The possible protective role of bone marrow transplantation on irradiated mothers and their fetuses

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Abstract: This work was conducted to evaluate the possible protective role of bone marrow transplantation (BMT) against whole body γ-irradiation (2Gy) in pregnant albino rats and their fetuses at two different gestation periods. Different treatments were performed on days 7or 14 of gestation and examined at the end of the gestation period (day 20).Pregnant rats irradiated at 2Gy γ-rays on day 7 or 14 of gestation showed unequal distribution of implantation sites between the two horns, reduction in the number of implantation sites and one case of complete abortion on day 7 of gestation, but on day 14 of gestation, there were lots of resorbed embryos. Fetuses showed very thin skin layers, subcutaneous hemorrhage, severe growth retardation and malformed rostrum, eyes and eye lids in addition to malformed fore and hind limbs and tails. Bone marrow transplantation showed no detectable changes in morphology of the fetuses. Also, radiation caused many histological and histochemical changes in the lung tissue of mothers and their fetuses on day 7 or 14 of gestation, but bone marrow transplantation post-irradiation highly improved the histological and histochemical architecture of the liver tissues.
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Keywords: bone marrow transplantation (BMT); γ-irradiation (2Gy); pregnant albino; fetus

1. Introduction:

The damaging effects of ionizing radiation lead to cell death and are associated with increased risk for number of diseases **(Halliwell and Aruoma, 1991).**

Gamma-rays caused high incidence of intrauterine mortality, resorption of embryos and inhibition of gestation to pregnant rats **(El-Naggar *et al.*, 1996)**.

The development of new concerns comprises immune function and / or radiation induced genetic damage **(Trosko, 1996).**

Leadon (1996) stated that ionizing radiation has been considered as a source for causing physical damage to living organisms. Exposure to ionizing radiation eventually results in injuries to the biological system depending on the dose, duration and type of radiation exposure besides the radio-sensitivity of different tissues **(Pecaut *et al*., 2001).** Ionizing radiation forms radicals in the DNA and in the surrounding water molecules of the hydration shell of the DNA, which in turn destroy DNA **(Kopjar *et al.*, 2006; Moss, 2012).**

De Santis *et al.* (2005) suggested that ionizing radiation represented a possible teratogen for the fetus, but this risk has been found to be dependent on the dosage and the effects correlatable to the gestation age at exposure.

Embryonic death occurred at the early stages of gestation by ionizing radiation (**Devi and Hande, 1990**).

Abu Gabal *et al.* (1994a) mentioned that 2Gy gamma – rays provided to rats at day 4 of gestation caused prenatal death beside a small percentage of resorbing bodies, subcutaneous haemorrhage and diminution in size with clubbed fore and hind limbs. **Salama (2004)** estimated abnormal implantation sites when pregnant rats were exposed to whole body gamma rays (1Gy/3 times) on the 7^{th}, 11^{th} and 15^{th} days of gestation**.**

Ramadan (2007a) exposed pregnant rats to whole body gamma rays at a dose of 0.5Gy for 4 times on gestational days 9, 10, 11 and 12, she detected a significant decrease in fetal numbers. She added that radiation exposure induced malformations including excencephaly, diminution of size and kypophyis**.**

Bone marrow is a complex tissue composed of two compartments, haematopoietic and stromal one. The stromal compartment is the structural basis of the haematopoiteic microenvironment which is a complex tissue that contains a subset of cells termed mesenchymal stem cells (MSCs) **(Caplan , 1991).**

Bone marrow transplantation at different time periods after radiation exposure induces its restorative effect when the treatment is done post irradiation asconfirmed by raiozinc uptake **(Kafafy , 1993)**

Abu- Sinna *et al.* (2005) cited that BMT is known to cause regeneration of thymus, spleen and bone marrow after lethal whole body irradiation.

Bone marrow transplantation to pregnant mice post-exposure to γ-rays improved the developmental and

structural changes in the fetus **(Wang, 2001; Mansour, 2012)**

The lung of fetuses obtained from mothers exposed to 3Gy on day 6 of gestation showed severe degenerated alveolar cells, narrow alveolar intercepts with presence of many pyknotic nuclei **(Abu El Naga, 1989).**

Lung damage post-irradiation was also detected by **Liao *et al*. (2000)** in mice exposed to single doses of X-rays ranging from 12 to 20 Gy. They observed increased morbidity from radiation pneumonitis and lethality between 12 to 32 weeks after irradiation. They also noticed many histological changes in the lung tissue.

Radiation pneumonitis was observed in lung of mice exposed to radiation **(Yan *et al*., 2004)** and in Brown Norway rat **(Eveline *et al*., 2009)**. They also stated that radiation exposure with a high dose rate (0.8Gy/ min) or low dose rate (0.05 Gy/ min) caused many pathological changes in lung of rats such as severe aplasia of hemopoietic and lymphoid tissues with increased hemorrhagic areas.

El-Khatib *et al*. (2009) irradiated mice with X-rays doses of 5 to 14Gy for sex weeks. They noticed that mice irradiated with 5 and 7Gy exhibited no changes in lung density, but those exposed to doses greater than 10Gy exhibited marked increases in lung density. They also noticed pneumonitis in the lungs of exposed mice.

In **2010, Kirsch *et al*.,** noticed increased lung cancer in mice post-irradiation (15.5Gy).

The present study aimed to evaluate the possible protective effect of BMT against radiation injury in pregnant rats and their fetuses.

2. Material and Methods

A. Experimental animals:

Mature albino rats (*Rattus albinus*) ranging from 120-150 gm body weight were housed in cages, six females per cage. The males were kept separated from females until mating. Females of proestrous and estrous periods were housed with males (2:1).

All rats were kept under normal conditions and fed pellets concentrated diet and vitamin mixtures.

Pregnancy was assured next morning by the presence of vaginal plug. The presence of spermatozoa in smears of vaginal content confirmed that mating had taken place and that day was taken as the first day of pregnancy.

B. Radiation facility:

Irradiation was performed by Gamma-cell 40 (^{137} Cesium) belonging to the National Center for Radiation Research and Technology "NCRRT", Atomic Energy Authority, Cairo, Egypt. The ^{137} Cesium source activity provided a dose rate 0.48Gy/min.

C. Bone marrow transplantation:

Bone marrow transplantation (BMT) donors and recipients were chosen of the same inbred strain. Sisters to sisters (syngenic transplantation). The donors were sacrificed by cervical dislocation and femur bones were cleaned and both ends were chipped by bone nibbling forceps. The marrow was blown of the femur into saline solution under sterilized conditions surrounded by ice cubes, and mixed by drawing and expelling it several times from the syringe without needle in order to avoid mechanical damage to the cells. Total viable of cells about 75 x 10^6 ± 5 were injected one hour post irradiation **(Decleave *et al*., 1972).**

D. Experimental design:

Pregnant animals were divided into the following groups (6 females each):

1- Normal untreated control pregnant rats (C). 2- A group of pregnant rats exposed to 2Gy on day 7 of gestation (R_7). 3- A group of pregnant rats exposed to 2Gy on day 7 of gestation and received freshly drawn BMT (75 × 10^6 ± 5 cells) by i.p. injection 1 h post-irradiation (R_{7+BM}). 4- A group of pregnant rats exposed to 2Gy on day 14 of gestation (R_{14}). 5- A group of pregnant rats exposed to 2Gy on day 14 of gestation and received one dose of (BMT) by i.p. injection 1 h post-irradiation (R_{14+BM}). All animals were sacrificed on day 20 of gestation.

E. The morphological study:

The pregnant females were dissected and the uterine horns were removed and immediately photographed. The uterine form and resorptions, fetuses malformations were carefully examined grossly for anatomical abnormality in rostrum, eye, digits, limbs and tails.

F. The histological and histochemical studies:

On day 20 of gestation, pregnant rats were sacrificed, small pieces of mothers and fetal's lung tissues were quickly removed and fixed in 10% neutral buffer formol and Carnoy's fluid for the histological and histochemical studies. Specimens were washed and dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were then cut at 5µ thickness and stained by haematoxylin and eosin stain according to the method of **Drury and Wallington (1980)**, Mallory's trichrome stain for demonstrating collagen fibers **(Pearse, 1977)**, periodic acid Schiff's technique for demonstrating polysaccharides in the lung **(Pearse, 1977)**, mercuric bromophenol blue method for detecting total protein **(Mazia *et al*., 1953)**.

3. Results:

Morphological observations:

Anatomical observation of the uteri of the control rats showed healthy bright appearance and normal distribution of implanted fetuses between the two horns **(plate 1A)**

Irradiated pregnant rats on the 7th day of gestation showed reduction in the number of implantation sites **(Plate1B),** but R_{14} group showed resorption of most fetuses which gave the uterus a dark color **(Plate1D)** with reduction in number of fetuses **(Plate1E).**

Animals irradiated at 2Gy on the 7th day of gestation and intraperitonealy injected with BM cells 1 h post-irradiation revealed normal distribution of implantation sites in the two horns of the uterus **(Plate 1C)**. A slight improvement in the number and distribution of implantation sites was noticed in R_{14+BM} group**(Plate 1F)**.

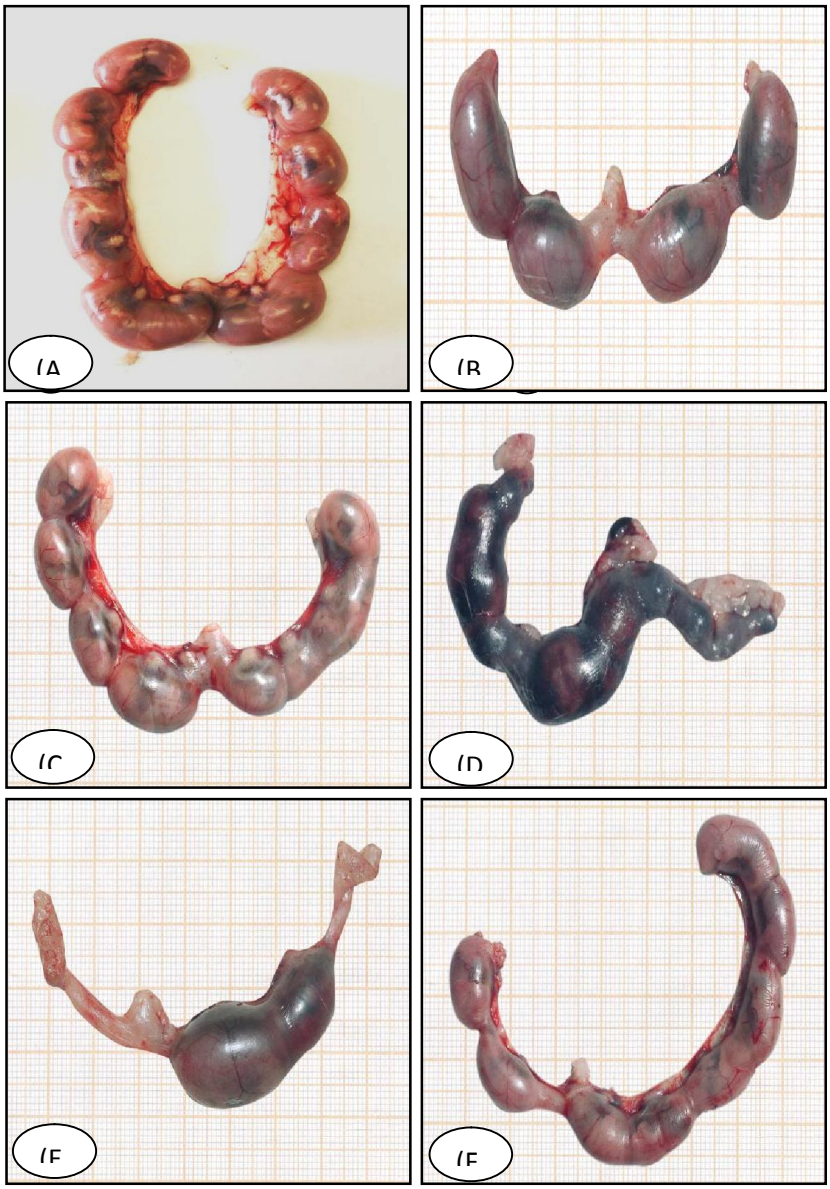

Plate (1): photomicrographs of uteri of rats sacrificed on day 20 of gestation showing: A; control group, **B**; reduction in the number of implantation sites(R_7 group), **C;** normal distribution of implantation sites (R_{7+BM} group), D&E (R_{14} group); resorption of most fetuses which gave the uterus a dark color with reduction in number of implantation sites respectively, E; a slight improvement in the number and distribution of implantation sites(R_{14+BM}group)**.**

