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REVIEW ARTICLE

Effect of ionizing radiation on the male reproductive system

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Abstract

Introduction and objective. In the light of current data concerning the growing exposure to ionizing radiation (IR) originating from atrificial sources, especially from medical ones, and also related to occupational exposure, it is justifiable to systematize the state of knowledge concerning the effect of IR on the male reproductive system.

Brief description of the state of knowledge. There is no basis for the application of the hypothesis of hormesis in the area of male reproductive health. Regarding the impact of IR on spermatogenesis, spermatogonia are less susceptible to the occurrence of DNA damage after exposition to IR, but are characterized by slower DNA repair compared to somatic cells. Damage to the genes after exposure to IR is possible at each stage of spermatogenesis; however, haploidal spermatids show the highest radiosensitivity in this respect. The genetic risk of the cells differentiating during spermatogenesis is limited to one cycle of spermatogenesis, whereas the genetic instability may persist for the whole period of life, and DNA damage induced by IR may be transmitted to future generations. The minimum dose causing detectable DNA damage was 30 Gy. While exceeding this dose, the number of single-strand DNA breaks increases. Among males exposed to IR, a decrease was observed in sperm motility and in the percentage of morphologically normal spermatozoa as well as in an intensification of vacuolization. The genetic material in the sperm of these males showed higher fragmentation and methylation of genomic DNA.

Conclusion. In the context of the epidemiological situation concerning the prevalence of infertility, while assessing the health effects of exposure to IR from artificial, including medical sources, the reproductive risk should be considered.

Key words

radiation protection, ionizing radiation, male fertility, human reproductive function, medical exposure, natural exposure

INTRODUCTION AND OBJECTIVE

Radioactivity (radioactive decay) is the capability of atomic nuclei for radioactive disintegration, which is related to the emission of alpha particles, beta particles, and gamma radiation. As a result of this process, new atomic nuclei are created the intensity of which is determined by indicating the activity of the radioactive source. Radiation accompanying nuclear transformations, passing through the medium – the surrounding environment, causes ionization which consists in knocking electrons out of atoms. Ionizing radiation (IR) contains alpha, beta, gamma, UV and X radiation. Radioactivity is an inseparable element of the human environment. In the environment, we are dealing with radioactive isotopes of natural and of artificial origin, which result from human activity (mainly caesium and strontium). Natural radioactivity comes from radioactive isotopes created as a result of the effect of cosmic radiation on the atomic nuclei of nitrogen, oxygen, and argon contained in the atmosphere. Natural isotopes are also present in soil and the earth's crust. The problem of exposure to radioactivity of such an origin occurs primarily in the mining of coal and other minerals, where exposure to radon occurs and its decay of products in the mine air, as well as gamma radiation coming from natural isotopes, mainly radium contained in the rocks of the rock mass, and exposure to water with an elevated content of radium isotopes [1]. It should be emphasized that the aircrews of aircraft achieving high flight ceilings of 10,000 meters are also an occupational group exposed to IR.

An important contemporary problem is exposure to IR associated with medical diagnostics, concerning both patients and medical staff. During the period 1987–2006 in the USA, exposure to IR from medical sources increased on the population scale by 300% up to the level of 3.0 mSv *per capita* annually (without considering oncologic radiotherapy). In this category, exposure related with performance of computed tomography (CT), with significant contribution of its cardiologic applications, occupied the leading position [2]. It is estimated that exposure due to medical reasons is currently equivalent to that from the natural background radiation [2, 3]. During the period 2007–2011 in South Korea, the estimated collective dose of radiation of the Korean population from medical diagnostic examinations increased by 50% [3]. In the light of these data, radiation exposure of employees in the medical sector is an important problem. It satisfies the criteria of occupational exposure, i.e. exposure to IR related with the performance of occupational duties. An increase is also observed in the exposure of patients who receive doses of radiation in association with diagnostic procedures.

