Pathological and biochemical studies on the negative economic effects induced by the more competitive air pollutant on laying chickens farms in El-Sharkia province

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Abstract
One hundred, four months old ISA brown, laying hens were tested for their reproductive efficacy on a base of good health and regular ovulatory pattern. They were housed in a farm located among the rice crop agriculture field and subjected to the rice straw burn gases during the whole experimental period with an average time of 7 hours/day for 60 days. Hens were used for studying the pathological and biochemical effects of gases elevated from rice straw burn process on ovulation and egg production. The experiment began from the day before the burn process in Autumn season at which blood samples were collected 1-2 hours prior to oviposition for hormonal assay and arterial blood gases analysis as a control, other blood samples were collected and analyzed at 15, 30, 45 and 60 day PE (post exposure) , Specimen from trachea, lungs, liver, ovary, heart and kidneys were collected and handled with histological technique then examined microscopically .Negative effects were detected and discussed.

Key words: *ovulatory pattern, laying hens , oviposition .*

Introduction

The open field burning is the cheapest disposal method for clearing the straw from fields in preparation for the succeeding planting season to release nutrient for the next growing season, in addition it considered the method of choice for controlling fungal stem-rot disease of rice **(Hsieh *et al*., 1981**).

Biomass burning introduces several compounds into the atmosphere including carcinogenic and mutagenic compounds as polycyclic aromatic hydrocarbons (PAHs) which can be formed by anthropogenic process (mainly the combustion process) or from nature of sources **(Hayakaw *et al*., 1995)**.

Open burning of biomass is a common technique for agriculture residue disposal which represents a considerable source of atmospheric pollutants. Emissions from biomass burning contribute a major part of trace gases and particulates that comprise carbon monoxide, hydrocarbon, carbon dioxide and sulfur oxide and significantly impact the photochemistry of the atmosphere **(Korenaga *et al*., 2001)**.

Rats exposed to PAHs, 5 hours daily for 15 and 30 days showed apnea during smoke inhalation and increase respiration immediately after exposure then gradually decrease and dyspnea occurred while increase nasal secretion and mouth breathing, wheezing in late stage were seen, lowering of the blood pH, significant increase in $PaCO_2$ and decrease in PaO with decrease level of HCO_3^- ions had been noticed **(Samah, 2008)**.

Ovarian follicle growth and development are controlled by a myriad of endocrine, paracrine and autocrine factors, including gonadotropins and growth factors **(Li and Johnson, 1993)**.

Growth of ovarian follicles in the domestic hen is precisely regulated, but the nature of this regulation is unknown. The follicles on the single ovary are arranged in a hierarchy, with largest follicle (F1) destined to ovulate on the next day, the second largest (F2) on the day after, and soon. Previous research had elucidated many of the endocrine events associated with ovulation. We know that pre ovulatory peak of LH, progesterone, testosterone and estrogen precede ovulation by 6-8 hrs **(Wilson and Sharp, 1973** and **Johnson and Van**

Tienhoven, 1980).
Because of the scarcity of avian FSH, there are few studies characterizing the pattern of secretion of FSH during the hen's ovulatory cycle. It has been reported that a rise in plasma FSH occurs in 14-15 hrs prior to ovulation **(Scanes *et al*., 1977)**. But the Radioimmunoassay(RIA) used for this result has been criticized as lacking sensitivity **(Sakai and Ishii, 1983)**.
Luteinizing hormone (LH) is a key hormone in the control of reproduction in birds. The secretion of LH by the anterior pituitary is regulated by gonadotropin releasing hormone. In females, the secretion of LH and follicle stimulating hormone (FSH) control the secretion of estrogen **(Bacon *et al*., 1980)**.
The steric resemblance of PAHs to steroid molecules led to the postulation that they would be able to bind to the same receptors as steroid hormones .Both invivo and in-vitro studies have demonstrated that PAHs have both oestrogenic and antioestrogenic activities **(Charles *et al*., 2000)**.
Slight hyperplasia of tracheal epithelium ,trachea exhibited a more severe mucociliary hyperplasia and small areas of squamous metaplasia, acute inflammation (edema and/or granulocyte infiltration), subacute inflammation (mononuclear infiltration and increase fibroblasts) fibrosis and hyalinization were seen with all PAHs in respiratory mucosa **(Topping and Martin, 1978)**.
Hubberd chicks exposed to PAHs 7 hrs daily for 15, 30, 45 days showed congested lung which became hard in texture with dark areas at 30 and 45 days Post exposure(PE), markedly swollen liver in some cases while microscopically anthracosis with congestion of pulmonary, hepatic blood vessels at 15 days PE, hyperplasia of the lining bronchial epithelium, periductal fibrosis around the proliferated bile duct at 45 days PE were noticed **(Rehab *et al*., 2012)**.
Our work aimed to study the pathological and biochemical effects of PAHs resulting from rice straw burn process on ovulation and egg production of ISA brown hens.