Normal control fetuses taken on the 20th day of gestation showed normal size and length **(Plate 2A).** Reduction in the size of fetuses of R_7 group was recorded with very thin skin layers **(Plate 2B).** Fetuses of R_{14} group showed subcutaneous haemorrhage in the neck region, dead fetuses, growth retardation, reduction in length, kyphosis and excencephaly **(plate 2D&E).**

Animals of group R_{7+BM} showed that size and length of fetuses were near to the control group **(Plate 2C)**. A slight improvement in size and length of fetuses was recorded in fetuses of group R_{14+BM} **(Plate 2F).**

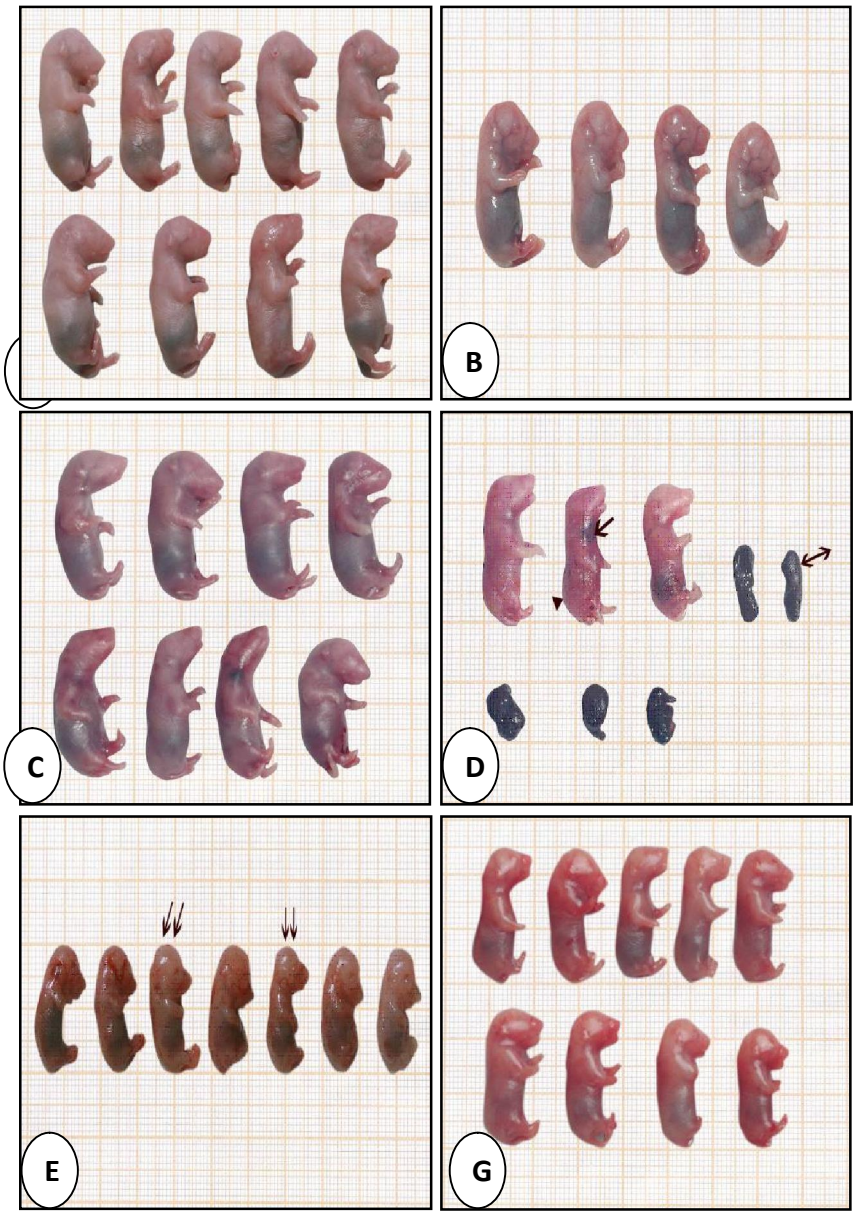

Plate (2): Photomicrographs of fetuses of rats sacrificed on day 20 of gestation showing: A; control group, **B,** (R_7 group); reduction in the size of fetuses with very thin skin layers, **C;** improvement in the size and length compared with the control one (R_{7+BM} group), D&E,(R_{14} group);verythin skin and reduction in length in all fetuses, subcutaneous haemorrhage in the neck region (↑), dead fetus (↕), kyphosis (▲) and exencephaly (↑↑) ,E; a slight improvement in the size and length of fetuses ($R_{14.+BM}$group).

Normal upper and lower jaws were recorded in the fetuses of R_7 and R_{7+BM} groups compared with the control fetuses **(Plate 3A),** but**,** unequal jaws were detected in all fetuses of R_{14} group and some fetuses of R_{14+BM} group **(Plate 3B).**

Normal eye lids and protrusion of eye ball in fetuses of the control group **(Plate 3C)**. Also, this was observed in fetuses of groups R_{7+BM}& R_{14}**.** Fetuses of group R_7 were devoid of eye lids with exophathalamus **(Plate 3D).** No protrusion of eye ball was noticed in fetuses of R_{14+BM} group **(Plate 3E).**

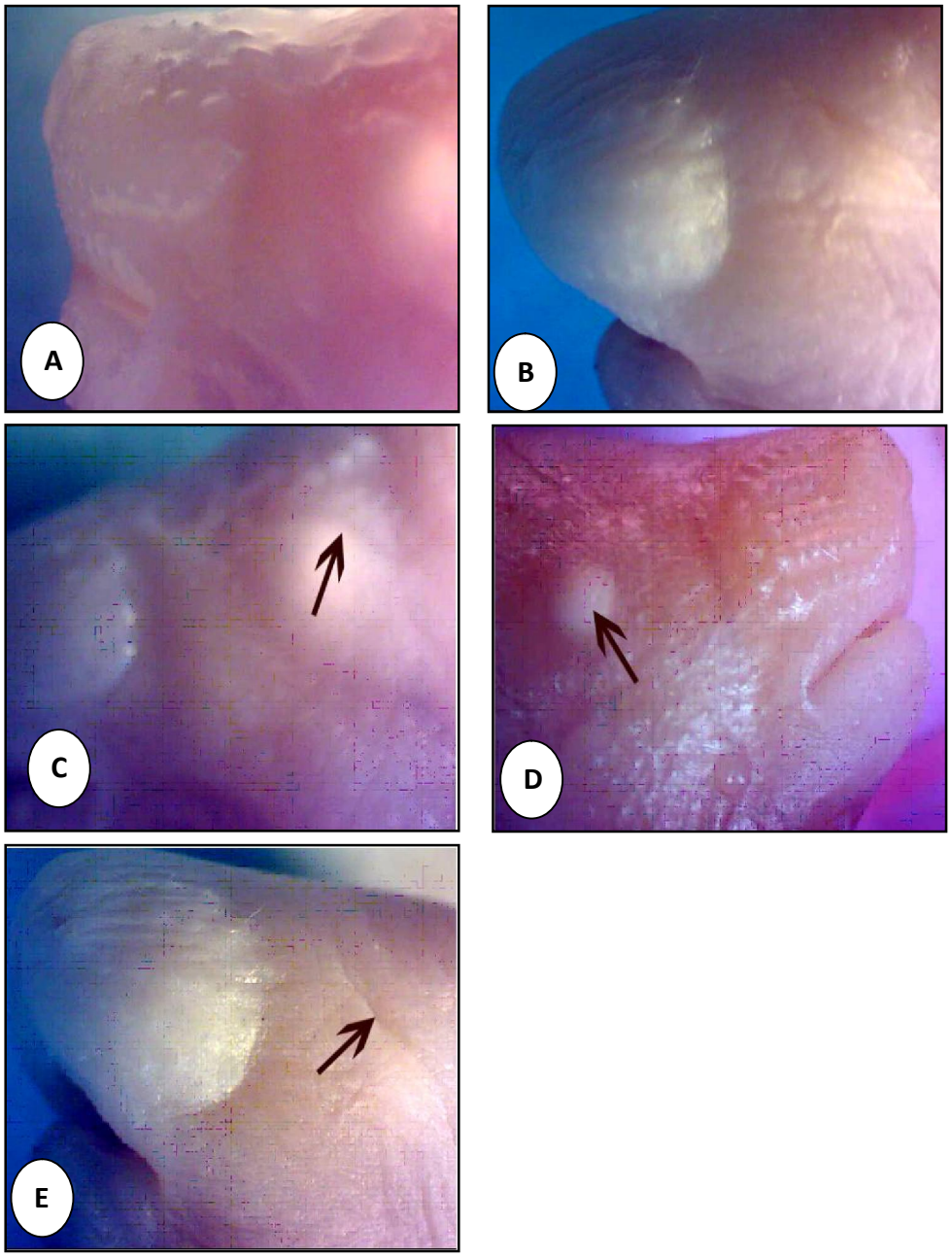

Plate (3): photographs of the rostrum and eye regions of 20-day old fetuses showing: A; normal upper and lower jaws (control group), **B;** unequal jaws (R_{14} or R_{14+BM} groups), **C;** presence of eye lids (↑) (control group), **D;** devoid of eye lids with exophathalamus (↑) (R7 group), **E;** no protrusion of the eye ball (↑) (R_{14+BM} group).

Fetuses of the control group showed presence of five digits or toes in the fore and hind limbs **(plates 4A&5A)**. In fetuses of R_7 group, malformed fore limbs were observed. Digits were smaller than the control with presence of syndactly in the other cases **(plate 4B&C)**. Adactly was observed in most fetuses of R_{14} group **(Plate 4D).** No malformations or change in the number of toes were noticed in the fore limbs of the fetuses in R_{7+BM}& R_{14+BM} groups when compared to fetuses of the control group. Ectrodactly and adactly were detected in fetuses of R_7 group **(Plate 5B&C).** All fetuses of R_{14} group and some fetuses of R_{14+BM} group showed presence of syndactly or meromelia **(Plate 5D, E& F)**.

Normal tails were detected in fetuses of the control group and R_{7+BM} groups **(Plate 6A).** In some cases of each treated groups, the tail was malformed such as twisted and short& thin tail in fetuses of R_7 group **(Plate 6B, C&D)**. Also, the short and thin tail was observed in fetuses of R_{14} group **(Plate 6E)**. One case of reversed tail was found in a fetus of group R_{14+BM} **(Plate 6F)**.

