Original Investigations

The immunohistochemical and histologic effects of contrast medium on uterus, fallopian tubes and ovaries, given during hysterosalpingography: rat study
Pek et al. Effects of hysterosalpingography on internal genital tract

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Abstract
Objective: We aimed to show that endometrial and tubes epithelium suffered free radical damage during HSG in line with previously made many studies on different systems.
Material and Methods: A total of forty rats were evaluated in five different groups. Only ionized radiation was applied to the two groups. In another two groups, iohexol was applied together with ionizing radiation. One group was determined as the control group. Groups were evaluated after seven and forty -two days. Inflammation and cellular changes were evaluated histopathologically. Cellular activities of antioxidant enzymes were measured immunohistochemically.
Results: Ionizing radiation and iohexol had serious negative effects on endometrium and tubes in both periods, especially in the late period.
Although there is a lot of research done before, there is no definitive method yet to protect against the harmful effects of iodinated contrast agents and ionizing radiation.
Conclusion: New methods need to be explored to protect cells and tissues from reactive oxygen radical damage caused by HSG.
Keywords: Free oxygen radicals, hysterosalpingography, iohexol

Introduction
Infertility is a condition of the reproductive system that prevents the conception of children. The diagnosis of infertility is usually given to couples who have been attempting to conceive for at least 1 year without success. (1,2) It is estimated to affect between 8 and 12% of reproductive-aged couples worldwide. In female infertility, approximately 30% to 40% of

cases involve ovulatory dysfunction, and 30% to 40% involve tubal and pelvic pathology; 30% of cases are attributed to other unexplained causes, of which reproductive age may be an important contributor. (3) Therefore, evaluation of tubal patency and uterine cavity is very important for treatment. Hysterosalpingography (HSG) is one of the oldest techniques (since 1914) used for tubal patency testing. It should be quoted that this technique has limited accuracy (positive predictive value <38%), and it is currently replaced by specific ultrasound procedures, especially with air/saline, foam, and Doppler. (4) However, in many places, it is still a basic method and recommended as the first-line diagnostic tool because it does not require highly qualified practitioners. (5,6,7) As can be seen from previous studies, it is clear that the rapidly dividing cells of the reproductive system will be damaged during HSG. (8,9) There are many researches on reproductive cells to reduce the cellular damage caused by HSG. Of course, there are studies on other systems to reduce the negative effects of ionizing radiation and iodinated contrast media. (10,11,12,13) However, any method that can provide protection from them has not been reported yet. In previous studies, early and late cellular effects of iohexol and ionizing radiation on the uterus, tubes and ovaries have not been clarified. Considering that hysterosalpingography is a diagnostic method used for women with low reproductive capacity, we aimed investigate the effects to HSG on endometrium, tubes and ovary epithelial cells.

Materials and Methods

This study was performed in the Laboratory Center for Experimental Studies of Çanakkale Onsekiz Mart University with the approval of the University' s Laboratory Animals Ethics Committee (2016/01-02).

Animals: Forty Wistar albino, 12- 14 week-old female rats with regular cycles and weighing 250- 300 g were kept under a 12-hour artificial light- dark cycle at a temperature of 20-24 ^{o}C, in groups of five per cage, and were fed with standard pellets and tap water. All rats in the estrous cycle (estrus phases of rats were confirmed with vaginal cytology) were randomly divided in to five (n=8) following groups. The number of rats in each group was set as the minimum number that could be statistically significant to prevent animal wastage.

Chemicals: Clinical substances were obtained from GeneTex glutathione S-transferase pi 1 antibody GSTP1 GTX31766-100, GeneTex glutathione reductase antibody N2C2 GTX114199-100, Novusbio superoxide dismutase antibody SOD1 NBP224915 and Novusbio catalase antibody CAT NBP2-24916. DAKO automatic dyeing machine was used for immunohistochemical staining. Iohexol (Omnipaque 350 mg/100 mL) was used as radio-contrast medium.

Experimental design: The procedure steps were performed as shown in Table 1.

Surgical, Radiation and Iohexol Application Procedure: 400 mg/kg/intraperitoneal dose of chloral hydrate was administered to the rats for provide to the anesthesia. (8) First day, after skin cleaning the rats with 10% batticone, a midline insicion (approximately 2 cm) was made to access the abdominal cavity (performed to all groups) and than, eight rats were directly sacrificed, and the uterus, tubes, and ovaries were removed (group A). After then, 1 - 2 ml of iohexol was injected via an injector from their cervix to groups C and E rats (figure 1A) and next, except for group A, other groups were exposed to radiation (figure 1B and 1C). All-body irradiation was applied at a dose of 15–20 miliRad three times with 3-min intervals to the rats after opening of the abdomen (in group B and D). In group C and E, the first dose radiation was given while iohexol was injected. The other two doses were given by the same procedure. The abdomens of the rats in all groups were closed continuously using absorbable suture materials (4.0 vicryl-rapide) after surgical procedures have been completed. For the evaluation of acute (early) effects seven days later laparotomy was performed again to group

B and C and similarly, group D and E were relaparatomized forty-two days later for the evaluation of chronic (late) effects and the uterus, tubes and ovaries were removed.
Preparation for pathological and immunohistochemical evaluation: Two different sections with a thickness of 3 -5 microns were prepared from all tissues removed. The pathological materials were preserved in 10% formaldehyde and fixed. One group of tissue sections were stained with hematoxylin and eosin for histopathological evaluation under the light microscope. The other group of tissues were embedded in paraffin and 4 –5 mm-thick sections were taken for examined and were stained with glutathione S-transferase, glutathione reductase, superoxide dismutase and catalase with immunohistochemically.
Histopathological scoring: Vascular ectasia, inflammation, epithelial cytological and architectural features were evaluated and scored with using objective criteria by the same pathologist. (8,14,15) Epithelial architectural features; (a) tafting, (b) stratification, (c) chromatin disorganization, (d) irregularity in nucleus contour, (e) increases in nucleus size and ratio of nucleus/cytoplasm, (f) pleomorphism, (g) presence of nucleoli, (h) mitosis and (i) hyperchromasia. All the criteria evaluated except inflammation and vascular ectasia were reported as cellular changes. A minimum of five fields were evaluated of each tissue slide with 40 and 400 magnification and assigned scores for severity of changes as follows: no effect or no staining (0), mild effect or poor staining in localized areas (1), the presence of moderate influence or moderate staining (2) and severe effect or strong staining (3). The other sections which were prepared for immunohistopathological assessment (glutathione S-transferase, glutathione reductase, superoxide dismutase, and catalase) again evaluated by the same pathologist and the same scoring system.

Statistical Analysis
For statistical analysis, we used SPSS v. 20.0. Results are presented as means ± standard deviation. Significance was accepted at $p < 0.05$. Statistically, the Kruskal– Wallis H-Test was used to determine whether mean differences were significant in terms of group variables, and Mann–Whitney U test was used to determine the group from which the differences originated.

