Effect of Intraperitoneal Injection of Bisphenol-A on Egg Production and Quality Traits in Japanese Quail

Abdel-Rahim, W. M.; A. M. Hanafy; A. M. Abdel-Ghany* and H. A. Khalil
Animal Production Department, Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt

Received: *25/9/2017*

Abstract: This experiment was carried out during winter season 2015, at the Poultry Farm, Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. A total of 40 immature female Japanese quail exposed to different levels of Bisphenol-A (BPA) was examined. Three-week-old, quails were individually weighted and divided into four groups 10 females' birds in each. The BPA was dissolved in corn oil and intraperitoneally injected weekly in the abdominal cavity of quail for three consecutive weeks (3^{rd} through 5^{th} wks old). The experimental groups were treated by a solution containing (0, 1, 5 and 10 mg BPA/kg BW). During the experimental period, eggs were collected from each group to estimate egg production and quality traits. At 14 weeks of age, all hens were weighed and then slaughtered in order to collect blood samples to determine serum concentrations of estradiol-17β hormone. The result showed that exposure of female quail before/at puberty to BPA delayed initiation of egg production. While the age at first egg in control female was at 47 days of age, consequently, the BPA treated females were slower to reach 50% of their final laying rate. The laying rate of eggs in the control not significantly higher than the rate of egg production in all the BPA treatment groups, with the exception of the 10 (mg BPA /kg BW group). BPA treated females showed drastically reduced egg quality compared to controls even with a minimal dose of 1 (mg BPA /kg BW group). Shell thickness and internal quality units were significantly linearly decreased, but egg shape index was significantly linearly increased with increased BPA dose compared with control group. The lowest ovarian follicle number and oviduct length were detected in 10 (mg BPA/kg BW group), while the highest values were obtained in 5 (mg BPA/kg BW group). All tested BPA concentrations significantly influenced the egg production and egg quality in female quail indicating a lowest observed effect concentration of 10 (mg BPA/kg BW). Significant increases of female's body weight and reproductive organ after BPA administration were recorded, as females produced pre-ovulatory follicles at all tested BPA levels it is presumed that BPA did not affect oogenesis but affect the process of ovulation, the highest values of body weight and reproductive organs of females in the 5 (mg BPA/kg BW group), estradiol concentration was increased in BPA-treated groups. It could be concluded that the BPA treatment decreased body weight and delayed puberty and initiation of egg production in female quail. The effect of BPA exposure is proportional to the dose and varies depending on the time of administration.

Keywords: Bisphenol-A (BPA), egg production, egg quality, estradiol, Japanese quail

INTRODUCTION

It is known that sex steroids hormones secreting mainly from gonads and, to a degree, from the adrenals (Ruiz-Cortés, 2012). Reproductive and productive processes depend mainly on gonadal steroids, because of that exogenous/ecological estrogens which target the reproductive system is very risky either economically, vigor and healthiness. Therefore, natural and synthetic estrogen exposure leads to disrupt the homeostasis/balance of sex hormone (Abdelnabi and Ottinger, 2003). In all vertebrates, estradiol is the core potent estrogenic hormone. A common characteristic of the estrogenic compounds is their ability to bind to estrogen receptors (ER) and get the estrogenic responses in target cells (Tollefsen *et al.,* 2002). Pre-puberty events/encounters of birds is probably the most important and critical stages that affects birds' production capabilities later in life (*e.g.* the onset of sexual maturity in terms of maturation of oocytes, spermatozoa formation, courtship behavior and length and persistence reproduction time).

Although wild birds' populations are normally exposed to combinations of chemical pollutants, it is essential for scientists to determine which of these components are prevailing and responsive to the observed adverse effects. Bisphenol-A (BPA), which is one of these chemical pollutants derived from plastic fabrication, has strong antagonistic effects in birds, even at low doses, causing numerous malformations in the reproductive tract that finally may result in a robust reduction in survival of exposed living birds and animals (Panzica *et al.,* 2009). Since plastic items are normally utilized as a part of the poultry farms, BPA, or one of its metabolites may emerge and be consumed by the growing birds (Hanafy *et al.,* 2005; Escande *et al.,* 2006).

