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Research Article

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Estrous Cycle of Mice (*Mus Musculus* L.) Exposed by Repeated Gamma Rays Radiation

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ABSTRACT

This study aims to determine the effect of a repeated gamma radiation on the estrous cycle in mice (Mus musculus L.). This research used a completely randomized design (CRD) of gamma ray treatments with a dose of 2 gray (2 gy) i.e. P0 (control), P1 (dose of 1 x 2 gray), P2 (dose of 2 x 2 gray), and P3 (dose of 3 x 2 gray). Estrous cycle was observed on the vaginal swabs with topical method. Parameter measured was estrous cycle length (days). The ANOVA test results of female mice (Mus musculus L.) estrous cycle exposed to repeated gamma radiation showed a significant difference (P = 0.002). After followed by Duncans test, there were no significant differences between control and treated mice P1 and P2, however, there was significant difference of the P3 treatment (treatment with a dose of gamma radiation 3x 2 gy). Estrous cycle becomes longer in mice (Mus musculus L.) treated with repeated gamma radiation.

Keywords: *estrous cycle, radiation, gamma, Mus musculus L.*

INTRODUCTION

The gamma rays have been used in medicine, industry, hydrology, food preservation and other fields. These rays belong to the ionizing radiation with high penetrating power as well as x-rays. Ionizing radiation is a radiation that the rays will ionise the materials in its path [1]. In the medical field, especially nuclear medicine, radioactive preparations which emits gamma radiation were used to diagnose diseases and cancer therapy. With the technique of this radioactive therapy, the life expectancy of patients suffering from cancer was increased [2]. However, everything that has a positive side, there is also a negative side effect. Nor has the gamma rays, in addition to give a positive benefit, their negative effects on health were also often discussed. Therefore these rays belong to the ionizing radiation, the rays will ionise the materials in its path, including biological materials such as cells. During the therapy, not only cancer cells were killed, but also healthy cells could die or damaged. If the cells did not die immediately, but the nucleus has been changed, most likely within a certain time, it will be changed to a new cancer cells[3].

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Cells and tissues have different sensitivities to the effects of radiation. Highly sensitive cells to radiation exposure, commonly are actively dividing cells i.e. blood cells, embryonic cells and cells of the gonads (ovaries and testes). The repeated x-ray radiation significantly affect the quality of sperm in mice exposed to X-ray radiation[4].. Likewise, the research of Suharjo[5], showed male mice that were irradiated with X-ray radiation dose of 200 rads, had a decrease number and diameter of seminiferous tubules of the testes. Weight and testes of male rats irradiated with a dose of 2 to 5 gray were also decreased[6]. The research of Zhang *et al.*[7] showed that human sperm motility in acrosomal reaction given the high radiation 16, 32, and 64 gray combined with ion 16O + 6 experienced a sharp decline. The purpose of this study was to determine the effect of repeated gamma radiation to the length of the estrous cycle in *mice (Mus musculus* L.).

MATERIALS AND METHODS

This research used dissecting sets, hand counters, scales, paraffin, measuring cups, bowls, dishes, pipettes, glass slide and cover glass, light and stereo microscopes, Radio Gamma equipment (Stabilipan artificial Siemens) at Radiology Unit, Sanglah Hospital. The materials used in this study were 0.9% NaCl, Geimsa 2% in distilled water, Eosin, Hematoxylin, 10% Neutral Buffer Formalin, absolute alcohol, 70% alcohol and xylol. Experimental animals used in this study were 40 female mice *(Mus musculus* L.) strains Switzerland, aged 3 months, body weight 25-30 grams, fed standardized pellets for chicken and water ad libitum.

This research used a Completely Randomized Design (CRD) i.e. 30 irradiated female mice and 10 female mice as controls. The first group of 10 animals were irradiated with gamma rays in a dose of 1 x 2 gray, the second group of 10 animals were irradiated with gamma rays in a dose of 2 x 2 gray once a week for two weeks, and the third group of 10 animals were irradiated with gamma rays in a dose of 3 x 2 gray once a week for three weeks. Radiation treatments performed with gamma rays at the Radiology Unit of Sanglah Hospital.