Material and Methods

Experimental hens
One hundred, 4 months old ISA brown, laying hens were selected on a base of good health and reproductive efficacy noticed in its regular oviposition. Hens were housed in a farm located among the rice agriculture field and treated according to an ideal program included balanced ration, suitable temperature, light and sufficient pure water supply.
These hens subjected to the experiment (rice straw burn gases) which lasts for 2 months with an average time of 7 hrs/day.
Arterial blood gases analysis
The day before burn process as a control and at 15, 30, 45 and 60 days post exposure, ten arterial blood samples were collected from brachial artery for arterial blood gases analysis (PaO_2, $PaCO_2$ and HCO_3^-ions) and pH were determined by gas analyzer, model **AVL990 (Samah, 2008)**.
Hormonal analysis
Ten blood samples were collected from branchial vein for analysis of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone by radioimmunosorbant assay (RIA) at a special laboratory in Zagazig city **(Bacon and Long, 1996)**.
Pathological studies:
Ten birds were necropsied at 15, 30, 45 and 60 days PE and five birds at The day before the beginning of the experiment. Specimens from trachea, heart, lung, liver, ovary and kidneys were collected and fixed in 10% neutral buffered formalin solution. Paraffin sections of 5 µ thick were prepared and stained with haematoxylin and eosin for histopathological examination **(Bancroft and Stevens, 1996)**.
Statistical analysis
The data obtained from this investigation were statistically analyzed by F-test **(Tamhane and Dunlop, 2000)** using MSTAT-C computer program.

Results and Discussion

Clinical signs
One day before the beginning of the burn process, the hens were healthy with normal ovulatory pattern and mortality rate (2%). At 15

days PE, mild respiratory manifestation appeared, more birds involved by time, these manifestations became severe at 45 and 60 days (PE) expressed as rapid respiratory rate, dyspnea, nasal secretion with 6% mortalities and 91% morbidity that could be attributed to inhalation of suspended smoke particle matter, from biomass burning of rice straw **(Favez *et al.*, 2008).** Changing the ovulatory pattern which became irregular with changing of the oviposition intervals to 28-39 hrs at 30 and 45 days PE and 40-52 hrs at 60 days PE instead of 24hrs as normal pattern which lead to economic losses in egg production.

Arterial blood gases analysis

As shown in table (1), compared with normal control group (one day before exposure) there were a highly significant decrease in pH at 15 > 30 > 45 > 60 days PE. There were a highly significant increase in $PaCO_2$ at 15 > 30 > 45 > 60 days PE. There was no significant change in PaO_2 in all groups when compared with control and with each other. Meanwhile HCO_3-ions showed highly significant decrease at 30 days PE when compared with control and 15 days PE and also highly significant decrease at 45 and 60 days PE when compared with each other groups. These results were mostly agree with those obtained by **(Rehab *et al.*, 2012)** and disagree with those obtained by **(Samah, 2008)** which could be attributed to the differences in species used and time of exposure.

Hormonal assay

In table (2), FSH compared with control group (one day before exposure) showed highly significant increase when compared with other groups, meanwhile at 15 days PE there was no significant change when compared with other groups.At 30 days a highly significant decrease was noticed when it compared with the control . Meanwhile, at 45 and 60 days PE only significant decrease in comparison with control. LH showed highly significant decrease between those data obtained at control and 15 days PE when compared with those obtained at 30, 45 and 60 days PE while there were no significant change between those at control and 15 days PE and between 45 and 60 days PE. Highly significant decrease at 30 days PE was observed when compared with all other groups. These results were partially agreed with those obtained by **Samah (2008) and Rehab *et al.*, (2012)**.

Estradiol showed regular and gradual highly significant decrease among all groups with each other meanwhile these results could be attributed to anti estrogenic effect of PAHs **(Bui *et al.*, 1986)**. Progesterone showed highly significant decrease at 15 and 30 days PE when compared with control and also at 45 and 60 days PE when compared with the other groups, on the contrary there is no significant change between these obtained data at 15 and 30 days PE and also between these at 45 and 60 days PE. These results could be attributed to the reproductive toxicity induced by PAHs **(WHO, 2001)** and also may be due to hypoproteinemia induced by PAHs **(Rehab *et al.*, 2012)**.