Effects of BPA on body weight have variant trends; Vandenberg *et al.* (2007) reported increasing of body weight, reproduction and development in humans. While, Furuya *et al.* (2006) could not be able to report any detectable differences among birds in all evaluated ages. On the contrary, Hanafy *et al.* (2006, 2007) demonstrated that in birds, upon the BPA injection, among other estrogenic chemicals, to ova or immature male quail, it gives rise to major reprogramming of hepatical yolk precursor genes expression. Halldin *et al.* (2001) reported that BPA had no considerable effects on the number of eggs produced compared to controls. But interestingly a higher frequency of females with a retained right oviduct in BPA treated groups was reported.

*Corresponding author e-mail: dr.abdelghany.ahmed@gmail.com

This manuscript focuses on and targets to reveal the probable potential, later in life, anatomical, physiological and histological effects as a result to intraperitoneal injection on immature Japanese quail with BPA as a simulation to bird's exposure to environmental contaminants that possess endocrine/gonadal disrupting activity (EDCs).

MATERIALS AND METHODS

Birds and treatments

This study was carried out during winter season 2015, at the Poultry Farm, Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Immature female Japanese quails received intraperitoneal injection of BPA for three consecutive weeks started from 3 weeks of age through 5 weeks. Effect of immature exposure to BPA on later productive and reproductive performance was examined.

A total of 100 hatched chicks of Japanese quails (*Coternix coternix japonica*) were raised on a temperature controlled floor brooder, provided with waterers and feeders. Quails were fed *ad libitum* on a commercial grower quails' diet during the growing period, contained 24% crude protein (CP) and 3000 KCal ME/Kg diet. The birds were given continuous light from the initial brooding period until the end of the 5^{th} week. At two-weeks old, a total of 40 immature female quails, approximately similar in body weight (100 g ± 3.20) and free from apparent clinical ailments, were placed on metal cages (120 x 60 cm and 50 cm height).

After one week from acclimatization, at three-week-old, these females were individually weighed and divided randomly into four groups of 10 females each. The BPA was dissolved in corn oil and intraperitoneally injected into the abdominal cavity. Birds of the first group received only the vehicle solvent (corn oil) and served as controls. The volume of corn oil injected was kept at 0.1 ml per 100 g body weight (BW). The other three experimental groups were treated with three graded levels of BPA (*i.e.* 1, 5 and 10 mg BPA/kg BW).

From the 6^{th} week, all groups were offered *ad libitum* water and quail breeder ration (CP= 18%, ME= 2800 KCal) with daily illumination for 16 h. photoperiod-schedule. During the experimental period that extended for extra 8 weeks eggs were collected from each group in order to estimate egg production and quality traits. All experimental groups were managed in accordance with the institutional guideline concerning ethics and animal use.

Traits studied

Female quails were individually weighed at the start of experiment (3 wks.) and at 8, 10, 12 and 14 weeks of age to the nearest 1 g using an electronic analytical balance. Ages at first egg and at 50% of hen/day per group production, were determined. Eggs were collected daily to calculate hen/day laying rate. Eggs were weighed individually (~ g) to estimate egg weight for all treatment groups.

For sake of egg quality measurements, eggs from all experimental groups during the period of 8 through 14 weeks of age were collected biweekly for three consecutive days per investigational group to measure whole egg and egg component weights (egg shell, albumen and yolk). Eggs were broken onto a glass plate to measure both internal and external egg quality characteristics. Before egg breaking, egg length and width were determined by a digital caliper. The heights of yolk and albumen were measured using a micrometer. Hence, egg yolk was carefully separated from albumen by clean towel and weighed. Egg shells were cleaned of any adhering albumen and allowed for dryness. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight. Shell thickness was measured after egg shell membranes removal from mid-section of the egg shell using a micrometer. Egg shape index, internal Haugh quality unit, yolk index were calculated using the following formulas:

Egg shape index (ESI) = (Egg width / Egg length) x 100.