After each treatment according to the experimental design, vaginal smear preparations with topical methods were made to observe the estrous cycle of irradiated female mice. Vaginal smears made by inserting the tip of a cotton bud dipped in a solution of 0.9% NaCl solution, twisted around in the vagina then smeared on a glass object that have been etched with 0.9% NaCl. The object glass then fixed in 70% alcohol for 5 minutes then stained with Giemsa for a 10 minutes. The slide then washed with water and wind-dried. Vaginal smear slides were observed under a microscope with a magnification of 100x and photographed by optilab camera.

If a female mice is in an estrus phase, 75% of her superficial cells of vaginal epithelial would be found in cornification form and there were no leucocyte cells [8]. The length between two estrous phases was calculated by observing the vaginal swabs of two cycles after the appropriate radiation dose trial.

Data processing

Quantitative data processed statistically by One Way ANOVA using SPSS program version 22. If there is a significant difference, it will be further analyze with Duncan test. Qualitative data will be presented in the form of pictures and description tables.

RESULTS AND DISCUSSION

From observations it was found that the exposure of repeated gamma irradiation at dose of 2 gy influenced significantly on the length of estrous cycles (shown in the following tables and figures).

Table 1: The length of estrous cycle (day) of female mice *(Mus musculus* L.) exposed to repeated gamma radiation

No	Treament	Length of the estrous cycle (day)
1	K (control)	4.40 ± 0.29 **a**
2	P1 (30 days)	3.70 ± 0.30 **a**
3	P2 (50 days)	5.10 ± 0.24 **a**
4	P3 (70 days)	7.20 ± 0.96 **b**

The results showed that the weight of the ovaries and uterus of female mice (Mus musculus L.) exposed to gamma radiation repeatedly showed the difference was not significant (P = 0.60). While the results of the ANOVA test against sklus estrus female mice (*Mus musculus* L.) exposure to gamma radiation repeatedly showed significant differences (P = 0.002).

The length of estrous cycles of female rats exposed to repeated gamma radiation showed significant differences. This is likely due to damage of ovarian cells exposed to repeated doses of radiation. As it is known that gamma radiation is an ionizing radiation, which means that this radiation is able to ionise the material in its path, in this case the cells of the ovaries. Cells which actively divided as blood cells and ovarian gametes, are very sensitive to the effect of radiation. The research results of Qomariah[9] showed a decrease in the quantity of haemopoietic cells i.e. cells CD34 and B220 that were irradiated with gamma rays, this decrease of quantity parallel with the increasing doses of gamma rays. Quantity of these cells decreased due to damage of the DNA in the chromosomes so that the cells become necrotic[10].

Radiation changed the characteristic of nucleus and cytoplasm. Cells of de Graaf follicle are very sensitive to the effects of radiation. Radiation also trigger changes in the production of hormones produced by the gonadal glands that contribute to the development of ovarian cells. Damage to cells in the ovaries affected the p roduction of reproductive hormones such as estrogen and progesterone. Decreasing concentration of estrogen and progesterone in the plasma will affect the estrous cycle in mice. Low levels of estrogen hormone will lengthen the estrous cycle in mice. This is consistent with the results of Lee[11] that there was a decrease level of estrogen and progesterone in blood plasma of irradiated mice. The decrease was due to hormonal regulation i.e. inactivated enzyme or hydroxy steroid dehydrogenase and isomerase enzyme in the theca cells, and also blocked the activity of granulosa cells in the follicle.