Iohexol, Ionizing Radiation and Measured antioxidant enzymes
Understanding the mechanism of damage caused by ionizing radiation and iodinated contrast media to on the cells and tissues will be useful here. First, let's talk about iodinated contrast agents. We used the iohexol as iodinated contrast medium in our study. Iohexsol is a water-soluble, non-ionic, monomeric and low-osmolarity iodinated contrast media. It may show direct cellular toxicity. Or it may cause indirect toxic effects by causing the formation of reactive oxygen radicals. It performs this effect by causing the release of vasoconstrictor substances such as adenosine, endothelin, vazopressin, angiotensin 2 and dopamine. The result is the release of hypoxia and free oxygen radicals. (16,17) Especially osmolarity is thought to be an important factor in these effects. However, osmolarity alone is insufficient in explaining this situation. Because the osmolarity of mannitol is similar to some iodinated contrast agents, but the effects are not the same. (18) Can the question to be considered at this stage be protected from this effect by the use of antioxidants and vasodilating agents? Many studies have been carried out for this question's answer. Melatonin, L-carnitine, vitamin C, vitamin E, amifostine, amlodipine, curcumin, N-acetylcysteine and trichloroacetic acid have been tried many times in this regard. (8,9,10,11,15,19,20,21,22,23,24) On the other hand, the effects of radiation on living things are examined in 4 stages. In the first step, energy is transferred to the substance. This stage is known as the physical step and causes ionization of the substance. The products that appear after the first stage are unstable and cause reactive products. This stage is the physico-chemical stage. In the chemical stage, which is the third

stage, reactive products react with cellular structures. The result is free radicals. The biological step is the final stage and starts with enzyme reactions that cause various damage. Meanwhile, DNA molecule damages. However, some of them can be repaired. Damages that cannot be repaired lead to cell death. (25) Physico-chemical changes caused by ionizing radiation in the cell take less than a second. On the other hand, it may take hours, days, months or even years for biological results to occur. Ionizing radiation causes the breaking of chemical bonds in intracellular molecules, especially chromosomes. If these genetic damages, called mutations, are not corrected by repair mechanisms, they lead the cell to death. However, if there is no cell death, they cause cancer years later. The effects seen through energy absorption were direct effects. On the other hand, there are indirect effects that occur through the formation of free oxygen radicals. (26,27) Water molecules be an ionize when the cell is exposed to radiation. A positively charged water molecule and free electrons are formed. Free electrons combine with another water molecule to form a negatively charged water molecule. Positive and negative water molecules are unstable and break down to form ions and free radicals. (28,29,30) Reproductive ability is faster in cells (such as genital system and reproductive cells), which is more sensitive to radiation. Cells are most susceptible to cell death during the G2 stage and mitosis.(31,32) The first response to oxidative stress damage occurring in the cell is given by antioxidant enzymes. In this context, the most important enzymes are known to be glutathione peroxidase, superoxide dismutase, catalase and glutathione reductase. Another non-enzymatic defense line consists of antioxidant compounds such as vitamin E, vitamin C, beta carotene, transferrin, ceruloplasmin, haptoglobin and albumin. (33,34) The most important activity belongs first to the superoxide dismutase enzyme for the breakdown of superoxides. When superoxides are broken down, hydrogen peroxide is formed. (35) The hydrogen peroxide formed continues to break down with catalase. (36) During these events, glutathione s-transferases act as catalysts. (37,38) Glutathione reductase is an enzyme that converts oxidized glutathione (GSSG), which occurs during reactions catalyzed by glutathione S-transferase, to reduced glutathione (GSH). (39) Therefore, in our study, we aimed to measure the activity of these enzymes in the cell immunohistochemically.

Results

Considerable cellular and histopathological changes were observed for all criteria compared to the control group. First, the normal glandular and columnar epithelium sections of the control group were examined. Figure 1D shows a normal section from the control group that evaluated without any procedures were performed. The entire assessment was carried out by the same pathologist. The status of the basal metabolic activity of a normal cell was evaluated immunohistochemically and histopathologically. This group, where the evaluation took place, was called the control group. For this basal metabolic condition, '0' score was given corresponding to the 'no effect or no staining' condition.

Inflammation: Inflammation was detected intensely in all groups and the statistically significant difference was found compared to the control group ($p=0.000 < 0.05$). Ionizing radiation -induced inflammation was more evident in acute phase (group B) than in chronic phase (group D). When iohexol was added to the ionizing radiation, inflammation was increased. But the inflammation was stronger in acute phase (group C and B). (figure 2A,2B and 3A,3B)

Vascular Ectasia: Statistically significant difference was found ($p=0.009 < 0.05$). Vascular Ectasia was more evident in group C. (figure 3A, 3B).

Celluar Changes: When compared to the control group, serious changes were observed in the structure of the cell in all groups ($p=0.000 < 0.05$). The most severe period is the chronic phase. The most striking result obtained when each group is evaluated separately is that

iohexol increases the effect power of ionizing radiation. The fact that the changes observed in the C group are more severe than the D group, shows this. (figure 2A, 2B)

Immunohistochemical evaluation results: The activity of all antioxidant enzymes that function in defense against free oxygen radicals has been measured higher in the chronic period. And of course their activities were much more in groups where ionizing radiation was applied together with iohexol.

- Glutathione Reductase: When compared with the control group, a statistically significant difference was found all groups ($p=0.000 < 0.05$). (figure 4A, 4B)

- Catalase: For catalase activity, we found the statistically significant differences compared to the control group ($p=0.000 < 0.05$). (figure 5A)

- Superoxide dismutase: Superoxide dismutase activity was more intense in all study groups rather than control group ($p=0.000 < 0.05$). (figure 5B)

- Glutathione S-transferase: When all groups were compared with the control group, the statistically significant difference was found for glutathione S-transferase activity ($p=0.000 < 0.05$). (figures 6A, 6B)

Interpretation of Results: It should not be surprising that the group in which inflammation is the least monitored is the group E. The first response of the cell to trauma is of course inflammation among the parameters which we evaluated. At the end of the inflammatory process, the cell will either rescue itself, either drift into apoptosis and commit suicide in a controlled manner, or enter a necrotic uncontrolled process. Groups E and D were testing chronic periods in our study. Therefore, the expected result is more severe monitoring of these inflammatory process in groups C and B. This situation is also seen in our study. It is not easy to explain vascular ectasia as much as to explain inflammation. Vascular ectasia was observed more dense in the acute period just like inflammation. This can be seen from the ranking. Indeed, the following conclusion can be drawn here. Vascular ectasia and congestion are a natural part of the inflammatory process. This may be partially true. But the group D, in which the late period is evaluated, spoils the ranking.And we cannot explain this situation. As can be seen from Table 2, enzymatic activities that develop against free oxygen radicals are more intense in the late (chronic) period. And the use of iohexol is an additive factor to the formation of free oxygen radicals caused by ionizing radiation. As a result of all this, it is natural to see the destructive effects (cellular changes) observed in the cell, in late period and especially in groups where ionizing radiation is applied together with iohexol. These results proved once again that HSG, which is used in infertility, is not an innocent method. Each parameter taken into evaluated and their results are shown in Table 2 in detail.

Discussion

Hysterosalpingography, which should be done in the follicular phase of the cycle, evaluates the contour of the uterine cavity, cervical canal, and tubal lumina. It is one of the basic tool for infertility diagnosis and has become standard test for evaluation of infertility worldwide. This procedure is also useful for evaluation Mullerian system anomalies, recurrent pregnancy losses, abnormal uterine bleeding or amenorrhea and cervical insufficiency. A thin tube is threaded through the vagina and cervix and a substance known as contrast material is injected into the uterus. A series of X-rays, or fluoroscopy, follows the dye, which appears white on X-ray, as it moves into the uterus and then into the tubes. If there is an abnormality in the shape of the uterus, it will be outlined. But the short- and long-term effects of ionizing radiation and contrast medium on tissues are not known. The aim of this study was to examine the effects of iohexol and ionizing radiation induced cellular injury in reproductive tissue during hysterosalpingography.

