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Ionizing Radiation Effects In Vitro Study; Using The Rat Whole Embryo Culture Model

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ABSTRACT

Background: Ionizing radiation poses a threat to the early embryo possibly leading to prenatal death, growth retardation, organ malformation, or mental retardation. It is important, assessment of any adverse effects of radiation upon the embryo. This study aimed to evaluate the outcomes of embryos irradiated with 1Gy doses in vitro and investigate hematopoiesis in the yolk sac of the irradiated embryos.

Materials and methods: In the study, the experimental group of rats was be exposed to total body ionizing radiation on days 8.5th of gestation. All embryos in the control and radiation group cultured from gestation day 9.5 to 11.5 were alive at the end of the culture period. After 48 hours culture period, the embryos from each group were harvested and analyzed morphologically. Histological evaluation of the vWF+ cell number was performed in vivo.

Results: The results showed that the embryonic growth and development during organogenesis decreased in the radiation exposed embryos when compared to control embryos. Additionally, the immunofluorescent examination showed that the vWF+ cell number reduced in the yolk sac of embryos exposed to ionizing radiation.

Conclusion: Consequently, these findings support the conclusion that 1 Gy ionizing irradiation may increase prenatal death, intrauterine growth restriction on embryonic development when ionizing irradiation decreases the vWF+ cell number in the yolk sac compared to control embryos. This research related to radiation was the first study using the in vitro embryo culture technique; thus, future studies that will be performed by using different doses of radiation will contribute to the literature.

Keywords: Embryo culture, Ionizing irradiation, Rat, vWF, Yolk sac

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ÖZET

Giriş-Amaç: İyonlaştırıcı radyasyon, erken embriyo için muhtemelen doğum öncesi ölüme, büyüme geriliğine, organ malformasyonuna veya zeka geriliğine yol açan bir tehdit oluşturmaktadır. Radyasyonun embriyo üzerindeki herhangi bir yan etkisinin değerlendirilmesi önemlidir. Bu çalışma, in vitro olarak 1Gy dozları ile ışınlanan embriyoların sonuçlarını değerlendirmeyi ve ışınlanmış embriyoların yumurta sarısında hematopoeziyi araştırmayı amaçlamaktadır.

Materyal-Metod: Çalışmada deney grubu sıçanlara gebeliğin 8.5. Günlerinde total vücut iyonlaştırıcı radyasyon uygulanmıştır. Kontrol ve radyasyon grubundaki tüm embriyolar, hamileliğin 9.5 ila 11.5. Günlerinden itibaren kültür dönemi sonunda canlıydı. 48 saatlik kültür süresinden sonra, her gruptan embriyolar toplandı ve morfolojik olarak analiz edildi. VWF + hücre sayısının histolojik değerlendirmesi in vivo gerçekleştirildi.

Bulgular: Sonuçlar, organogenez sırasında embriyonik büyüme ve gelişmenin, kontrol embriyolarına kıyasla radyasyona maruz kalan embriyolarda azaldığını gösterdi. Ek olarak, immünofloresan inceleme, iyonlaştırıcı radyasyona maruz kalan embriyoların yumurta sarısında vWF + hücre sayısının azaldığını gösterdi.

Sonuç: Sonuç olarak, bu bulgular, 1 Gy iyonize radyasyonun doğum öncesi ölümü artırabileceği, iyonize radyasyonun kontrol embriyolarına kıyasla yumurta kesesindeki vWF + hücre sayısını azalttığı zaman embriyonik gelişimdeki intrauterin büyümenin kısıtlı olduğu sonucunu desteklemektedir. Radyasyonla ilgili bu araştırma, in vitro embriyo kültürü tekniğinin kullanıldığı ilk çalışmadır; böylelikle ileride farklı dozlarda radyasyon kullanılarak yapılacak çalışmalar literatüre katkı sağlayacaktır.

Anahtar kelime: Embriyo kültürü, İyonlaştırıcı radyasyon, Sıçan, Vitellüs kesesi, vWF

1. INTRODUCTİON

Radiation is crucial for diagnosing diseases. It is also used for therapeutic techniques including various organs' anti-oncogenic therapy (Connell and Hellman, 2009). Radiation therapy approaches employ ionizing radiation delivered either externally by linear accelerators or internally with the use of radioisotopes to destroy cancer cells. Despite the advancement in radiation therapy, pregnant women and their fetuses are considered to be particularly vulnerable to the negative effects of radiation. Accidental expos embryo or fetus in the mother's womb, causing disability, and even death (Shimura, 2011).

The most critical period of embryonic development is the period in which the main organ drafts are evolved (15-60 in humans, 9.5-11.5 days in rats). The exposure to radiation may lead to physiological anomalies, dysfunction of organs or termination of pregnancy in earlier periods as the main organ systems are shaped later. Radiation exposure, which is one of these external factors, may cause significant malformations in pregnancy. In a study, it was stated that the radiation dose that may be harmful to the fetus is 50 mGy and that the fetus is exposed to radiation under this amount is less likely to be damaged. Therefore, the growing and developing tissues and the tissues of the embryo see the most damage. However, these effects show duration and dose-dependent effects (Brent and Mettler, 2004). Experimental animal studies have gained importance to detect the effects of drugs and elucidate the etiology of congenital malformations. For this purpose, in vivo, and in vitro studies are carried out simultaneously (Hacıalioglulları and Ulger, 2010).