Internal Haugh quality unit (IQU) = 100 log (H + 4.18 – 0.8989 x $W^{0.6674}$).

Where: H = albumen height (mm) and W = egg weight (g).
Yolk index (YI) = (Yolk height / Yolk diameter) x 100

Blood samples

All birds were weighed before slaughter at 14 weeks of age. To collect blood samples, birds were slaughtered by cutting the jugular vein and blood were collected individually in heparinized glass tubes and centrifuged at 3000 rpm for 15 min. Separated plasma was then stored at -20°C until subsequent estradiol-17β analysis. Concentration of estradiol-17β hormone was determined by human ELISA kits manufactured by DiaMetra, Spello-Perugia, Italy. The sensitivity of the assay was 8.7 pg/ml, the percentage of recovery was 95-100% and the intra- and inter-assay for estradiol-17β were 9 -10%.

Histopathology of oviduct

The abdominal cavity of the female was opened promptly after slaughtering and the ovary and oviduct were removed and weighed. The number of yellow follicles and the length of oviduct were measured. To assess BPA induced changes in gonadal development, female isthmus and uterus were processed for histological inspection in contrast to controls.

Statistical analysis

Separate one-way-ANOVA, was applied using SPSS 18 (SPSS, 2009). Differences among means were detected using Duncan's new multiple ranges test (Duncan 1955).

The following model was applied: $\mathbf{Y_{ij} = \mu + T_i + e_{ij}}$; ***Where***: μ = the overall mean; T_i = the observation on the i^{th} BPA Treatment (levels = 0, 1, 5 and 10 mg); e_{ij} = The random error related to the j^{th} individual from the i^{th} BPA Treatment and for testing procedure it is necessary to assume that e_{ij}'s are independently and approximately normally distributed, with zero mean and variance equal σ^2e, {*i.e.* ~NID (0, σ^2e)}.

RESULTS AND DISCUSSION

Egg production and egg quality traits

The effect immature intraperitoneal administration for three consecutive weeks (at 3, 4 and 5-wk-old) by graded levels of BPA on egg production traits (*i.e.* age at first egg, age at 50 percentage of production and percentage hen/day laying rate); and egg quality (averages of egg weight, shell thickness, egg shape index, yolk index, and internal Haugh quality units), as well as on the 14th week of age female slaughter weight (g), ovary weight (g), ovary (%), oviduct weight (g), oviduct (%), number of ovarian follicle, oviduct length (cm), estradiol concentration (E_2 pg/ml) traits are shown in Table (1).

Table (1): Effect of immature intraperitoneal injections with different concentration of Bisphenol-A (BPA) on subsequent female Japanese quail average egg production, egg quality traits and reproductive (genital organs weights and percentages) traits