The ionizing radiation imaging methods play an important role in the early diagnosis and treatment of diseases. In diagnosis and treatment, there is the possibility of radiation-induced

damage to the patient despite the radiation dose being kept as low as possible and radiation precautions being taken. (40) There is no realistic prediction of the diversity and size of health problems that will occur in living organisms at low doses (≤10 cGy) of ionizing radiation. In this respect, the low-dose ionizing radiation should not be viewed as safe or tolerable under any circumstances. Because, due to radiation independent of the dose, somatic mutations may develop that may lead to neoplastic and non-neoplastic diseases. (41) Ionizing radiation may directly and / or indirectly produce various effects on the DNA by reactive free radical production. (42,43,44) The effects of ionizing radiation can occur in two different ways as sitochastic and deterministic. Sitochastic effects are independent of the exposure dose. It may even occur at very low doses. (40) These types of damage are important for after the HSG. In addition, the presence of many factors such as the total radiation dose received, the defense mechanisms demonstrated by the organism,the dose taken in each session and the duration of exposure, simultaneous chemicals, and other factors that may lead to proto-oncogen activation increases ionizing radiation-related damage. When cells are exposed to ionizing radiation during mitosis and G0-G1 phases, the frequency of unstable dicentric chromosomes increases and chromosome aberrations may occur due to incorrect regulation of chromosomal fragments. (40,45,46,47) In this respect, the low-dose ionizing radiation should not be viewed as safe or tolerable under any circumstances. Because, due to radiation independent of the dose, somatic mutations may develop that may lead to neoplastic and non-neoplastic diseases. DNA alterations and breaking to double-strand occur in cells exposed to ionizing radiation. Activation of phosphorylase and kinases prevent DNA repair, and consequently G1, S, G2 cell cycles cannot proceed, leading to cellular death models including apoptosis, mitotic catastrophy, and terminal binding. (48) HSG is done in the proliferative phase of the cycle in order to sure that woman is not pregnant when the procedure is performed. So, it is a clear fact that basal cells which will start mitosis, will be more affected by radiation, during this process.

Analysis methods at the cell and tissue level are very important to explain the possible early and late effects of radiation on different tissues. The total radiation exposure to the patient during HSG withdrawal was calculated as 713 cGy/cm² (range: 247–1.623 cGy/cm²) by Fernandez et al. (49) In another study, the average radiation dose exposure of the reproductive organs during an HSG procedure was found to be 500 -1000 mRad. (50) An avarage human being has 17,000-20,000 cm² surface area and a rat that weighs 250-300 g has 300-400 cm² average surface area. In line with these previous studies, we determined the dose that should be applied to rats as 15- 20 mRad. (9) The development of the first follicular wave in the rodents to the antral follicle occurs in about 3 weeks. The developmental stage of primordial follicle to secondary follicular may take >30 days. (51) Well-developed secondary follicles are observed on the seventh day. (52,53)

Pala et al showed that the HSG treatment caused a significant increase in epithelial degeneration in the rat endometrium at 3 hours after HSG withdrawal. (9) Lee et al investigated the primary and primordial follicular damage after exposure to gamma radiation in rats and found that the most significant damage occurred after 3 hours with a reduction after 6–12 hours following radiation exposure. (54) But, Can et al have chosed a period of 3 hours to examine possible radiation damage in the early period and a 1-month period to investigate possible late period radiation damage. (19) Our aim here was to go further than previous studies. For this reason, we have chosen a period of seven day to examine possible ionizing radiation and contrast medium damage in the early period and a six-week period to investigate possible late period ionizing radiation and contrast medium damage. To our knowledge, no other study on tubes and endometrium cells has previously reported such pronounced changes in cellular effects for iohexol and ionizing radiation. However, for the first time, this is an experimental model of HSG process (separately of iohexol and ionized

radiation) in which the effects on ovarian, tubes and endometrium histopathology are studied, and this is the first pilot study in this area. So, this is the strength of our study.
Although, there have been previous studies investigating the damage caused by iodine contrast medium on cells and tissues (esp. renal tubuler system), there is no study that clearly shows its effects on the female internal genital system. Therefore, the effects of iohexol are not as clear as ionizing radiation. Our current knowledge is only as limited as research done on other systems. Solomon et al have investigated the mechanism of action of iodinated contrast agents on the renal tubular system and reported the results as that; the mechanisms responsible for the pathogenesis of contrast induced nephrophaty are thought to be a combination of the direct tubular toxicity of contrast media, reduction in medullary blood flow, and generation of reactive oxygen species (ROS), in which ROS play a central role. (55) ROS can cause vascular endothelial injury and may further intensify tissue parenchymal hypoxia by causing endothelial dysfunction and dysregulation of membrane transport. (56,57,58) Iohexol has high viscosity and osmolality among the LOCMs. Viscosity and osmolality are important and both contribute to cell and tissue-toxicity. Iohexol decreases extracellular volume contraction. The direct vasoconstrictor effects of iohexol and further exacerbation of ischemia are significant because the vasoconstrictor hormones (e.g., rennin, endothelin, and adenosine) increase and the vasodilator hormones (e.g., prostaglandin and nitric oxide) decrease. (59)
Iohexol is a non-ionic monomeric iodinated contrast medium. Heinrich et al compared different contrast media with the MTT assay, and when ICM were compared at equal iodine concentrations (75 mg I/mL), they found that dimeric contrast media showed a slightly weaker effect on inhibition of mitochondrial dehydrogenases than monomers, but this difference was not statistically significant. In the same study, the contrast media were also compared at molar basis and it was shown that dimeric ICM were significantly more cytotoxic than monomers on cultured renal cells. (60) Carlisle et al. exposed the embryonal cancer cells to iohexol, iopamidol and metrizamide at concentrations below those used for clinical myelography. And they had examined by light and electron microscopy the results. In their study, cytologic changes consisting of swelling and vacuolation of mitochondria and other cytoplasmic organelles were observed within 1 hours of exposure to the contrast media.They observed that after 12 hours, changes in shape and cell death in the cells. And they followed the same study in neuron cultures derived from embryonic stem cells and rat dorsal stem ganglion cell cultures. They reported as a result indicate that iohexol and other iodine contrast medium are cytotoxic to cells in culture at less than 20% of the concentration used for myelography and this could contribute to the adverse reactions to myelography seen in people and animals. (61) Jensen et al. in their studies with water soluble iodinated contrast agents, the iso-osmolal contrast medium iodixanol was found to be less toxic than the iohexol in cultured cells of rat proximal tubule origin. (62) It may be thought that using iodixanol during HSG may be more beneficial with the results of these three studies that we exemplified, but it should not be forgotten that iodixanol is in dimeric form iodinated contrast medium. Berg at showed that iohexol, iodixanol, ioxaglate and diatrizoate (contrast medium from all classes of iodinated X-ray contrast media) all possess antioxidant properties in vitro. The reason for this is unknown, but it is possible, although speculative, that the antioxidant properties of the iodinated contrast medium may contribute to the lower cell death at early time points. (63) The results of our study likewise show that all effects, except inflammation, are more severe in the late period. In this regard, it will be useful to do more research.
After this stage, it will not be beneficial to discuss the results of our study separately as ionized radiation or iodinated contrast agents. As we have already mentioned, it is important in that it is the first study to evaluate both factors separately. Previous studies have either evaluated ionizing radiation alone or iodinated contrast media (on the other cell and tissue

systems). Also in a limited number of studies evaluated together, cells and tissues of the entire internal genital system were not evaluated. As in the studies that Can et al evaluated ovaries. (19)

