Chapter 9

The Role of Human Semen as an Early and Reliable Tool of Environmental Impact Assessment on Human Health

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Additional information is available at the end of the chapter

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Abstract

Several studies have shown a dramatic reduction of semen quality in many industrialized countries and infertility is becoming a public health top priority, whose incidence is associated to late-onset adult diseases, especially cancer, shorter life expectancy and trans-generational effects. The male reproductive system is particularly sensitive to a broad variety of reproductive and developmental toxicants, including many environmental pollutants and recent studies suggest that human semen is an early and sensitive environmental and health marker. A set of semen biomarkers is described for reproductive health effects in relation to environmental exposure, where human semen seems to be an early and sensitive source of biomarkers than blood to monitor high environmental pressure on human health. Environmental health should consider reproductive health and development, from intrauterine life to childhood and puberty: these are both vulnerable targets and high-value protection goals, inasmuch as they represent the future of our societies. Hence, biomarkers of reproductive health should be exploited as early signals of environmental pressure and increased risk of adverse chronic health effects so that the use of "*human seminal model*" might be the main objective to be considered in the agenda of public prevention policies for early detection and innovative programs of health surveillance in environmental risk areas.

Keywords: semen quality, pollution, DNA sperm damage, environmental marker, health marker, endocrine disruptors, sperm telomere, redox status, epigenetic, aneuploidies, reproductive health, environmental health

1. Introduction

Since the early 1950s, in several demographic surveys a steady decline of birth rates in all European countries has been observed [1]. In particular semen quality was highly decreased in many industrialized countries [2–4] and in many European, Japanese and American young people poor semen quality was associated with subfertility or even infertility [5, 6]. The risk is that semen quality of a significant proportion of young men in developed countries will impair the fecundity potential causing on a short-term basis just a longer waiting time to pregnancy without to considerably family sizes of modern couples [7, 8], but on a middle-, long-term basis, strongly contributing (along with socio-economic factors) to the already observed European decrease in the birth rate. While there was a considerable variability in trends in sperm counts over the past 20 years, several recent studies have reported that 20–30% of young men today have sperm concentration below 40×10^6/ml, which is associated with reduced fecundity [9–11]. Among life-style changes that contribute to a reduced birth rate, affecting semen parameters and/or semen quality, there are: increased age at conception of both parents (although as a consequence of socio-economic factors), the increase in obesity, physical inactivity and the exposure to environmental and dietary environmental and chemical contaminants, including drugs. Exposure to man-made chemicals, in particular in the workplace, is recognized as major risk factors for male infertility in both epidemiological and experimental studies [12–16]. Individuals exposed for professional reasons to environmental contaminants show a reduction of concentration, motility, morphology and/or sperm DNA damage. In addition, toxicological studies in animal models are reporting DNA damages or epigenetic alterations within the germline: exposure to environmental xenobiotics during the fetal development and in early post-natal life, caused congenital malformations or reproductive tissue alterations or reduced fertility or signs of reproductive syndromes, such as the testicular dysgenesis syndrome, in particular when multiple *in utero* exposure to chemicals are tested. Furthermore, gene expression of genes mediating hormone (e.g. sex steroid hormones) actions is affected by epigenetic alterations even after some generation from the exposure to chemicals showing that the adverse effects can be eventually recorded only in next generations. A milestone in understanding the pathogenesis of testicular tumor has been the discovery of the fact that its onset in adults results from cancer cells *in situ*, which are transformed germ cells of the gonocyte type, which have failed to differentiate into spermatogonia during the fetal period [17, 18]. More strikingly, especially in industrialized countries, the reduction of semen quality and/or semen count present differences in areas within the same country or even in the same region supporting the idea that environmental factors, present in some areas but not in others, may be responsible for the decline in semen quality and sperm count [19–27]. Furthermore, different studies have reported that in high environmental pressure areas there is both an increase of infertility, urogenital malformation and chronic disease (cancer, diabetes, etc.) [28–32]. These epidemiological data are important to understand the shared biological mechanisms mediated by contaminants. In fact, infertility is becoming a public health top priority because, in addition to psychological distress and high economic costs, there are more and more evidences of diseases associated with poor semen quality [33] including cross-generational effects [34, 35], shorter life expectancy [36], testicular cancer [37–41] and overall other types of cancer [42, 43]. However, the first systematic study regarding environmental

pollution and human reproduction has been conducted in the Czech Republic within the research program "Teplice" [44]. In particular, with regard to the impact on the semen quality, it has been proved a positive correlation between the increased concentration of polycyclic aromatic hydrocarbons (PAHs) in atmospheric pollution as well as of airborne particulate matter (PM), with an aerodynamic diameter smaller than 10 μm (PM10), mainly in winter, and an increase in fragmentation of sperm chromatin, DNA-PAHs adducts, abnormal sperm shapes and in the rate of sperm aneuploidies [45]. Other human biomonitoring studies have documented widespread human exposure to chemicals [46, 47] and actually the European Commission has financed the Human Biomonitoring Initiative (HBM) (https://ec.europa.eu/research/conferences/2016/hbm4eu/index.cfm) to promote the generation of current HBM data throughout Europe as well as the development of new biomarkers of exposure for chemicals. However, knowing the environmental pollutant concentrations in the environment and their seasonal variability, is essential to consider each source of exposure related to individual lifestyle (including living places, dietary habits, use of cosmetics, plastic bottles, personal computers, wireless internet and much more), and the plausibility of the cause-to-effect relationship among the real life mixture of dietary and environmental contaminants, the tissue/biological fluid levels at which chemicals (or their metabolites) are present in the human body and human disorders and/or pathologies. From this stage onwards, how much chemical values are measurable in fluids or tissues (biomarkers of exposure) and to which extent they are associated with a biological effect (biomarkers of effect) depending on specific, individual response (markers of genetic susceptibility, polymorphisms, etc.) will define a complete risk assessment founded on a reliable Adverse Outcome Pathway (AOP) in which each sequential step is linked to the other. Furthermore, in order to adopt an effective primary prevention strategy, it will be important to identify not only the source and extent of the exposure but also the tissue or organ most sensitive to such exposure and, simultaneously, the biological tool more sensitive and reliable to predict future alterations and to detect the earliest clinical risk indices. Dietary and environmental chemicals exposure may influence human endocrine and metabolic homeostasis and, especially, the reproductive system. Among the reproductive system targets, the male reproductive system could be considered a general health check detector since it is particularly and uniquely sensitive to a broad variety of reproductive and developmental toxicants, including many environmental pollutants, throughout the lifespan. Indeed, spermatogenesis and secretory fluids of the differentiated accessory glands of the male reproductive system are continuously renovated starting from newly differentiating staminal cells, thus making them a feasible target to study both short- and long-term effects of chemical exposure. The male germline accumulates mutations faster than the female one [48, 49]. For instance, it is thought that sperm cells are more susceptible than eggs to the effects of oxidative damage [50] and recent studies have demonstrated the association between semen quality and state of health, correlating the semen quality with either chronic degenerative diseases, comorbidities and even mortality [51–53]. Thus, spermatogenesis is a cycle extremely complex and vulnerable to endogenous and exogenous stress and that human semen can become an important "environmental and health marker". In this way, the qualitative assessment of human semen might be envisaged as a potential focus for future development of public prevention policies. Therefore, the use of reproductive biomarkers as environmental health risks was proposed as a promising/ innovative strategy for the early detection and prevention of environmental health [54].

