EFFECTS OF X-RAY RADIATION EXPOSURE ON SPERM MOTILITY AND MORPHOLOGY CHANGES OF WISTARSTRAIN RATS

[1]Bangun Oktavian Hariyoto, [2]Bambang Soeprijanto, [1]Doddy M. Soebadi, [1]Soetojo, [1]Fikri Rizaldi.

[1] Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.
[2] Department of Radiology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.

ABSTRACT

Objective: *To analyze the differences of sperm motility and viability changes in Wistar Rats that exposed to X-ray radiation compared to Wistar Rats that were not exposed to X-ray radiation.* ***Material & Methods:*** *Experimental laboratory research within vivo design using Wistar strain rats as subjects. There are three treatment groups in this study: rats exposed to 50 mGy X-ray radiation, 100 mGy, and 200 mGy and one untreated control group. Radiation exposure was calibrated using the RTI Piranha dosimetry. After radiation exposure, at week 4, orchidectomy was performed in rats. Sperm analysis was carried out to determine sperm motility by direct observation and sperm viability by Hematoxylin Eosin (HE) staining observations using a light microscope. One Way ANOVA was used to compare motility and viability of rats spermatozoa in the treatment group compared to control group with a significant value of $p<0.05$. The analysis was continued with a Post Hoc test to determine the differences in each group.* ***Results:*** *The percentage of motile sperm decreased in all treatment groups compared to control group ($p<0.05$). There was no significant difference in mean sperm motility on rats exposed to 200 mGy X-ray radiation compared to 100 mGy X-ray radiation ($p>0.05$). Viability counts decreased in all treatment groups compared to control group ($p<0.05$) and the spermatozoa viability in 200 mGy radiation group was not significantly different from 100 mGy group ($p>0.05$).* ***Conclusion:*** *X-ray radiation exposure decreases sperm motility and viability in Wistar rats with the optimum doses 100 mGy.*

Keywords: *X-ray, radiation, sperm motility, sperm viability.*

ABSTRAK

Tujuan: *Mengetahui perbedaan perubahan motilitas dan viabilitas pada tikus Wistar yang terpapar radiasi X-Ray dibandingkan dengan tikus Wistar yang tidak terpapar radiasi X-Ray.* ***Bahan & Cara:*** *Penelitian laboratorium eksperimental dengan desain in vivo menggunakan subjek tikus strain Wistar. Terdapat tiga kelompok perlakuan pada penelitian ini, yaitu: tikus yang terpapar radiasi sinar-X 50 mGy, 100 mGy, dan 200 mGy dan satu kelompok kontrol yang tidak diberi perlakuan. Paparan radiasi dikalibrasi menggunakan RTI Piranha dosimetry. Pasca paparan radiasi pada minggu keempat dilakukan orcidectomy pada tikus. Analisa sperma dilakukan untuk menentukan motilitas sperma dengan pengamatan langsung dan viabilitas sperma dengan pewarnaan Hematoxylin Eosin (HE). Pengamatan dilakukan menggunakan mikroskop cahaya. Analisis ANOVA One Way digunakan untuk membandingkan motilitas dan viabilitas sperma tikus dalam kelompok perlakuan dengan kelompok control dengan nilai signifikan ($p<0.05$). Analisis dilanjutkan dengan Post Hoc untuk menentukan perbedaan masing-masing kelompok.* ***Hasil:*** *Persentase sperma motil menurun pada semua kelompok perlakuan dibandingkan dengan kelompok kontrol ($p<0,05$). Analisis lebih lanjut menunjukkan tidak ada perbedaan yang signifikan motilitas sperma tikus pada paparan radiasi sinar-X 200 mGy dibandingkan dengan radiasi sinar-X 100 mGy ($p>0,05$). Pada analisa viabilitas, paparan sinar-X pada kelompok perlakuan memberikan penurunan yang signifikan dibandingkan dengan kelompok kontrol ($p<0,05$). Pengamatan lebih lanjut menunjukkan viabilitas spermatozoa tikus yang terpapar radiasi 200 mGy tidak berbeda secara signifikan dengan kelompok 100 mGy ($p>0,05$).* ***Simpulan:*** *Paparan radiasi sinar-X menurunkan motilitas dan viabilitas sperma pada tikus Wistar dengan dosis optimum 100 mGy.*

Kata Kunci: *X-ray, radiasi, motilitas sperma, viabilitas sperma.*

Correspondence: Fikri Rizaldi; c/o: Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya. Jl. Mayjen. Prof. Dr. Moestopo 6-8 Surabaya 60286. Phone: +62315501318; Fax: +62315024971. Mobile phone: +628563096336. Email: fikririz@gmail.com.