Our results were similar to studies in which ionized radiation was evaluated alone, and ionized radiation together with iodinated contrast agents was evaluated in a particular part of the female genital system, or tissues of other systems. As seen from all studies, both ionized radiation and iodinated contrast agents cause harmful effects at the cellular level. The results we obtained in our study were in this direction. The point we want to draw attention to is that all cellular changes, except inflammation and increased vascularization following inflammation, are more and more severe in the late period. And again, cellular changes and the increase of reactive oxygen radicals in the cell are always more severe in combination with ionizing radiation and iohexol. The question that needs to be considered here is the necessity of finding methods to protect the reproductive cells of infertile-subfertile women, or to ensure minimal damage during hysterosalpingography. For this, antioxidant substances can be applied during the process as in many studies where ionizing radiation and iodinated contrast agent are used together. This stands as an option. Pala et al investigated vitamins C and E for the prevention of endometrial cell damage induced by hysterosalpingography and presented them as an option. (9) Yılmaz et al also contributed to the literature with their studies that pre-HSG melatonin use can protect on ovarian surface epithelium. (20) Gülle et al proposed different approaches with two different studies. They reported that L-carnitine or Curcumin may be beneficial to protect against the negative effects of ionizing radiation on ovaries. (21,22) Can et al used amifostine to prevent ovarian damage caused by ionizing radiation, and announced it as an option. (19) Yurut-Caloglu et al compared of the protective roles of L-carnitine and amifostine against radiation-induced acute ovarian damage, on this subject. (23) Sapmaz et al examined of the effect of trichloroacetic acid attachment and instillation methods on dysplastic changes in ovarian surface epithelium. (15) However, it was reported that more research and studies should be done in all these studies. Kılcıksız et al supported the researches on this subject with a study using N-acetylcysteine to prevent the negative effects of ionizing radiation and oxidative stress. (11) Karaman et al reported that as a novel approach, agomelatine can be used to prevent nephrotoxicity caused by the use of iodinated contrast media. (10) Duan et al investigated the protective effect of amlodipine to nephrotoxicity of high- and low-osmolar contrast media. (24) Also, researches have been made on the urinary system with the use of theophylline, sodium bicarbonate or similar materials. It can be thought that the results of these studies related to the urinary system of the same embryological origin may be valid in the genital system. (64) This may be partly true in a way. But, to our knowledge, no proven benefit has been found for the use of other renal protective agents such as N-acetylcysteine, sodium bicarbonate, diuretics, and theophylline. (65) Sapmaz and colleagues used lipiodol (iodinated ethyl esters of fatty acids of poppyseed oil) in their studies as a different approach. And they reported the results of their work as follows; lipiodol significantly reduces dysplastic modifications and increases fusiform structures in the myometrium. Lipiodol plus melatonin restore all the negative changes. (8) But lipiodol is a water-insoluble iodinated contrast media. It is possible to use both oil and water soluble contrast media during HSG. There are a few differences between the two contrast agents in the evaluation of intra-uterine pathology and in the evaluation of the tubal patency. (66) Lipiodol contains mostly linoleic acid and omega series of polyunsaturated fatty and it is a potent antioxidant that performs many positive events in the body. (67,68) Considering the results of this study, it can be thought that the use of lipiodol may be reasonable. But, water-soluble contrast agents are associated with decreased complications and better radiographic quality as compared to the lipo-soluble contrast media. (69,70,71) For this reason, the hydro-soluble media achieved widely used with HSG. As a result, it is clear

that a reliable method should be found. This is also evident in previous studies which were done to protect the cell from the negative effects of both ionizing radiation and iodinated contrast media. This situation can be seen once again when all the negative effects that have been revealed in our study are examined.

For now, it seems more logical to use hysterosalpingo-foam sonography (HyFoSy) to evaluate tubal patency. (19) Findings of the study of Dreyer et al suggested that in case a HyFoSy procedure is performed as the first-line tubal patency test during the fertility work-up, a HSG can be avoided in the vast majority of cases. Perhaps it may be considered to be use before HSG as a first step assessment. In addition to the previously stated advantages of HyFoSy, the procedure appears to be less expensive than HSG. In general, HyFoSy is a less painful and less time-consuming tubal patency test compared to HSG. It also appears to be an accurate and safe test that can be performed by a single operator in an outpatient clinic setting without the need for radiation exposure, making it a far more patient-friendly first-line tubal patency test. (72) Future research should focus on whether tubal patency testing during the fertility workup using HyFoSy leads to the same diagnostic outcomes, subsequent management decisions, and ongoing pregnancy rates as tubal testing using HSG. (73,74) But not yet no large trials have been published comparing HSG with HyFoSy.

Conclusion

As seen in both previous studies and in our study, women are exposed to many harmful factors during HSG. For this reason, more innocent methods should be researched and applied in order to evaluate infertile women. For now, HyFoSy seems to be usable in this regard, at least because it eliminates exposure to ionizing radiation.

References

1. Thoma ME, McLain AC, Louis JF, King RB, Trumble AC, Sundaram R, Buck Louis GM. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertil Steril. 2013;99(5):1324-1331.e1.
2. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, Vanderpoel S. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril. 2009;92:1520–1524.
3. Borght MV, Wyns C. Fertility and infertility: Definition and epidemiology. Clinical Biochemistry. 2018;62:2-10.
4. Ludwin I, Ludwin A, Wiechec M, Nocun A, Banas T, Basta P, Pitynski K. Accuracy of hysterosalpingo-foam sonography in comparison to hysterosalpingo-contrast sonography with air/saline and to laparoscopy with dye. Hum Reprod. 2017;32(4):758-769.
5. Fayez JA, Mutie G, Schneider PJ. The diagnostic value of hysterosalpingography and hysteroscopy in infertility investigation. Am J Obstet Gynecol. 1987;156(3):558-60.
6. Roma DA, Ubeda B, Ubeda A, Monzón M, Rotger R, Ramos R, Palacio A. Diagnostic value of hysterosalpingography in the detection of intrauterine abnormalities: a comparison with hysteroscopy. AJR Am J Roentgenol. 2004;183(5):1405-9.
7. Copobianco G, Piredda N, Maiore M, Cherchi PL, Dessole M, Virdis G, Dessole S, Meloni GB. Hysterosalpingography in infertility investigation protocol: is it still useful ? Clin Exp Obstet Gynecol. 2015:42(4):448-451.
8. Sapmaz E, Akpolat N. Examination of the effect of pre-Hsg melatonin on endometrial ablation and uterine dysplastic cell development associated with radiation. Fırat Medical Journal. 2012;17(1):1-5.