2. Main

With the release of the Silent Spring in 1962 [55] the issues related to chemical pollution have begun to become a topic of political and scientific debate by laying the basis of environmental chemistry and ecotoxicology as we know them. Environmental toxicology concerns the way in which toxic substances reach the organism and affect human health. At present many chemicals [56] have been detected in tissues and biological fluids of human body (**Figures 1** and **2**).

2.1. Organic pollutants and reproduction

Persistent organic pollutants (POPs) are very durable toxic chemicals which include polychlorinated dibenzodioxins polychlorinated dibenzofurans polychlorinated biphenyls (PCBs), chlorinated organic pesticides, PAHs, hexachlorobenzene and many other substances that we find in daily life such as polybrominated diphenyl ethers (PBDEs), perfluorooctane sulfonate, Perfluorottanoic acid ammonium salt, brominated flame retardants, food additives such as bisphenols and phthalates (plasticizers) and parabens (preservatives), according to recent experimental acquisitions, are known as endocrine disruptors (Endocrine Disrupting Chemicals). They are able to interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones of the body responsible for maintaining the homeostasis and the setting of endocrine reproductive processes [57–59]. They can also alter the cellular oxido-reductive homeostasis (redox status), resulting in a condition known as biochemical oxidative stress [60–62] a genotoxic action featuring a genetic and epigenetic damage transmissible through the germ line to the offspring (transgenerational effect). This last aspect is definitely very disturbing to future generations' public health and justifies the growing interest of the scientific community in the study of the reproductive system in recent years [63–65]. These substances, very stable and soluble in fats, are found in semen that has a considerable lipid amount [66, 67].

2.2. Inorganic pollutants and reproduction

Metals toxicity depends on several factors, including their ability to bonds to reactive groups of enzymes and proteins (e.g. thiol groups) thus altering their structure and/or function. They may also interfere with the bioaccumulation of essential metals (e.g. iron, calcium and zinc) thus negatively affect those physiological mechanisms depending upon their bioavailability. Heavy metals accumulation in living organisms, in particular lead, cadmium, arsenic, mercury, depend upon the exposure to contaminated environment and may trigger acute and chronic degenerative diseases: In particular, genotoxic elements (Arsenic, Cadmium and Nickel) may damage the DNA structure either directly (through the production of oxygen radicals) or indirectly (via the alteration of enzymes responsible for DNA repair) and they may interfere in the activities of regulators of proliferation, apoptosis, differentiation and cell transformation [68–71]. Metals also include "trace metals," such as zinc, copper, iron, manganese, present in humans under physiological conditions, which are toxic at high concentrations. The risk assessment of the exposure to metals is achieved through human biomonitoring studies and their quantification in human biological fluids such as blood, serum and urine,

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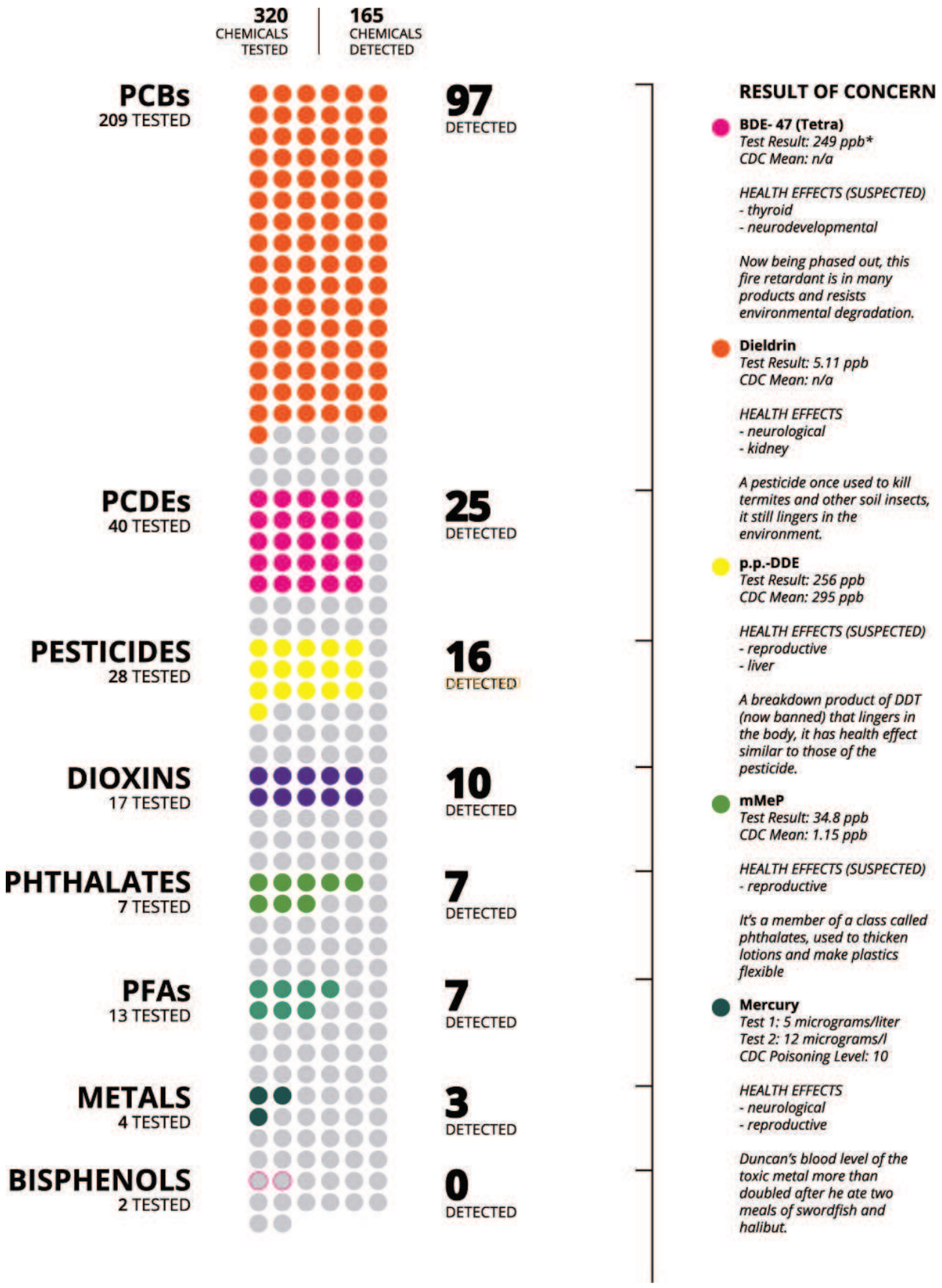

Figure 1. List of some of the chemicals tested and detected.