Table (1). Arterial blood gases (mean values ±S.E) of hens at different times (n = 5)

Parameters / Treatments	pH	$PaCO_2$ (Kpa)	PaO_2 (Kpa)	HCO_3- Mmol/L
Control	7.356 ±0.037[a]	44.91 ±0.22[e]	12.13 ±0.083	24.59 ±0.18[a]
15 days PE	7.140 ±0.047[b]	48.81 ±0.47[d]	12.21 ±0.186	24.72 ±0.11[a]
30 days PE	6.96 ±0.021[c]	50.97 ±0.29[c]	12.19 ±0.105	24.15 ±0.05[b]
45 days PE	6.7 ±0.047[d]	51.92 ±0.29[b]	11.96 ±.02	23.81 ±0.05[c]
60 days PE	6.12 ±0.031[e]	52.92 ±0.09[a]	12.14 ±0.14	23.55 ±0.10[c]
F-test	**	**	N.S	**

N.S: Non significant at P<0.05 * *: Highly significant at P<0.01

Table (2). Reproductive hormones (mean values ±S.E) of hens at different times (n = 5)

Parameters / Treatments	FSH mlU/ml	LH mlU/ml	Esradiol Pg/ml	Progesterone ng/ml
Control	0.159±0.01[a]	0.104±0.001[a]	131.86±1.13[a]	0.106±0.007[a]
15 days PE	0.120±0.003[bc]	0.102±0.005[a]	117.86±0.99[b]	0.091±0.004[b]
30 days PE	0.121±0.004[b]	0.09±0.001[c]	99.12±0.78[c]	0.087±0.001[b]
45 days PE	0.105±0.002[bc]	0.094±0.002[b]	54.13±1.97[d]	0.080±0.002[c]
60 days PE	0.108±0.004[bc]	0.096±0.005[b]	42.95±2.5 l[e]	0.074±0.002[c]
F-test	**	**	**	**

*: Highly significant at P<0.01.
Means in the same column followed by different letters were statistically significant and the highest values were represented with the letter (a).

Pathological studies

Macroscopically, exposed hens showed congestion of trachea and lung at 15 and 30 days PE while severe congestion with black pigmentation and hard texture were noticed in lung at 45 and 60 days PE **(Fig. 12)**. Some cases showed marked swollen liver and heart at 15 and 30 days PE. Ovary showed congestion at 15 days PE and hypotrophy was observed at 45 and 60 days PE.

Microscopically,

At 15 days PE sacrificed hens showed congestion of tracheal blood vessels (**Fig. 1**), severe congestion of pulmonary blood vessels (**Fig. 2**) and congestion of ovarian blood vessels with normal ovarian follicle and ova (**Fig. 3**), congestion of hepatic vein (**Fig. 4**) and myocardial blood vessels, while kidneys showed normal renal tissue.

At 30 days PE, sacrificed hens showed coal particles inside tracheal epithelium (Anthracosis) **(Fig. 5**) and inside the alveoli with severe congestion of pulmonary blood vessels (**Fig. 6**). Heart showed diffused mononuclear leukocyte cell infiltration **(Fig.7)** and hypertrophy of mycocardial fiber, ovary showed atrophied ova inside it's ovarian follicle **(Fig. 8**) while focal mononuclear leucocytic cell infiltration of hepatic parenchyma in addition to diffused fatty changes were seen (**Fig. 9**). Kidney showed congestion of cortical blood vessels in addition to perivascular fibrosis (**Fig.10**).

At 45 days PE, trachea of sacrificed hens showed mucinous degeneration of tracheal epithelium **(Fig.11)**, meanwhile lung showed hyperplasia of bronchial epithelium **(Fig**. **13**) with anthracosis. Perivascular mononuclear leukocyte cell infiltration and focal fibrosis of hepatic parenchyma were noticed **(Fig. 14**). Kidney showed mononuclear leukocyte cell infiltration in addition to mild cloudy swelling (**Fig. 15&16**). Ovary showed congestion of ovarian blood vessels and perioval fibrosis (**Fig. 17&18**) that could be attributed to PAHs toxicologic effects on reproductive system **(WHO, 2001)**. Heart showed broken myocardial fiber with mononuclear cell infiltration.

At 60 days PE, sacrificed hens showed absence of tracheal epithelium **(Fig. 20)** while lung showed congestion of pulmonary blood vessels, focal mononuclear leukocyte cell infiltration with severe anthracosis and hyaline degeneration (**Fig. 21**). Ovary showed atrophied follicle (complete absence of ovarian follicles) (**Fig. 22).** Liver showed hyperplasia of epithe-

lial lining of bile duct and periductal fibrosis around the proliferated bile ductule that could be attributed to the hepatotoxic effect of PAHs **(Brodie *et al*., 1971)**. Kidneys showed the mononuclear cell infiltration.