The yolk sac, a membranous sac attached to an embryo has a significant role in early embryonic survival. It is involved in the first blood-cell formation; and in charge of the generation of germ cells, which are the common precursors of sperm and oocytes. Moreover, it is a promising source for stem cells since the yolk sac comprises hematopoietic as well as mesenchymal cell niches during the embryo and fetus development (Samokhvalov, 2014; Wenceslau et al. 2011). It is also known that the yolk sac is the first area of blood vessel development. The yolk sac has the first red blood cells and is a possible source for stem cells. The primitive multipotent stem cells from hemangioblasts, a precursor cell pool that can vary in hematopoietic and endoepithelial lineages (Hyttel, 2009).

Hematopoiesis is a time and field incident in the course of vertebrates' ontogeny. The first of the hematopoietic activity occurs in the ventral blood islands of the vitellus sac, where primitive core erythrocytes are formed to control the oxygen demand of the growing embryo. The second of primitive hematopoietic activity is followed by a shift to the fetal liver, where the production of all hematopoietic cells is initiated. The production of blood cells from birth to the end of life takes place in the bone marrow. Therefore, embryonic, fetal and adult hematopoiesis is related to a common stem cell that reaches the specific environment permitted for the programmed and consecutive expression of various shapes of hematopoiesis during each migration event (Tavassoli, 1991).

It is well known that cellular organization has an important part in adjusting normal hematopoietic stem cell variation, care, and reproduction (Heissig et al. 2005). Although fetal hematopoiesis has a high self-renewal capacity, the hematopoietic system is one of the most responsive tissues to radiation. Besides, medium-dose radiation has been reported to damage hematopoietic progenitor cells (Velardi et al. 2018).

Patients undergoing imaging for diagnosis, medical staff working near the radioisotopes, and nuclear power plant workers are often exposed to ionizing radiation. Therefore, the evaluation of ionizing radiation is both a medical and a social issue. This study was intended to search for the impact

of 1 Gy radiation in embryo organ development and embryonic vitellus sac vessels in the period covering 9,5-11,5 days. As far as we know, this study is the first report showing the effect of radiation on embryo organ development and embryonic vitellus sac vessels in vivo and in vitro.

2. MATERİALS AND METHODS

2.1. Animals and Experimental Design

The study was approved by Erciyes University Animal Ethics Committee. Wistar albino female rats were acquired from Erciyes University Experiment Research Applicationand Research Center. All procedures were carried out in accordance with ethical rules. In the study, 16 female and 8 male Wistar albino rats were exploited. A male rat and two female rats that can be fertilized were placed in a cage at overnight and successful mating was confirmed by checking vaginal smears the next morning. Female with sperm in their vaginal smear became pregnant for 0.5 days and kept in a separate cage for 9 days (Hacıalioglulları and Ulger, 2010). So as to evaluate the impact of ionizing radiation in embryonic growth, rats considered to be pregnant were separated into two groups as the radiation and control group. The pregnant rats were maintained in plastic cages at 19-21ºC constant temperature and 12 h light/dark cycle environment during the research, in specially crafted standard laboratory conditions, and the air conditioner rooms.

2.2. Radiation Application

Rats determined to be pregnant for 8.5 days were irradiated in a special box of 28x24 cm length and 2.5 cm depth by using the Cobalt-60 teletherapy (GWXJ80-Co60 Teletherapy Unit) X-ray in Erciyes University Faculty of Medicine.To allow the rats to breathe during irradiation and to provide fixed SSD (source skin distance), the density of the top and bottom of the box was placed on the perforated tray. The physical calculation of the radiation dose was calculated as a single dose of 1 Gy from the two opposing and two posterior areas at an area of 28x24 and 2.5 cm depth. When calculating the irradiation time, the tray factor was considered.

2.3. The Culture Medium Preparation

The abdomens of the rats under anesthesia were cleaned with 70% alcohol. The skin was folded to the head level by making a V-shaped incision. The abdominal organs were pushed to one side and the abdominal aorta was made visible. With the help of the injector, approximately 8-10 ml of blood was collected. Blood was centrifuged at 3500 rpm for 5 minutes. The resulting serum was kept in the 56 °C water bath for 30 minutes for protein inactivation. Filtered through a 0.22 dum filter. Penicillin (100IU/ml) and streptomycin (100 µg / ml) were added to prevent contamination in serum (Hacıalioglulları and Ulger, 2010).

2.4. Whole Embryo Culture

The abdomen of 9.5-day-old pregnant rats were opened under anesthesia, then the embryos lined up in the uterus horn were transferred to a sterile petri dish with Hanks Balanced Salt Solution (HBSS) using a sterile forceps and scissors. All operations after this stage were carried out in Laminar-air cabin, under semi-sterile conditions, and under a stereomicroscope. Holding with the help of forceps, the uterine muscle was carefully cut along the antimesometric edge. The decidual tissue mass was separated and the embryo was made visible. Then, the Reicherts membrane surrounding the embryo was removed from the embryonal pole with the help of forceps (Figure 1/Figure 2). It was placed in a small sterile petri dish containing an embryo medium. Damaged embryos were not included in the study. Robust embryos were divided into groups of five and placed in 50 cc sterile glass culture bottles (1 embryo/1 ml serum) with a Pasteur pipette containing 5 ml normal rat serum.