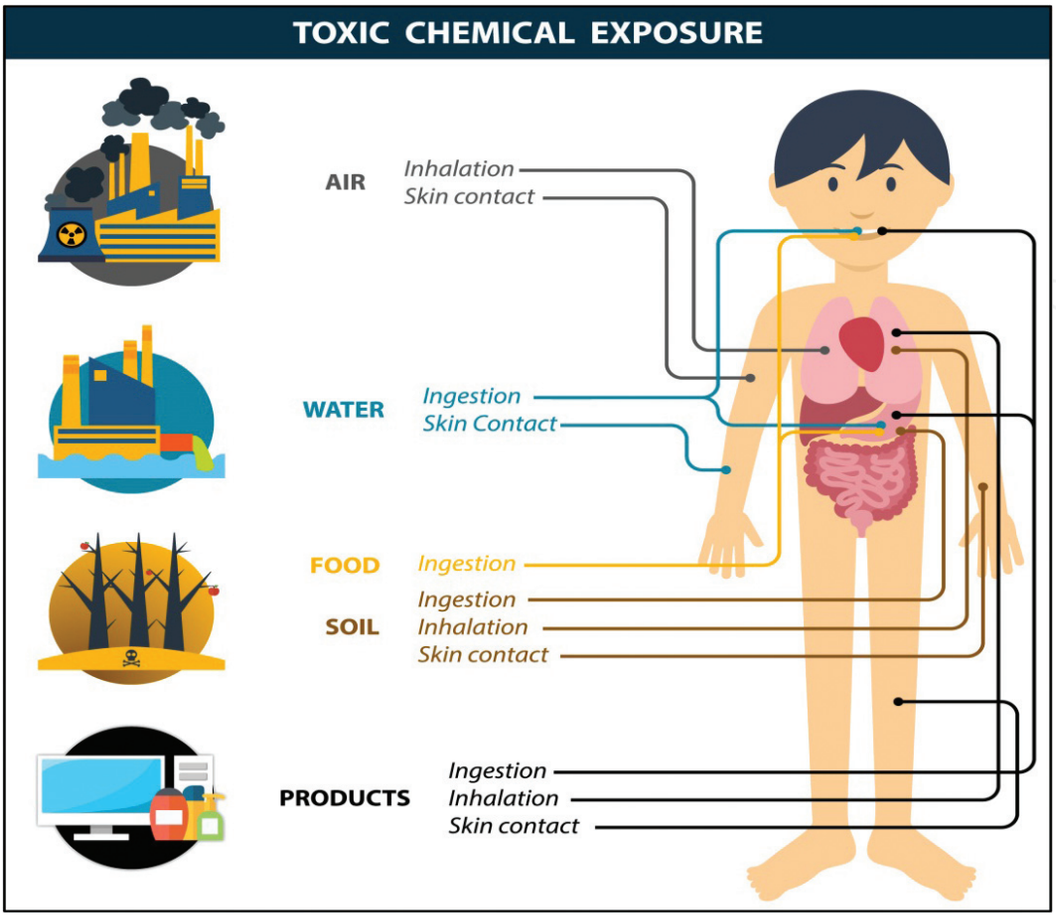

Figure 2. The entry routes of the contaminants into the body.

being an indispensable tool to evaluate the possible influence of environmental determinants on human health. The level of metals in human fluids reflects the amount entering the body *via* all exposure routes (ingestion, inhalation and dermal absorption). Moving into the bloodstream, they are compartmentalized in organs or tissues, where they carry out their harmful effects according to the concentration and to their inherent toxicity. Even though several papers have covered the report of qualitative parameters of the seminal fluid with the occupational exposure to metals [72, 73], environmental impact studies in urban areas are still unsatisfactory [74]. An Italian study [68] has compared, through statistical methods, the sperm counts with the geochemistry distribution of heavy metals in soils of the metropolitan area of Naples, observing a strong correlation in the case of lead, whereas a lesser correlation has been found in the case of mercury and zinc. In addition, data have been reported regarding the effects of changes in concentration of zinc, magnesium and calcium on semen quality parameters and infertility [75].

2.3. Mechanisms involved in male reproductive dysfunction

2.3.1. Oxidative stress

Oxidative stress plays an important role in the etiology of male infertility by impairing negatively the quality and the function of the sperm [76] although the relationship between the

bioaccumulation of environmental pollutants and the alteration of the seminal redox status has not been elucidated yet, and neither the possible mechanism of action. The imbalance of antioxidant defenses and detoxification processes provides a logical explanation to the onset of diseases caused by oxidative stress in men [77] and increases the organism susceptibility to pollutants toxicity [78]. After all, the balance between oxidation and anti-oxidation is critically important in maintaining healthy any biological system.

The fact remains however, that pro-oxidant activity of PM [79] PAHs [60] on human health has been demonstrated in clinical data, whereas the harmful effects caused by toxic heavy metals or pesticides organophosphates [80] have been proved in animal studies. Reactive oxygen species (ROS), at low physiological levels, play an important role in sperm maturation and function [81]. On the contrary, excessive amounts of ROS produced by leukocytes and immature spermatozoa can damage mature sperm and DNA integrity [82–84]. The mechanism of DNA damage by ROS is mainly due to the high susceptibility of spermatozoa to ROS for their high content of polyunsaturated fatty acids, major components of cellular and intracellular membranes (**Figure 3**). An increase in oxidative stress has been found in 80% of infertile men clinically tested, and it seems that exposure to environmental toxicants contributes to this increment [60, 78–80]. In addition, a positive correlation between ROS and sperm DNA fragmentation has been reported in studies [85]. However, the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), plays a key role in the modulation of antioxidant response, which basically modulates both synthesis and the recycling of the main cellular antioxidant, that is the reduced glutathione. The reduced activity of glutathione reductase, has been associated with oxidative stress-related diseases [77], just like an increased susceptibility to adverse effects induced by pollutants [78] has also been associated with increased expression of p53 [86] (**Figure 4**).

Notably, detoxifying/antioxidant defenses can be modulated by diet. However, it is known that improper eating habits (*i.e.*, increased intake of carbohydrates, high protein and total fat) have been linked to poor sperm quality [87], although the protective effects of a proper diet towards pro-oxidants effects caused by bioaccumulation of environmental pollutants have not yet been demonstrated. In summary, although supplementation with antioxidants may improve pregnancy and birth rates for infertile couples [88] the efficacy of dietary supplements in improving the quality of male sperm is still controversial [89] and the link among bioaccumulation of environmental pollutants, diet and semen quality remains to be demonstrated.

2.3.2. Genetic alterations

Endocrine Disrupting Chemicals affect spermatogenesis both through alterations in the hypothalamic–pituitary axis, and direct damage to spermatozoa [90–92]. In recent decades, several studies have shown disorders of spermatogenesis due to genetic causes (15–30% of infertile males) [93, 94] and chromosomal aberrations, either numerical or structural, can profoundly affect fertility. It is estimated that the frequency of chromosomal aberrations in the general population is about 0.6% [95], and 2–14% in infertility male [96]. In particular, chromosomal aberrations increase with the increasing severity of infertility. Moreover, some genetic polymorphisms involved in the metabolism and detoxification activities as well as in DNA repair capacity influence individual susceptibility to environmental exposure leading to changes in