Our results were partially agreed with those previously recorded by **(Samah, 2008)** and mostly agree with **(Rehab *et al*., 2012)**.

We concluded that our results confirmed our notifications about the negative effect of straw burn smoke or gases on the ovulation and egg production of brown laying hens.

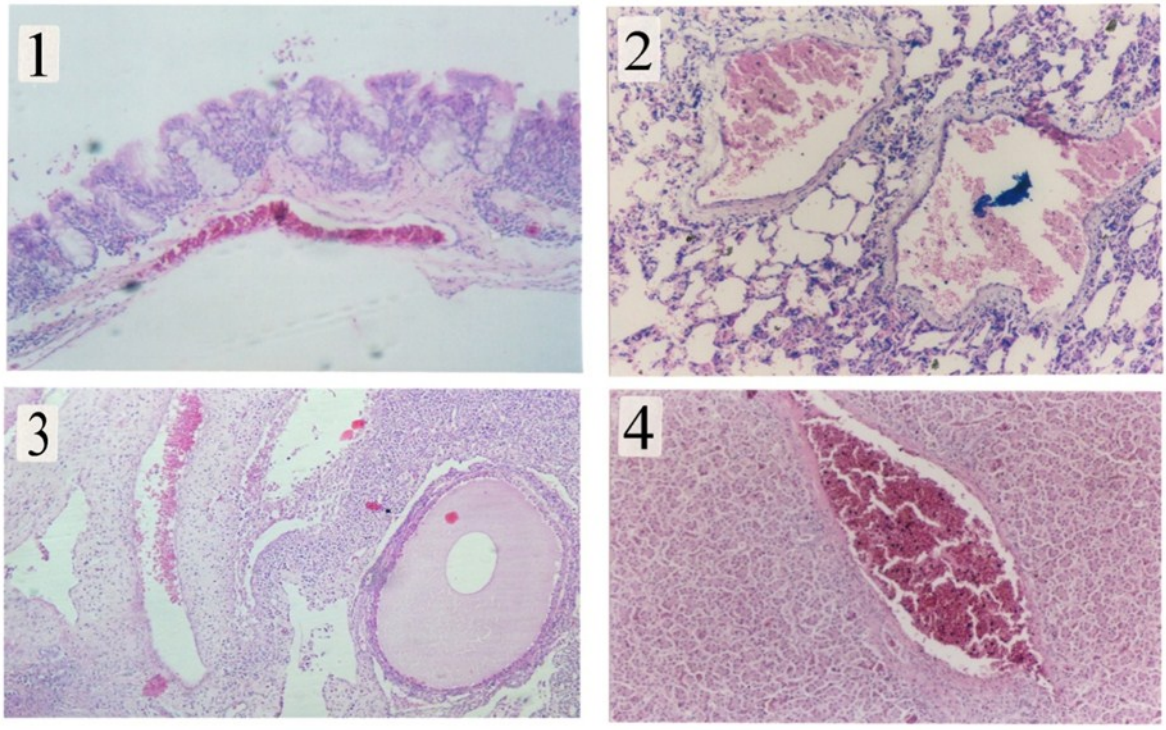

Plate 1 (From Fig. 1-4)

Fig.1. Photomicrograph of trachea of smoke exposed sacrificed hens at 15 days PE showing severe congestion of tracheal blood vessels. H&E x 130.

Fig.2. Photomicrograph of lung of smoke exposed sacrificed hens at 15 days PE showing congestion of pulmonary blood vessels. H&E x 520.

Fig.3. Photomicrograph of ovary of smoke exposed sacrificed hens at 15 days PE showing congestion with normal ovarian follicle and ova. H&E x 130.

Fig.4. Photomicrograph of liver of smoke exposed sacrificed hens at 15 days PE showing severe congestion of hepatic vein. H&E x 520.