9. Pala S, Atilgan R, Kuloglu T, Kara M, Baspinar M, Can B, Artas G. Protective effects of vitamin C and vitamin E against hysterosalpingography –induced epithelian degeneration and proliferation in rat endometrium. Drug Des Devel Ther. 2016;10:4079-4089.
10. Karaman A, Diyarbakir B, Durur-Subasi I, Kose D, Özbek-Bilgin A, Topcu A, Gundogdu C, Durur-Karakaya A, Bayraktutan Z, Alper F. A novel approach to contrast-induced nephrotoxicity: the melatonergic agent agomelatine. Br J Radiol. 2016;89(1061):20150716.
11. Kilciksiz S, Demirel C. Oxidative stress, radiation-induced damage and the potential role of N-acetylcysteine as a radioprotector. Turkish Journal of Oncology. 2008;23(4):200-207.
12. Bibault JE, Fumagalli I, Ferte C, Chargari C, Soria JC, Deutsch E. Personalized radiation therapy and biomarkerdriven treatment strategies: A systematic review. Cancer Metastasis Rev. 2013;32(3-4):479-92.
13. Liu N, Lei R, Tang MM, Cheng W, Luo M, Xu Q, Duan SB. Autophagy is activated to protect renal tubular epithelial cells against iodinated contrast media induced cytotoxicity. Molecular Medicine Reports. 2017;16(6):8277-8282.
14. Nieto JJ, Crow J, Sundaresan M, Constantinovici N, Perrett CW, MacLean AB, Hardiman PJ. Ovarian epithelial dysplasia in relation to ovulation induction and nulliparity. Gynecologic Oncology. 2001;82(2):344-349.
15. Sapmaz E, Akpolat N, Gurates B, Celik A, Atilgan R. Examination of the effect of Trichloroacetic Acid attachment and instillation methods on ovarian reserve and dysplastic changes in ovarian surface epithelium. Fırat Medical Journal. 2012;17(2):71-75.
16. Goldenberg I, Matetezy S. Nephropathy induced by contrast media: pathogenesis, risk factors and preventive strategies. CMAJ. 2005;17(11):1461-1471.
17. McCollough PA. Radiocontrast-Induced Acute Kidney Injury. Nephron Physiol. 2008;109:61-72.
18. Haller C, Istvan H. The cytotoxicity of iodinated radiocontrast agent on renal cells in vitro. Investigative Radiology. 2003;39(3):149-154.
19. Can B, Atilgan R, Pala S, Kuloglu T, Kiray S, Ilhan N. Examination of the effect of ovarian radiation injury induced by hysterosalpingography on ovarian proliferating cell nuclear antigen and the radioprotective effect of amifostine: an experimental study. Drug Des Devel Ther. 2018;12:1491–1500.
20. Yılmaz ES, Sapmaz T, Kazgan H, Yildiz SM, Kocamaz D, Akpolat N, Sapmaz E. Examination of the antioxidant effects of pre-HSG melatonin use on ovarian surface epithelium in rats: an experimental study. Advances in Clinical and Experimental Medicine. 2018;27(7):907–911.
21. Gulle K, Akpolat M, Oz ZS, Bakkal HB, Arasli M, Kokturk F. Protective effect of L-carnitine on morphological alterations and occurrence in developing follicles exposed ionising radiation in rat ovary. Official Journal of Health Sciences. 2017;8(1):33-37.
22. Gülle K, Pala I, Akpolat M, Bakkal BH. Protective effect of curcumin on folliculogenesis, and PARP-1 (Poly ADP-ribose polymerase) expression exposed ionising raditation in rat ovary. Official Journal of Health Sciences. 2018:9(2):102-111.
23. Yurut-Caloglu V, Caloglu M, Eskiocak S, Tastekin E, Ozen A, Kurkcu N, Oz-Puyan F, Kocak Z, Uzal C. Comparison of the protective roles of L-carnitine and amifostine against radiation-induced acute ovarian damage by histopathological and biochemical methods. J Cancer Res Ther. 2015;11(2):447-453.

24. Duan SB, Liu FY, Luo JA, Wu HW, Liu RH, Peng YM, et al. Nephrotoxicity of high- and low-osmolar contrast media. The protective role of amlodipine in a rat model. Acta Radiol. 2000;41:503–507.
25. Pouget JP, Mather SJ. General aspects of the cellular response to low and high-LET radiation. Eur J Nucl Med 2001;28:541-561.
26. Eric J. Hall, Amato JG. Radiobiology for the Radiologist, Sixth Edition. Lippincott Williams & Wilkins. 2006:135.
27. Li L, Story M, Legerski RJ. Cellular responses to ionizing radiation damage. International Journal of Radiation Oncology*Biology*Physics. 2001;49(4):1157-1162.
28. Little JB. Radiation carcinogenesis. Carcinogenesis. 2000;21:397:404.
29. Huang L, Grim S. Ionizing Radiation Induces Delayed Hyperrecombination in Mammalian Cells. Mol Cell Biol. 2004;24(11)5060-5068.
30. Oıkawa S. Sequence-Specific DNA Damage by Reactive Oxygen Species. Environ Health Prev Med. 2005;10(2):65–71.
31. Hong WK, Bast RC, Hait WN, Holland JF, Frei E. Holland Frei. Cancer Medicine. Andreeff M, Goodrich DW, Koeffler P (Ed), Cell Proliferation and Differentiation. USA: Eighth Edition, AACR. 2009:26-27.
32. Baskar R. Emerging role of radiation induced bystander effects Cell communications and carcinogenesis. Genome Integr. 2010;1(13):1-8.
33. Diplock A.T, Charleux J.-L, Crozier-Willi G, Kok F. J, Rice-Evans C, Roberfroid M, Stahl W. and Vina-Ribes J. Functional food science and defence against reactive oxidative species. British Journal of Nutrition. 1998;80(Suppl 1):77-112.
34. Mates JM. Effect of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000;153:83-104.
35. Buettner GR, Ng CF, Wang M, Rodgers VGJ, Schafer FQ. A new paradigm: manganese superoxide dismutase influences the production of H_2O_2 in cells and thereby their biological state. Free Radical Biology Medicine. 2006;41:1338–1350.
36. Scibior D, Czeczot H. Catalase: structure, properties, functions. Postępy Higienyi Medycyny Doświadczalnej. 2006;60:170-180.
37. Mannervik B, Board PG, Hayes JD, Listowsky I, Pearson WR. Nomenclature for mammalian soluble glutathione transferases. Methods in Enzymology. 2005;401:1-8.
38. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annual Review of Pharmacology and Toxicology. 2005;45:51-88.
39. Candas R, Sohal S, Radyuk SN, Klickhko VI, Orr WC. Molecularorganization of the glutathione reductase gene in Drosophila melanogaster. Archives of Biochemistry and Biophysics. 1997;339:323-334.
40. Manisaligil YA, Yurt A. Cellular and molecular effects of ionizing radiation. Duzce Medical Journal. 2018;20(2):50-53.
41. Prasad KN. Rationale for using multiple antioxidants in protecting humans against low doses of ionizing radiation. Br J Radiol. 2005;78:485–489.
42. Tewari S, Khan K, Husain N, Rastogi M, Mishra SP, Srivastav AK. Peripheral blood lymphocytes as in vitro model to evaluate genomic instability caused by low dose radiation. Asian Pac J Cancer Prev. 2016,17(4):1773-1777.
43. Bulat T, Keta O, Koricanac L, Zakula J, Petrovic I, Ristic- Fira A, et al. Radiation dose determines the method for quantification of DNA double strand breaks. An Acad Bras Cienc. 2016;88(1):127-136.
44. Nikitaki Z, Nikolov V, Mavragani IV, Mladenov E, Mangelis A, Laskaratou DA, et al. Measurement of complex DNA damage induction and repair in human cellular