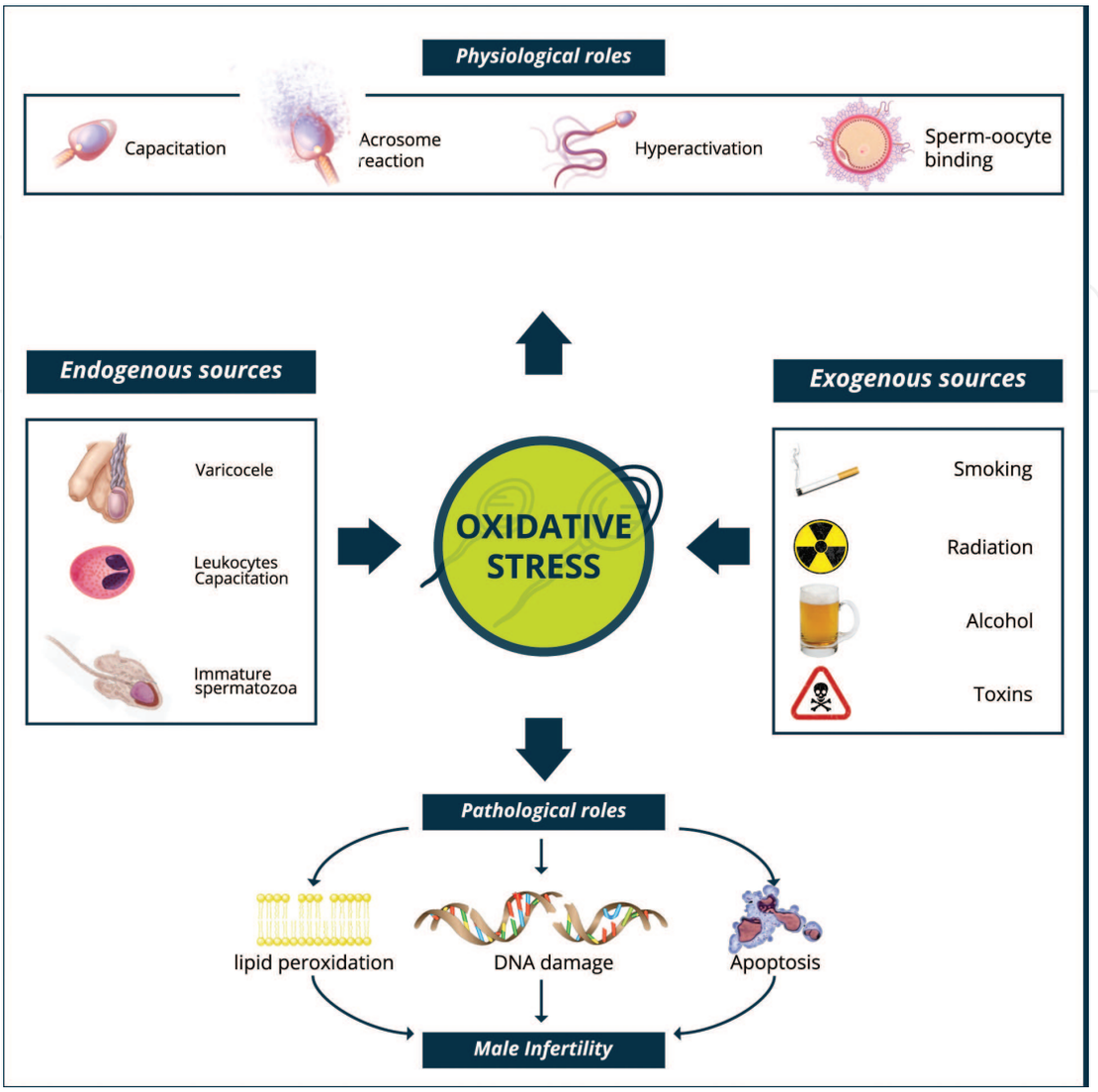

Figure 3. The positive and negative effects on spermatogenesis and steroidogenesis of controlled or uncontrolled oxidative stress.

sperm quality [60]. The main alteration responsible for male infertility is represented by DNA and chromatin alterations, highly sensitive to exogenous contaminants [97]. Some studies have suggested that environmental toxins affect sperm DNA's integrity and it has been observed that exposure to air pollutants such as PM, is capable of producing disomy of sexual chromosome in nemasperm DNA [98]. In fact, most chromosomal abnormalities are lethal and so they either manifest as a sperm's inability or as miscarriage [99].

In particular, aneuploidy defined as structural and numerical aberrations of chromosomes [100], is an informative effect biomarker, for male reproductive toxicants and a hallmark of cancer [101–104]. There are some substances known that induce sperm aneuploidy and can be carcinogenic [105, 106] and for this reason sperm aneuploidy is associated with both increased risk of cancer and reproductive toxicity. Fortunately, sperm aneuploidy assessment has become very easy and this opens up to a growing use of health risk assessment from chemical hazard [107] so that, it could be integrated with current aneuploidy and chromosome imbalance

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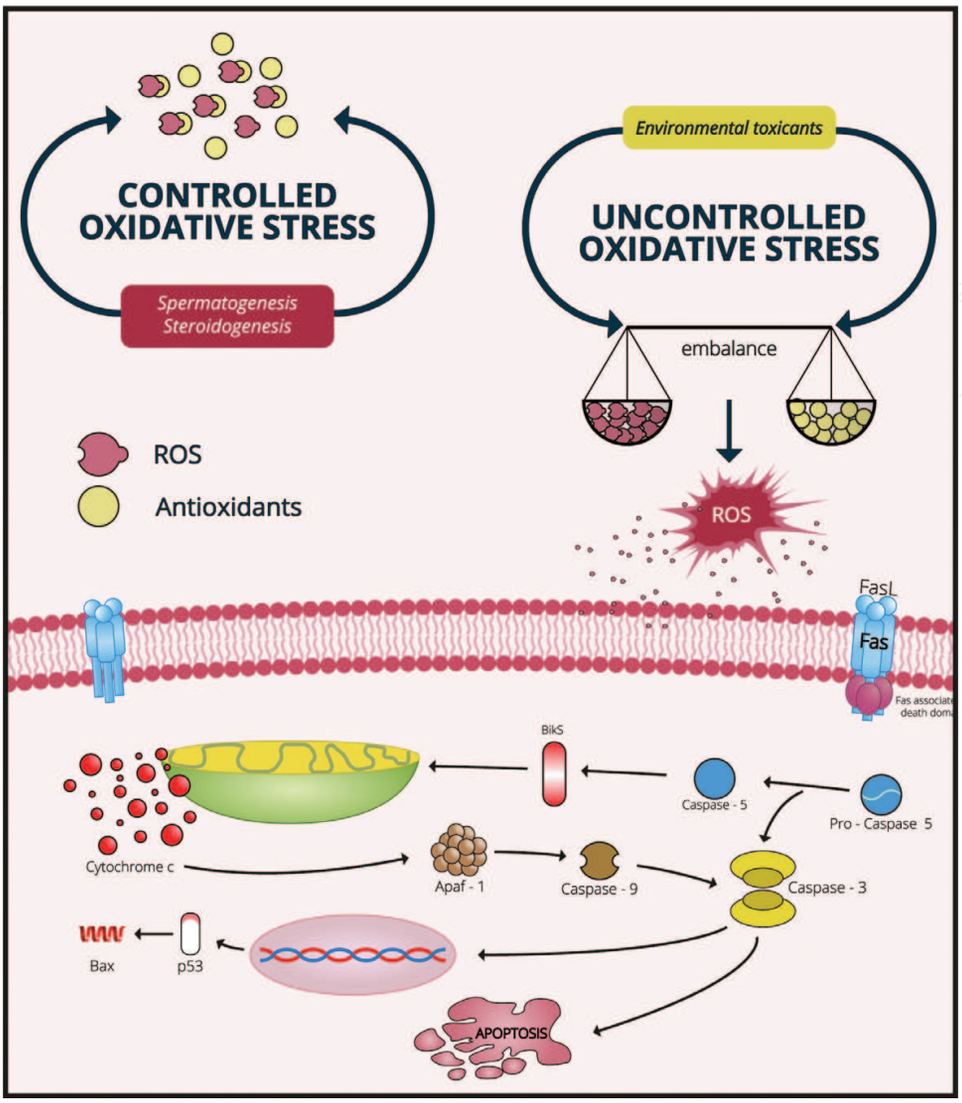

Figure 4. Exogenous and endogenous factors inducing oxidative stress: Pathological and physiological roles on sperm function.

assessments in place for somatic cells [108]. In conclusion, sperm aneuploidy evaluation is informative well beyond the standard sperm parameters (number, motility, morphology) useful for comprehensive evaluation of carcinogenicity and reproductive toxicity.