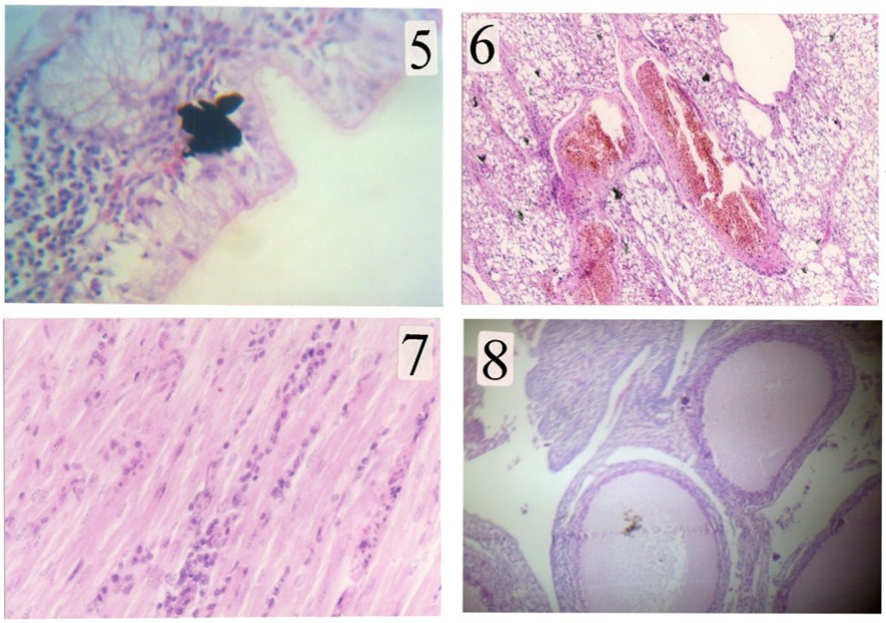

Plate 2 (From Fig. 5-8)

Fig.5. Photomicrograph of trachea of smoke exposed sacrificed hens at 30 days PE showing coal particles inside the tracheal epithelium (Anthracosis). H&E x 520.

Fig.6. Photomicrograph of lung of smoke exposed sacrificed hens at 30 days PE showing severe congestion together with anthracosis. H&E x 130.

Fig.7. Photomicrograph of heart of smoke exposed sacrificed hens at 30 days PE showing diffuse mononuclear leukocyte cell infiltration. H&E x 520.

Fig.8. Photomicrograph of ovary of smoke exposed sacrificed hens at 30 days PE showing atrophies eggs inside its ovarian follicles. H&E x 520.

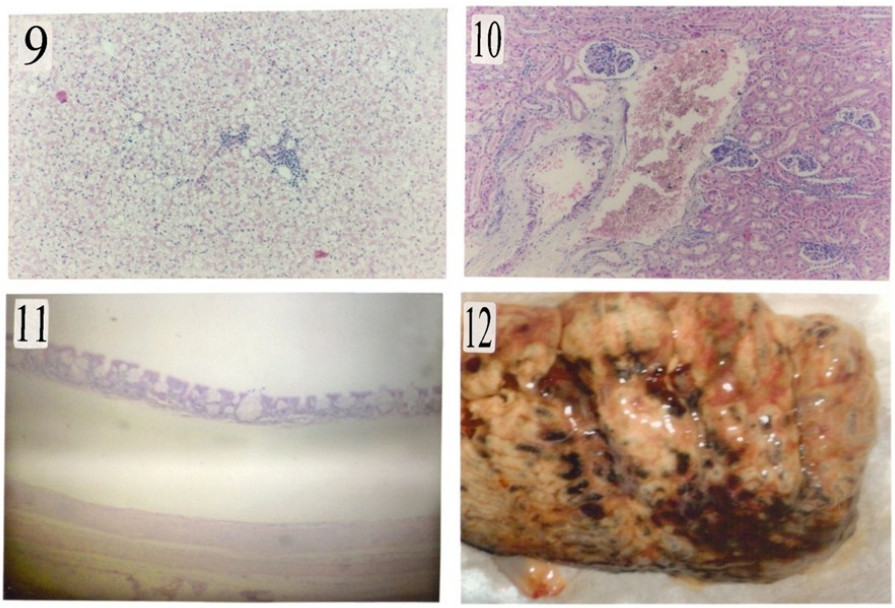

Plate 3 (From Fig. 9-12)

Fig. 9. Photomicrograph of liver of smoke exposed sacrificed hens at 30 days PE showing focal mononuclear cell infiltration and diffuse fatty change. H&E x 130.

Fig.10. Photomicrograph of kidney of smoke exposed sacrificed hens at 30 days PE showing congestion of cortical blood vessels and perivascular fibrosis. H&E x 130.

Fig.11. Photomicrograph of trachea of smoke exposed sacrificed hens at 45 days PE showing mucinous degeneration of tracheal epithelium. H&E x 130.

Fig.12. Lung of smoke exposed sacrificed hens at 45 day PE showing severe congestion, areas of black pigmentation (arrows).