Trait	Bisphenol-A (mg/kg BW)				*P*-value
	0	1	5	10	
	(Control)	(T1)	(T2)	(T3)	
Egg production and egg quality traits					
Age at First Egg (day)	47	48	50	52	--
Age at 50% (day)	55	60	61	66	--
Laying Rate (%)	54.36 ± 3.83 a	46.63 ± 3.42 a	54.39 ± 4.03 a	28.01 ± 2.79 b	0.00
Egg Weight (g)	12.09 ± 0.34 b	11.92 ± 0.12 b	13.00 ± 0.31 a	11.41 ± 0.43 b	0.01
Shell Thickness (µm)	17.00 ± 0.55 a	15.36 ± 0.55 b	15.13 ± 0.41 b	14.09 ± 0.41 b	0.00
Egg Shape Index‡	77.50 ± 0.69 b	80.13 ± 0.84 a	80.42 ± 0.82 a	80.99 ± 1.09 a	0.02
Yolk Index‡‡	42.15 ± 0.75 a	44.10 ± 0.47 a	43.80 ± 0.65 a	39.80 ± 1.12 b	0.00
Haugh Unit†	98.12 ± 0.50 a	96.79 ± 0.68 ab	95.63 ± 1.02 b	92.26 ± 1.17 c	0.00
Reproductive traits the 14th week of age					
Female slaughter Weight (g)	262.4 ± 4.96 b	267.2 ± 6.12 ab	287.2 ± 7.71 a	261.2 ± 11.15 b	0.05
Ovary Weight (g)	7.87 ± 0.34	7.17 ± 0.42	9.75 ± 1.35	6.95 ± 0.95	0.14
Ovary¥ (%)	3.01 ± 0.17	2.69 ± 0.16	3.36 ± 0.40	2.68 ± 0.38	0.35
Oviduct Weight (g)	8.04 ± 0.32	7.52 ± 0.45	10.35 ± 2.05	8.09 ± 0.92	0.35
Oviduct¥ (%)	3.07 ± 0.11	2.81 ± 0.14	3.54 ± 0.61	3.07 ± 0.25	0.51
Nº of Ovarian Follicle	4.00 ± 0.00 ab	4.00 ± 0.00 ab	4.60 ± 0.24 a	3.80 ± 0.37 b	0.05
Oviduct Length (cm)	39.40 ± 1.50 ab	39.80 ± 1.20 ab	43.40 ± 3.43 a	36.3 ± 0.86 b	0.05
Estradiol (pg/ml)	105.26 ± 10.94	161.93 ± 39.50	174.71 ± 67.59	190.26 ± 56.32	0.64

a,b Means at any row with no common superscript differ significantly ($P \leq 0.05$) using Duncan's Multiple Range test (Duncan, 1955).
†Internal Haugh quality unit (IQU) = 100 log ($H + 4.18 - 0.8989 \times W^{0.6674}$). Where: H = albumen height (mm) and W = egg weight (g).
¥ Percentages are calculated relative to relevant female's slaughter weight. ‡ Egg shape index (ESI) = (Egg width / Egg length) x 100.
‡‡ Yolk index (YI) = (Yolk height / Yolk diameter) x 100

From Table (1), egg production and quality traits (*i.e.* Laying Rate%; Egg Weight, g; Shell Thickness, µm; Egg Shape Index; Yolk Index) altered significantly, with the intraperitoneal injection of juvenile female quails with graded concentration of Bisphenol-A.

Haugh Unit Outcomes, seems to be fluctuated with the administered of juvenile female quail doses of BPA and seems to have dose-independent competence of egg production and quality traits (Table 1).

Results revealed also that immature BPA exposure of female quail caused 1-5 days delaying of produce eggs in treated groups, in per-group-sexual-maturity (*i.e.* Age at First Egg). While the per-group age at first egg in controls was at 47 days of age, BPA treated females were slower (48, 50 and 52 days) to begin egg production as group 1, 5 and 10 (mg BPA /kg BW groups), respectively. Consequently, the BPA treated females were slower to reach 50% of their hen/day laying rate, while control group reached 50% percentage rate hen/day egg production at 55 days of age. However, females in 1, 5 and 10 (mg BPA /kg BW groups) did not reach their 50% laying rate until they were 60, 61 and 66 days of age, respectively.

BPA administrations proved that, as a pollutant, it can influence many forms of reproduction and production activities and likewise has impacts at low doses, in the light of the fact that even little changes in hormone concentration can have biologically important consequences. Similar results have been reported by Ottinger *et al.* (2002) who showed that female Japanese quail exposed to *in ova* 20 µg/egg estradiol benzoate has a more prolonged period.

Also, Oshima *et al.* (2012) found that bisphenol-A have the dose-independent ability to cause ovotestis in the Japanese quail embryo. In fish, the percentage of ovulated brown trout females was similar for the control group and the groups exposed to BPA concentrations of 1.75 and 2.40 µg/L, whereas at BPA concentrations of 5.00 µg/L females did not ovulate through the investigation (Lahnsteiner *et al.,* 2005).