INTRODUCTION

Radiation is an energy emitted in the form of particles, electromagnetic waves or light (photons) from the source of radiation. X-rays are one of the ionizing radiation that grouped into the type of electromagnetic waves with short wavelengths. X-rays have high translucent power due to their short wavelength. Roentgen X-ray included in ionizing radiation, which is a rapidly growing diagnostic tool. In the medical field, the use of X-ray for diagnostic imaging has utilized for more than a century.[1]

In addition to providing benefits to people, radiation also contains a potential hazard. Deterministic effect on the reproductive organs or gonads can interfere with the process of forming the resulting sperm cell. The radiation dose of 0.15 Gy can already result in a decrease in sperm cell count (oligospermia).[2] The decline in the number of sperm may affect fertility.[3]

Infertility defined as a failure of couple to achieve pregnancy after having routine sexual intercourse without contraception for twelve months. About 30% of infertility cases caused by male factors as a single factor. In addition, 20% of infertility cases due to a combination of male and female factors.[4]

Infertility in humans depends on the maturation of germ cells through the process of meiosis. This process is very sensitive to radiation. The influence of radiation exposure to infertility is thought to derive from DNA damage by free radicals due to radiation ionization. DNA damage resulted in cell death (apoptosis) or could induce some mutations.[5] X-ray is one of the main modalities in patient diagnostics, the exposure effect of X-rays to medical personnel is not extensively studied. One phenomenon that often observed in medical personnel who are often associated with radiation is infertility. The use of radiodiagnosis tools such as X-ray, CT-SCAN, C-ARM is still a major focus in the diagnostic of various types of diseases. The use of X-ray radiation in small amounts but carried out continuously can provide an accumulative effect that can cause DNA damage and other epigenetic abnormalities.[6] X-ray doses, duration, and safe distance to reduce X-ray impact for Medical personnel also still need to be further investigated.

OBJECTIVE

To analyze the differences of sperm motility and viability in young aged Wistar strain rats after exposed to X-ray radiation.

MATERIAL & METHODS

The design of the experimental study laboratory with post-test only control group design. The samples used in this study were the white Rats (Rattus norvegicus) of the young Wistar strains which had the following criteria:2-3 months of age; weight 150-250 grams; healthy without disability.[7, 8] Samples were obtained from the Animal Laboratory of Faculty of Veterinary, Airlangga University, Surabaya.

Samples are grouped into four random groups. Calculation with Federer's formula showed that the sample size in each group was at least 6 samples. In this study, there was a chance that the sample would be dropped out, so it was decided to take seven samples per group so that the overall samples in this study were 28 samples of White Mouse (Rattus norvegicus) Wistar strain. This research has obtained approval from the Ethics Commission of the Faculty of Veterinary Medicine of Airlangga University with no certificate: 2.KE.028.02.2019.

Samples were first carried out in the process of adaptation in the cage/research environment (in the cage section of the Veterinary Medicine Faculty of Airlangga University, Surabaya) for 1 week. Furthermore, samples were randomly divided into four groups: P1 is a group with X-ray radiation exposure at a dose of 50 mGy, P2 is a group with X-ray radiation exposure at a dose of 100 mGy, and P3 is a group treated with radiation exposure X-ray at a dose of 200 mGy. The control group in this study included a negative control group (CN), where there was no treatment at all in this group. After 30 days, the rats sacrificed and the epididymis was taken for morphological and motility analysis of the rat's sperm cell.

X-ray radiation is obtained from the X-ray generator in the veterinary hospital of the faculty of veterinary medicine, Airlangga University. The radiation treatment is carried out using portable generators with specifications, Model: TX plus 4 ION 5 duty cycles OFF; Maximum current: 58 A; Voltage: 100-240 V ~ 10% 1ph; Frequency: 50-60 Hz; Input Power: 5 kVA; Output Power: 4 kW; Serial number: MCC 12674. The X-ray exposure dose emitted by the generator is measured and calibrated using RTI's dosymmetry with the Piranha series. The rats were irradiated for 25, 50, and 100 shots for a single day to obtain the radiation dose. Radiation exposure dose obtained from the combination of probe distance to the sample and the size of kVA/mAS produced by radiation equipment. The

results obtained from the dosimetry are used to determine the optimal dose equation that can be used for radiation rays exposure in the treatment group.