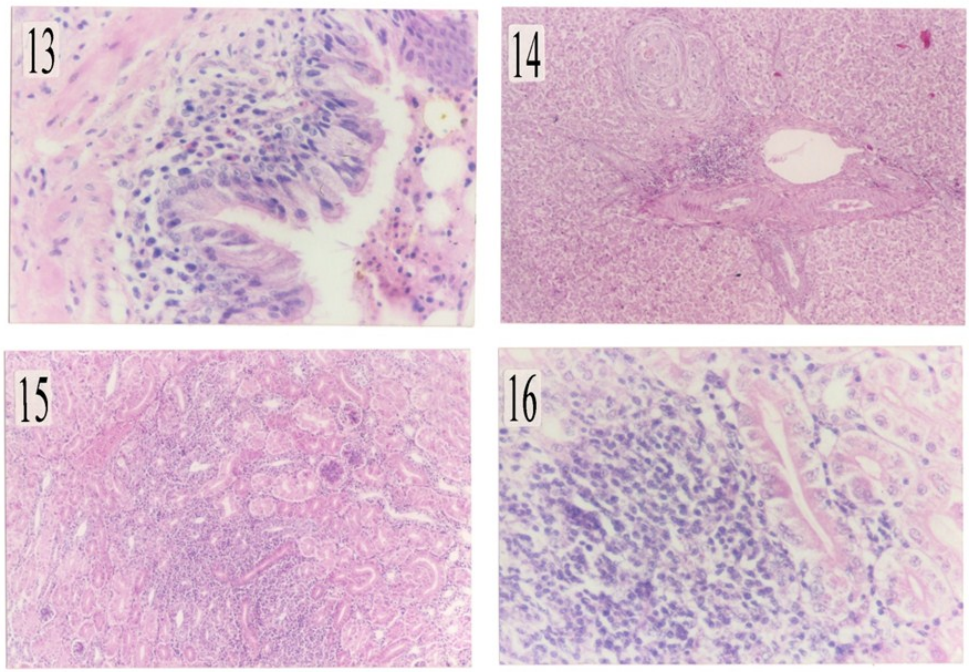

Plate 4 (From Fig. 13-16)

Fig.13. Photomicrograph of lung of smoke exposed sacrificed hens at 45 days PE showing hyperplasia of bronchial epithelium. H&E x 130.

Fig.14. Photomicrograph of liver of smoke exposed sacrificed hens at 45 days PE showing perivascular mononuclear leukocyte cell infiltration and focal fibrosis of hepatic parenchyma. H&E x 130.

Fig.15. Photomicrograph of kidney of smoke exposed sacrificed hens at 45 days PE showing mononuclear leukocyte cell infiltration and cloudy swelling. H&E x 130.

Fig.16. A high power of figure (15) showing mononuclear leukocyte cell infiltration. H&E x 130.

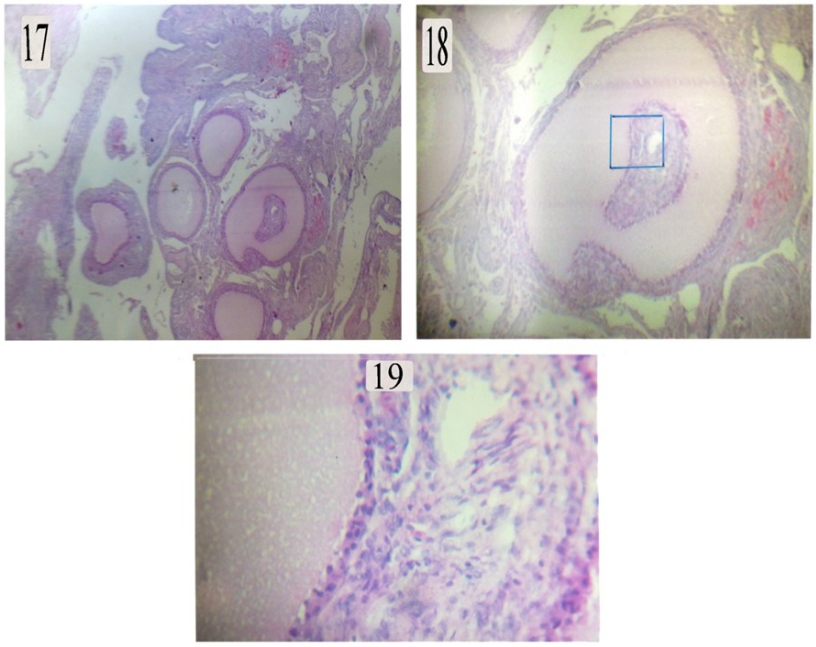

Plate 5 (From Fig. 17-19)

Fig.17. Photomicrograph of ovary of smoke exposed sacrificed hens at 45 days PE showing congestion of ovarian blood vessels and perioval fibrosis. H&E x 130.