systems after exposure to ionizing radiations of varying linear energy transfer (LET). Free Radic Res. 2016;50(sup1):64-78.
45. Gregoire E, Roy L, Buard V, Delbos M, Durand V, Martin BC, et al. Twenty years of FISH-based translocation analysis for retrospective ionizing radiation biodosimetry. Int J Radiat Biol. 2018;94(3):248-258.
46. Tello Cajiao JJ, Carante MP, Bernal Rodriguez MA, Ballarini F. Proximity effects in chromosome aberration induction: Dependence on radiation quality, cell type and dose. DNA Repair (Amst). 2018;64:45-52.
47. Andersen KJ, Vik H, Eikesdal HP, Christensen I. Effects of contrast media on renal epithelial cells in culture. Acta Radiol Suppl. 1995;399:213-218.
48. Faulhaber O, Bristow RG. Basis of cell kill following clinical radiotherapy. Application of Apoptosis to Cancer Treatment. 2005;125: 293–320.
49. Fernández JM, Vañó E, Guibelalde E. Patient doses in hysterosalpingography. Br J Radiol. 1996;69(824):751–754.
50. Shirley LH. Ovarian radiation dosage during histerosalpingogram. Fertil Steril. 1971;22(2):83–85.
51. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev. 2000;21(2):200-214.
52. Mazaud S, Guigon CJ, Lozach A, Coudouel N, Forest MG, Coffigny H, Magre S. Establishment of the reproductive function and transient fertility of female rats lacking primordial follicle stock after fetal gamma-irradiation. Endocrinology. 2002;143(12):4775-4787.
53. Gaytán F, Morales C, Bellido C, Aguilar E, Sánchez-Criado JE. Ovarian follicle macrophages: is follicular atresia in the immature rat a macrophage-mediated event? Biol Reprod. 1998;58(1):52-59.
54. Lee CJ, Park HH, Do BR, Yoon Y, Kim JK. Natural and radiation-induced degeneration of primordial and primary follicles in mouse ovary. Anim Reprod Sci. 2000;59(1-2):109-117.
55. Solomon R, Dauerman HL. Contrast-induced acute kidney injury. Circulation. 2010;122:2451–2455.
56. Heyman SN, Rosen S, Khamaisi M, et al. Reactive oxygen species and the pathogenesis of radiocontrast -induced nephropathy. Invest Radiol. 2010;45:188–195.
57. Idée JM, Bonnemain B. Reliability of experimental models of iodinated contrast media-induced acute renal failure: From methodological considerations to pathophysiology. Investigative Radiology. 1996;31(4):230-241.
58. Solez K, Racusen LC, Olsen S. The pathology of drug nephrotoxicity. The Journal of Clinical Pharmacology. 1983;23(10):484-490.
59. Solomon R. Contrast media: are there differences in nephrotoxicity among contrast media? BioMed Research International. 2014; Article ID 934947:1- 8.
60. Heinrich MC, Kuhlmann MK, Grgic A, Heckmann M, Kramann B, Uder M. Cytotoxic effects of ionic high-osmolar, nonionic monomeric, and nonionic iso-osmolar dimeric iodinated contrast media on renal tubular cells in vitro. Radiology. 2005;235:843–849.
61. Carlisle CH, Pass MA, Lowndes HE, Reuhl KR. Toxicity of the radiographic contrast media iopamidol, iohexol and metrizamide to cell cultures. Veterinary Radiology and Ultrasound. 1995;36(3):207-211.
62. Jensen H, Doughty RW, Grant D, Myhre O. The effects of the iodinated X-ray contrast media iodixanol, iohexol, iopromide, and ioversol on the rat kidney epithelial cell line NRK 52-E. Journal Renal Failure. 2011;33(4):426-433.

63. Berg K, Skarra S, Bruvold M, Brurok H, Karlsson JO, Jynge P. Iodinated radiographic contrast media possess antioxidant properties in vitro. Acta Radiol. 2005;46:815-822.
64. Acién P, Acién MI. The history of female genital tract malformation classifications and proposal of an updated system. Human Reproduction Update. 2011;17(5):693–705.
65. Beckett KR, Moriarity AK, Langer JM. Safe use of contrast media: what the radiologist needs to know. RadioGraphics. 2015;35(6):1738–1750.
66. Piccotti K, Guida D, Carbonetti F, Stefanetti L, Macioce A, Cremona A, David V. Comparison of diagnostic quality in hysterosalpingography between iodinated non-ionic contrast media with low and high osmolarity. La Clinica Terapeutica. 2015;166(2):e91-97.
67. Abe S, Otsuki M. Styrene maleic acid neocarzinostatin treatment for hepatocellular carcinoma. Curr Med Chem Anti-Canc Agents. 2002;2:715-726.
68. Brown JM, McIntosh MK. Conjugated linoleic acid in humans: regulation of adiposity and insulin sensitivity. J Nutr. 2003;133:3041-3046.
69. Bhoil R, Sood D, Sharma T, Sood S, Sharma J, et al. Contrast intravasation during hysterosalpingography. Pol J Radiol. 2016;81:236–239.
70. Fang F, Bai Y, Zhang Y, Faramand A. Oil-based versus water-based contrast for hysterosalpingography in infertile women: a systematic review and meta-analysis of randomized controlled trials. Fertility and Sterility. 2018;110(1):153-160.e3.
71. Nunley WC Jr, Bateman BG, Kitchin JD 3rd, Pope TL Jr. Intravasation during hysterosalpingography using oil-base contrast medium –a second look. Obstet Gynecol. 1987:70(3 pt 1):309–312.
72. Dreyer K, Out R, Hompes PGA, Mijatovic V. Hysterosalpingo-foam sonography, a less painful procedure for tubal patency testing during fertility workup compared with (serial) hysterosalpingography: a randomized controlled trial. Fertility and Sterility. 2014;102(3):821-825.
73. Emanuel MH, van Vliet M, Weber M, Exalto N. First experiences with hysterosalpingo-foam sonography (HyFoSy) for office tubal patency testing. Human Reproduction. 2012;27(1):114-117.
74. Dreyer K, Mijatovic V, Emanuel MH, Hompes PGA. Hysterosalpingo-foam sonography (HyFoSy), a new technique to confirm proximal tubal occlusion after treatment of a hydrosalpinx by an Essure device prior to in vitro fertilization (IVF). Fertil Steril. 2012;98:224.

Table 1. This table has gives an information about the which methods applied to the groups			
group	**n/n**	**time (in days)**	**procedure**
A	8/8	0	Control group. There is no procedure was implemented. The uterus, fallopian tubes, and ovaries were removed after under the anesthesia.
B	8/7	0-7	After the laparotomy rat abdomens were closed without any intervention under the anesthesia. Then, 15–20 mRad / dose radiation was administered three times (in the 1st, 3rd, and 15th min) to the rat's. After that procedure, 7 days later laparotomy was performed again, and the uterus, tubes, and ovaries of rats were removed under the anesthesia.
C	8/6	0-7	After the laparotomy, iohexol (10ml/kg) was administered by a canule from the cervix of rat's under the anesthesia. The procedure was terminated after the tubes were filled, and when contrast matter was observed in abdomen. Then, the abdomens were closed and exposed to X-ray (at the same dose and time), similar to other rats. After that procedure, 7 days later laparotomy was performed again, and the uterus, tubes, and ovaries of rats were removed under the anesthesia.
D	8/7	0-42	After laparotomy, rat abdomens were closed without any intervention under the anesthesia. Then, 15–20 mRad / dose radiation was administered three times (in the 1st, 3rd, and 15th min) to the rat's. After that procedure, 42 days later laparotomy was performed again, and the uterus, tubes, and ovaries of rats were removed under the anesthesia.
E	8/7	0-42	After the laparotomy, iohexol (10ml/kg) was administered by a canule from the cervix of rat's under the anesthesia. The procedure was terminated after the tubes were filled, and when contrast matter was observed in abdomen. Then, the abdomens were closed and exposed to X-ray (at the same dose and time), similar to other rats. After that procedure, 42 days later laparotomy was performed again, and the uterus, tubes, and ovaries of rats were removed under the anesthesia.