With regard to sperm DNA's integrity, it is a great indicator of male fertility, since men with normal sperm parameters may also have a high degree of DNA fragmentation, leading cause of undiagnosed /inexplicable infertility. In fact, the damage to sperm DNA contributes not only to infertility, but also on the frequency of miscarriages and birth defects in the offspring. The data supporting this are primarily derived from animal toxicology studies, which unequivocally demonstrate that the genetic integrity of the male germ line play an important role in determining the normal embryonic development [109].

The results of studies by toxicologists using several compounds in increasing doses prove adverse effects on the development of the embryo, on animal behavior, postnatal growth, longevity of progeny, as well as increased susceptibility to cancer. These toxicology animal-data

support the hypothesis that toxic substances can act on the male germ line by interfering with the development of human pregnancies and the health of the unborn. To support this, there are associations between paternal smoking, oxidative DNA damage of sperm and the incidence of cancer in children. The origins of sperm DNA damage are not yet clearly defined, but in light of recent discoveries, six main mechanisms are hypothesized: (1) apoptosis during the process of spermatogenesis; (2) breakage of DNA strands created from the sperm chromatin remodeling during the process of spermatogenesis; (3) post-testicular DNA fragmentation induced mainly by oxygen radicals, including nitric oxide and hydroxyl radicals, during the transport of spermatozoa through the seminiferous tubules and epididymis; (4) DNA fragmentation induced by endogenous caspase and endonuclease; (5) DNA damage induced by radiotherapy and chemotherapy; (6) DNA damage induced by environmental toxins [110]. The damage in testicular sperm DNA is statistically lower than what is found in ejaculated sperm [111]. Sperm nuclear DNA fragmentation is the last phase of apoptosis, a highly controlled programmed cell death program that plays a key role in different biological processes such as embryonic development and maintenance of homeostasis. High cell proliferation rate and cell differentiation processes occur during maturation from stem cell to haploid mature sperm. Apoptosis is needed to avoid the excess of cell proliferation and it seems to have a role in germ cells differentiation. This process might also be induced by several environmental stimuli or damages [112]. In case of DNA damage within the male germ line, the adverse outcome(s) will depend either from the type of damage or from the genomic region affected or from the timing of the damage itself and, as an overall consequence, from the ability of the embryo repair system to properly counteract any damage earlier than the first mitotic division will occur. In any case, the embryo could not always effectively repair damages carried on from male germ as it occurs in genetic dominant diseases, such as achondroplasia [113]. Furthermore, healthy children born with assisted reproduction from DNA-damaged sperm [114] may possess genetic or epigenetic alterations generating a phenotypic change in the next generation(s) due to double recessive gene expression or in the birth of a male upon chromosome X mutations. Finally, it is also possible that DNA-damaged sperm can cause offspring defects not recognized at birth. The recent discovery that DNA damage in sperm of males due to aging is associated with the onset of epilepsy, schizophrenia, autism, and bipolar illness [115, 116].

Strikingly, an increased risk of sperm DNA fragmentation was associated to high levels of air pollution, in fact seems that the classical sperm parameters -motility, concentration, morphology- do not change related to high smog levels, while sperm DNA fragmentation appeared to be much more sensible [98]. In this direction, also in Campania Region (Southern Italy), preliminary data of EcoFoodFertility initiative [54], indicated an increased sperm DNA damage associated to environmental pressure, measured with two techniques. In fact, healthy, no-smoking, no-drinker, no professionally exposed to environmental stresses males (n = 175, mean age 30 $\pm$ 4) were enrolled in areas of High or Low environmental impact. According to their stable residence in "Land of Fires", a wide area between the towns of Naples and Caserta (High Environmental Impact Area—HIP; n = 70) or in Alto-Medio Sele in Salerno province (Low Environmental Impact Area – LIP; n = 105), data of the enrolled men were compared by their DNA Fragmentation Index (DFI). DFI was evaluated by using the sperm DNA fragmentation Kit (Halosperm®, Halotech DNA SL). Furthermore, the spermatic p53 levels were also assessed by using the DuoSet® ELISA (R&D) [117]. The results obtained so far support the

effectiveness of the considered markers in the quantification of DNA damages as well as the relationship between the extent of the observed sperm DNA damage and the environmental characteristics of the area of residence (HIP versus LIP areas). In conclusion, these data showed sperm DNA damage measured as DFI by SCD and p53 overexpression to be an early and sensitive marker of environmental pollution [118].

In recent years, an increasing interest has been directed on other biomarkers of DNA integrity in male germinal cells: telomere. Telomeres are noncoding double-stranded DNA repeats (in humans, TTAGGG sequences extended 10–15 kbIn dividing cells, the synthesis of new telomeric DNA repeats requires the activity of telomerase, a protein complex composed of the TERT enzyme and of the telomere-associated proteins, able to recognize the 150–200 nt 3′-single stranded (G-strand) overhang. During aging in most adult somatic cells, a progressive telomere shortening occurs and, in turn, telomerase activity decrease or completely disappear. In contrast to such adult somatic cells, germ cells maintain high telomerase activity, long telomeres and high proliferative potential [119, 120]. In particular, the sperm telomere length (STL) seems to be of fundament importance for fertilization and early embryo development [121]. To date, the relationship between telomere function and aspects of semen quality is an area of great attention. Indeed, it has been reported that sperm TL is lower in oligozoospermic than in normozoospermic men [122]. Furthermore, spermatozoa from elderly males have significantly longer telomeres than those from younger males, but the biological implications of this paradoxical effect are unknown [123]. Additionally, telomere dysfunction is a relevant mechanism driving cancers in humans [124]. Indeed, critical telomere attrition results in chromosomal aberration which in the absence of normal cellular DNA repair and apoptosis can lead to genetic instability. On the other hand, long telomeres may permit cells to escape growth arrest and increase the chance of acquiring mutations, especially in the presence of an external exposure, i.e. smoking and sun exposure. In fact, longer telomeres have been associated with some types of cancers, especially melanoma and lung cancer [125]. Recently, a Mendelian randomization study reported that longer telomeres were associated with increased risk of several cancers but reduced risk of some non-neoplastic diseases [126].

Interestingly, accumulating evidence indicates that leukocyte telomeric DNA may be one important target of environmental [127–132]. Accordingly, a very recent study has shown a possible association between high environmental pressure in polluted area and the STL [133]. In particular, a preliminary study was carried out evaluate the influence of environmental exposure to the telomere length (TL) of leukocytes (LTL) and of STL. This pilot study was conducted on young healthy men living in HEI or in LEI area and the data obtained showed that STL was significantly greater in subjects while no significant difference was observed between LTL and HEI in the LEI group and no correlation between STL and sperm parameters was found [134]. These findings support the view that STL is a more sensible marker than LTL to environmental pollution and it is a further evidence that the genetic structure of spermatozoa is particularly sensitive to environmental insults.