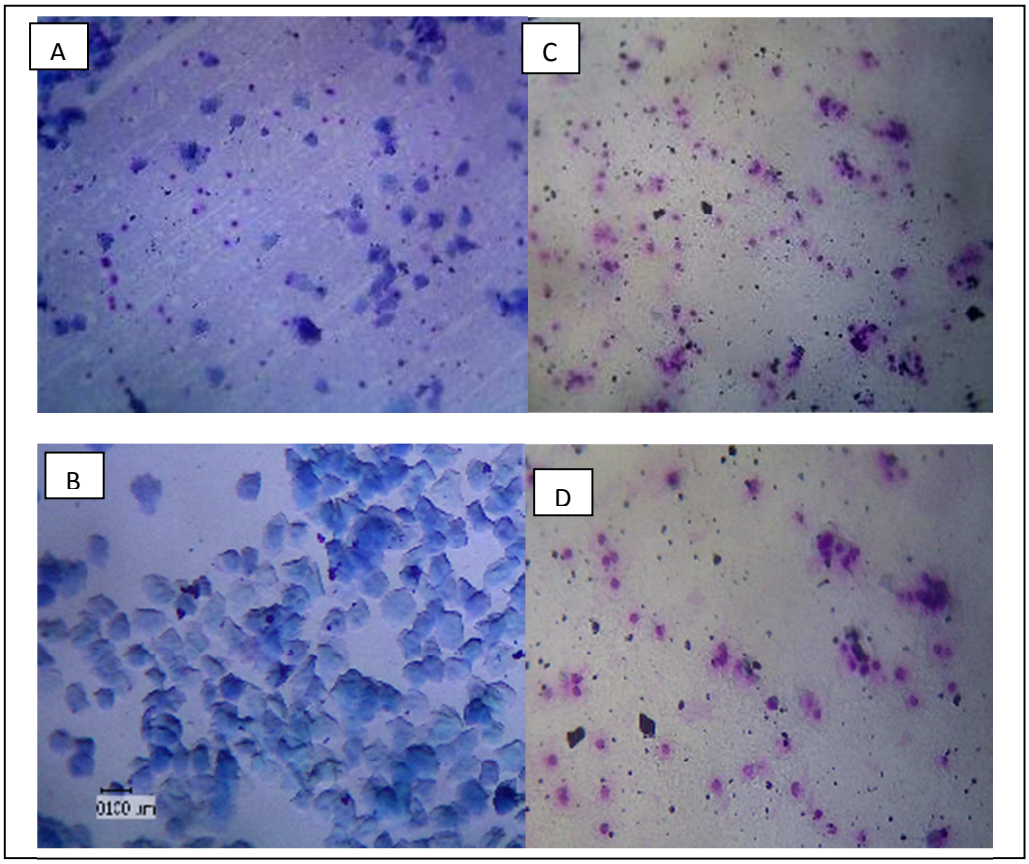

Fig. 1: Stages of the estrous cycle in vaginal smear of female mice (*Mus musculus* L.) irradiated with gamma-rays. Description: A. Proestrus phase, B. Estrus phase, C. Metestrus phase, and D. Diestrus phase (magnification 100x, Giemsa staining)

The results study of Cahyati *et al.*[12] was also in line with the above statement that the dysfunction of the ovary and stress as well as decrease in estrogen hormone of mice due to radiation exposure, and also another radiation effects was premature menopause. The decline of these hormones due to an increase in free radicals from radiation exposure resulting in an increase usage of gamma oxidant enzymes then cells become damaged. Likewise, the results of research of Dicu *et. al.*[13] showed an increase number of ovarian cells that died after exposure of cytotoxic gamma radiation. The number of dead cells of radiation-induced ovarian affected the production of hormone i.e. estrogen and progesterone. Estrogen and progesterone hormones produced an effect on the epithelial layer of vaginal walls dan estrous cycles. Low estrogen levels will prolong the estrous cycle, or in other words, lowering fertility, and too low estrogen levels can trigger early menopause.

CONCLUSION

Estrous cycle becomes longer in mice (*Mus musculus* L.) treated with repeated gamma radiation.

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