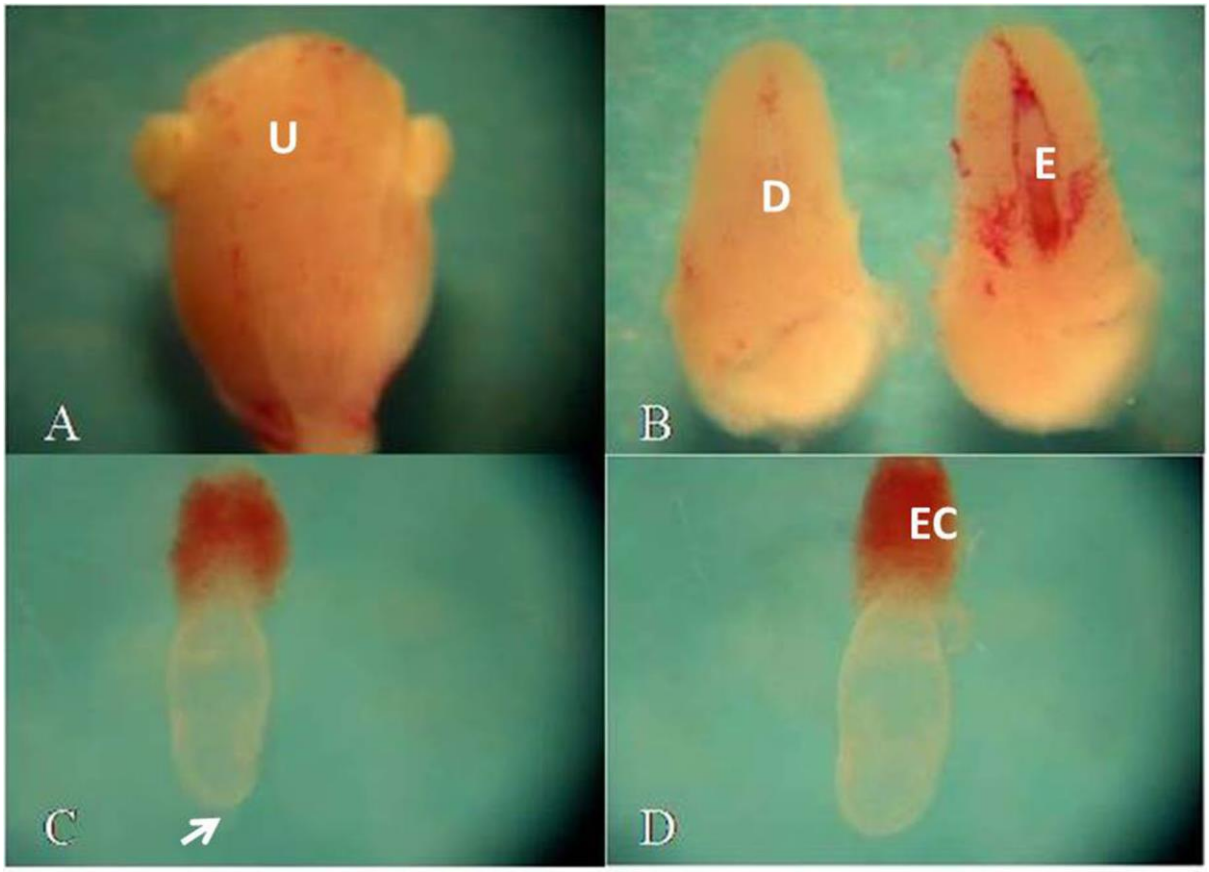

Figure 1: Removal of embryos from extraembryonic tissue. (A) Part of the uterus (U), (B) Decidua (D), Embryo (E), (C) Reichert's membrane (Arrow), (D) Reichert's membrane removed embryo (control group) (EC; Ectoplacental cone), Control group X2.

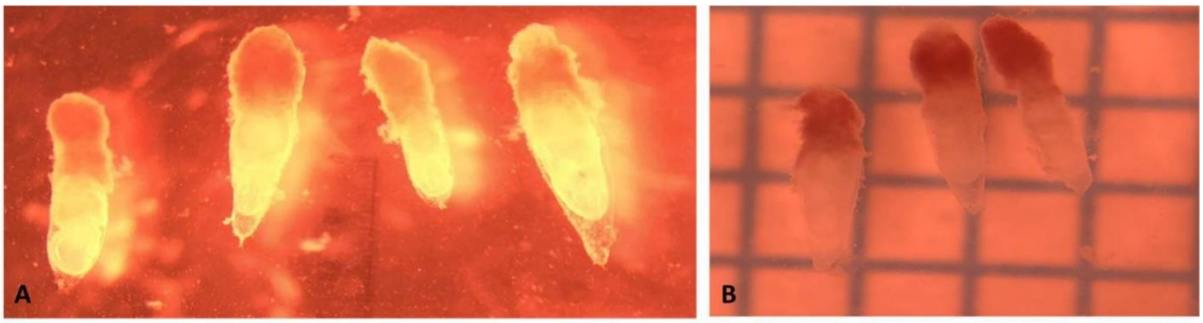

Figure 2: The appearance of 9.5-day embryos (A) Embryos belonging to the control group, (B) 1Gy radiation group embryos, X2.

2.4.1. Occurring The Experimental Groups

To evaluate the effect of ionizing radiation on total embryonic growth, embryos were divided into 2 groups (n = 10), control and radiation. The control and radiation group of embryos was cultured in whole rat serum.

2.5. Embryo Culture Process

The first gas mixture (5% O2, 5% CO2 and 90% N2) was given to the bottles with embryos for 1 minute. The bottles were closed with corks, and they were placed in a roller rotating at approximately 30 rpm in a 37°C incubator. After 24 hours from explantation, the culture bottles were removed from the incubator and corks were opened. Then, the second gas mixture (20% O2, 5% CO2 and 75% N2) was applied for 1 minute and the culture bottles were placed back in the incubator. 4 hours before the morphological scoring of embryos, the third gas mixture containing 40% O2, 5% CO2 and 55% N2 was given to bottles for 1 minute. The embryos grown in culture medium were transferred to petri dishes with HBSS solution. It were scored according to the

technique described. After the 48 hour culture period, embryos were examined under a dissecting microscope. Morphological scores, somite number, egg yolk diameter and crown-rump length data were analyzed statistically (Van Maele-Fabry et al. 1990).