Table 2. Comparison of parameters by groups						
variable	**group**	**n**	**mean ±sd**	**Kw**	**p value**	**effects**

Cellular Changes	A	8	0.250 ±0.463	20.163	0.000	E>C>D>B chronic>acute
	B	7	1.571 ±0.535			
	C	6	2.167 ±0.753			
	D	7	2.143 ±0.690			
	E	7	2.286 ±0.756			
Vascular Ectasia	A	8	0.625 ±0.518	13.538	0.009	C>D>B>E acute>chronic
	B	7	1.571 ±0.535			
	C	6	2.000 ±0.894			
	D	7	1.857 ±0.690			
	E	7	1.429 ±0.535			
Inflammation	A	8	0.375 ±0.518	21.383	0.000	C>B>D>E acute>chronic
	B	7	1.857 ±0.690			
	C	6	2.333 ±0.516			
	D	7	1.714 ±0.488			
	E	7	1.286 ±0.488			
Glutathione Reductase	A	8	0.750 ±0.463	21.206	0.000	E>D>B>C chronic>acute
	B	7	2.143 ±0.690			
	C	6	2.000 ±0.633			
	D	7	2.286 ±0.488			
	E	7	2.714 ±0.488			
Catalase	A	8	0.625 ±0.518	23.258	0.000	E>D>B>C chronic>acute
	B	7	1.857 ±0.378			
	C	6	1.833 ±0.753			
	D	7	2.000 ±0.000			
	E	7	2.571 ±0.535			
Superoxide Dismutase	A	8	0.875 ±0.354	20.950	0.000	E=D>C>B chronic>acute
	B	7	1.857 ±0.690			
	C	6	2.000 ±0.633			
	D	7	2.571 ±0.535			
	E	7	2.571 ±0.535			
Glutathione S-transferase	A	8	0.875 ±0.354	21.675	0.000	E>D>C>B chronic>acute
	B	7	1.714 ±0.488			
	C	6	1.833 ±0.408			
	D	7	2.000 ±0.577			
	E	7	2.571 ±0.535			

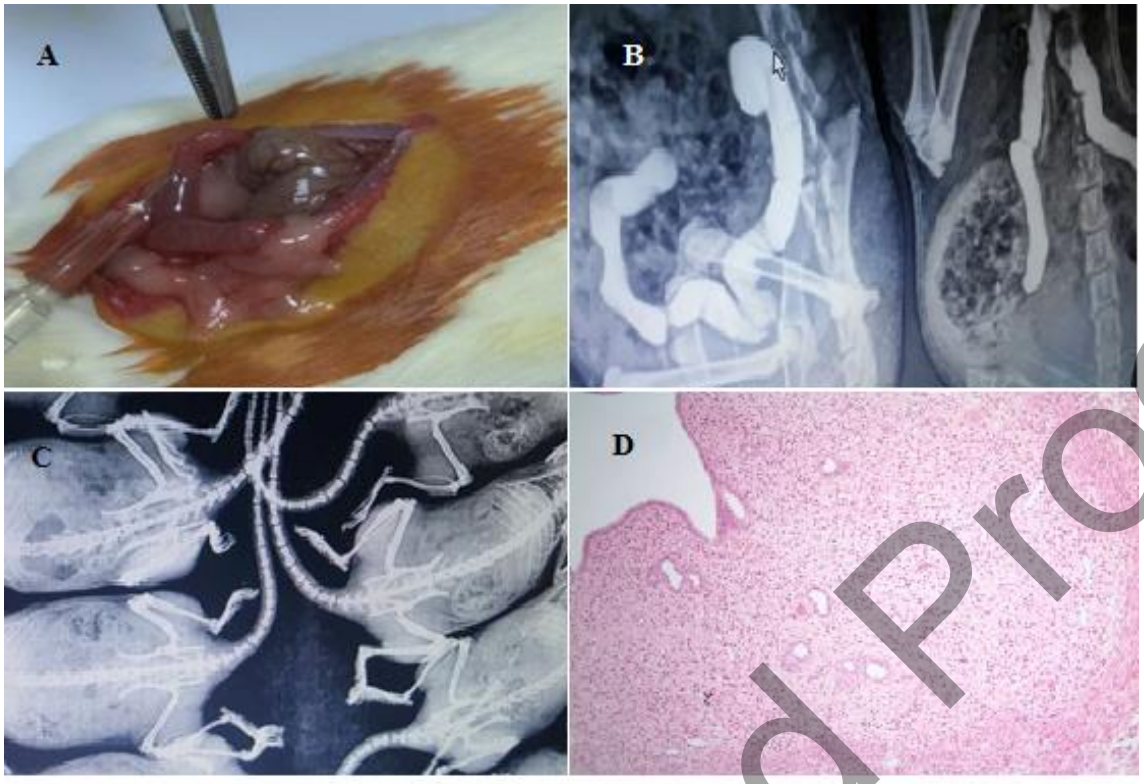

Figure 1. (A) uterine injection of radiocontrast material from the rat's cervix with a tuberculin injector. **(B)** the hysterosalpingography image after the iohexol injection. **(C)** the hysterosalpingography image which the group without radiocontrast agent. **(D)** the normal appearance of endometrium and columnar epithelium and glandular epithelium (hematoxylin -eosin staining x 40) (the image of histological section of group A)

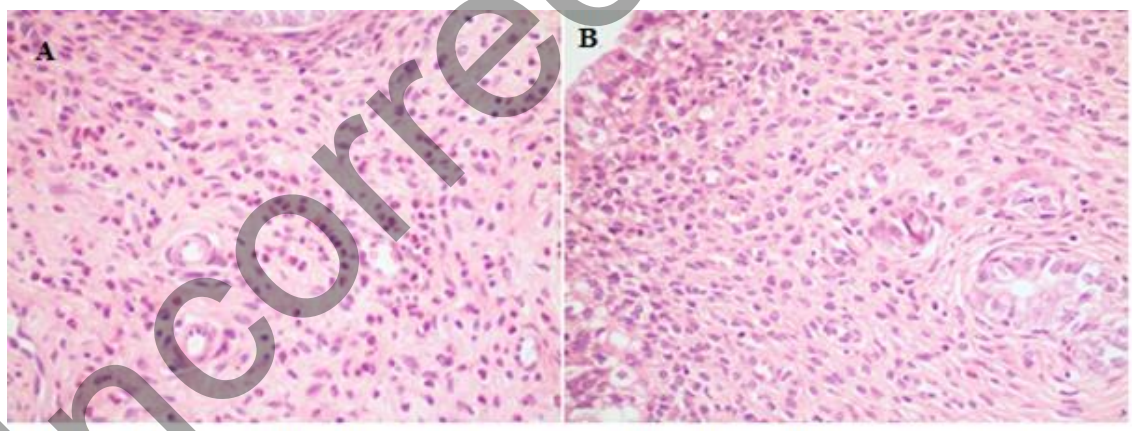

Figure 2. (A) mix type intense inflammation in endometrial stroma; mainly consisting of the eosinophilic leukocytes (group B) (hematoxylin -eosin staining x 400) **(B)** mild reactive changes and mild inflammation in superficial cells (group E) (hematoxylin -eosin staining x 400)