The laying rate showed likewise oscillated or dose liberated effect and in the controls was 54.36%, and this was not significantly higher than the rate of egg production in all the BPA treatment groups, with the exception of the 10 mg BPA /kg BW group (Table 1). The only dose of BPA that highly significantly ($P\leq 0.001$) impaired egg production was the 10 mg/kg BW and that was probably because of greater endocrine-disrupting mechanism leading to extensively reduced the total number of ovarian follicles with a higher apparent malformation to the oviduct (Figures 4 and 8).

Berg *et al.* (2001) injected quail eggs by 200 µg of BPA/g egg and found an abnormal oviduct in females suggested that mechanism of endocrine-disrupting may led to these effects and that reproduction was probably to be impaired. On the other hand, embryonic treatment of female quails to 67 or 200 µg/g egg of BPA had no significant effects on egg production as compared with controls (Halldin *et al.,* 2001).

As revealed in Table (1) BPA treated females showed variable degrees of reduction in egg quality traits (*i.e.* Shell Thickness, µm; Egg Shape Index; Yolk Index and Haugh Unit), compared to controls even with the minimal dose of 1 mg BPA /kg BW group. These inhibitory impacts showed in a fluctuated dose-independent manner. In this respect, Shell thickness and internal quality (Haugh units) were generally decreased significantly, but egg shape index, however and apart from the various BPA doses, significantly increased with the administration of the BPA.

Reproductive traits

From reproductive traits at the 14th week of age of Table (1) female slaughter weight (g) number of ovarian follicle and oviduct length (cm) altered significantly ($P\leq 0.05$), with the intraperitoneal injection of juvenile female quails with graded concentration of Bisphenol-A. On the other hand, ovary weight (g); ovary (%); oviduct weight (g); oviduct (%); estradiol (pg/ml) were not affected significantly, with the intraperitoneal injection of juvenile female quails with graded concentration of Bisphenol-A.

All traits that have been altered significantly (*i.e.* female slaughter weight; number of ovarian follicle and oviduct length), seemed to have dose-independent response and have no obvious trend that can be stated herein. This inconsistent fluctuated drift may be characteristic to this pollutant (Berg *et al.,* 2001); or it is because of the low sample size applied herein (Number of observation per group = 10). Another explanation is that this fluctuated effect is recognized as non-monotonic-dose-response-curves (NMDRCs), which are usually reported in pharmacology in response to treatment with EDCs that interact with receptors (Magyar, 2011).

Though immature intraperitoneal administration of Bisphenol-A caused significant alteration on the whole length (cm), it has not reveal same effect on oviduct weights or percentages. This may mean that this augment in length was not associated similar boost or amplification on the cellular level of the different component of oviduct, or perhaps inconsistent cellular growth, which may be revealed when dealing with the histological sections from isthmus and uterus that will be presented afterwards.

Similarly, Hermansson *et al.* (2007) reported that body weight in hens exposed to 60 µg/g egg Ethinyl estradiol (EE) was significantly higher compared to control hens. In contrast, embryonic treatment of female quail to 67 or 200 µg/g egg of BPA had no significant effects on body weight and weight of the left oviduct as compared with control (Halldin *et al.,* 2001; Furuya *et al.,* 2006). They concluded that the body weight did not show any differences among all ages of birds, though the growth of different organs was affected significantly in chicks even with at low doses of 2 µg BPA/kg. Oral administration of 0.01 to 0.1 mg/kg Diethylstilbestrol**,** which was began with the first day of hatching had no effect on the body weights of both male and female adult quails (Yoshimura *et al.,* 2000). In a similar study, comparison of doses from BPA and DDT did not produce any impacts on the tested female quails (El-Gawish *et al.,* 2008).

Though of insignificant effect on E2 concentrations, (Table 1) Intraperitoneal injections of BPA, as an estrogen disrupting compound, proved that it can have biologically important consequences on the reproductive system appearance (*i.e.* number of ovarian follicle; oviduct length) and body metabolism (female slaughter weight at the 14th week of age), even at miniature doses, which reflects its environmental impacts. These impacts should be considered in mind as its consequences not only on the productivity of domesticated birds, but also on the wild birds and the world biodiversity.