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In the light of the report by the Polish National Atomic Energy Agency (PAA) of 2014, the radiation situation in Poland is that in the structure of the annual total effective dose of ionizing radiation in 2014 of 3.31 mSv, the exposure from natural sources constituted 73.6% of the total radiation exposure (2.438 mSv/year), whereas exposure from artificial sources constituted 26.4% of the total exposure – 0.874 mSv/year, the vast majority of which was exposure related with medical diagnostics (26% of the total annual dose; 0.860 mSv/year) [1]. In turn, in 2017, in the structure of the annual total effective dose of radiation of 3.56 mSv, exposure from natural sources was 68.7% (i.e. 2.449 mSv), while exposure from artificial sources – 31.3% (1.114 mSv), including that from medical sources – 30.9% (1.102 mSv) [4]. Therefore, a clear upward tendency has been observed in the contribution of exposure from medical sources (within 4 years, an increase from 26% to 30.9% of exposure from medical sources in the total annual effective dose) [1, 4]. While considering exposure from natural sources, it should be mentioned that in Poland it is 1.5–2 times lower than in countries such as Finland, Sweden, Italy or Romania. There are regions in the world which are characterized by an extremely high level of natural radioactivity, related with the presence of radionuclides in rocks, or occurring in the form of gases, such as radon. These regions include: Ramsar in Iran, Morro do Ferro in Brazil, Mombasa in Kenya, Lodeve in France, and Kerala in India [5]. Reports by the Polish National Atomic Energy Agency for 2014 and 2017 state that in the pool of exposures from medical sources in the Polish population, there were primarily radiological tests, especially CT (0.33 mSv/year in 2014 and a more than twofold increase up to 0.67 mSv in 2017), as well as conventional radiography and fluoroscopy associated with the use of visual tracking (0.38 mSv/year in 2014 and decrease to 0.17mSv/rok in 2017). During both periods analyzed, the exposure from medical sources constituted the vast majority of exposure from artificial sources. Apart from medical exposures, it also included, among others, exposure to radiation from artificial radionuclides in food and the environment, mainly isotopes of caesium and strontium, and exposure from items in everyday use, etc. [1, 4].

Occupational radiation exposure in Poland concerns tens of thousands of people, of whom, as estimated by the Polish National Atomic Energy Agency, approximately 50,000 persons qualified into Category B of exposure to IR in 2014 were covered by the supervision of personal radiation doses. In this group, staff employed in the medical sector – 30,000 persons employed in approximately 4,000 radiological diagnostic laboratories in Poland, constitute a high percentage [1]. In 2014, the Category A of persons exposed to IR, burdened with the risk of exposure to the effective dose on the level of over 6 mSv annually, covered by compulsory personal supervision and the obligation to keep the register of doses, included 1,565 people. However only 41 persons in this group had received in that year an effective dose exceeding 6 mSv [1].

The data presented in this study contribute to the advancement of knowledge concerning the etiopathogenesis of male infertility. Referring to this issue, Winters and Walsh [6] presented the premise that '*the true nature of male infertility incidence remains elusive* […]'. The above-quoted researchers indicated that the scale of the phenomenon has been increasing within the last decades; however, they paid attention to the fact that '*the extent and causes of declining male reproductive health remain largely unknown*'. This results from the fact that the majority of cases of male infertility result from the poor quality of the semen, the causes of which are also unknown. In addition, it should be emphasized that in as many as nearly 12% of infertile couples, attempts to detect the cause of infertility have been unsuccessful [acc. to 6].

The scale of the phenomenon of male infertility is difficult to estimate due to a number of methodological difficulties. Winters and Walsh, based on a review of available data, found that the component of 'male infertility may range widely from 6% to 50%', whereas a number of studies indicate that the percentage of the male factor is from 30% to 50%. These data are most probably underestimated because in a certain percentage of males, in infertile couples, diagnostics is not performed (this percentage is from 18 to even 27%). In addition, male infertility is not a reportable disease, and in many countries it is also diagnosed and treated outside the system of public health insurance [6].