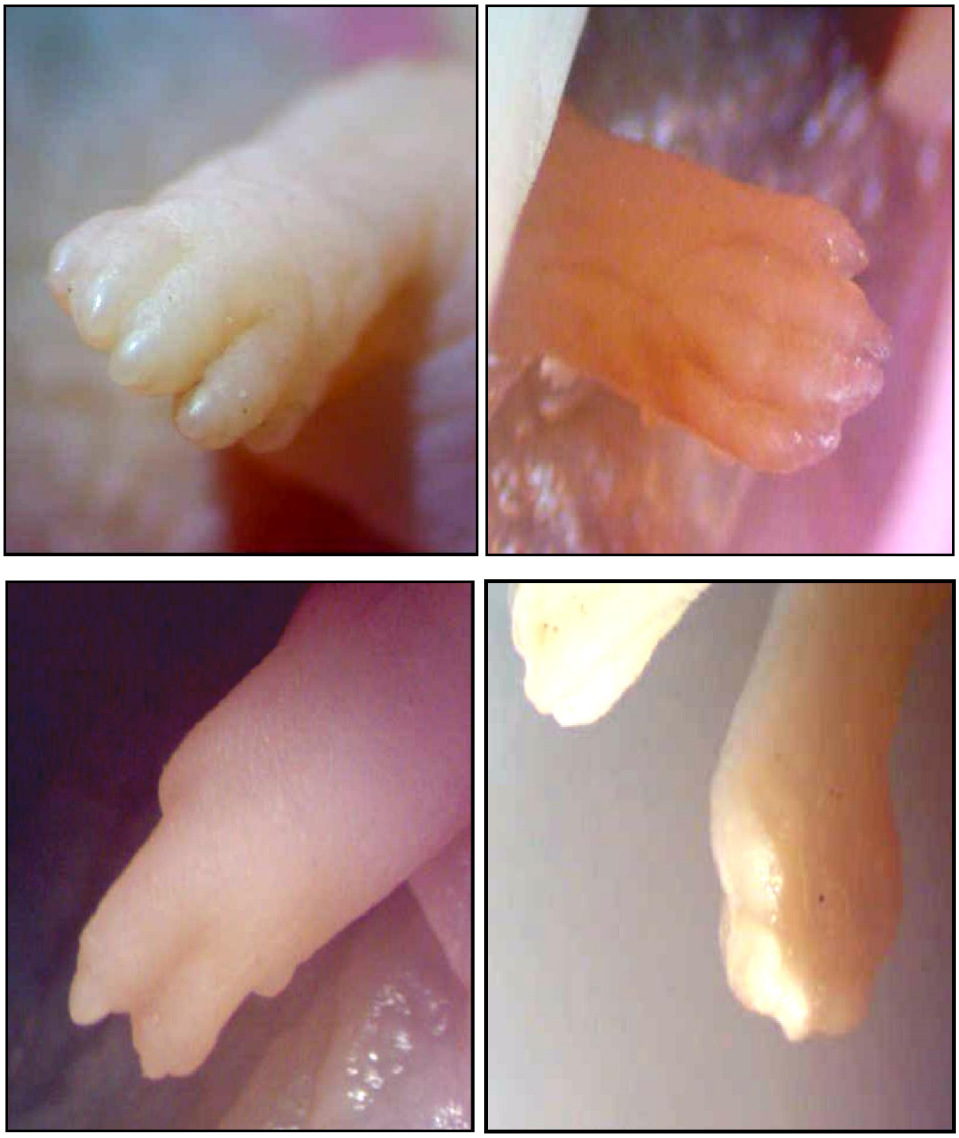

Plate (4): photographs of the fore limb region of 20-day old fetuses showing: A; presence of five toes (control group), **B;** digits are smaller than the control (R_7 group), **C&D** (R14 group)**;** presence of syndactly and adactly respectively.

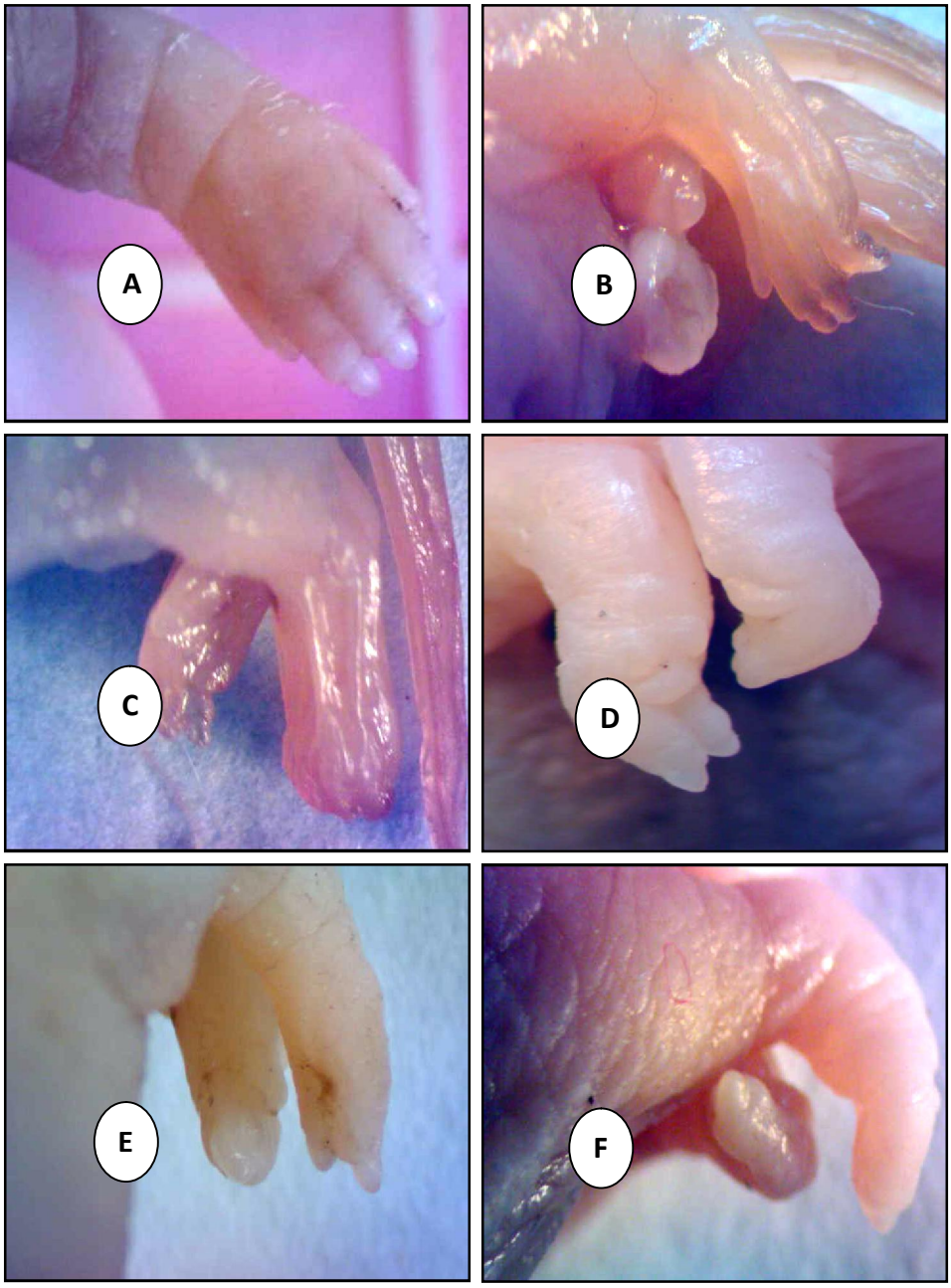

Plate (5): photographs of the hind limb region of 20-day old fetuses showing: A; presence of 5 toes (control group), B, C (R_7 group); presence of ectrodactly and adactly respectively, D, E&F (R_{14} and R_{14+BM} groups); presence of syndactly or meromelia.

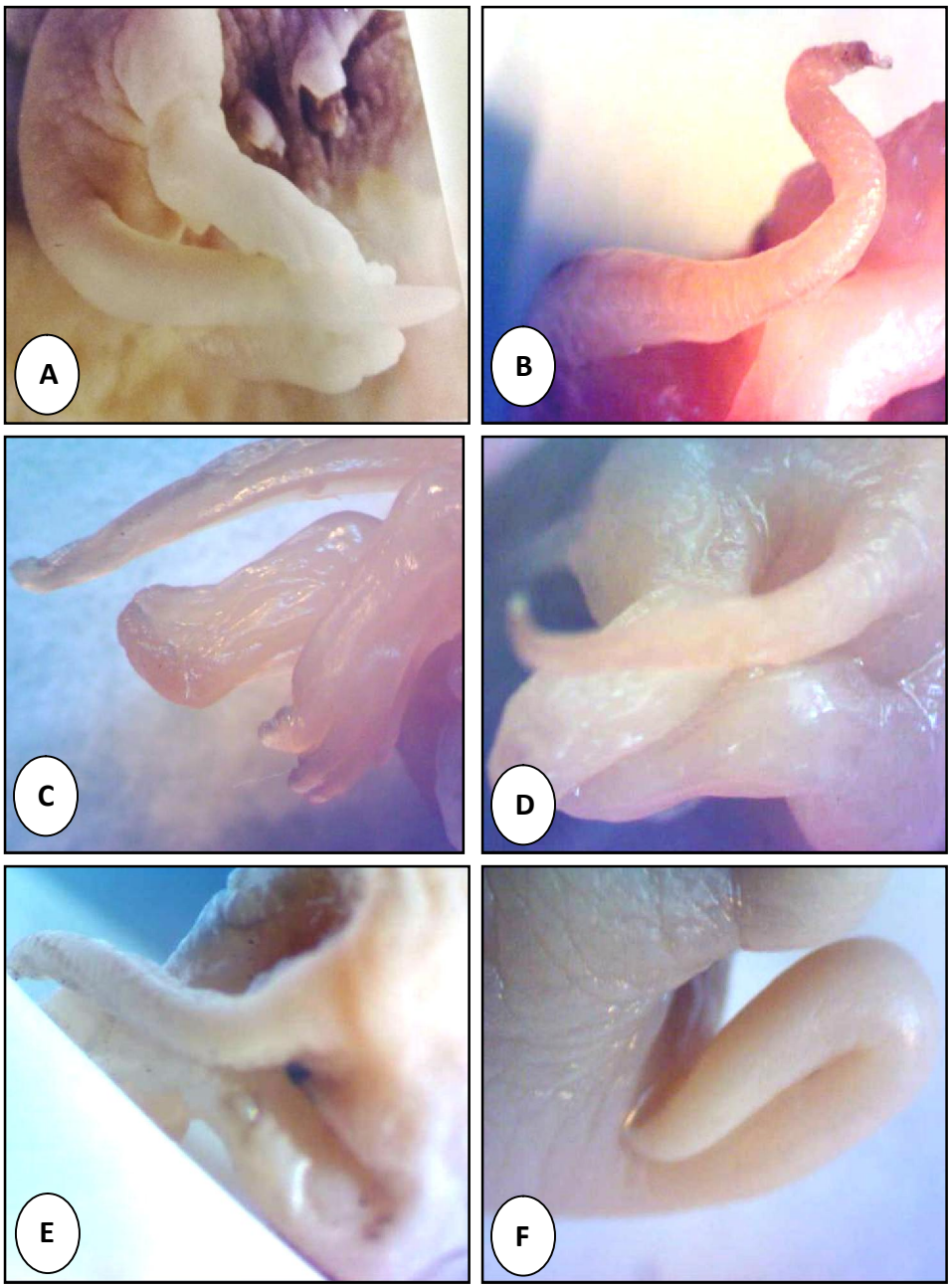

Plate (6): **photographs of the tail region of 20 day old fetuses showing: A;** control group, B, C&D (R_7 group); twisted tail, short and thin tail respectively, E; short and thin tail (R_{14} group), F; reversed tail (R_{14+BM} group).