2.3.3. Epigenetic alterations

In recent years, interest has grown on new acquisitions that regulate gene expression and epigenetic mechanisms. In fact, if the interaction between genes and environment in

determining human phenotypes has been known for many years, the real innovation provided by epigenetic studies concerns specific gene expression changes without any change in their sequence. Therefore, as genetic variants make the organism vulnerable to certain environmental insults, epigenetic alterations induced by the environment may have the same effect and especially could be transmitted to the offspring. Thus, birth defects, greater susceptibility to diseases in adulthood, may be the result of a gene/environment interaction that occurred in one of the parents, not the subject itself. Studying the sperm epigenome represents a new frontier in the field of human reproduction, and numerous studies have shown the importance of epigenetic mechanisms as potential biomarkers in hazard identification and risk assessment attributable to environmental exposures. Epigenetic mechanisms responsible for these alterations are represented by DNA methylation, histone modifications and noncoding microRNAs [135]. The association between sperm DNA methylation and idiopathic male infertility is already documented with studies [136–139]. Other studies have shown that DNA hypermethylation of gene promoters (like *MTHFR*, *PAX8*, *NTF3*, *SFN* and others) plays a crucial role in determining male infertility. On the contrary, hypomethylation of other genes, including the check zone *IGF2*/*H19 1* (ICR1), is found in patients with lower sperm concentration and motility compared to controls with normal sperm kinetics [140–145]. Nuclear condensation in the spermatozoon represents the most delicate and sensitive stress related event, inducing genetic and epigenetic alterations. During this phase, in fact, about 85% of histones (rich in lysine) bound to DNA, are replaced with proteins of transition and arginine-rich proteins: the protamine [146, 147]. In contrast to histones, which form a ring-like association with DNA (nucleosomes), protamines are linked to DNA helix grooves, wrapping themselves tightly around the DNA strands (about 50 kb of DNA and protamines), to form tight loops highly organized. The spermatozoon's nuclear condensation is obtained by the intramolecular disulfide bonds between cysteine-rich protamines resulting in the reduction of about 10% of the size of the nucleus. The bromodomain testis-specific protein is the key factor mediating the chromatin compaction promoting nuclear remodeling ensuring the transition between a histone chromatin organization, which is somatic, and the protamine one typical of the mature sperm. The sperm genome is protected from physiological and environmental stresses by this peculiar nuclear compaction, but also from genetic mutations and chromosomal abnormalities that can interfere with the mechanisms of spermatogenesis [148]. These alterations may result in an abnormal chromatin structure, a feature incompatible with fertility. The resulting genomic material defects that are found in mature sperm may be packing defects (defective replacements of histones-protamines), defects in the maturation of the nucleus, DNA fragmentation defects (that is, single or double strand breaks), sperm DNA integrity defects or chromosomal aneuploidy and changes in gene expression (epigenetic modifications). In fact, an increasing amount of data now supports the hypothesis that in the mature spermatozoon of mammals the DNA is actually not homogeneously rich of protamine [149]. Defects in the action of protamine affect the transcription of genes. For example, in mice, the deregulation of the protamine action process results in premature chromatin condensation, interruption of the transcription, and failure of spermatogenesis [150]. The human sperm's nucleus preserves 10–15% of its original histone content, which is distributed heterogeneously in the genome [142]. An analysis of the entire genome of seven infertile patients has clearly demonstrated that five out of seven infertile men had a random process of protamine action in comparison with normal fertile

men where the preservation of histone quota was programmatic [143]. Specific errors in the epigenetic control, damaging male fertility and embryonic development, can occur at each stage of spermatogenesis [144]. At the mitotic level, epigenetic alterations can affect the expression of specific genes involved in the early stages of spermatogenesis, decreasing the overall differentiation process. At the meiotic level, epigenetic alterations can trigger double strand breaks or chromosomal nondisjunction and, during the spermiogenesis, protamine replacement errors may induce, in turn, epigenetic alterations due to defects in the above described histone-protamine transition [144]. Taken together, these facts suggest that the different characteristics of male infertility, including alterations in sperm count or morphology, DNA fragmentation chromosomal, aneuploidy, alterations in the chromatin density, could all be related to epigenetic mechanisms that occur at different stages of spermatogenesis. Great attention is then lately directed to the role of microRNA (miRNA) and so to the posttranscriptional regulation. Increasing evidence has shown that miRNAs play a critical role in mitosis and meiosis as well as in spermatogenesis [151–153]. MiRNAs are expressed specifically during spermatogenesis and participate in the control of every phase of the male germ cell differentiation. Genetically altered rat models have shown the importance of miRNA's pathway for the development of a normal spermatogenesis and functional studies have been conducted to establish the roles of specific miRNAs [154]. Finally, clinical studies have shown that spermatozoa from patients with sperm alterations present an altered miRNA profile [155, 156]. Hence, a strong emphasis on the crucial role of miRNA in spermatogenesis: indeed, the miRNA profile expression can be also seen as a new reliable and non-invasive diagnostic biomarker for the study of male fertility. Recently, a pool of sperm samples obtained from fertile and infertile men was examined and shown that alterations in miRNA profiles both in azoospermia and asthenozoospermia conditions can be found [157]. In particular, the level of seven miRNAs was significantly lower in patients with azoospermia and higher in the asthenozoospermia, compared to fertile subjects considered as case–control, leading to the hypothesis that these seven miRNAs may have confirmatory molecular diagnostic value for male infertility. Furthermore, miR-I9B and let-7 bis expression pattern was analyzed in patients affected by idiopathic infertility, azoospermia or non-obstructive oligozoospermia: it was showed that both miRNAs were expressed at higher levels in infertile patients compared to fertile individuals [158]. Therefore, it was concluded that miR-I9B and let-7 bis may be considered good diagnostic molecular markers for non-obstructive azoospermia cases with primary infertility or oligozoospermia. Similarly, it was recently identified miR-155 serum level as a potential biomarker of male fertility [159]. Interestingly, the miR-155 serum has been associated with male subfertility regardless of the systemic inflammation grade or androgenic alteration. Ultimately, the damage assessment to the spermiogenesis caused by pollution, of genotoxic, genetic and epigenetic type, are a major concern not only for the susceptibility to chronic diseases in adulthood, but also and especially for the vulnerability to diseases of future generations (transgenerational effects). (**Figures 5** and **6**).

2.4. The semen as an early marker of environmental exposure (environmental sentinel)

Semen qualitative and quantitative changes observed by several epidemiological studies, by Carlsen and latest ones [2–5], show how these changes are induced by individual lifestyle and