The aim of the study is systematization of the state of knowledge concerning the effect of ionizing radiation on the function of the male reproductive system in order to formulate premises concerning radiological protection of reproductive health.

DESCRIPTION OF THE STATE OF KNOWLEDGE

Selected controversies concerning pathomechanisms of the effect of ionizing radiation on living organisms. The effects of ionizing radiation on living organisms directly depend on the size of the dose and type of radiation. Biological effects of this process also depend on the conditions of irradiation, such as dose rate, method of dose fractionation, mass, type and oxygenation of the irradiated tissues (hypoxia protects against damage as a result of irradiation). The effects of exposure to radioactivity also depend on individual biological properties of the organism. Deterministic effects of exposure to IR are distinguished: those which always occur if the dose exceeds the threshold level, characteristic of high radiation doses, which are the result of death or delayed division of cells, and stochastic effects, related with lower radiation doses – the effect of modification of cells which may manifest themselves in the population after a period of latency. This type of the effects of radiation, covering, among others, the occurrence of neoplasms, occurs in association with a whole range of doses, and the probability of its occurrence is proportional to the dose received [7, 8]. The deterministic effects of radiation include, apart from dermal effects, hair loss, cataract, as well as transitional male infertility occurring from threshold value of 0.1 Gy, and permanent male infertility occurring from the threshold dose of 5–6 Gy [8].

It is suggested that paradoxically there may occur effects of exposure to low radiation doses which are beneficial for the organism. This phenomenon is handled in the definition of hormesis by Calabrese and Baldwin [9], who paid attention to the dual reaction of an organism to the dose, consisting in the stimulation of the body within the range of low radiation doses, and inhibition of life functions of the organism within the range of high doses. Such a reaction is described by the U curve (Fig. 1), dealing with the relationships between such parameters as mortality, or incidence of cancerous diseases and graded exposure to radiation. The dotted line in the Figure describes the reaction of the body

not exposed to the effect of the considered doses, and thus concerns the reaction observed in the control groups. The described phenomenon is basically different from the threshold reaction, i.e. the reaction which starts not earlier than beyond a certain minimum radiation dose.

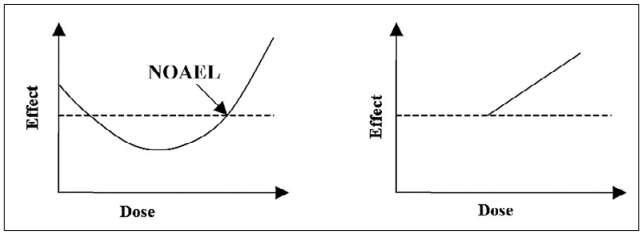

Figure 1. Dose-effect U curve indicating the effect of hormesis (to the left) and threshold reaction (to the right). The NOAEL (No Observed Adverse Effect Level) point means the dose below which the negative effects of radiation are not observed

The theory of hormetic positive effects of radiation on living organisms is subjected to doubt in the meta-analysis by Møller et al., in which it is emphasized that spatial heterogeneity of the intensity of radiation from natural sources, manifested by a nearly thousand-fold geographical differentiation of exposure to radiation, results in a documented negative influence of radiation on the occurrence of many health effects (occurrence of mutations, damage to DNA and the processes of DNA repair, effect on the immune system, including cancer). In the interpretation of the researchers, these data deny the occurrence of hormetic positive effects of low levels of radiation in the case of humans [5].