The sperm analysis performed on the 31st day is calculated from the 1st day of treatment. Sperm analysis is performed with the motility examination by direct preparation. Observations were carried out using a light microscope with 400x magnification. The assessment is performed quantitatively as the percentage of sperm motility that followed the calculation method by WHO. The classification used in this study is: Progressive Motility (PR) which is an active movement of spermatozoa, either linear movement or large circle movement regardless of the speed, Non-Progressive Motility (NP) which are all forms of motility that have no progressive movement, such as the rotating movement in the small circle or just the visible flagella's movement, and the Immotility (IM) which does not show any movement of spermatozoa.

Morphological/viability examination is performed by making eosin-nigrosin staining preparations, evaluation of living spermatozoa is carried out based on the WHO Laboratory Manual (2010). The number of living/viable and moving/motile spermatozoa is expressed in percent.

Data counting of the sperm motility and morphology will be carried out with normality test and variance test. If data distributions are normal and data variances are equal, then the One Way Anova hypothesis test is used. Data is considered normal if $p<0.0$. To determine the Post Hoc Test that will be used, the data homogenization test is carried out. All data processing techniques were analyzed computerically by using statistical product and service solution 20 for windows software (SPSS 20).

RESULTS

In this study, a homogeneity test was carried out on the body weight of the subject aimed at assessing the randomization of the research subjects. The results obtained were the rat's body weight that normally distributed after randomization with $p>0.05$. The sample characteristics are shown in table 1.

In this study, the data normality test was performed using the Shapiro Wilk test and obtained the percentage of sperm motility in each group is normally distributed with $p>0.05$ (Table 2). Furthermore, the One Way ANOVA parametric test was conducted to see the mean difference between groups. One Way ANOVA test results showed a significant mean difference between groups at $p<0.05$ (Table 3). Mean data between groups were analyzed further to determine whether there are

Table 1. Study sample characteristics.

Group	n	Mean ± SD	Normality	p value
Control	6	182 ± 10.463	0.873	0.086
P1	6	172 ± 9.908	0.894	
P2	6	171 ± 5.707	0.738	
P3	6	172 ± 3.082	0.755	

P1: Rats exposed to 50 mGy X-ray radiation
P2: Rats exposed to 100 mGy X-ray radiation
P3: Rats exposed to 200 mGy X-ray radiation

Table 2. Comparison of the motility percentage on radiation exposure.

Group	n	Mean ± SD	Normality	p value
Control	6	44 ± 9.174	0.149	0.000 *
P1	6	20 ± 10.206	0.092	
P2	6	5 ± 6.325	.110	
P3	6	5 ± 6.325	.110	

P1: Rats exposed to 50 mGy X-ray radiation
P2: Rats exposed to 100 mGy X-ray radiation
P3: Rats exposed to 200 mGy X-ray radiation

differences in variability using Levene's Test and the results are no differences between groups variance with p>0.05. Bonferroni post hoc test was performed to compare the mean differences between the study groups (Table 4).

From the results of Bonferroni Post Hoc statistical analysis, there was a significant difference in sperm motility of spermatozoa in each treatment group when compared to the control group with a value of p<0.05. In addition, there was a significant difference in sperm motility in the treatment group exposed to 50 mGy X-ray radiation compared to the group that received 100 mGy and 200 mGy X-ray radiation exposure. From further analysis, there was no difference in mean spermatozoa motility in the treatment group with 100 mGy X-ray radiation exposure compared to 200 mGy with p>0.05.

Data viability of spermatozoa collected from microscopic observations were statistically tested to determine normality. Result of normality test Shapiro Wilk obtained normally distributed datawith p>0.05.

After being tested with normality test,data distribution of viability spermatozoa was obtained normal, then the parametric test One Way Anova was done. The analysis result is the significant difference was obtained between quantity mean between each of the groups, with the value of p<0.05. Levene test was used to analyze the variability of the data, homogeneous data was obtained with p>0.05. So the test was followed by post hoc Bonferroni.

Table 3. Comparison of sperm motility percentage in each group.