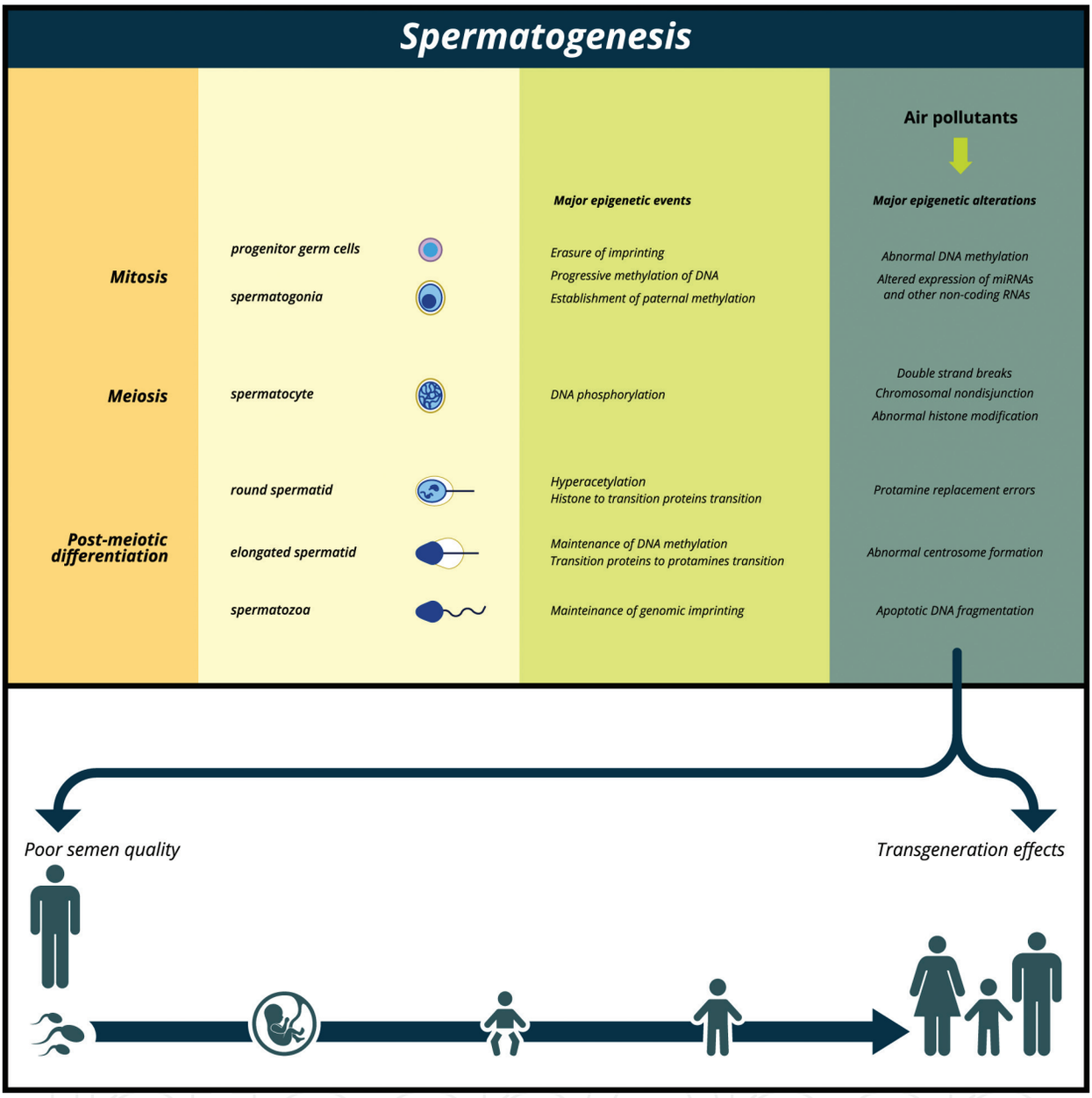

Figure 5. Epigenetic alterations by environmental factors affects sperm quality and when fertilization occurs, transgenerational epigenetic effects may compromise embryo development, favoring congenital diseases at birth and diseases in adulthood.

from the environment. Epidemiological studies on individuals exposed for professional reasons or living in contaminated areas and nearby settlements, demonstrate significant alterations of the semen: reduction of the motility, concentration, of sperm's morphology, sperm DNA damage, sperm aneuploidies, alteration of sperm epigenome that result in increased cases of infertility, recurrent miscarriage, congenital malformations. Toxicological studies conducted on mice, show how some of the major environmental organic and inorganic contaminants reduce seminal quality. Significant changes of semen quality are noticed in different environments [22–27]. Exposure to air pollution has been associated with abnormalities in sperm parameters. In recent studies the negative effect on sperm motility was estimated, in

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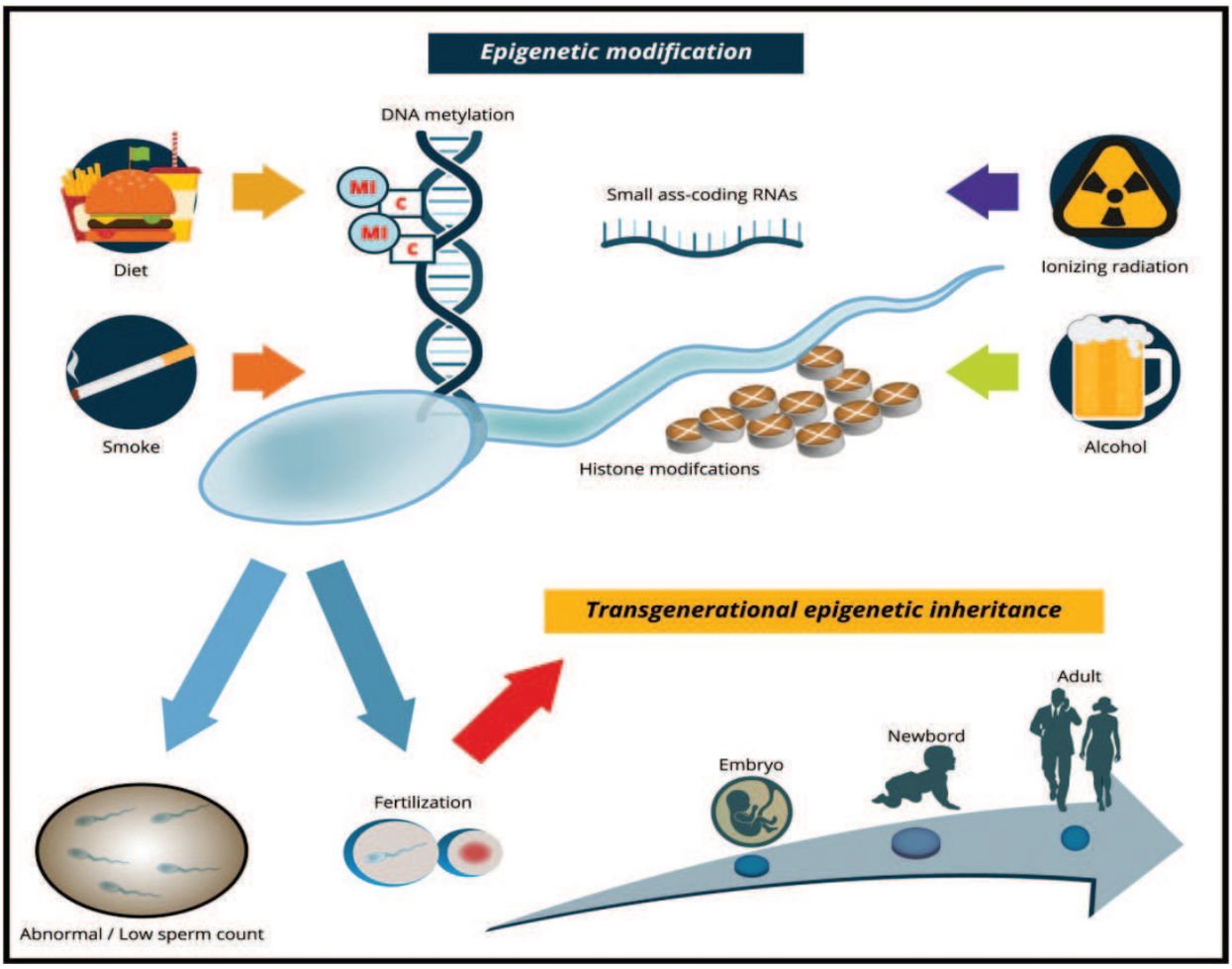

Figure 6. Environmental, life style and diet factors causing with different epigenetic mechanisms (histone modifications, DNA methylation, small ass-coding RNAs) alterations of the sperm epigenome and subsequent transgenerational effects.

particular on sperm DNA's integrity from carbon monoxide, nitrogen dioxide, sulfur dioxide, ozone, lead and PM 2.5, the latter being of particular interest, because it contains several trace elements and PAHs, powerful endocrine disruptors [160].