In scientific literature, there are no reports concerning beneficial effect of radiation on the male reproductive system; therefore, there is no basis for the application of the hypothesis of hormesis in this area. This may result from the fact that mature spermatozoa do not have the capability to repair their own genetic material. Considering the effect of radiation on a single cell, with respect to the reproductive system, it may be expected: below the NOEL point – lack of reaction and temporary functional or morphological changes, and above the NOEL point – permanent changes and necrotic cell death as a result of damage to the cell membrane, or apoptosis. The basic pathomechanism of cell damage results from the formation of free radicals following IR (radiolysis of water). Oxidative stress is the cause of DNA damage and damage to other molecules, and its effects depend on the radiation dose, DNA being considered as the most important cellular shield against the effect of IR. In addition, there occurs, among other things, damage to lysosomal membranes and the release of Fe ions to the cytoplasm, which increases the amount of DNA damage and cell death. This is the so-called indirect effect of radiation, consisting in the absorption of radiation by the medium and formation of indirect products damaging microparticles, the participation of which in the biological effects of radiation, is estimated at 60–70%. The remaining part (30–40%) is a direct effect related with direct radiation deposition in the cell [8]. The effect of radiation on DNA takes place on the pathway of both effects and, as a result, both DNA components may be subject to damage: sugar backbone, as well as the nitrogenous bases. The most frequently occurring damages are: oxidative damage to DNA bases, loss of the base, DNA strand breaks and DNA cross-linking (more comprehensively, see Tab. 1).

Table 1. Effects of radioactivity on DNA according to radiation dose

Type of damage	Dose
DNA single-strand breaks (break in a single strand sugarphosphate chain)	500–1,000/Gy
Rupture of double-stranded DNA	40/Gy
Damage to nitrogenous bases	1,000–10,000/Gy
Carbohydrate damage in DNA	800–2,000/Gy
Formation of cross-linking of nuclear proteins and DNA within one or two strands	150/Gy
Formation of cross-links between DNA molecules	30/Gy

According to the law of Bergonié and Tribondeau, the radiosensitivity of mammalian cells is directly proportional to their reproductive activity, and inversely proportional to their degree of differentiation. This is due to the fact that cell death takes place primarily at the moment of division, which results in the tissues with a considerable dynamics of regeneration being more sensitive to the effect of radiation. As a result, bone marrow and lymphatic tissue, germ cells and intestinal epithelial cells are the most sensitive to radiation. In turn, muscle cells, parenchymal organs, nervous and connective tissues are less sensitive [8]. The radiosensitivity of cells depends also on the phase of the cell cycle; thus, radiosensitivity of cells is a function of their proliferative activity. This results from the fact that the repair of DNA damage and proliferative cell activity decide about the final effect of exposure; therefore, the processes which take place after irradiation [8]. Somatic cells may be at various phases of the cell cycle simultaneously – at the phase G_0, i.e. cell cycle arrest, G_1 – phase of preparation for DNA synthesis, subsequently at S phase – phase of DNA synthesis, G_2 – preparation for mitosis, and M – mitosis. Cells show the highest radiosensitivity at the late G_2 phase and mitosis, whereas the lowest – at late S phase. During the G_1 phase, radiosensitivity of the cells increases [8]. Exposure of cells to the dose of 5 Gy contributes to the inhibition of mitosis, which means that the number of dividing cells will decrease and, consequently, the speed of tissue regeneration will also decrease. In addition, the resistance of tissues to radiation depends on the mass of the irradiated tissue, and on whether, in the mass not subjected to irradiation, there remained a sufficient amount of mature cells which would sustain the function of the organ/tissue [8].

Effect of radioactivity on spermatogenesis. Spermatogenesis consists in the formation of mature male gametes (sperm) from germinal stem cells – the spermatogonia. The precursors of the spermatogonia are gonocytes, i.e. precursor germ cells. Spermatogonia are subject to divisions and evolution into first-order spermatocytes, which are subject to meiotic division leading to the formation of second-order spermatocytes, and then spermatids which are subject to differentiation and transformation into spermatozoa (sperm) [10]. Due to the fact that meiosis is the key process in the formation of sperm, which conditions the reduction in the number of chromosomes in the cell nucleus which are indispensable for the formation of a functional, haploidal reproductive cell, all germ cells may be divided into 3 types, and spermatogenesis into 3 stages: premeiotic, meiotic, and postmeiotic. Premeiotic cells include spermatogonia, meiotic cells are first-order spermatocytes, while into postmeiotic

cells are classified second-order spermatocytes, spermatids, and spermatozoa [10]. The final stage of spermatogenesis is transformation of spermatids into spermatozoa without a division (spermiogenesis). Four stages are distinguished in this process, leading to the formation of the acrosome, formation of the strand, condensation of sperm chromatin, and reorganization of organelles and cytoplasm. The stages of spermiogenesis are as follows: the Golgi phase, cap phase and early and late acrosomal phases [10].