Between-group comparisons of Sperm Motility	Mean Difference	Confidence interval 95%		p value
		Lower bound	Upper bound	
Control vs P1	23.333 *	9.49	37.17	0.000
Control vs P2	39.167 *	25.33	53.01	0.000
Control vs P3	39.167 *	25.33	53.01	0.000
P1 vs P2	15.833 *	1.99	29.67	0.019
P1 vs P3	15.833 *	1.99	29.67	0.019
P2 vs P3	0.000	-13.84	13.84	1.00

P1: Rats exposed to 50 mGy X-ray radiation
P2: Rats exposed to 100 mGy X-ray radiation
P3: Rats exposed to 200 mGy X-ray radiation

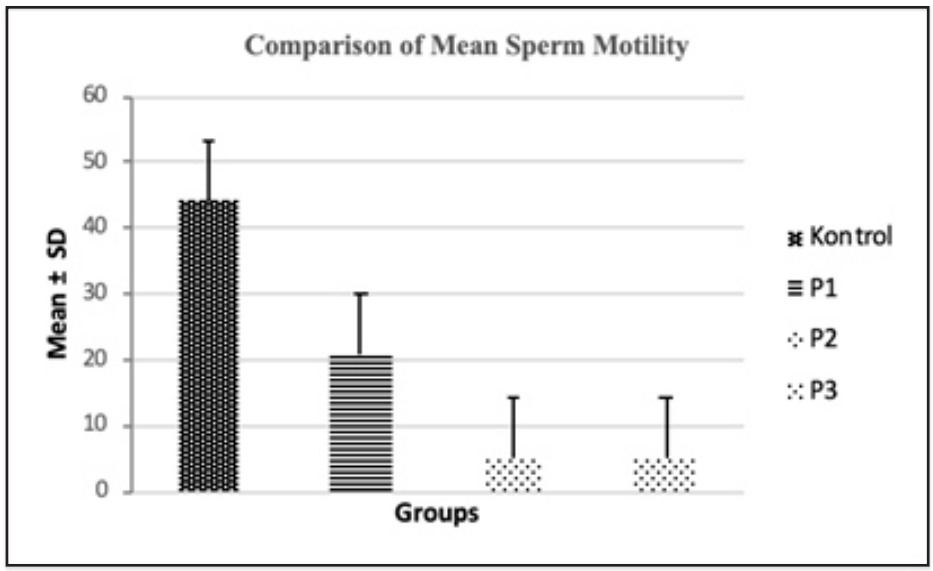

Figure 1. A comparison of the spermatozoa motility.

Table 4. Comparison of the percentage viability of spermatozoa in radiation exposure.

Group	n	Mean ± SD	Normality	p value
Control	6	79 ± 2.994	.790	0.00 *
P1	6	71 ± 4.885	0.805	
P2	6	57 ± 4.502	0.404	
P3	6	58 ± 6.306	0.355	

P1: Rats exposed to 50 mGy X-ray radiation
P2: Rats exposed to 100 mGy X-ray radiation
P3: Rats exposed to 200 mGy X-ray radiation

Table 5. Comparison of spermatozoa motility in each group with treatment.

Comparisons of Sperm Motility Between the Groups	Mean Difference	Confidence interval 95%		p value
		Lower bound	Upper bound	
Control vs P1	8.167 *	0.02	16.31	0.049
Control vs P2	22.500 *	14.36	30.64	0.000
Control vs P3	21.667 *	13.52	29.81	0.000
P1 vs XP2	14.333 *	6.19	22.64	0.00
P1 vs P3	13.500 *	5.36	21.64	0.01
P2 vs P3	-0.833	-8.98	7.31	1.00

P1: Rats exposed to 50 mGy X-ray radiation
P2: Rats exposed to 100 mGy X-ray radiation
P3: Rats exposed to 200 mGy X-ray radiation

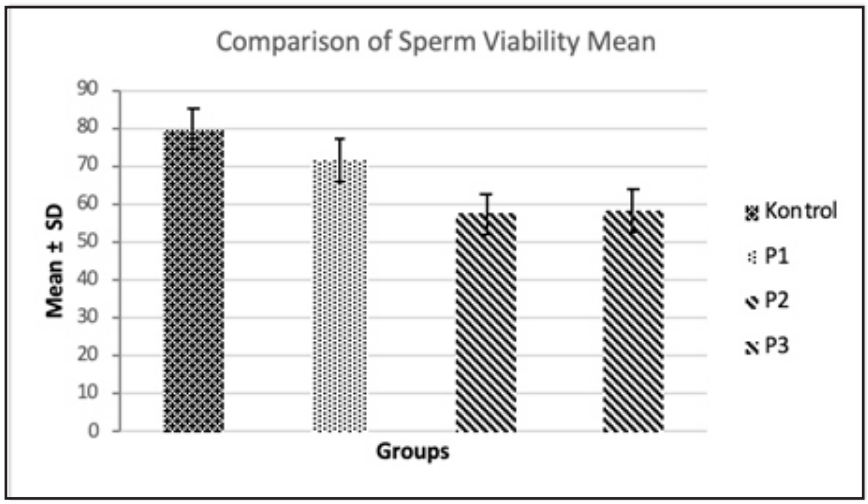

Figure 2.Comparison of Mean Sperm Viability.