2.6. Histological Analysis

Histological analyzes were carried out in the Department of Histology and Embryology at Erciyes University Medical School. 11.5 day embryos were fixed in 4% paraformaldehyde for 24 hours. Then, they were quickly frozen in liquid nitrogen, blocked and stored at -80°C. 7 µm sections were taken from the samples, stained with Hematoxylin-Eosin and examined under the Olympus BX51 microscope.

2.7. Immunofluorescen Staining

Immunofluorescence staining was used to find out the Willebrand factor (vWF, factor VIII related antigen) in the yolk sac of embryos in control and radiation groups. Embryo sections (7 µm) stored at -80°C were firstly dried at room temperature for 10 minutes. It was fixed in acetone for 10 minutes at -20°C. After the slides were washed 3 times with phosphate-buffered saline (PBS) solution for 5 minutes, they were incubated with normal goat serum for 20 minutes. Primary antibody vWF (1:100, ab9378, Abcam) was applied directly to the sections and the slides were incubated overnight at 4°C in a humidity chamber. After washing 3 times for 5 minutes in PBS the next day, the sections were incubated for 45 minutes with secondary antibody (1: 200; Jackson Immuno Research, Newmarket, UK). Cell nuclei were stained with DAPI (Sigma, St. Louis, USA) and closed with Fluoromount-G (Southern Biotechnologies, Birmingham, USA). Nuclear counterstaining was performed with DAPI (Sigma, St. Louis, USA) to stain the cell nuclei and mounted with Fluoromount-G (Southern Biotechnologies, Birmingham, USA). Primary antibodies were removed as a negative control for immunostaining assays. To obtain numbers for vWF was calculated by using Image J software at high power fields (×400). To get numbers for vWF-positive cells, the yolk sac per each region was analyzed.

2.8. Statistical Analysis

The analysis of the data was performed in TURCOSA (Turcosa Analytics Ltd Co, www.turcosa.com.tr) statistical software. Shapiro-Wilk test was used to determine the normality of continuous variables. In the analysis of variables that were not normally distributed, non-parametric Kruskal-Wallis and Mann-Whitney-U tests were used. Data were shown as median (Q1-Q2) and p-value below 0.05 were considered significant

3. RESULTS

3.1. Whole Embryo Culture

All embryos belonging to the control group were alive at the end of the 9,5-11,5 culture period. In addition to normal growth and development, no abnormalities were set downed macroscopically. Particularly, the yolk sac was vascularized and red blood cells were easily visible. The embryonic development of all groups was compared according to the morphological scoring system on day 11.5 (Table 1). All embryos exposed to radiation showed violent growth and development retardation in all embryonic drafts compared to the embryos in the control group. Radiation affected the embryos increased the retardations of embryonic growth and development. Besides these findings, there was also poor yolk sac vascularization, failure in the fusion of nerve folds, deficiency in embryonic flexion, delayed development of branches bar, maxillary and mandibular protrusions and limbs, as well as otic, optic and olfactory systems. In addition to; yolk sac diameter, somite numbers and crown-rump length in the embryos of the irradiated group diminished significantly when it was compared to the control group ($p < 0.05$) (Table 1).

The yolk sac diameter was 3.47 ± 0.20 mm in the control group, 1.83±0.16 in the radiation group. While in the control group, the mean crown-rump length was 3.21 ± 0.19 mm, in the radiation group it diminished gradually (1.22±0.08). In addition, the mean value of the somite numbers was 27.20 ± 1.81 in the control group and it was 6.20± 0.40 in the radiation group.

Whereas the yolk sac of embryos grown in the control group had an exactly developed yolk sac plexus of vessels, the yolk sac of embryos grown in radiation group had no vessels and only a form of blood islands was seen (Figure 3 A and C). Additionally, a decrease in neural tube formation was detected in the radiation group compared to the control group. At the same time, morphological analysis results demonstrated that all embryos growing in the radiation group had open neural tubes in both cranial and caudal regions, and the nervous system was less developed (Figure 3B and 3D). While the three-chambered heart of embryos grown in the control group was normal (two atrial, one ventricular), the heart development in embryos grown in radiation had not reached this level (Table 1). When all the data obtained were evaluated, it was revealed that exposure to 1 Gy radiation caused growth retardation in all embryos.

Table 1: Developmental characteristics of rat embryos exposed to radiation embryonic viability, morphometric parameters, total morphological scores and abnormalities.

	Median (Q_1-Q_2)	**Median (Q_1-Q_2)**	**p**
YSC	5(5,00-5,00)[a]	2(2,00-2,75)[b]	.001
ALLATOİS	3(3,00-3,00)[a]	1(1,00-1,00)[b]	.001
FLEKSİYON	4,5(4,00-5,00)[a]	2(1,25-2,00)[b]	.001
HEART	4(3,25-4,00)[a]	2(1,25-2,00)[b]	.001
CNT	4(4,00-4,75)[a]	1,5(1,00-2,00)[b]	.001
HB	4(4,00-4,75)[a]	2(2,00-2,00)[b]	.001
MB	4(4,00-4,75)[a]	2(2,00-2,00)[b]	.001
FB	4(4,00-4,75)[a]	2(2,00-2,00)[b]	.001
OTİC	4(4,00-4,75)[a]	2(2,00-2,00)[b]	.001
OPTİC	4(4,00-4,00)[a]	2(2,00-2,75)[b]	.001
OLFACTOR	2(2,00-2,00)[a]	1(1,00-1,00)[b]	.001
BB	2(2,00-2,00)[a]	1(1,00-2,00)[b]	.001
MAXİLLARP	2(2,00-2,00)[a]	1(1,00-1,75)[b]	.001
MANDİBULARP	2(2,00-2,00)[a]	1(1,00-1,00)[b]	.001
FL	2(2,00-2,00)[a]	1(1,00-1,00)[b]	.001
HL	1(1,00-1,00)[a]	1(1,00-1,00)[b]	.001
SOMİT	5(5,00-5,00)[a]	1(1,00-1,00)[b]	.001
SN	28(27,00-28,00)[a]	6(6,00-6,00)[b]	.001
TS	57,50(56,00-62,25)[a]	25,50(25,00-27,25)[b]	.001
MYSD	3,50(3,35-3,62)[a]	1,80(1,70-2,00)[b]	.001
CRL	3,20(3,07-3,40)[a]	1,20(1,175-1,30)[b]	.001
Immunofluoresence			
vWF+cell number	3(2,00-4,00)[a]	1,50(0,75-2,25)[b]	.001