The effect of radioactivity on spermatogenesis has been best investigated on animal models with the use of rodents. The consequences of irradiation in rodents are both macroscopic changes (dose-related decrease in the mass of the nuclei on days 16 and 45 after exposure to the dose of 4–1 Gy), and microscopic changes (decrease in cells of the tubules in the testes, and the reduction in the number of spermatozoa occurring on day 45 after exposure to 0.25 Gy [11, 12, 13, 14]. For differentiating spermatogonia, the mean lethal dose is 0.5 Gy [14]. The effect of irradiation to spermatogenesis was investigated by administration of fluorescent dye Hoechst 33342 to rodents [15]. The use of Hoechst 33342 dye metabolized by ABCG2 carriers enabled identification of spermatogonia in mice, including spermatogonial stem cells (SSCs) in the side population. A day after the period of irradiation with the dose of 4 Gy, the SSCs population decreased by nearly a half. This was caused by the high sensitivity of spermatogonia to radiation, which induced their death. In the cells which did not die on the first day after irradiation, atrophy of the first-order spermatocytes was observed after 2 weeks, and of spermatids – after 3 weeks, which resulted in temporary infertility. However, the parenchymal tissue of the nucleus quickly regenerated from the SSCs pool, and as a result, 2 weeks after exposure an increase was observed in the number of spermatogonia and the return of spermatogenesis [15].

Spermatogonia are less radiosensitive from the aspect of susceptibility to the occurrence of DNA damage, compared to the somatic cells [16, 17]. Due to their tight spatial organization in seminiferous tubules and, in consequence, limitation of oxygen supply, spermatogonia are in a state of specific hypoxia, which protects them against radiation [18]. However, compared to the somatic cells, spermatogonia are characterized by slower DNA repair and, in their population, the repair of some DNA damages are skipped-over [16, 17].

Damage to the genes resulting from exposure to radiation is possible at each stage of spermatogenesis; however, haploidal spermatids show the highest radiosensitivity in this respect [16, 17, 19]. During spermatogenesis, spermatids undergo a series of morphological changes until they achieve the stage of a mature spermatozoon. During spermatid elongation, histones are replaced with protamines in order to enable more chromatin compaction, which leads to the inactivation of transcription in elongated cells. Analysis of DNA damage in the Comet Assay (CA) demonstrated that this compaction may protect mouse sperm during their irradiation with the dose of 4 Gy [11]. An increase in this dose up to 5 Gy *in vitro* did not change the properties of sperm, whereas after the use of these spermatozoa for *in vitro* fertilization, a considerable reduction was observed in the numbers of blastocysts obtained during the *in vitro* procedure [20]. Nevertheless, it may be expected that human sperm may be more susceptible to damage related to irradiation than the gametes of rodents, due to less tightly compacted chromatin [21]. DNA damage detected during irradiation more frequently occurred in less mature mouse cells [16, 22]. Among radiation-induced DNA damage there dominated DNA double-strand breaks (DSB), which are very difficult to repair. The numbers of this type of damage increased together with an increase in the radiation dose [16, 22, 23, 24]. The majority of DNA damages detected by the CA test were repaired within 2 hours after irradiation [16, 22]. During the same time, the number of DSB in the round spermatids rapidly declined; however, there also remained permanent foci, which did not undergo the repair for several days [16, 17, 22, 23, 25]. The damaged double-strand DNA, to a small extent was repaired in the haploid cells, despite the presence of protein responsible for the repair of this type of damage. While the haploid spermatids possess only one chromatid, the damaged double-strand DNA undergoes repair consisting in non-homologous end joining (NHEJ). This process consists in the joining of the free DNA ends after the resection of a part of DNA in the region of the lesion, in order to obtain homologous ends, which may lead to the loss of some of the genetic information and to mutation. This is an error-susceptible pathway of DNA repair [26], opposite to the repair pathway by the homologous recombination, which is a process not burdened with the risk of the occurrence of errors, although dependent on the presence of the second identical DNA strand (sister chromatid) [8].