The significant difference was obtained between mean viability of spermatozoa between control group and all treatment groups with pvalue<0.05. There are differences between the mean treatment group who received 50 mGy X-ray exposure compared with the group who received 100 mGy and 200 mGy with pvalue p<0.05. Further analysis, there were no significant differences between the group received the X-ray exposure of 100 mGy compared with 200 mGy at p>0.05 (Table 5).

DISCUSSION

Radiation is one of the main causes of hypermethylation on sperm DNA, which resulted in the emergence of pathological defects and abnormalities in sperm morphology.[9] Radiation also result in epigenetic abnormalities, which may lead to chromosomal instability. Hypermethylation of DNA and chromosomal instability resulting in destabilization of nucleosomes because of the disruption of the

histone-protamine conversion. Further deteriora-tion in the level of genomic damage leads to a fragmentation of the DNA and vacuoles resulting in abnormalities of morphology (viability) sperm cells.[10]

Free radical is a condition when an element contains one unpaired electron. Free radical element is highly reactive and unstable because the imbalance of its unpaired electron.[11] Free radicals are the side effects of physiological processes that can occur in humans. Free radicals in the human body can be produced by processes such as metabolism of endogenous or exogenous like exposure to radiation, ozone, smoke, and exposure to other pollutants.[12] Excessive radiation on the human body can induce genetic instability, which in turn leads to the activation of the apoptotic pathway or failure of cells to reproduce. One of the largest sources of free radical exposure for medic or paramedic workers is procedure-based radiation such as diagnosis and therapy. The continuous radiation exposure of medical personnel by the tools-based low dose radiation such as X-ray picture, CT SCAN, C-Arm, and fluoroscopy, resulted in their cumulative effect of radiationthey were received.[13]

Repeated exposure toward free radicals that were obtained from a medical procedure results in the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) on cells. Increasing levels of ROS resulted in the emergence of oxidative stress. Oxidative stress will eventually induce the formation of superoxide radicals (O2) and peroxide (H2O2), which is highly reactive to the cell membrane. The most susceptible cell toward radiation exposure is sperm cells.

The sperm cell membrane is composed of a polyunsaturated fatty acid (PUFA), which is a highly susceptible derifat toward free radicals. Damage of PUFA causing the sperm cells will have abnormalities in various pathways of metabolism such as Glyceraldehyde-3-phosphate (GAP). GAP is very important protein anabolism cells in the process of glycolysis, malfunctions of these proteins lead to the disruption of glycolysis. As a result, the cell will decrease the amount of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). Then there was disturbance on axoneme phosphorylation in sperm that led to the disruption of sperm motility.[14]

In this study, significant differences were obtained on the motility of spermatozoa between the treatment group who received radiation exposure compared to the control group at $p<0.05$. On further analysis, significant differences were also found between each of the treatment groups with exposure toward X-ray (50 mGy vs 100 mGy and 50 mGy vs 200 mGy). But this study did not find significant difference in cell motility of spermatozoa between treatment groups who received X-ray radiation exposure 100 mGy compared with the treatment group who received radiation exposure 200 mGy. A study which similar to this research was done by Kumar et al, [6] Kumar et al's study analyzed changes that occur in health personnel whom exposed to radiation, with a total sample of 51 patients compared with those who not exposed to radiation of 83 patients. Analysis of risk factors that possibility leads to confound the result such as alcohol consumption, smoking, diet, and marital status was also observed in this study. Kumar et al. proved there was significantly different on sperm cell motility ($p<0.001$) in humans that were often exposed to radiation compared with normal control humans. The result of Kumar et al. study are consistent with the results of this study which showed a decrease in sperm motility in rats who exposed to X-ray radiation. The difference between this study compared with the study conducted by Kumar is the research subjects, this study was using rats while Kumar et al's study used human for research subjects. In this study, the detail radiation dose delivery only used X-ray radiation and each dose was calculated by Dosimetry so that the radiation dose was given to research subjects could be controlled and more measurable.[6]

