogens around us

Increased rates of cutaneous scrotal cancer are seen in chimney sweeps. Chronic helicobacter gastritis is associated with the development of gastric lymphomas and carcinomas, and thereby *Helicobacter pylori* is listed as a human carcinogen. Exposure to aniline dyes is related to bladder cancer. A definitive cause–effect relationship exists between tobacco and cancer. Benzo[a] pyrene, the most potent carcinogenic agent of tar, is present in the environment as a result of cigarette smoke and automobile exhaust fumes. Other carcinogens include acetaldehyde, arsenic, asbestos, benzene, cadmium, chromium, coal tar, dioxins, oestrogen, ethanol, ionising radiations, radon, vinyl chloride, etc.[30]

Carcinogenesis

The concepts of initiation, promotion and progression have been identified in the process of carcinogenesis.[31] Initiators interact with host DNA or macromolecules to induce specific changes. Promotion, through promotors,

Table 2: Environmental stressors linked to potential outcomes in females

Air pollution	Hypertensive disorders of pregnancy
	Polycystic ovarian syndrome
	Subfertility
	Miscarriage
	ART failure
Metals	Hypertensive disorders of pregnancy
	Uterine fibroids
	Subfertility
	Miscarriage
	Birth defects, immune system dysfunction
PFAS (perfluoroalkyl and polyfluoroalkyl sabstances)	Liver damage
	Breast cancer
	Obesity/lipid and insulin dysregulation
Persistent pesticides (DDT and DDE)	Lactation impairment
	Breast cancer risk in mother and female offspring
Persistent pollutants (dioxin/PCBs –polychlorinated biphenyl)	Breast cancer risk in mother by 50 years of age
	Impairement of immune system and endocrine system
EDCs (e.g., phthalates, phenols)	Uterine fibroids
	Neurological impairement of developing foetuses
	obesity, fertility and carcinogenesis

is a multistep carcinogenic process that enhances the development of neoplasms in the background of initiated cells. Promoters include agents such as drugs, plant products and hormones that do not interact directly with host cellular DNA (are not genotoxic) but somehow influence the expression of genetic information encoded in the cellular DNA. Progression is that part of the multistep neoplastic process associated with the development of an initiated cell into a biologically malignant cell population. Tumour cell heterogeneity is an important characteristic of tumour progression.[32]

Following are the popular assays for the assessment of genotoxic potential:

- Ames assay
- Chromosome aberration assay
- Mouse lymphoma TK
- Micronucleus (in vitro)
- Micronucleus (in vivo)
- 33p-post-labelling immunological assays
- Comet assay

We have come a long way in identifying the molecular basis of diseases around us and the role of certain agents in the causation of infertility, and the cancer is well established. The environmental exposure and domestic use of certain agents are inevitable in today's day and age, but with the available techniques, we can identify the population at risk, educate the masses about the hazardous risks, to keep the use of potential genotoxins to a minimum and more importantly by replacing them with a safe alternative.

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Conflicts of interest

There are no conflicts of interest.

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