The same letters on the same line refer to the similarities between the groups, and the different letters refer to the differences between the groups. **YSC:** Yolk sac circulation, **CNT:** Caudal neural tube, **HB:** Hindbrain, **MB:** Midbrain, **FB:** Forebrain, **BB:** Brancial bars, **MAXİLLARP:** Maxillar processes, **MANDİBULARP:** Mandibular processes **FL:** Forelimbs, **HL:** Hindlimbs, **SN:** Somit number, **TS:** Total score, **MSD:** Mean yolk sac diameter, **CRL:** crown-rump length

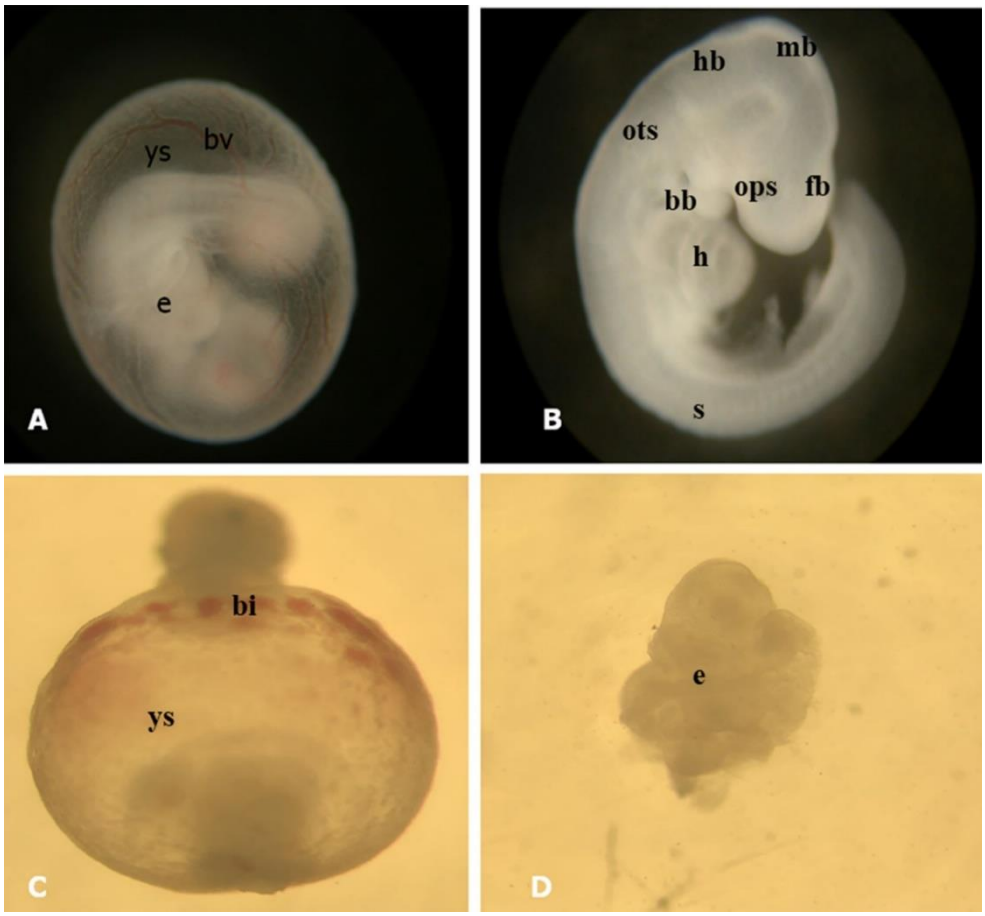

Figure 3: (A) 11.5-day control rat embryo and prominent vitellus sac, (B) Side view of the embryo belonging to the control group, (C) Radiation-exposed 11.5-day rat embryo, vitellus sac, (D) Radiation-exposed embryo side view. bv: blood vessel, ys: vitellus sac, e: embryo, bi: blood islands, fb: forebrain, mb: midbrain, hd: back brain, ots: otic system, ops: optic system, bb: branch bar, h: heart, s: somit.

3.2. Histological Findings

Embryos in the experimental group showed different features than those in the control group in histological examination. The vitellus sac of the embryos in the control group had a regular structure. The new cells formed with the onset of hematopoiesis caused a bold appearance of the vitellus sac wall. However, the vitellus sac of embryos in the radiation group was seen as an irregular and thin layer (Figure 4).