Fig.18. A high power of figure (17) showing congestion of ovarian blood vessels and fibrosis. H&E x 300.

Fig.19. A high power of figure (18) showing the fibrosis. H&E x 620.

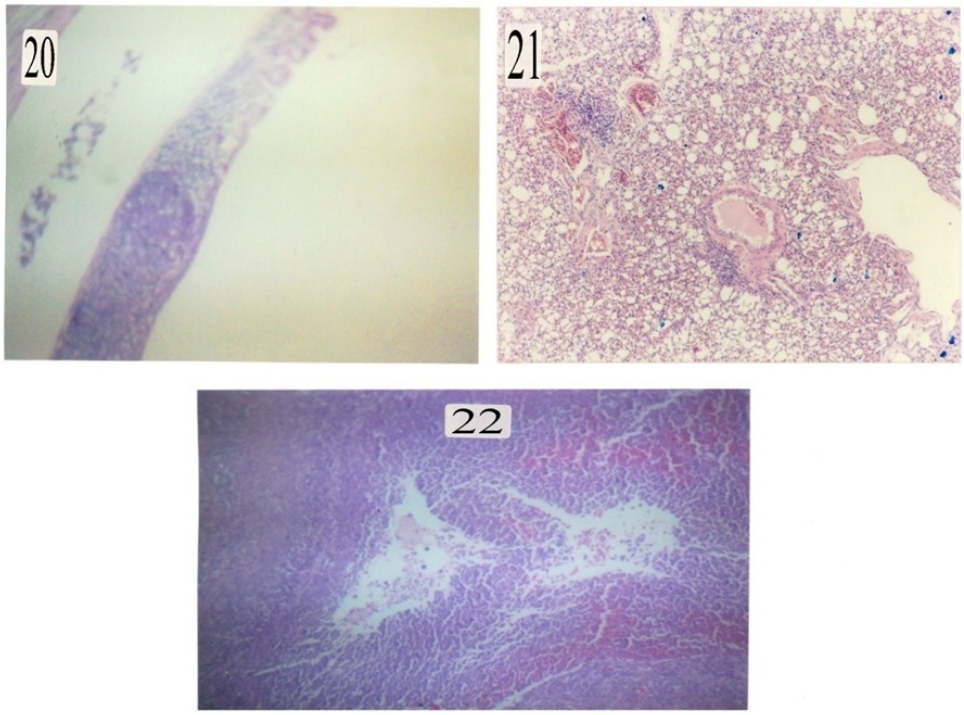

Plate 6 (From Fig. 20-22)

Fig. 20. Photomicrograph of trachea of smoke exposed sacrificed hens at 60 days PE showing absence of tracheal epithelium. H&E x 130.

Fig.21. Photomicrograph of trachea of smoke exposed sacrificed hens at 60 days PE showing congestion of pulmonary blood vessels, focal mononuclear cell infiltration, anthracosis and hyaline degeneration. H&E x 130.

Fig.22. Photomicrograph of ovary of smoke exposed sacrificed hens at 60 days PE showing atrophied follicles (complete absence of ovarian follicle). H&E x 200.

References

Bacon W.L. and Long D.W. (1996). Secretion of luteinizing hormone during a forced molt in turkey hens. Poult. Sci., 75: 1579-1586.

Bacon W.L., Brown K.I. and Musser M.A. (1980). Changes in plasma calcium, phosphorus, lipids, and estrogens in turkey hens with reproductive state. Poult Sci., 59 (2). 444-52.

Bancroft J.D. and Stevens A. (1996). Theory and Practice of Histological Technique. 4th Ed., Churchill, Livingston, New York, London, San Francisco, Tokyo.

Brodie B.B., Reid W.D., Tho A.K., Sipes G., Krisjna G. and Gillett J.R. (1971). Possible mechanism of liver necrosis caused by aromatic organic compounds. Proc. Nati. Acad. Sci. USA, January 1968 (1). 160-164.

Bui Q.Q. *et al*., (1986). A competitive study of the reproductive effects of methadone and benzo (a) pyrene in the pregnant and pseudo-pregnant rat. Toxicology, 42: 195-204.

Charles G.D., Bartels M.J., Zcharewski T.R., Gollapudi B.B., Freshour N.L. and garmey E.W. (2000). Activity of bezo (a) pyrene and it'shydroxylated metabolites in an oestrogen receptor- α-receptor general assay. Tociol. Sic., 55: 320-326.

Favez O., Cachier H., Sciare J., Alfaro S.C., El-Araby T.M., Harhash M.A. and Abdelwahab M.M. (2008). Seasonality of major aerosol species and their transformations in Cairo megacity. Atmospheric Environment, 42 (7). 1503-1516.