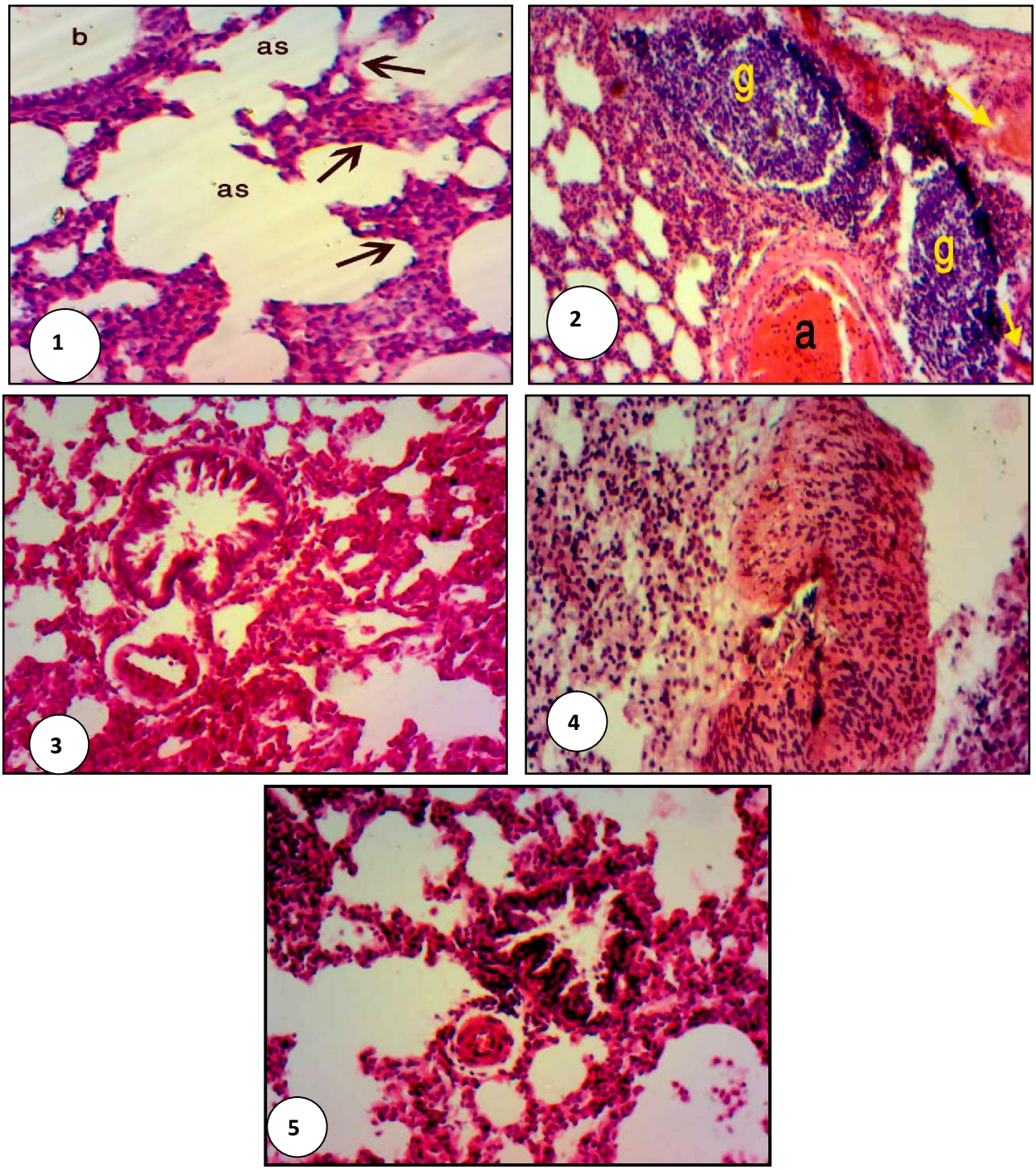

Fig.(1):showing lung tissue of a pregnant control rat. Bronchiole(b), alveolar sac(as),alveolar septa (↑). **(H &E x 100)**
Fig.(2): showing lung tissue of a pregnant rat of R_7 group. Notice: areas of granuloma cells (g), highly congested and dilated artery (a) and congested alveolar septae (↑). **(H &E x 100)**
Fig.(3): showing somewhat lung tissue of pregnant rat of R_{7+BM} group, but, some alveolar septae were thickened. **(H &E x 100)**
Fig.(4):showing lung tissue of a pregnant rat of R_{14} group. Notice: highly thickened, corrugated and distorted arterial wall. Most nuclei of cells of the lung tissue appeared **(H &E x 100)**
Fig.(5): showing lung tissue of a pregnant rat of R_{14+BM} group. Some alveolar septae were highly thickened with numerous pyknotic nuclei in their epithelial cells and cells of the bronchiole. Nearly the lumen of the artery was oblitrated due to the thickened arterial wall. **(H &E x 100)**

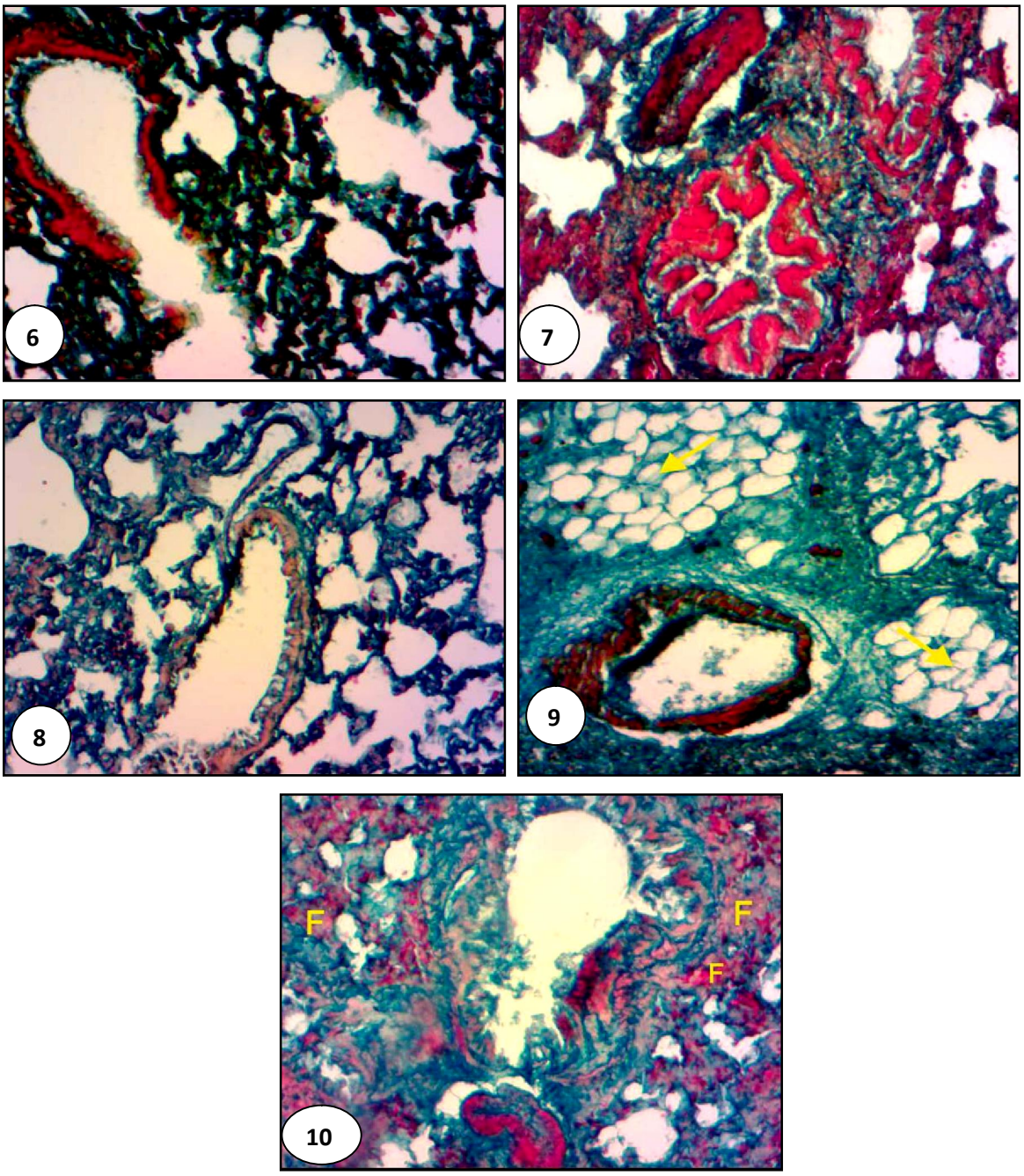

Fig.(6): showing normal distribution of the collagen fibers in the lung tissue of a control pregnant rat. **(Mallory's trichrome stain x 100)**

Figs.(7):showing lung tissue of a pregnant rat of R_7 group. Notice: highly fibrotic walls of the bronchioles, arteries and veins with highly increased collagen fibers in the thickened alveolar septae and around the walls of the bronchioles. **(Mallory's trichrome stain x 100)**

Fig.(8):showing normal distribution of collagen fibers in the lung tissue of a pregnant rat exposed of R_{7+BM} group. **(Mallory's trichrome stain x 100)**

Figs.(9): showing collagen distribution in the lung tissue of a pregnant rat exposed of R_{14} group. Notice: fibrotic walls of the arteries and veins with highly increased collagen fibers in the distorted walls of the bronchioles and thickened alveolar septae. Notice also fatty degeneration (↑). **(Mallory's trichrome stain x 100)**

Fig.(10): showing collagen distribution in the lung tissue of a pregnant rat of R_{14+BM} group. Notice: numerous fibrotic areas (F) and increased collagen fibers in the highly thickened alveolar septae. **(Mallory's trichrome stain x 100)**