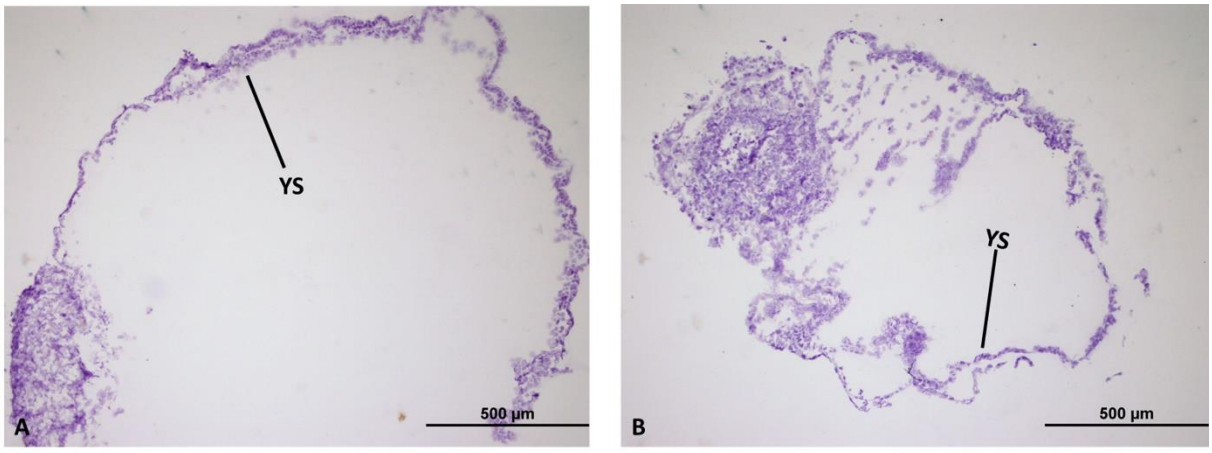

Figure 4: Light microscopic view of the yolk sac (YS) belonging to the Control (A) and Radiation (B) groups, H & EX10.

3.3. Immunofluorescent Findings

In this part of the study, vWF immunofluorescence staining in yolk sac was detected and vWF+ cell number in both control and radiation group of this antibody was calculated (Table 1, Figure 5). Histological examination approved the reduced presence of the vWF+ cell number in the yolk sac of embryos exposed to ionizing radiation. In summary, radiation affected vWF+ hematopoietic stem cells during vitellus sac hematopoiesis, thereby inducing anemia in rat embryos. A number of vWF+ cells in the control group were significantly higher than the 1 Gy radiation group ($p < 0.05$). The number of vWF + cells in the control group was significantly higher than that in the group exposed to 1 Gy radiation ($p < 0.05$)

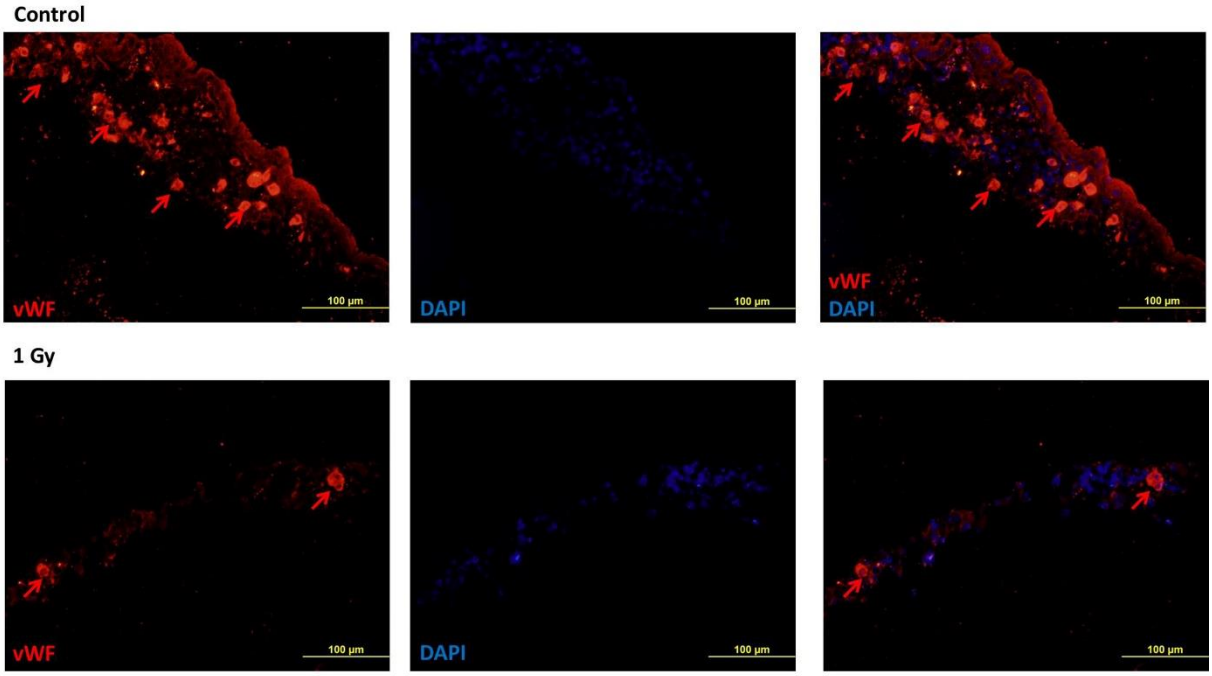

Figure 5: Immunofluorescence localization of vWF in the vitellus sac of the experimental group exposed to control and 1 Gy radiation (vWF immunoreactivity, x40).

4. DISCUSSION

Radiation and radioactive substances are widely used in medical research, diagnosis, cancer treatment and crucial for the diagnosis of diseases (Fendler et al. 2017). Radiation is applied for therapeutic techniques involving the anti-oncogenic treatment of different organs (Connell and Hellman, 2009). Potential biological effects of radiation exposure of a developing fetus include prenatal death, intrauterine growth restriction, organ malformation, and childhood cancer. The risk of each effect depends on the gestational age at the time of exposure, fetal cellular repair mechanisms, a dose of absorbed radiation (Mc Collough et al. 2007). When the fetal radiation dose is below 0.05 Gy, non-carcinogenic risks such as abortion and malformation can be ignored. However, it has been suggested that the minimum effect value of the central nervous system may be 0.06 – 0.3 Gy (Canman et al. 1998). Even with low dose irradiation ranging from 0.25 to 1 Gy, 1-cell embryos were found to have a dose-response relationship and increased the frequency of fatal events (Muller et al. 1994).