Nunez *et al.* (2001) stated that in adult ovariectomized female rats treated with 4 or 5 mg/day BPA for 15 days, showed a significant decrease of body weight gain with normal food intake. The difference between the results among the previous literature might be attributed to the difference in exposing period and age sensitivity to BPA toxicity and the cytochrome *P450* enzyme level among the different species. The level of cytochrome *P450* enzymes in humans, chicken, and quail livers is not high, thus they have lower catalytic activity than rats. Therefore, the compound stays a longer period in the body leading to greater access to target tissues (Hansen *et al.,* 2011). Hence, quails are more sensitive to BPA compared with other species. Collectively, the time of exposure is important factor to provoke the effect of EDCs on birds.

As females produced pre-ovulatory follicles at all tested BPA levels it is presumed that BPA did not affect oogenesis but the process of ovulation Table (1). Similarly, Ethinylestradiol (EE), another endocrine disruptor, stimulated egg production at low doses and inhibited egg production at higher doses in the fathead minnow (*P. promelas*) (Jobling *et al.,* 2003).

There is, however, no such organ or system in the body that is not controlled in some way or another by the endocrine system. Hence, while the estrogenic hormone is often studied for its effects on the reproductive system of female and secondary sex characteristics, in male, estrogens are likewise responsible for keeping females' reproductive health, bone density, cardiovascular health, and the immune system (Wend *et al.,* 2012). Natural hormones are recognized according to follow certain biological rules, and it consequently follows that substances that mimic or block (EDCs) the activities of hormones would likewise follow similar rules. The question is how long the study of EDCs will continue to benefit from progress in science of environmental health, which works at the intersection of multiple area counting ecology, endocrinology, and toxicology. In the current study, the highest values of body weight and reproductive organs of females in the 5 (mg BPA/kg BW group) may be attributed to the dose effect of BPA. This effect is recognized as non-monotonic dose response curves (NMDRCs), which are usually reported in pharmacology in response to treatment with EDCs that interact with receptors (Magyar, 2011). However, yet for a considerable years there has been discussion about NMDRCs from EDCs exposures and whether they are safe in their different current uses (Vandenberg *et al.,* 2009), including that NMDRCs could be specific to only a little EDCs and accordingly without broader importance. Previous author discovered cases of NMDRCs in laboratory animals and in human populations for more than 70 EDCs from a range of chemical classes and suggested that BPA is one of these substances (Vandenberg *et al.,* 2013).

Histology of oviduct

Isthmus

Sections from isthmus of the control group (Figure 1). No changes were seen on administration of 1 (mg BPA /kg BW group) (Figure 2). Figure (3) showed effect of administration of 5 (mg BPA/kg BW group) on isthmus. Superficial atrophic single layer of lining epithelium (Red arrow), with several hyperplastic disorganized basal cells (Arrow heads). Edematous chronically inflamed lamina propria is evident (Long black arrows), disfigurement of some papillary fronds is seen (about 30%), in which lining epithelium changed into a single layer of superficial atrophic epithelial cells, with eosinophilic cytoplasm and small dark nuclei, with several hyperplastic disorganized basal cells. Abundant lymphocytic infiltrate extends from underlying edematous inflamed lamina propria, permeating the overlying epithelium (Figure 3).

On administration of 10 (mg BPA/kg BW group), about 80% of papillary fronds show disfigurement changes, in which epithelial lining changed into a single layer of atrophic epithelial cells, with eosinophilic cytoplasm and small dark nuclei. Moreover, marked inflammatory cell infiltrate, congested blood vessels and prominent edema are seen in underlying lamina propria. Epithelial lining changed into a single layer of atrophic superficial epithelial cells, with eosinophilic cytoplasm and small dark nuclei (Red arrows), with several hyperplastic disorganized basal cells (Black arrows). Marked inflammatory cell infiltrate, congested blood vessels (Arrow head) and prominent edema are seen in underlying lamina propria (Figure 4).