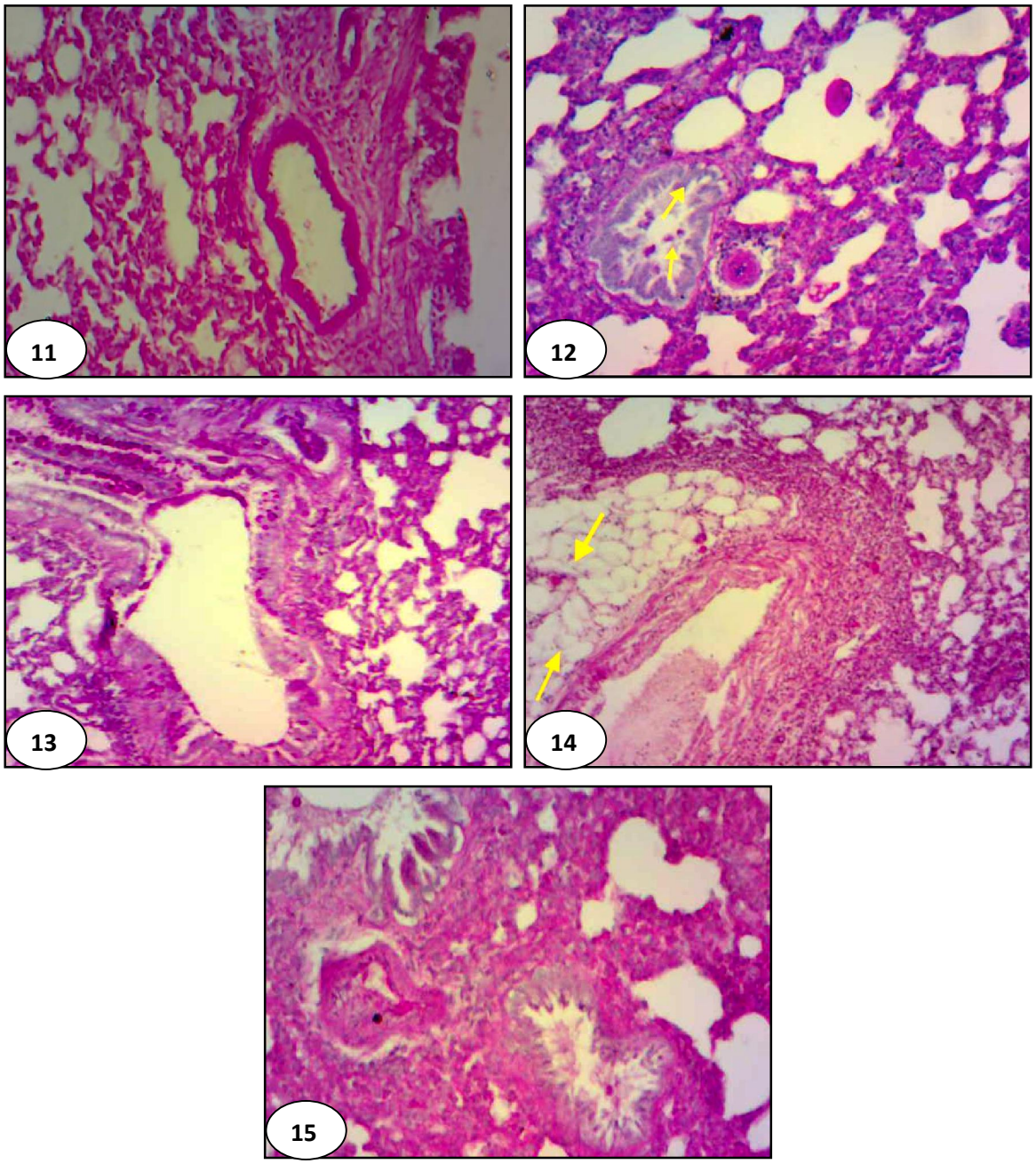

Fig.(11): showing normal distribution of polysaccharides in the lung tissue of a control pregnant rat. **(PAS X 100)**
Figs.(12): showing increased stain affinity of polysaccharides in the highly thickened walls of arteries, alveolar septae and bronchioles. Debris of the epithelial cells inside the lumen of the bronchioles acquired moderate stain affinity (↑).Granuloma cells acquired dense stain affinity (g) in the lung tissue of R_7 group**.** **(PAS x 100)**
Fig.(13): showing normal polysaccharides content in the lung tissue of a pregnant rat of R_{7+BM} group. **(PAS X 100)**
Fig.(14): showing polysaccharides distribution in the lung tissue of a pregnant rat of R_{14} group. Notice: depleted areas of fatty degeneration (↑), haemolysed RBCs inside the highly corrugated and elongated arterial wall appeared faintly stained, while, some thickened alveolar walls, granuloma cells and thickened arterial walls were deeply stained. **(PAS x 100)**
F**ig.(15):** showing polysaccharides distribution in the lung tissue of a pregnant of R_{14+BM} group. Notice that alveolar septae, arterial wall and the fibrous layer encircling the bronchiole acquired a dense stain affinity. **(PAS X 100)**

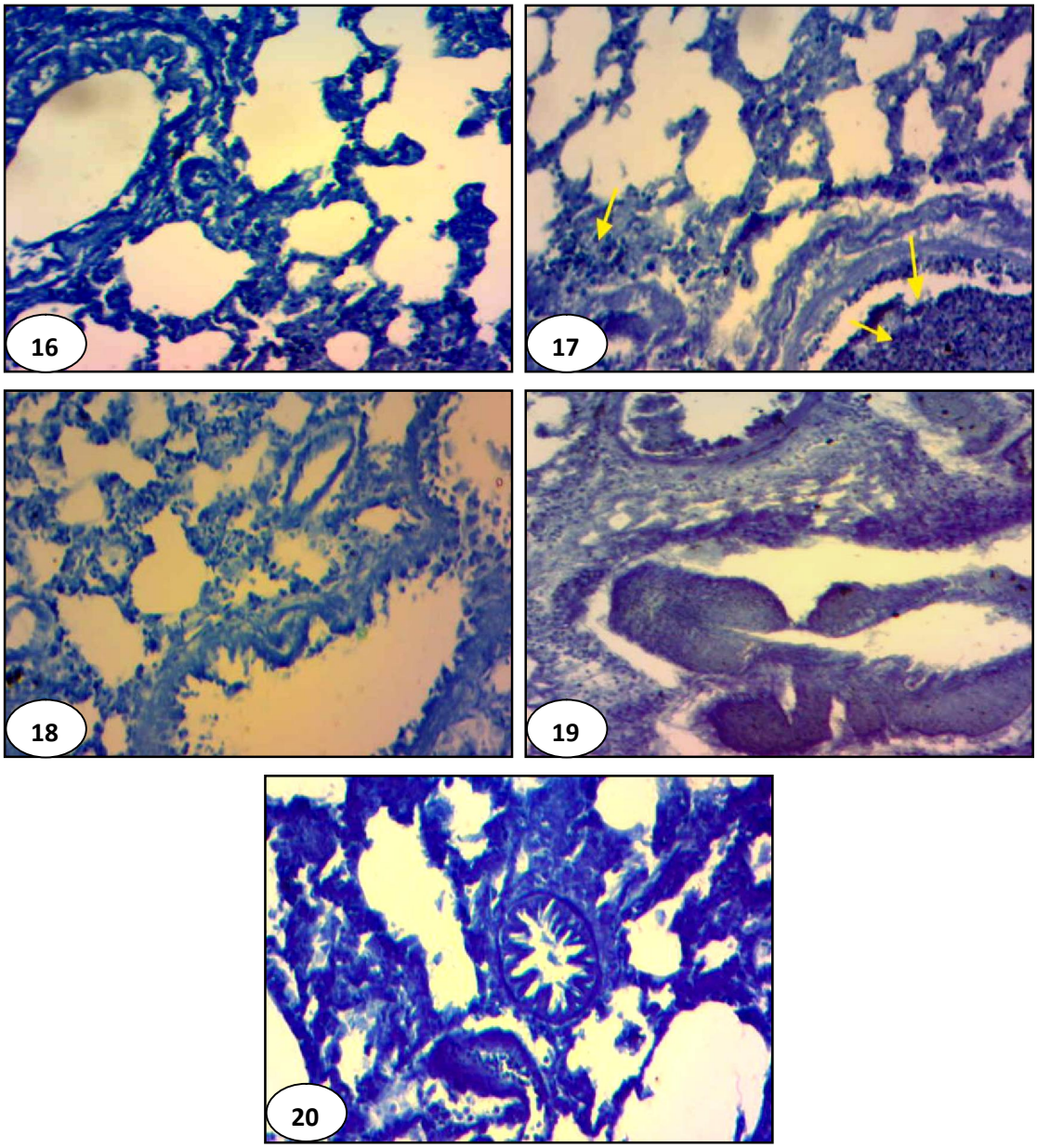

Fig.(16): showing normal total protein content in the lung tissue of a control pregnant rat. **(Mercuric bromophenol blue x 100)**
Fig.(17): showing densely stained total protein in the granuloma cells inside and outside the bronchiole (↑), the arterial wall and also in the thickened alveolar septae in the lung tissue of a pregnant rat of R_7 group **(Mercuric bromophenol blue x 100)**
Fig.(18): showing somewhat content of total protein in the lung tissue of a pregnant rat of R_{7+BM} group. **(Mercuric bromophenol blue x 100)**
Figs.(19): showing total protein in the lung tissue of a pregnant rat of R_{14} group. Notice: deeply stained arterial and bronchial walls. Some alveolar septae appeared densely stained. **(Mercuric bromophenol blue x 250)**
Fig.(20): showing deeply stained total protein in the walls of bronchioles and arteries. Also some alveolar septae were densely stained in the lung tissue of a pregnant rat of R_{14+BM} group. **(Mercuric bromophenol blue x 100)**

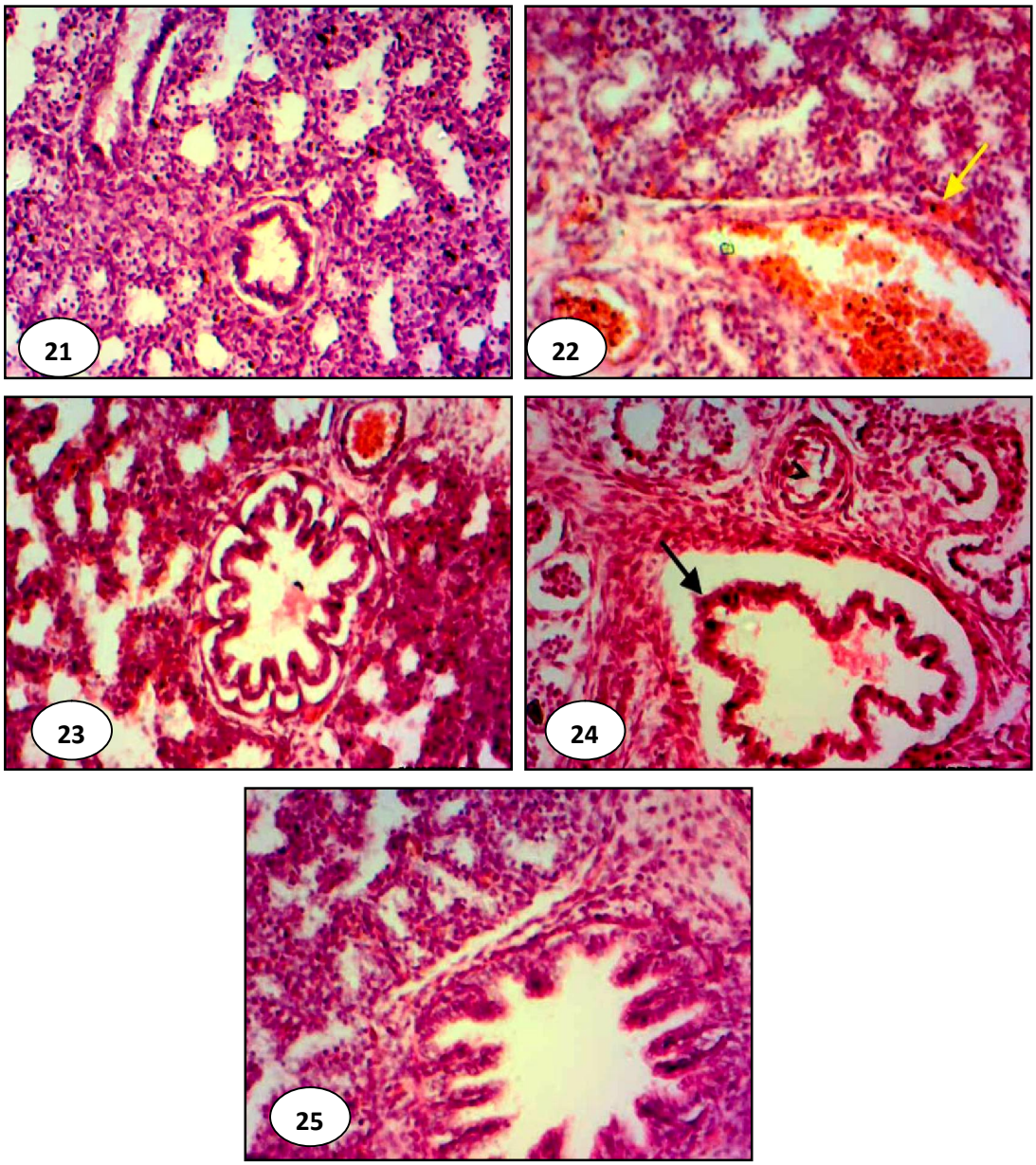

Fig.(21): showing fetal lung tissue of a control pregnant rat. **(H &E x 100)**

Figs.(22):showing fetal lung tissue of R_7 group. Notice: highly dilated and congested blood vessels with numerous haemorregic areas around the bronchiole and alveolar septae with presence of micronucleus (↑). Fibrosis was detected beside the wall of bronchiole (f). **(H &E x 100)**