The embryo culture is a very useful technique for teratological studies of the rat embryo culture system. It is also used to detect morphogenetic events that occur during early organogenesis, especially in mammalian embryos (Torun et al. 2014). In this research, we assessed the effect of 1-Gy radiation on embryo organ development and embryonic vitellus sac hematopoietic stem cells in vitro embryo culture. We observed that 1-Gy exposure during the 9.5-11.5 days period of development will result in clinically significant fetal damage. There was a substantial decrease in the total morphological score such as mean morphological scores, vitellus sac diameter, head-aft length, and somite number in the radiation group. The yolk sac important source of stem cells for research due to the hematopoietic and mesenchymal cell niches that are present in this structure during the development of the embryo and participate in the formation of the first blood cells (Samokhvalov, 2014).

Blood islands are initially characterized by the maturation of primitive erythroid cells in the vitellus sac at E7.5. Absolute erythroid-myeloid precursors also occur in the vitellus sac at E8.5. Eventually, around E10.5 – E11.5, the first definitive hematopoietic stem cell can reconstruct the adult recipient's hematopoietic system is identified, and possibly these precursors are involved in lifelong blood production (Medvinsky et al. 2011). Hematopoiesis present as committed progenitors on the yolk sac cannot reconstitute the entire hematopoietic system. Multipoint hematopoietic stem cell takes form just before the establishment of the hematopoietic liver. It then expands and colonizes the

hepatic tissue and finally the newly formed bone marrow (Isern et al. 2008).

Exposure to ionizing irradiation results in not only lower numbers of hematopoietic stem cells, but also the induction of apoptosis. Accidental exposure to medium or high doses of radiation poses an important threat to human health, especially the hematopoietic system (Shao et al. 2014). Hematopoietic stem cells are primarily responsible for hematopoietic recovery. They are highly sensitive to ionizing radiation and are the most vulnerable system to the harmful impacts of total body irradiation (Coleman et al. 2004). Hematopoietic stem cell injury is the main cause of death after exposure to radiation (Shao et al. 2014). The dose range of ionizing radiation 1–7 Gy (human) is at risk of damaging the hematopoietic system in humans. This causes a decrease in blood cells and platelet count, an increase in susceptibility to infection and bleeding (Coleman et al. 2004). In addition, radiation doses beyond 3.5 Gy can cause bone marrow failure due to severe injury to hematopoietic stem cells, which can turn into long-term bone marrow damaged by complete ablation of hematopoietic stem cell reserves and functions (Waselenko et al. 2004). The interaction of hematopoietic and stromal cells is known to be critical for hematopoiesis; hematopoietic cells differentiate directly from the endothelium through a hematopoietic transition (Boisset et al. 2010). von Willebrand factor is a multimeric glycoprotein produced by endothelial cells that can be secreted outside the cell or stored in cytoplasmic Weibel-Palade bodies. The vWF is an adhesion molecule expressed on the surface of endothelial cells, as well as in migrating normal and metastasized malignant cells (Epperly et al. 2002). Endothelial cells line the internal lumen of blood vessel walls and can directly release proteins into the bloodstream. These cells play a role in different tissue system functions, such as blood pressure control, interactions with immune cells, and nutrient uptake (Khoo et al. 2017). It has been reported that endothelial cells from various tissue origins and types, including the human coronary artery, may experience aging and apoptosis in cells after exposure to moderate to high doses (Heo et al.2016; Dong et al. 2015). Radiation-induced endothelial cells exhibit a variety of senescence-like phenotypes. These include changes in cell morphology, permanent cell cycle arrest, and elevated expression of p16 and p21. The cells are also defective in angiogenesis have reduced the ability to sprout and migrate (Imaizumi et al. 2010). Cellular aging and induction of apoptosis lead to the growth of genetically imbalanced cells, critically shortened telomeres or genetic instability and cell transformation, leading to permanent DNA damage. More importantly, increasing evidence suggests that stimulating cellular aging can stimulate the immune system to quickly eliminate these cells (Hoenicke et al. 2012; Kang et al. 2011). Due to these properties, induction of old age and apoptosis is an important function of radiation in cancer treatment.

5. CONCLUSION

In our study, the number of cells showing vWF + expression was also decreased. We can say that this decrease is caused by aging and apoptosis in cells exposed to radiation. In this study, we show that ionizing irradiation decreases the vWF + cell number in the yolk sac of the radiation group. The findings of this study are important to figure out the embryo development mechanism after 1-Gy dose irradiation.

6. REFERENCES

Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. (2010) In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. Nature. 464:116–120. doi:10.1038/nature08764.

Brent RL, Mettler FA. (2004) Pregnancy policy. AJR. Am. J. Roentgenol. 182:819-822. doi:10.2214/ajr.182.3.1820819.

Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K et al. (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science. 281:1677–1679. doi:10.1126/science.281.5383.1677.

Coleman CN, Stone HB, Moulder JE, Pellmar TC. (2004) Medicine. Modulation of radiation injury. Science. 304:693–694. doi:10.1126/science.1095956.

Connell PP, Hellman S. (2009) Advances in radiotherapy and implications for the next century: a historical perspective. Cancer. Res. 69:383–392. doi:10.1158/0008-5472.CAN-07-6871.