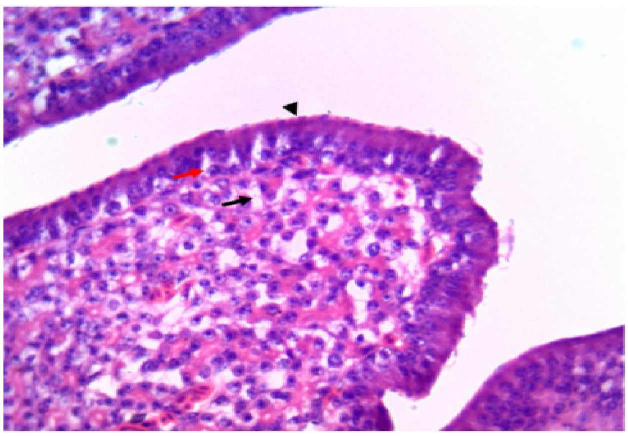
Figure (1): Isthmus from control group.

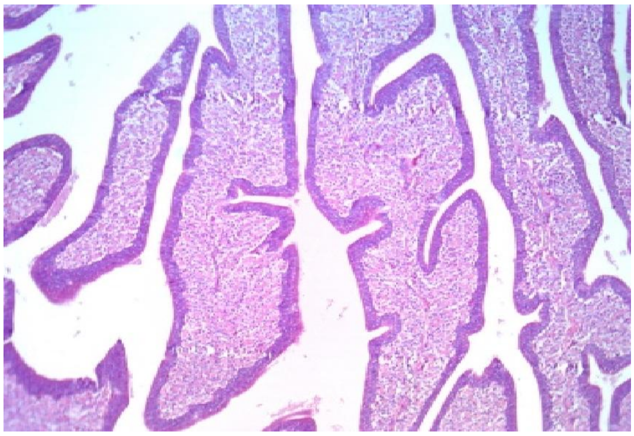
Figure (2): Isthmus from 1 (mg BPA /kg BW group).

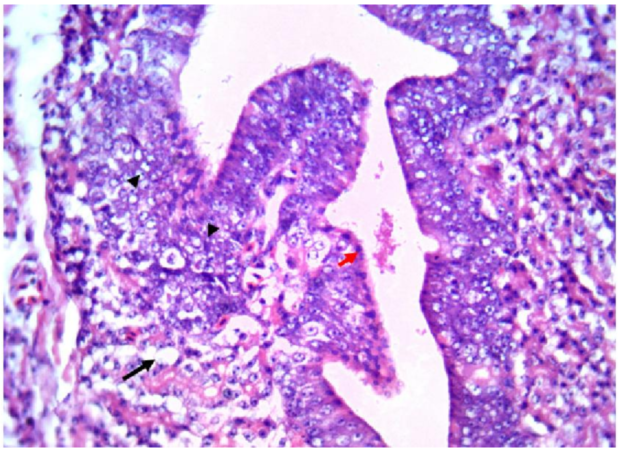

Figure (3): Isthmus from 5 (mg BPA /kg BW group).

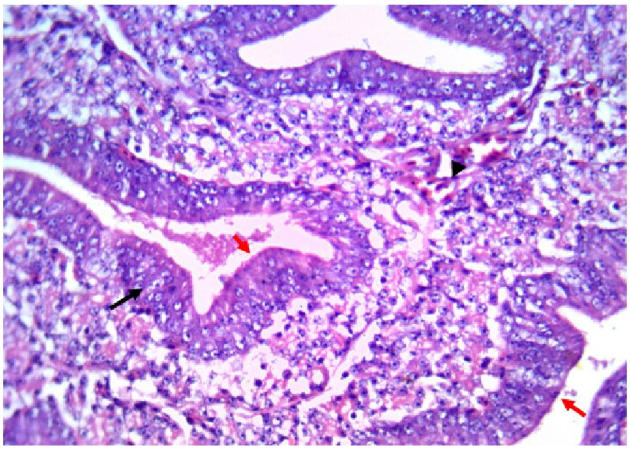

Figure (4): Isthmus from 10 (mg BPA /kg BW group).