Fig.(23): showing nearly normal fetal lung tissue of R_{7+BM} group with exception of the congested alveolar septae. **(H &E x 100)**

Figs.(24):showing fetal lung tissue of R_{14} group. Notice: delaminated epithelial layers of the bronchioles (↑) which lost their normal architecture and surrounded by complete fibrous layers, highly distorted arterial wall (^) which contained haemolysed blood cells and alveolar septae lost their normal architecture. The lumen of the bronchiole contained debris of degenerated epithelial cells.**(H &E x 100)**

Fig.(25): showing nearly normal bronchiole and alveolar septae in the fetal lung tissue of R_{14+BM} group, but some alveolar septae were congested .**H &E x 100)**

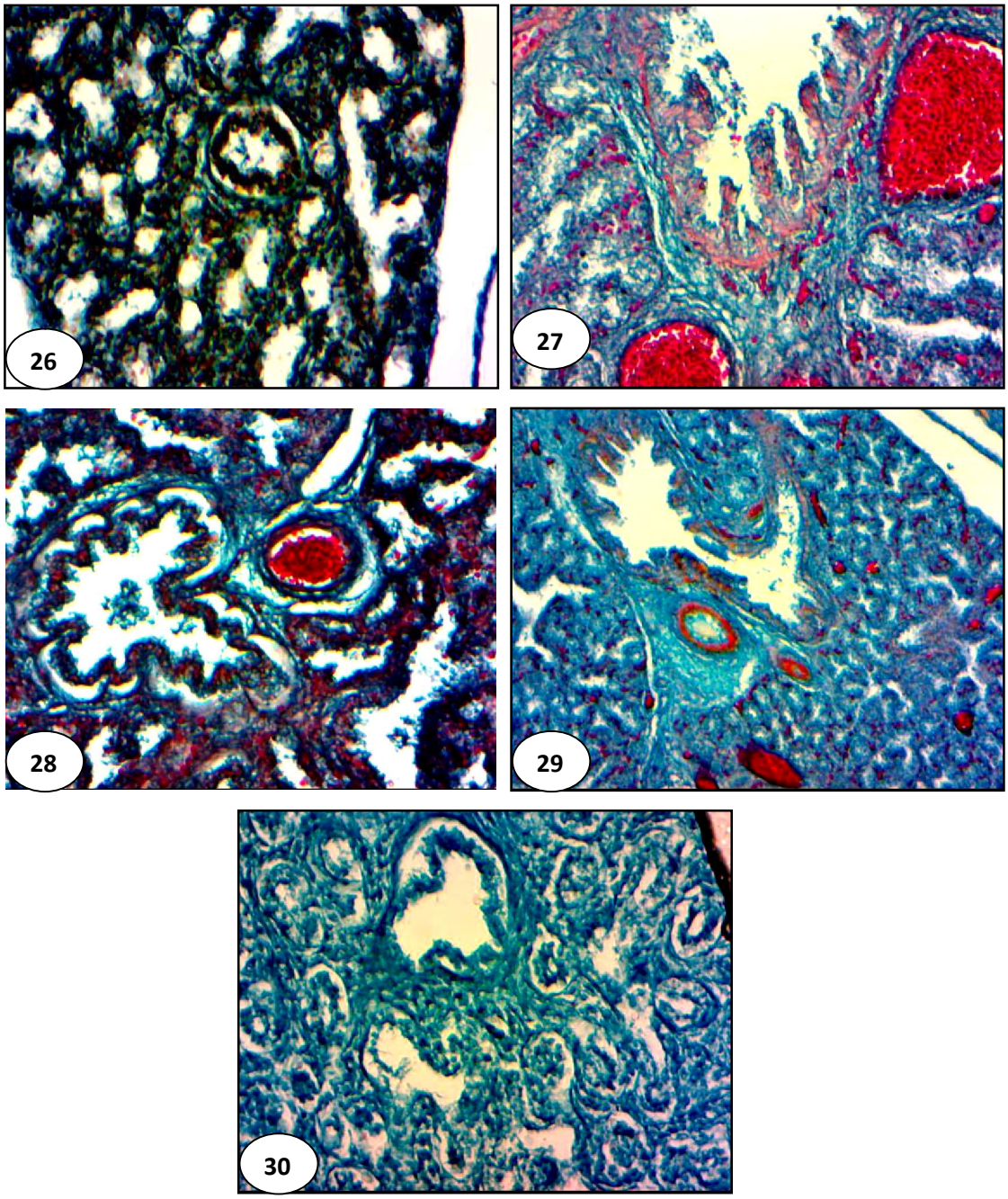

Fig.(26): showing normal distribution of collagen fibers in the fetal lung tissue of a control pregnant rat.**(Mallory's trichrome stain x 100)**

Figs.(27): showing collagen fibers in the fetal lung tissue of R_7 group. Notice: highly increased collagen fibrers in the walls of bronchioles, dilated blood vessels, thickened alveolar septae and blood vessels. **(Mallory's trichrome stain x 100)**

Fig.(28): showing collagen fibers in the fetal lung tissue of R_{7+BM} group followed by bone marrow transplantation one hour post-irradiation. Notice: a slight increase in collagen fibers in and around walls of bronchioles, blood vessels and alveolar septae. **(Mallory's trichrome stain x 100)**

Fig.(29):showing collagen fibers in the fetal lung tissue of R_{14} group. Notice: increased collagen fibers in the branched and thickened walls of the bronchioles, fibrotic areas and thickened alveolar septae. **(Mallory's trichrome stain x 100)**

Fig.(30): showing nearly normal content of collagen fibers in the fetal lung tissue of R_{14+BM} group. **(Mallory's trichrome stain x 100)**

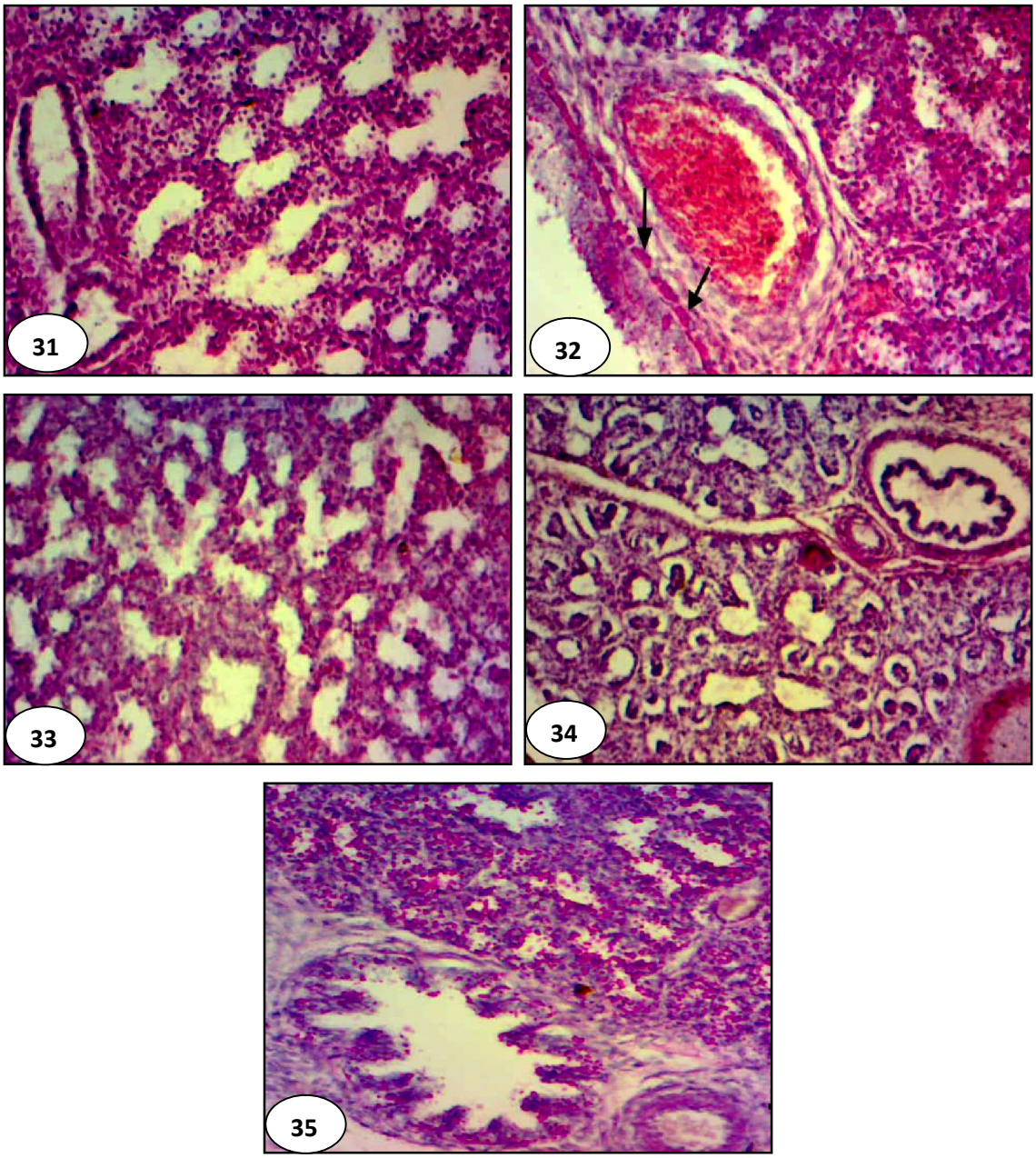

Fig.(31):showing normal distribution of polysaccharides in the fetal lung tissue of a control pregnant rat. **(PAS x100)**
Figs.(32):showing polysaccharides distribution in the fetal lung tissue of R_7 group. Notice: increased stain affinity of polysaccharides in RBCs found inside the congested blood vessels and haemorrhagic areas. Fibrous layers encircling walls of the bronchioles showed moderate stain affinity. Thickened alveolar septae showed increased stain affinity(↑). **(PAS x100)**
Fig.(33): showing normal distribution of polysaccharidesin the fetal lung tissue of R_{7+BM} group. **(PAS x100)**
Fig.(34):showing dense stain affinity of polysaccharides in the delaminated epithelial layer of the bronchiole, thickened alveolar septae and arterial wall of the fetal lung tissue of R_{14} group. **(PAS x100)**
Fig.(35): showing polysaccharides in the fetal lung tissue of R_{14+BM} group. Notice: a slight increase in stain affinity of polysaccharides in the wall of the bronchiole and alveolar septae due to increased RBCs.**(PAS x100)**