Dong X, Tong F, Qian C, Zhang R, Dong J, Wu G et al. (2015) NEMO modulates radiation-induced endothelial senescence of human umbilical veins through NF-kappaB signal pathway. Radiat. Res. 183:82–93. doi:10.1667/RR13682.1.

Epperly MW, Sikora CA, Defilippi SJ, Gretton JE, Bar-Sagi D, Carlos T et al. (2002) Pulmonary irradiation-induced expression of VCAM-1 and ICAM-1 is decreased by MnSOD-PL gene therapy. Biol. Blood Bone Marrow Transplant. 8:175–187. doi:10.1053/bbmt.2002.v8.pm12014807.

Fendler W, Malachowska B, Meghani K, Konstantinopoulos PA, Guha C, Singh VK et al. (2017) Evolutionarily conserved serum microRNAs predict radiation induced fatality in nonhuman primates. Sci. Transl. Med. 9:1-21. doi:10.1126/scitranslmed.aal2408.

Hacıalioguları M, Ulger H. (2010) Embryo cultur tecnic. Journal of Health Sciences. 19:216-225.

Heissig B, Ohki Y, Sato Y, Rafii S, Werb Z, Hattori K. (2005) A role for niches in hematopoietic cell development. Hematology. 10:247-253. doi:10.1080/10245330500067249.

Heo JI, Kim W, Choi KJ, Bae S, Jeong JH, Kim KS. (2016) XIA-Passociating factor 1, a transcriptional target of BRD7,

contributes to endothelial cell senescence. Oncotarget. 7:5118–5130. doi:10.18632/oncotarget.6962.

Hoenicke L, Zender L. (2012) Immune surveillance of senescent cells biological significance in cancer- and non-cancer pathologies. Carcinogenesis. 33:1123–1126. doi:10.1093/carcin/bgs124.

Hyttel P, Sinowatz F, Vejlsted M. (2009) Essentials of Domestic Animal Embryology. Elsevier Health Sciences: Edinburgh, London, UK. p.182-208.

Imaizumi N, Monnier Y, Hegi M, Mirimanoff RO, Ruegg C. (2010) Radiotherapy suppresses angiogenesis in mice through TGFbetaRI/ALK5-dependent inhibition of endothelial cell sprouting. PLoS One. 5:e11084. doi:10.1371/journal.pone.0011084.

Isern J, Fraser ST, He Z, Baron MH. (2008) The fetal liver is a niche for maturation of primitive erythroid cells. PNAS. 105:6662-6667. doi:10.1073/pnas.0802032105.

Kang TW, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D et al. (2011). Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature. 479:547–51. doi:10.1038/nature10599.

Khoo CP, Roubelakis MG, Schrader JB, Tsaknakis G, Konietzny R, Kessler B et al. (2017). miR-193a-3p interaction with HMGB1 downregulates human endothelial cell proliferation and migration. Sci. Rep. 7:44137–51. doi:10.1038/srep44137.

Mc Collough CH, Schueler BA, Atwell TD, Braun NN, Regner DM, Brown DL et al. (2007). Radiation exposure and pregnancy: when should we be concerned? Radiographics. 27:909-17. doi:10.1148/rg.274065149.

Medvinsky, A., S. Rybtsov, and S. Taoudi. 2011. Embryonic origin of the adult hematopoietic system: advances and questions. Development. 138:1017–1031. doi:10.1242/dev.040998.

Muller WU, Streffer C, Pampfer S. (1994) The question of threshold doses for radiation damage: malformations induced by radiation exposure of unicellular or multicellular preimplantation stages of the mouse. Radiat Environ Biophys. 33:63–68. doi:10.1007/BF01255274.

Samokhvalov IM. (2014) Deconvoluting the ontogeny of hematopoietic stem cells. Cell Mol. Life Sci. 71:957–978. doi:10.1007/s00018-013-1364-7.

Shao L, Luo Y, Zhou D. (2014) Hematopoietic stem cell injury induced by ionizing radiation. Antioxid. Redox. Signal. 20:1447–462. doi:10.1089/ars.2013.5635.

Shimura, T. (2011) Acquired radioresistance of cancer and the AKT/GSK3beta/cyclin D1overexpression cycle. Journal of Radiation Research. 52:539–544. doi:10.1269/jrr.11098.

Tavassoli M. (1991). Embryonic and fetal hemopoiesis: an overview. Blood Cells. 17:269-281.

Torun YA, Ozdemir MA, Ulger H, Nisari M, Akalın H, Patıroglu T et al. (2014) Erythropoietin improves brain development in short-term hypoxia in rat embryo cultures. Brain & Development. 36:1-6. doi:10.1016/j.braindev.2014.01.005.

Van Maele-Fabry G, Delhaise GF, Picard JJ. (1990). Morphogenesis and quantification of the development of post-implantation mouse embryos in vitro. Toxicol. 4:149-156. doi: 10.1016/0887-2333(90)90037-t.

Velardi E, Tsai JJ, Radtke S, Cooper K, Argyropoulos KV, Jae-Hung S et al. (2018) Suppression of luteinizing hormone enhances HSC recovery after hematopoietic injury. Nat Med. 24:239–246. doi:10.1038/nm.4470. Epub 2018 Jan 8.

Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley ALDickerson WE et al. (2004) Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Ann. Intern. Med. 140:1037–1051. doi: 10.7326/0003-4819-140-12-200406150-00015.

Wenceslau CV, Miglino MA, Martins DS, Ambrósio CE, Lizier NF, Pignatari GC et al. (2011) Mesenchymal progenitor cells from canine fetal tissues: yolk sac, liver, and bone marrow. Tissue Eng. Part A. 17:2165–2176. doi:10.1089/ten.TEA.2010.0678.