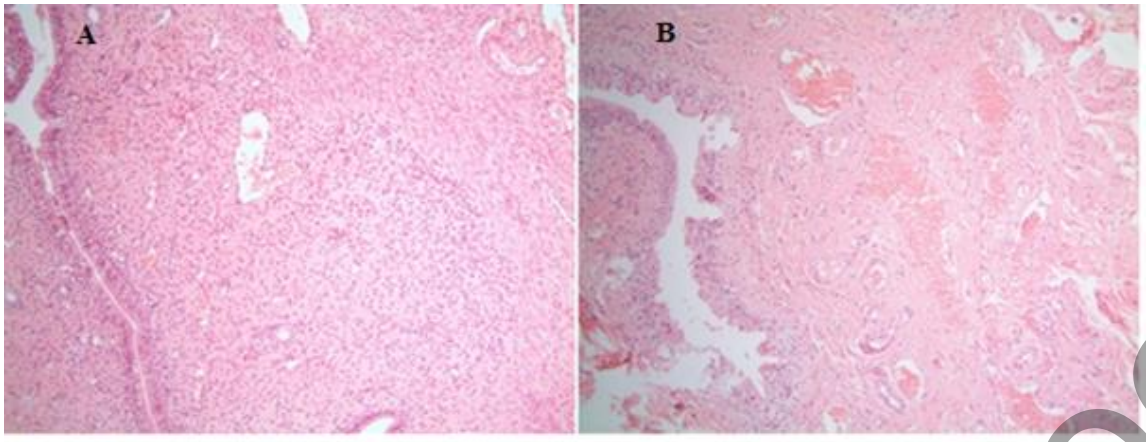

Figure 3. (A) reactive changes in superficial cells, mix type of mild inflammation in the stromal areas and moderate vascular ectasia (group D). (hematoxylin -eosin staining x 400) **(B)** intensive vascular congestion, mix type of mild stromal inflammation, reactive changes in superficial cells (group C). (hematoxylin -eosin staining x 40)

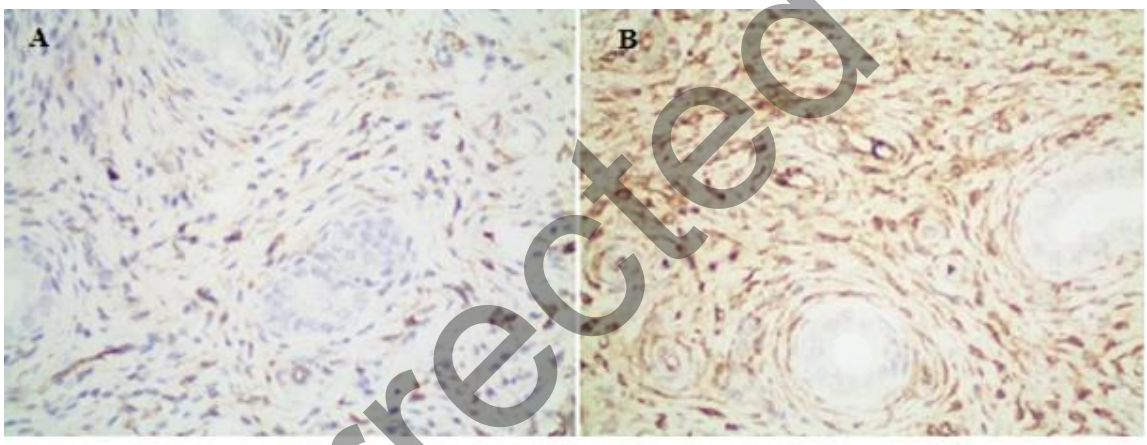

Figure 4. (A) mild immunohistochemical positivity seen that when the tissue stained with glutathione reductase (group B). (immunohistochemical glutathione reductase staining x 400) **(B)** severe immunohistochemical positivity with glutathione reductase (group E) (immunohistochemical glutathione reductase staining x 400)

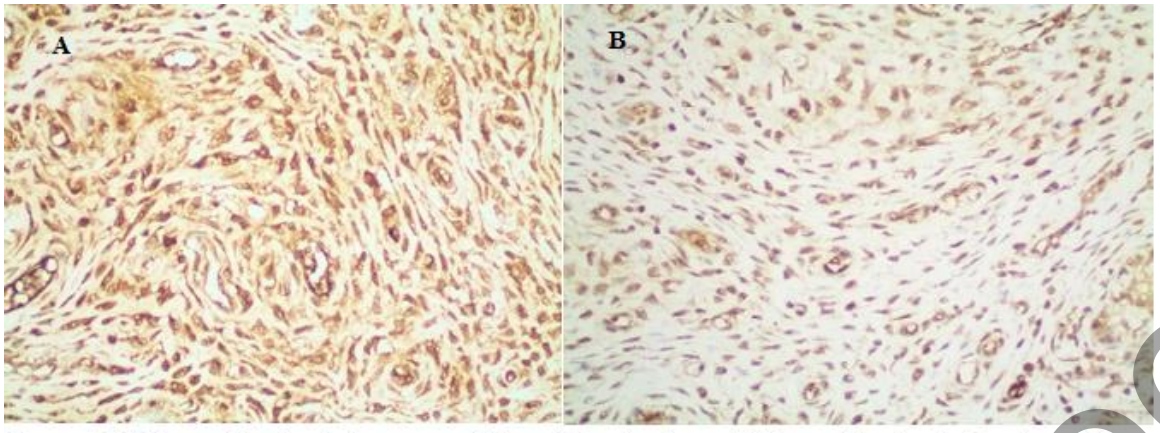

Figure 5. (A) immunohistochemically severe positivity with Catalase (group E) (immunohistochemical Catalase staining x 400) **(B)** immunohistochemically severe positivity with Superoxide dismutase (group D) (immunohistochemical Superoxide dismutase staining x 400)

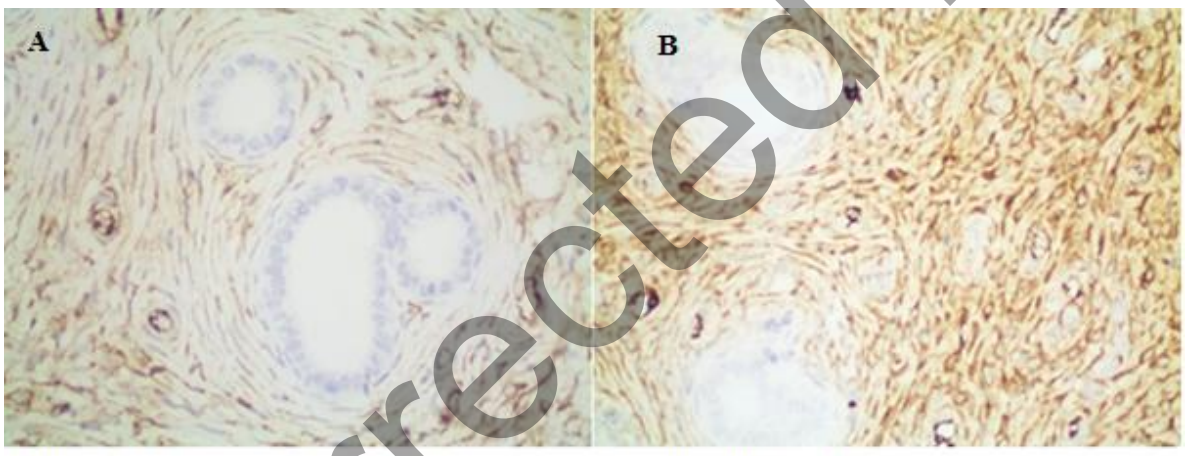

Figure 6. (A) immunohistochemically moderate positivity with Glutathione -S transferase (group B) (immunohistochemical Glutathione -S transferase staining x 400) **(B)** immunohistochemically severe positivity with Glutathione -S transferase (group E) (immunohistochemical Glutathione -S transferase staining x 400)