The repair of the damaged DNA strand by joining non-homologous DNA ends takes place due to the Ku protein, an important binding factor in the process of NHEJ [27, 28, 29]. Ku protein recruits the catalytic subunit of DNA-dependent protein kinase to the site of the strand break, and forms an active DNA-PK complex. The DNA-PK complex has the DNA binding site, and the dsDNA binding site within the ends. The kinase activity of DNA-PK is stimulated by the complex: free DNA·Ku·DNA-PK end. Thus, DNA-PK mediates in the formation of so-called synapsis between the opposite ends. When the synapsis is formed, DNA-PK may trans-fosphorylate the opposite Ku protein molecule, and DNA-PK molecule and, subsequently, DNA-PKCS bind to the opposite end. This leads to the displacement of DNA-PKCS and activation of Ku heliase, which unwinds DNA enabling the so-called microhomogenous base pairing. The unpaired 'tails' are digested away, and the gaps are filled with DNA ligase, which terminates the process of repair. Nevertheless, in round mouse spermatids, DSB repair is delayed after irradiation [17, 23]. It is worth mentioning that pathological syndromes characterized by the lack of DSB repair are also characterized by an increased risk of carcinogenesis [8].

An alternative pathway independent of Ku, may also be the activation of spermatids via protective PARP1 and XRCC1 expression [23]. The PARP-1 molecules are a part of the complex called PLX (PARP-1/ligase DNA III/XRCC1), which stimulates DNA repair by base excision (BER), and participates in the course of repair of two-strand DNA breaks. PARP1 participates in the initiation of Alt-EJ and XRCC1 at the final stage of ligation [30]. PARP-1 is automodified performing the function of the 'house-keeping' gene's product. The presence of inactive, not ADP-ribose molecules provides an instant response to the damage to genetic material. If the cells find themselves in adverse, stressful conditions, this enzyme synthesizes PAR chains, and their level increases approximately 500-fold. Half-life of PAR usually decreases from 6–7 minutes to a few seconds [23, 24, 29, 31].

Meiosis and radiosensitivity. During meiosis, cells seem to be better protected against exposure to IR [32, 33]. This results from the fact that all proteins participating in DNA repair, indispensable in the course of meiotic and homologous recombinations, are present in first-order spermatocytes, some of which play the key role in the maintenance of fertility in humans [34]. In physiological conditions, topoisomerase-dependent SPO11 begins meiotic division, generating DSB in early first-order spermatocytes (leptotene), and activating H2AX phosphorylation. The SPO11 and H2AX proteins are of great importance to fertility in mice [35, 36, 37, 38]. The basic level of DNA damage in the tetraploid mouse cells (mainly first-order spermatocytes with several spermatogonia in stage G2) does not change after exposure to the radiation dose of 4 Gy [39]. In turn, irradiation with the dose of 1 Gy causes a rapid increase in the frequency of occurrence of the gammaH2AX L-foci, biomarker for DSB in the first-order spermatocytes. In contrast, the gammaH2AX S-foci are generated by endogenous SPO11 activity [16, 40, 41]. In irradiated cells at the pachytene stage, gammaH2AX L-foci are removed within several hours [41]. The repair of radiation-induced DSBs takes place by the way of homologous recombination (HR). Accumulation of RAD51, i.e. the protein which is a member of the RAD51 family proteins participating in DSBs repairs, is normally limited to the stage of early first-order spermatocytes, but as a result of irradiation, it is prolonged to the late diploten [25]. Nevertheless, the role of HR repair mechanism, characterized by lack of the risk of mutation, decreases during meiosis, and HR is replaced with NHEJ in later meiotic cell divisions, which is burdened with the risk of occurrence of mutations [40]. In fact, endogenous expression of MRE11, which is a component of the MRN complex (MRE11-RAD50-NBS1) participating in the initiation of the process of DSBs repair on the pathway of both HR and NHEJ, begins the completion of DNA ablation in HR, and is reduced at the stage of late first-order spermatocytes (late pachytene stage) [27, 28, 29, 30]. After irradiation, the Ku 70 protein is not stimulated at the stage of early first-order spermatocytes (leptotene and zygotene), similar to 53Bp1, the task of which is the protection of ends of the DNA strand in the NHEJ process [25, 30]. The 53BP1 foci are present at the stage of late first-order spermatocytes, and there occurs expression of Ku 70 proteins [25, 30].