Hayakawa K., Kawahuchi Y., Murahashi T. and Miyazaki M. (l995). Distributions of nitropyrenes and mutagenicity in air borne particulates collected with an Anderson sampler. Mutation Research, 348: 57-61.

Hsieh D.P., Seiber J.N. and Fisher G.L.

(1981). Potential health hazards associated with particulate matter released from rice straw burning Sacramento. C.A: California Air Resources Board.

Johnson A.L. and van Tienhoven A. (1980). Plasma concentrations of six steroids and LH During the ovulatory cycle of the hen, Gallus domesticus. Biol. Reprod., 23: 386-393.

Korenaga T., LiuX. and Huang, Z. (2001). The influence of moisture content on polycyclic aromatic hydrocarbons emission during rice straw burning. Chemosphere Global Change Science, 3:117-122.

Li Z. and Johnson A.L. (1993). Regulation of P450 cholesterol side-chain cleavage messenger ribonucleic acid expression and progesterone production in hen granulosa cells. Biol. Reprod., 49 (3). 463-9.

Rehab E. Mowafy, Sahar N. Mohamady and Gehan H. El-Sakkar (2012). Pathological studies on the negative effects of rice straw burn on feed conversion rate and weight gain on broiler in El-Sharkia province. Zag Vet. J., 40 (4). 35-45.

Sakai H. and Ishii S. (1983). Radioimmunoassay of avian FSH in Mikami S. *et al*., (eds.), avian Endocrinology: Environmental and Ecological perspectives. Tokyo, Japan Sci. Soc. Press; Berlin Springer-verlag; 125-134.

Samah R.E. Khalil (2008). Risk assessment of some environmental pollutants in Sharkia province. A Thesis (Forensic Medicine and Toxicology), Faculty of Veterinary Medicine, Zagazig University.

Scanes C.G., Godden P.M. and Sharp P.J. (1977). An homologous radioimmunoassay for chicken follicle-stimulating hormone: observations on the ovulatory cycle. J. Endocrinol., 73 (3). 473-81.

Tamhane A.C. and Dunlop D.D. (2000). Statistics and Data Analysis from Elementary to Intermediate. Upper Saddle River, USA.

Topping D.C., Pl B.C. and Martin D.H., (1978). Pathogenic changes induced in respiratory tract mucosa by polycyclic hydrocarbons of differing carcinogenic activity. Am. J. Pathol., 93 (2). 311-324.

WHO (2001). Polynuclear aromatic hydrocarbons (PAHs) in WHO air quality guidelines for Europe. 2nd edition, World Health Organization, Regional Office for Europe Copenhagen.

Wilson S.C. and Sharp P. (1973). Variations in plasma LH levels during the ovulatory cycle of the hen, Gallus domesticus. J. Reprod. Fertil., 35 (3). 561-564.

دراسات باثولوجية على الآثار الإقتصادية السلبية الناتجة عن أكثر ملوثات الهواء تهديدا لمزارع البياض بمحافظة الشرقية

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الملخص العربى

يعتبر الحرق المكشوف لقش الأرز من أبرز ظواهر التلوث البيئى للهواء لما له من أضرار شديدة على مختلف أجهزة الجسم ويهدف هذا البحث لدراسة التأثير السلبى للدخان المتصاعد من عملية الحرق والذى يحوى العديد من الغازات والتى أبرزها وأكثرها خطورة مركب الهيدروكاربون على قدرة الدجاج البياض على إنتاج البيض فى المزارع والتى تقبع وسط المسطح الزراعى وتتعرض لعملية الحرق وخاصة أثناء فصل الخريف وذلك من خلال قياس مستوى غازات الدم الشريانى وهرمونات التبويض فى الدم وكذلك دراسة تأثيرها الباثولوجى على كل من القصبة الهوائية والرئة والكبد والكلية والقلب والمبيض.

وتم ذلك بإستخدام 100 دجاجه بياض عمر اربعة اشهر بإحدى المزارع القابعة وسط المسطح الزراعى القائم على زراعة الارز حيث تم تجميع عينات دم شريانى لقياس غازات الدم الشريانى وكذلك دم وريدى لقياس هرمونات التبويض قبل الحرق بيوم واحد كضابط للتجربة ثم تجميع العينات فى ايام 15 ، 30 ، 45 ، 60 يوم من بداية الحرق.

وقد أوضحت النتائج التأثير السلبى على التبويض ومعدل إنتاج البيض الذى إنخفض بنسبة ملحوظة جداً فى البيض نتيجة لحرق قش الارز.