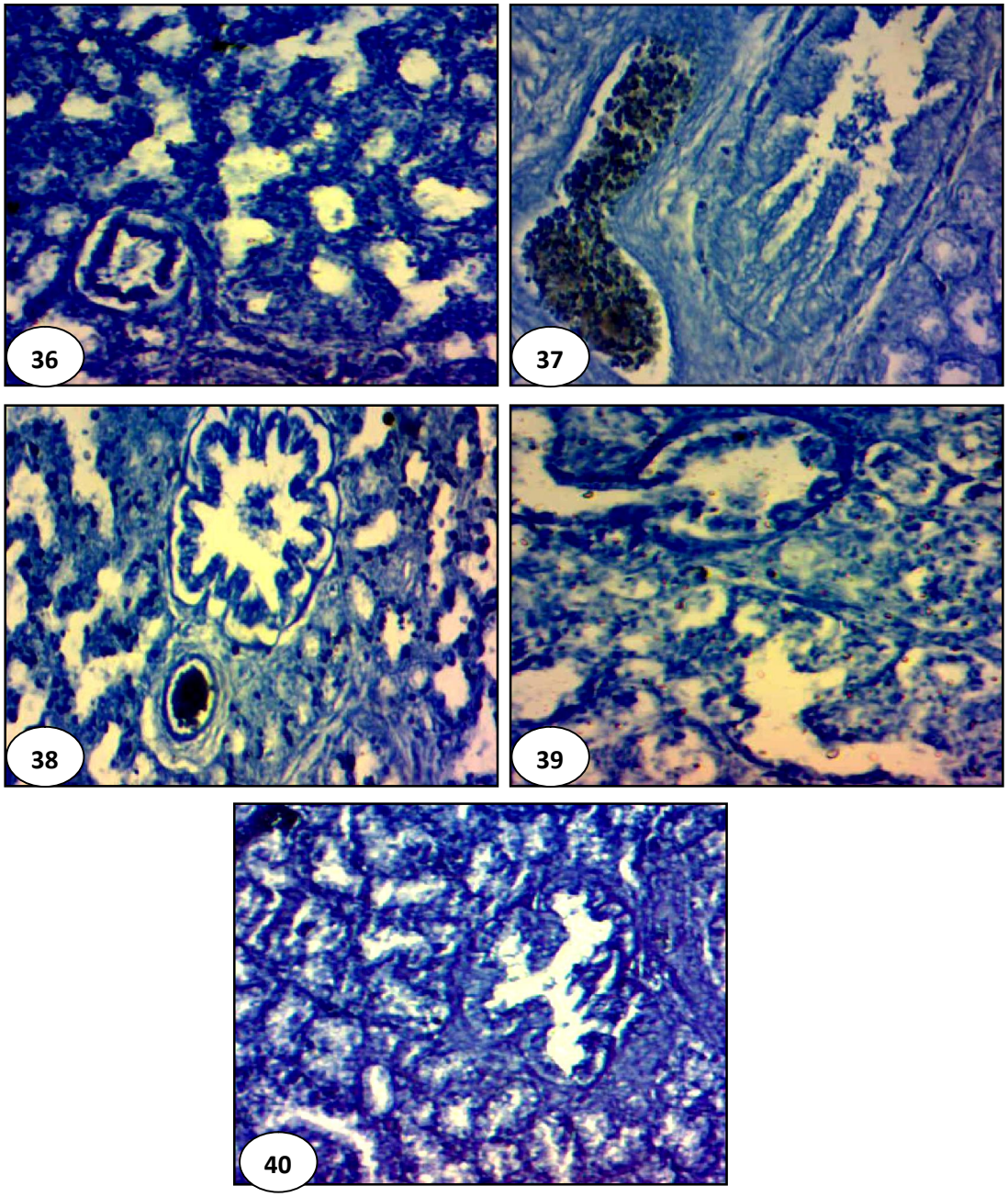

Fig.(36): showing normal distribution of total protein in the fetal lung tissue of the control group. **(Mercuric bromophenol blue x 100)**

Fig.(37):showing total protein in the fetal lung tissue of R_7 group. Notice: increased stain affinity of total protein in the highly congested and elongated arterial wall, but wall of the bronchiole and alveolar septae appeared less stained. **(Mercuric bromophenol blue x 100)**

Fig.(38): showing nearly normal content of total protein in the fetal lung tissue of R_{7+BM} group, but the congested artery contained deeply stained RBCs. **(Mercuric bromophenol blue x 100)**

Fig.(39): showing total protein in the fetal lung tissue of R_{14} group. Notice: faintly stained alveolar septae and walls of the bronchioles which were surrounded by densely stained fibrous layers **(Mercuric bromophenol blue x 100)**

Fig.(40):showing increased stain affinity of total protein in the thickened alveolar septae and the fibrous layer surrounding walls of the bronchioles of the fetal lung tissue of R_{14+BM} group**.** **(Mercuric bromophenol blue x 100)**

4. Discussion

The biological effect of radiation correlates with the given doses **(Fowler, 1994).** The most important target in living cells is DNA (direct target theory), but more often it indirectly damages DNA by including the formation of free radicals, particularly those that form the radiolysis of water (indirect target theory). Other cell molecules that may also be direct or indirect targets of radiant injury include lipids in cell membranes and proteins that function as critical enzymes. The transfer of energy to a target atom or molecules from the incident source of radiant energy occurs within micro fractions of a second, yet its biological effect may become apparent for minutes or, if the effect is on DNA, even decades **(Kumar *et al*., 2003).**

The interaction of ionizing radiation with the biological system resulted in generation of reactive oxygen species (ROS) or free radicals **(Gracy *et al.*, 1999; Strinivasan *et al.*, 2006; Mansour, 2012).** Free radicals cause oxidative stress where antioxidants decrease lipid peroxidation **(Basaga, 1990; Karbowink and Reiter, 2000; Nordberg and Arner, 2001).**

According to **Heibashy (1990)** ionizing radiation caused destructive effect on the cells of tissues which release enzymes from organells, moreover ionizing radiation caused alternation in the ability of enzymes to hydrolyse phosphate esters. Damage can occur due to direct ionization of DNA molecule itself or indirectly through the formation of toxic products, such as free radicals and free ions that interact with any molecule in their path **(ATSDR, 1999).** Several studies have suggested that infield DNA damage after lung irradiation is caused by both the direct effects of radiation and the indirect effects of the inflammatory response, whereas the out-of-field damage may be caused by the indirect effects of the inflammatory response alone. The exact mechanisms involved in the inflammatory response to radiation are unknown; however, it has been suggested that the generation of ROS (and RNS) immediately after irradiation, together with a cyclic (and chronic) up-regulation of inflammatory cytokines and the recruitment of inflammatory cells such as macrophages and neutrophils, is responsible for the damage seen in the lung after irradiation (**Khan *et al*., 2003; Fleckenstein *et al.,* 2007)**

Physiological alternations take place in the mother during pregnancy. These changes are profound and vital for the successful completion of gestation. Many of these adaptations are hormonally mediated and others are attributed to the effect of gravid uteri **(Bocking, 1994).**

Exposure to gamma rays during the intrauterine development can produce a broad spectrum of congenital abnormalities, growth retardation, developmental delays and functional defects (**Kiskova and Smajda, 2006)**.

Pompfer *et al. (*1992) concluded that exposure to 2.05Gy X-rays at day 8 after conception resulted in increasing malformations in the sensitive tissues and caused defects in the eye, skull and nervous system as results of delayed cell division.

High incidence of resorptions and fetal lethality may be due to the direct action of radiation on the fetuses or inhibitory action of radiation on the protein synthesis and placental dysfunction **(Abu Gabal *et al.*, 1994a,b; El Naggar *et al*., 1996).**

The degree of damage induced by irradiation depends on the degree of differentiation, state of the cell concerning it's cycle, the dose rate and the age of the animal at the time of irradiation (**Moustafa, 1997; Salama, 2004).**

Morphological changes:

1- Uterine form and resorptions:

In the present study different abnormalities in the uteri of pregnant female were noticed as unequal distribution of embryos in the two horns, implantation in one horn only, beside shrinkage of the two horns and complete abortion in irradiated pregnant rats on day 7 day of gestation. These results are in line with those of **Hong Wang *et al*. (1993) ; Kafafy *et al. (*2006).**

Ramadan (2007 b) found that pregnant albino rats exposed to 3Gy (1Gy/3 times) on the 7^{th}, 11^{th} and 15^{th} days of gestation showed severe abnormalities, frequent implantation sites and resorptions.

The present results obtained from pregnant rats irradiated at 2Gy gamma rays on days 7 or 14 of gestation and sacrificed on day 20 of gestation resulted in numerous abnormal features. The characters of variation are summarized as evidence of abnormalities, implantation in one horn only, beside unequal distribution of fetuses in the two horns, presence of resorption leaving resorbed bodies especially in the 14^{th} day of gestation and reduction in size of embryos. These results are supported by other authors who reported more or less similar results which varied with the variation of the dose or the stage of development at which radiation was performed **(Prakash Hande and Uma-Devi, 1993; El-Naggar *et al*., 1996; Ashry, 1997; Salama, 1998; Kafafy *et al.*, 2006., Ramadan, 2007a).**

Bone marrow transplantation post-irradiation in this study showed improvement in the uterine form with equal distribution of embryos in the two horns in the pregnant rats irradiated at 2Gy γ-rays on days 7 of gestation, but in the pregnant rats irradiated at 2Gy γ-rays on days 14 of gestation, unequal distribution of

embryos in the two horns were noticed. These results agree with those of **Hussein (2004).**

2- Embryonic malformations:

Teratogenitic effects observed in the present work due to radiation exposure ($R_{14}$and some cases in R_7 groups)were expressed as size diminution, growth retardation, clubbed limbs, malformed eye lids, malformed rostrum, and absence of some digits of hands or toes of legs, twisted tail, short tail and subcutaneous haemorrhage. These results agree with those of **Ramadan (2007b)** who found that pregnant albino rats irradiated with 0.5Gy 4 times on gestation days 9,10,11,12, showed fetal interauterine death together with serious teratogenic effects in the head, eye and extremities of surviving fetuses and **Moustafa (2000)** who reported that teratogenic effects of γ-rays observed mainly in the fore and hind limbs and tail region, ectodactyles and three metacarpals.

The growth retardation recorded in the present work agree with the results reported by many authors **(Hussein, 2004; Ramadan, 2007a).**

In the present study bone marrow transplantation post-irradiation showed a slight improvement in the size and length of fetuses on pregnant rats irradiated at 2Gy γ-rays on days 7 or 14 of gestation. These results agree with those of **Hussein (2004).**

2-Lung

A-Lung of the pregnant rats

In the present study exposure of the pregnant rats to 2Gy of γ-rays on day 7 of gestation showed many deleterious changes in lung tissue of the pregnant rats, but, these changes were more pronounced on day 14 of gestation**.** These changes include highly thickened and congested alveolar septae, highly elongated and branched bronchioles, their lumen contained debris of degenerated epithelial cells with ruptured epithelial lining of these bronchioles and thick fibrous layers surrounding them, also highly thickened, corrugated and distorted arterial walls were observed with different masses of granuloma.