In this study, the significant differences were obtained between the viability of sperm cells in the treatment group who received X-ray radiation exposure with the control group ($p<0.005$). On further analysis it was found that there was a significant difference of viability in each of the groups treated with X-ray exposure (50 mGy Vs 100 Vs mGy and 50 mGy 100 mGy). In addition, significant difference was not found between treatment groups who received X-ray exposure (100 mGy Vs 200 mGy). This result indicated a decrease in sperm cell viability after exposure to the X-ray. Analysis of the results also indicate the decrease of cell viability was already happening to the maximum at the dose of 100 mGy exposure, so three was no significant difference between the exposure dose of 200 mGy compared with 100 mGy. The explanation for the decreasing of sperm cell viability after exposure to X-ray is the hypermethylation that happening on sperm cells. This is consistent with the findings of research conducted by

Kumar et al. which showed an increase in sperm cell DNA hypermethylation of medical workers whom exposed to radiation compared with the medical workers whom not exposed to radiation with $p<0.01$. Furthermore, research conducted by Kumar et al. also compared the effects of hyperventilation that occured toward the morphology and chromosomal abnormalities of sperm cells. As a result, there are significant morphological differences in the number of spermatozoa in humans who often exposed to radiation compared with who seldom exposed to radiation.[6]

Hypermethylation is a silencing gene mechanism that included in epigenetic regulation. Epigenetic regulation is a non-structural genetic modification, this process could be happening because of methylation, acetylation, change the chain of non-coding RNA, chromatin remodeling, or histone modification.[12] Methylation occurs because of chemical modification of DNA after receiving a group of methyl. Methylation is initiated by the addition of methyl groups to the cytosine base DNA by DNMT enzyme and substrate S-Adenosine L-Methionine (SAM). In human, there are three types of enzymes DNMT namely DNMT 1, DNMT 2, and DNMT 3. DNMT-1 is anenzyme most commonly found in humans and suspected acts as the main regulator in cell maintenance.[15] Changes of morphology, DNA fragmentation, and damage to the vacuoles occur in sperm cells after radiation exposure are likely to occur because of hypermethylation resulting in destabilization of genetic material and cellular changes in histone-protamine.[6]

Hypermethylation occured in sperm cells may result in change of morphology and viability of sperm cells. Although several studies have shown no significant decrease in the concentration of sperm cells that were obtained after exposure to radiation. But this change was not fully result for sperm cells to lose its ability to perform fertilization. Epigenetic changes such as hypermethylation was potentially inherited in the next generation.[16] Moreover, the epigenetic change that will be inherited to the next generation was very dependent on epigenetic modifications carried by the paternal's sperm cells.[17] So basically, the damage that occurs in paternal sperm cells will lead to the accumulation of genetic abnormalities in the next generation. Fertilization that produce a zygote from the sperm cells who had hypermethylation has a high risk failed implantation and miscarriage.[18]

This study has shown low-dose radiation exposure that accumulates in rat resulted in changes in motility and viability of sperm cell. The dose that was given in this study clearly defined and measurable. In this study, the effect of hyper-methylation on rat sperm cells has not been observed with certainty so that the exact disruption mechanism of sperm viability and motility need to be observed furtherly. In the future, similar studies as this research with low-dose radiation exposure, but to be done in human is considered. Because it still needed further evaluation regarding the effects of low radiation doses that accumulate in human, especially medical personnel who almost daily exposed with radiation.

This research has focused on analyzing the effect of radiation on the motility and viability of sperm cells. The results of this study showed a decrease in both variables, but the main mechanism of X-ray radiation exposure which decrease sperm motility and viability are not observed in this study. To find out the fundamental mechanisms of the X-ray exposure and spermatozoa further research is still needed and may involve additional analysis on hypermethylation DNA, the sperm morphology count, as well as epigenetic change in sperm cells.

CONCLUSION

Exposure to X-ray by 50 mGy and 100 mGy are able to induce a decrease in motility and viability of sperm cell in rat. In this study, the most optimum dose of X-ray in inducing a decrease in motility and viability of sperm cells of rat was 100 mGy. Further research required to assess the main mechanism of X-ray radiation in inducing the transformation of the sperm cells of rat, and also further research with human subject that constantly and continuously exposed to X-ray to assess the effects of X-ray radiation on human spermatozoa.

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