Spermatogenesis unlike oogenesis from puberty onward is continuously and therefore more easily exposed to insults in his stages of continuous replication. Moreover, biologically a 20-year-old's sperm has undergone about 160 rounds of chromosome replication, a 40-year-old's has undergone 610 and many male germline mutations fall into the "replicative" category or the "non-replicative," such as those caused by environmental exposure, so male germline accumulates mutations faster than female one [48, 49]. For instance, it is thought that sperm cells are more susceptible than eggs to the effects of oxidative damage as a consequence of: (i) the limited cytoplasmic space where to host the enzymes involved in the antioxidant protection, and (ii) the higher amount of polyunsaturated fatty acids within the sperm membranes rendering them more susceptible to oxidative stress, such as lipid peroxidation [50]. Furthermore, in semen it is possible to measure simultaneously environmental contaminants and *in vivo* effects on sperm cells, which are readily available, with features sensitive to environmental pollutants such as motility, morphology and the integrity of the DNA strand.

In 2010, Rubes while assessing seasonal differences of exposure of police officers who worked in the Centre of Prague (Czech Republic), found that sperm DNA fragmentation was significantly higher in winter (high exposure) rather than in spring (low exposure) in samples of all men, including non-smokers [161]. Also in the metropolitan area of Naples studies support the relationship between low sperm motility and high environmental exposure to emissions of traffic or heavy metals [162]. In addition, significantly higher level of sperm DNA damage, measured by means two different techniques, was found in healthy male volunteers living in HIP area as compared with that measured in volunteers living in LIP [118]. Human semen sensitivity to pollution-induced alteration of semen redox status was recently confirmed in a recently published study [26]. In particular, it was demonstrated that semen is more susceptible than blood plasma to pollution-associated alteration of redox status and that STL, but not LTS, was significantly influenced by the environmental impact [134] Certainly, the possibility for measuring simultaneously in human semen the presence of environmental contaminants and checking *in vivo* effects on sperm cells, readily available, with sensitive features to environmental pollutants such as motility, morphology, integrity of DNA strand, semen redox status, sperm aneuploidies, STL, make it an ideal way to assess the adverse effects of environmental exposure for measuring the environmental impact on human health. In conclusion, human semen seems an earlier and sensitive source of biomarkers than blood to monitor high environmental pressure on human health, hence useful for innovative prevention programs and health surveillance, especially in risk areas.

2.5. The semen as an early marker of health (health sentinel)

The spermatogenesis cycle is extremely complex and vulnerable to endogenous and exogenous stress, so it is not surprising that it can be an important indicator of the state of well-being of the organism. Recent studies have demonstrated the association between semen quality and state of health, correlating the semen quality with either chronic degenerative diseases, comorbidities and even mortality [36, 42, 43, 51–53].

In a first study of Eisenberg [53] a group of 9387 men was examined, average age 38 years, which had been evaluated for infertility issues between 1994 and 2011. Within the group, 44% had at least one medical diagnosis not related to infertility. Using the Charlson Comorbidity Index, researchers have shown that men with a higher index of chronic conditions had a lower count of sperm volume and motility, of total number of sperms and of normal shape. Sperm abnormalities rates were significantly higher among men with endocrine-metabolic, circulatory or genitourinary disorders and skin diseases, compared to other men without these conditions. Vascular hypertension, cerebrovascular disease and ischemic heart disease were associated with higher rates of sperm abnormalities. On the other hand, about 15% of all human genes are directly involved in reproduction and the majority of these genes may also play an important role in other parts of the body.

In a second study of Eisenberg [42] 2238 men recruited in an infertility clinic of Texas were analyzed: 451 of which with azoospermia and 1787. It was compared the incidence of cancer on with that on the general population of Texas. At the first evaluation of infertility, the average age was 35.7 years. After a 6–7 years follow-up, it was shown that 29 of the infertile

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men developed a cancer, 10 (2.2%) among those ones with azoospermia and 19 (1.1%) among those ones without it. In comparison to the overall population of Texas, this subset of infertile men had a significantly higher risk of overall cancers and such a was significantly higher in men with azoospermia than in those without azoospermia.

The same Eisenberg linked semen quality with mortality rates [53] and found that men with damaged seminal parameters, including low sperm volume, concentration, sperm motility, had higher death rates than men with normal sperm parameters. Men with at least two abnormal sperm parameters had a 2.3-fold higher death risk (95% CI 1.12–4.65) than men with normal sperm. This further study of association, shows that men with poor semen parameters have an increased mortality rate in subsequent years and suggests that the fertility assessment may be an indicator of overall health.

3. Conclusion

A certain number of regions in all the world experience a higher incidence of health disorders (reproductive, pediatric, cancer, etc.) due to environmental pollution: the societal costs associated with poor health and the interventions to reduce pollution are stirring debates and concerns. It is important a science-based guidance for preventing/reducing health risks in many high environmental pressure areas.

Information about levels of exposure to contaminants (chemical, physical) is critical to evaluate and to manage environmental and professional risks and, as a result, as much as possible, to measure the biological risk expressed in terms of probability of reaching potential harm through the exposure to certain chemical and/or physical stress. There are new analytical tools today that first identify and measure biomarkers, quantitative end-point and intermediate pathways of biological tissue/fluid fluids to identify early signs of functional or structural modification before clinical damage. Therefore, in order to have greater preventive efficacy and raise the level of attention and protection especially to populations living in areas with greater environmental exposure, it is important consider to organofunctional "*sentinel*" systems more susceptible to endogenous and exogenous modifications, those that suffer effects before others. For this reason and in relation to the new primary prevention approaches, the endocrine-metabolic system, and in particular the male reproductive, considering "*double function*" of human semen (Health and Environmental marker), represent an ideal tool for investigating and promoting health surveillance. Human semen seems to be a time-effective, sensitive and informative source of biomarkers, providing information about the presence of biologically active exposures, useful for innovative prevention programs and health surveillance, especially in environmental risk areas. Furthermore, maintaining a good semen quality and fertility is a prevention coverage. Bad lifestyles and environmental contaminants can impair reproductive health and overall health, encouraging the development of chronic degenerative diseases affecting the adult and, through the sperm epigenome changes, future generations. Environmental health should consider reproductive health and development, from intrauterine life to childhood and puberty: these are both vulnerable targets and high-value

protection goals, inasmuch as they represent the future of our societies, in particular, biomarkers of reproductive health should be exploited as early signals of environmental pressure and increased risk of adverse chronic health effects. Hence, the use of reproductive biomarkers for early detection and prevention of environmental health risks represents a useful initiative for public health. Thus, identifying risk factors to improve the management of human wellness and health throughout standardized analysis, which correlates the toxic bioaccumulation of the seminal fluid with the multiple semen parameters, might be the main objective to be considered in the agenda of public prevention policies.

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