Conditioning of radiosensitivity of premeiotic cells. Cells at the phase of meiosis present a moderate radiosensitivity. At present, within the time necessary to isolate the spermatogonia, attempts to precisely quantitatively determine the scope of DNA damage in CA within the first hours after irradiation of mice with IR, have been unsuccessful [11, 16]. In consequence, the level of DNA damage is measured by the detection of DSB in spermatogonia (via gamma H2AX foci or 53BP1) several minutes after exposure to IR [17, 29]. The DNA repair proteins involved in homologous recombination (HR) (MRE11, RAD51) and in NHEJ (Ku 70) are sometimes active in the irradiated spermatogonia [28, 42, 43]. In physiological conditions, Ku 70 and 53BP1 are located only in spermatogonia, and the activation of NHEJ is observed after exposure to irradiation [17, 36, 44]. The use of a defined pathway of DNA repair depends on the phase of the cell cycle and the achieved developmental phases. DNA damage is still detected after the second cycle of spermatogenesis (120 days), which confirms the hypothesis that irradiation changes the genetic stability of the spermatogonial stem cells (SSCS) [45]. The genetic risk of the cells differentiating during spermatogenesis is limited to one cycle of spermatogenesis, whereas the genetic instability may persist for the whole period of life. Therefore, it may be expected that DNA damage induced by irradiation may be transmitted to future generations [46]. Despite the fact that the effects of irradiation with strong radiation doses have been relatively well investigated and described, there is still much controversy concerning the effect of small doses of radiation on the stability of DNA. It is rather difficult to find reliable scientific material pertaining to this range of doses, and a considerable part of the conclusions drawn are not based on observations of the effects of exposure to radiation, but rather on the lack of such observations. It is considered that within the range of low doses, the lethal effects of exposure to IR result from the accumulation of events which are not lethal, but become so in the situation where they occur next to each other, or when the number of lethal damages increases when the effectiveness of the repair processes declines [8].

Effect of IR on male fertility. The mature human spermatozoa practically do not have the capability to repair their own DNA [34, 47]. Experiments related with exposure of sperm to radioactivity showed that sperm DNA damage depends on the radiation dose applied. Exposure to radioactivity also exerts an effect on the course of spermatogenesis. Irradiation of the male gonads with the dose of 3.5–6 Sv may lead to permanent infertility and increased risk of the occurrence of congenital anomalies in the offspring. However, in the case of lower doses, higher than 150 mSv, there may occur transitional infertility [48]. Fernandez et al., in their study irradiated sperm with X-rays, increasing the dose from minimum to 80 Gy, and then sought for DNA breaks by the *in situ* hybridization (DBD-FISH) technique. CA was used as the reference method. The minimum dose of radiation causing detectable DNA damage was 30 Gy, which means that it was considerably higher than the dose received incidentally. While exceeding this dose, the number of single-strand DNA breaks increased, and positively correlated with the intensity of radiation [49]. This was also confirmed by an experiment related with irradiation of sperm with the dose of 10 Gy and evaluation of DNA fragmentation using the Dynhalosperm® method, which did not show DNA damage with this dose [50].