In this respect, **Fleckenstein *et al*. (2010)** stated that chronic production of reactive oxygen and nitrogen species is an underlying mechanism of irradiation induced lung injury, they also noticed increased lung damage and decreased immunity in female rats post-irradiation (28Gy).

In **2005, Shediwah** exposed male rats to 1Gy γ-rays per day for 10 days and noticed many histological changes in lung of these rats; these changes include different masses of granuloma, fatty degeneration, thickened and congested alveolar septae, many fibrotic areas and distorted, elongated and thickened walls of the bronchioles and the blood vessels. **Mahmod (2006)** noticed that radiation exposure caused many changes in the haemoglobin such as decreased peptide bound and he noticed many pathological changes in RBCs. He added that increased mutation and decreased DNA content in nuclei of the cells post-irradiation may be due to production of active oxygen which lead to oxygen pressure and increased free radicals which affect chains of DNA that lead to cancer.

Abdollahi *et al*. (2005) noticed damaged lungs of rats exposed to non-ionizing radiation (20Gy). Through radiotherapy **Petit *et al*. (2005)** noticed increased pneumonocytes 2-3 months in females aged 50-60 years post radiation exposure. In **2005, Van der Meeren *et al*.,** demonstrated increased immunological response in lungs of rats exposed to 15Gy.

Lung damage and lung injury pneumonitis post-irradiation was noticed by several authors **(Liao *et al*., 2000; El Khatib *et al*., 2009 ; Kirsch *et al*., 2010; Miyake,2012).**

Thickened and congested alveolar walls congested and dilated blood vessels with haemolysis, dilated bronchioles and appearance of different masses of granuloma were realized in mice lung by **El Salkh (2009)** post-exposure to EMF radiation. Neutrophils are the first responding cells in lung tissues injured by irradiation (**Chiang*et al*., 2005)**. Lymphocytic alveolitis is a well-recognized component that occurs in response to tissue injury caused by irradiation, and aprominent feature of post-radiation lung injury is the development of lymphocytic alveolitis (**Huang *et al.*, 2002).**

Dilated and congested blood vessels observed in this study may be due to increased pulmonary arterial pulse pressure noticed by **Grant *et al*. (1988)** in rats exposed to radiation. Degenerated alveolar septae and cells of the epithelial layer in the bronchioles observed in the present study may be due to highly affected DNA in the nuclei of their cells **(Simko, 2000 ; Zhang *et al*., 2006)**

Bone marrow transplantation post-irradiation in the present study showed a noticeable improvement in the lung tissue of the pregnant rats especially on day 7 of gestation**,** but highly thickened alveolar septae and arterial walls were still observed on day 14 of gestation.

The present results revealed that in case of injury, the stem cells from bone marrow are responsible for tissue regeneration and these cells have unique properties that make them attractive candidates for the treatment of diseases and injuries. Also stem cells can be transplanted to replace non-functional or lost stem cells in tissues to accelerate

tissue healing and restore the original function **(Burt *et al*., 2008).**

In this study, highly increased collagen fibers was detected in the lung tissue especially in the thickened walls of the arteries, veins, bronchioles and alveolar septae in the lung tissue of the pregnant rats exposed to 2Gy γ-rays on day 7 or day 14 of gestation.

Zhang *et al* .(2006) noticed many changes in the lungs exposed to irradiation and they reported that increased collagen post-radiation exposure may lead to rapid healing, rapid differentiation of cells and appearance of a new network of blood vessels.

According to **Ahmadian *et al*. (2006)** radiation exposure of rats lead to increased collagen in the skin. **Khaki *et al*. (2006)** reported that decreased collagen, reticular fibers, ribosomes, glycogen granules and cristae of mitochondria may led to corrugated membranes.

Increased collagen post-irradiation exposure in the different tissues was detected by several authors **(Shedwah, 2005; Al Gahtani, 2006; Eid and Al Dossary, 2007; El-Salkh, 2009).**

Rousovan *et al*. (1992) declared that the increase in the collagen fibers may be due to increased interstitial and white fibers under the effect of radiation, but, **Hassan *et al*. (1988)** reported that increased collagen fibers may lead to increase the defense reaction against toxic materials.

The results of the present study showed nearly normal collagen content in the lung tissue of the pregnant rats treated with bone marrow post-irradiation on day 7 of gestation**,** while, on day 14 of gestation increased collagen fibers was detected in the fibrotic areas and highly thickened alveolar septae. Walls of blood vessels, bronchioles and areas of granuloma cells showed increased affinity of polysaccharides in the lung tissue of the pregnant rats exposed to 2Gy of γ-rays on day 7 or day 14of gestation with less stained debris of epithelial cells inside the bronchioles and haemolysed RBCs.

Increased stain affinity of polysaccharides post-irradiation in this work was also noted by many authors **(Shedwah, 2005; Al Dossary, 2007 ; El Salkh, 2009).** Increased stain affinity in granuloma cells indicating the high content of polysaccharides in these cells and increased stain affinity inside walls of bronchioles, blood vessels, alveolar septae and fibrotic areas may be due to increased thickness of these components.

Decreased polysaccharides content in the degenerated epithelial cells of bronchioles and haemolysed RBCs was detected also by **Abu El Naga (1989)** in lung tissue exposed to γ-rays. Reduced glycogen in cells post-irradiation may be due to decreased T_3 and T_4 hormones of the thyroid glands, which lessen entrance of glucose to the cells

Results of the present study showed nearly normal polysaccharides content in the lung tissue of the pregnant rats treated with BM post-irradiation on day 7of gestation, but, on day 14, thickened alveolar septae, arterial walls and the fibrous layers encircling the bronchioles acquired a dense stain affinity of polysaccharides.

Concerning total protein, deeply stained walls of arteries and brochioles, alveolar septae and granuloma cells were observed in the lung tissue of pregnant rats exposed to 2Gy of γ-rays on day 7 or day 14 of gestation**.** This increase may be due to increased thickness of the different walls, fibrotic areas and increased areas of granuloma cells. Increased total protein in lung tissue post exposure to different types of radiations was noticed by many authors **(Gorczynsk and Wegrynowicz ,1991; Shedwah, 2005; Al Dossary, 2007; El-Salkh, 2009; Mansour, 2012).**

Highly affected protein and DNA post-radiation exposure may be due to response of hydrogen bounds of these materials to radiation.

Bone marrow transplantation post-irradiation in this study showed somewhat normal total protein content in the lung tissue of pregnant rats on day 7or day 14of gestation, but thickened a lveolar septae and corrugated walls of bronchioles were deeply stained.

B- Lung of the embryos

In the present studyexposure of pregnant rats to 2Gy of γ-rays on day 7 or 14 of gestation led to many histopathological changes in the fetal lung tissue. These changes were more drastic on day 14 of gestation. These changes include: highly dilated and congested blood vessels with numerous haemorregic areas and common fibrosis around the walls of bronchiole. The lumena of these bronchioles contained debris of degenerated epithelial cells; the congested alveolar septae lost their normal architectures.

In agreement with the present results **Abu El-Naga (1989)** studied the pathological changes in embryos exposed maternally to 3Gy of γ-rays on the 6^{th} and 10^{th} days of the gestation. She noticed thickened alveolar septae with reduced alveolar sacs and highly dilated and ruptured walls of blood vessels. She also noticed lots of haemrrhagic areas and karyolysis in numerous nuclei.

Abu Gabal *et al*. (1998) observed pyknotic nuclei in cells of the lung tissue of embryos maternally exposed to 1Gy γ-rays, while those maternally exposed to fractionated 2Gy showed some delay in the development with thickening of their inter alveolar septae.

Also, **Al-Dossary (2007)** observed many haemorrhagic areas covered the thickened alveolar septae in the fetal tissue maternally exposed to EMF radiation. She noticed many pyknotic nuclei in the epithelial cells of the bronchioles with highly thickened and corrugated arterial walls and haemolysed blood cells inside them.

In the control fetal lung tissue, thin collagen bundles are supporting the walls of the bronchioles, blood vessels and alveolar septae, highly increased collagen fibers was observed in the fetal lung tissue maternally exposed to 2Gy γ-rays on day 7 or day 14 of gestation. Dilated walls of blood vessels, thickened alveolar septae and walls of the bronchiole showed dense stain affinity of collagen.

Results of the present study showed somewhat normal distribution of collagen fibers was detected in the fetal lung tissue maternally treated with 2Gy γ-rays on day 7 or day 14 of gestation followed by bone marrow treatment, but a slight increase of these fibers was detected in the walls of the bronchioles, blood vessels and alveolar septae on day 14 of gestation.

The regenerative potential of stem cells was studied by several authors **(Ferrari *et al*., 1998; Pye and Watt, 2001; Kirsch *et al*., 2010).**
The improvement observed in the fetal lung tissue maternally exposed to γ-rays and treated with BM may be due to the ability of bone marrow cells to differentiate to mature, non-haematopoitic cells of multiple tissues **(Abedi *et al*., 2004).**

Concerning polysaccharides, the fetal lung tissue maternally exposed to 2Gy γ-rays on day 7 or day 14 of gestation showed increased stain affinity.

Increased stain affinity of polysaccharides in the fetal lung tissue exposed maternally to γ-rays may be due to increased RBCs in the congested sinusoidal spaces, blood vessels and haemorrhagic areas, since RBCs contained 10% of their weight polysaccharides (**Junqueira and Carneiro, 2003).** In accordance to the present results **Moustafa and Hafez (1998) and Moustafa (2000)** noticed an increase in PAS +ve materials in the fetal tissues post exposure to 2Gy γ-rays.

Eid *et al.* (1994) indicated that the frequency of changes in polysaccharides content was high in lung and ileum tissue of rat embryos exposed to 3Gy on days 6 and 12 of pregnancy. Fetal lung tissue taken from mothers exposed to 2Gy γ-rays on day 7 or day 14 of gestation followed by bone marrow transplantation restorted the normal polysaccharides content with a slight increase in stain affinity in walls of the bronchioles, and alveolar septae of the fetal lung tissue on day 14 of gestation.

In the present study increased stain affinity of total protein content was observed in the fetal lung tissue exposed maternally to 2Gy γ-rays on day 7 of gestation. Faintly stained alveolar septae and densely stained fibrous layers were detected on day 14 of gestation. This increase in stain affinity of total protein may be due to increased RBCs in the congested alveolar septae and blood vessels or may be due to appearance of the fibrous tissue, but reduced stain affinity of total protein may be due to damaged protein molecules by irradiation. This finding is in accordance with those of **Kapyaho *et al.* (1983)** who stated that ionizing radiation usually inhibits the protein synthesis and the decline of protein which they recorded could be attributed to the degeneration in the cellular tissues.

In the present study somewhat normal total protein content was detected in the fetal lung tissue maternally treated with the bone marrow post-irradiation on day 7 of gestation, while congested arteries contained deeply stained RBCs. But on day 14 of gestation, highly thickened alveolar septae and the fibrous layers surrounding the bronchiole**s** showed increased stain affinity of total protein.

It is clear that pregnant rats exposed on day 14 of gestation are more sensitive to γ-rays; BMT cannot completely overcome radiation injury and restore the normal content of collagen polysaccharides and total protein content in the fetal and maternal lung tissue.

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