Epidemiological studies concerning the effect of low radiation doses on male fertility were conducted in groups of males who were occupationally exposed to radioactivity. Such a group is the staff of X-ray laboratories. Among males exposed to contact with radioactivity, a decrease was observed in sperm motility, an increase in the percentage of pathological sperm (the abnormalities concerned primarily the head), as well as an intensification of vacuolization [51, 52]. In addition, examinations of the genetic material in the sperm of these males showed an intensification of fragmentation and total methylation of genomic DNA [51]. Similar damage to the DNA structure and an increase in pathological forms was observed in the sperm of males engaged in cleaning-up the site of the explosion of the nuclear reactor in Chernobyl [53, 54, 55].

To-date, only Premi et al. have published a study concerning the effect of natural background radiation (NBR) on sperm, focused on the effect of NBR on the genetic material of sperm.

The AZFc region was also analyzed using the DNA from blood and semen of 100 males living near the coastal peninsula in Kerala (India), exposed to NBR of an elevated intensity in this region associated with the occurrence of thorium-containing monazite sand, along with other 50 normal fertile males. This study confirmed the impact of natural background radiation on the human Y chromosome owing to its haploid status and clonal inheritance [56]. In another study, Premi et al. demonstrated the occurrence of tandem duplication and copy number polymorphism of the SRY gene in patients with sex chromosome anomalies, and males exposed to NBR [57]. Premi et al. and Pathak et al. also proved a mutagenic effect of NBR on sperm Y chromosome [58, 59].

A number of scientific reports concern the effect of radiation in the workplace related with radioactivity on the quality of semen. A study conducted by Kumar et al. [60] among occupationally exposed volunteers from various hospitals having diagnostic or therapeutic radiation (X/β/γ rays) facilities, showed an unfavourable effect of radioactivity on the motility characteristics, viability, and sperm morphological abnormalities. This study also demonstrated that among workers exposed to radiation there occurred a higher level of sperm DNA fragmentation, as well as a significant number of hypermethylated spermatozoa, compared to the non-exposed group [60]. Kumar et al. [60] showed also that occupational exposure to radioactivity leads to disorders in the sperm oxidation reduction system. Similar observations concerning the erythrocytes of persons occupationally exposed to radiation were presented by Kłuciński et al. [61]. The hazardous effect of gamma radiation was confirmed by the results obtained in the study by Alvarez et al. carried out on male sperm, which showed the effect of this radiation on the occurrence of sperm DNA damage [62]. Similarly, the adverse effects of gamma radiation were described on the animal model by Saiyad Musthafa et al., who observed disorders of the endocrine function of male gonads in fish after irradiation [63]. Also on the animal model, Verçosa et al. showed a decrease in fertility in insects *Panstrongylus megistus* after exposure to gamma radioactivity [64].

CONCLUSION

1) In the context of the deteriorating epidemiological situation concerning the prevalence of infertility, with a considerable contribution of the male factor, and taking into account the documented effect of IR on male fertility, it is justifiable to continue or implement the monitoring of exposure to IR among males at reproductive age who are occupationally active, and perform work in environments related with exposure to IR, including, among others, X-ray diagnostics in medicine, nuclear medicine, and oncologic radiotherapy, as well as in mining and aviation.
2) In coal mining and the extraction of other minerals, an increased exposure to IR related with natural radioactivity concerns practically all miners working underground [1].
3) While assessing the effects of exposure to IR from medical sources, the reproductive risk should be considered, resulting from the negative effect of IR on male fertility.
4) The optimization of the techniques of dose reduction in imaging diagnostics using IR, especially computed tomography, is very important from the aspect of radiological protection of reproductive health.

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