Uterus

Figure (5) showed uterus section from control group. In Figure (6) compared to control group, on administration of 1 (mg BPA /kg BW group), uterine mucosal glands show focal areas of irregular distribution, edema and hemorrhage, glandular distortion, hemorrhage and stromal edema (Black arrow). In Figure (7) on administration of 5 (mg BPA /kg BW group), similar pathological changes were noted, as observed with administration of 1 (mg BPA/kg BW group) glandular distortion, hemorrhage and stromal edema (Black arrow). In Figure (8) on administration of 10 (mg BPA/kg BW group), similar pathological changes were noted, as observed with administration of 1 and 5 (mg BPA/kg BW groups), glandular distortion, hemorrhage and stromal edema (Black arrow).

In avian species, female reproductive function depends upon the control of ovarian steroid hormones that enables follicular development, ovulation, and oviposition. It is suggested that exposure of the developing reproductive system to EDCs can disrupt oogenesis, folliculargenesis and/or oviduct development acting through estrogenic, ant-estrogenic, and/ or anti-androgenic effects.

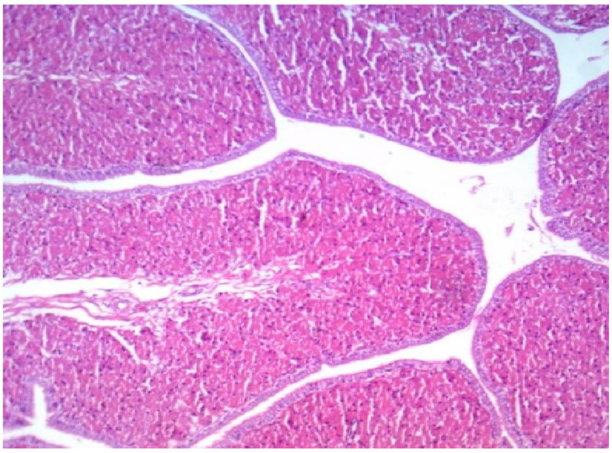

Figure (5): Uterine control group.

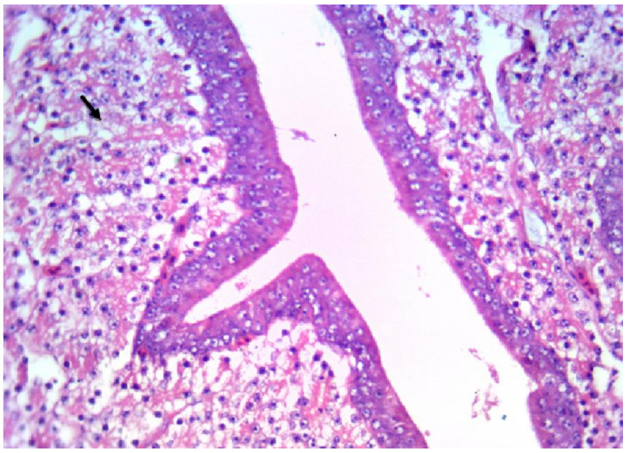

Figure (6): Uterus 1 (mg BPA /kg BW group).

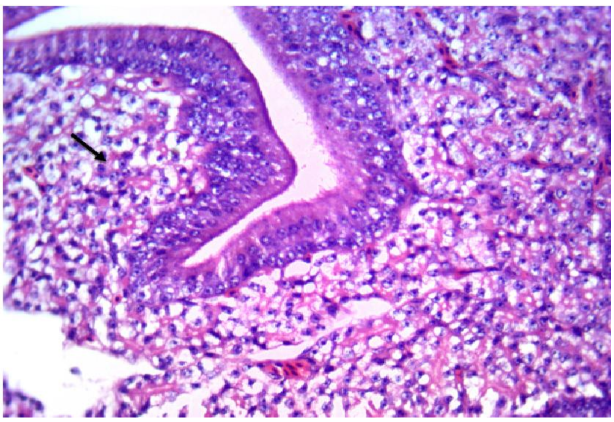

Figure (7): Uterus 5 (mg BPA /kg BW group).

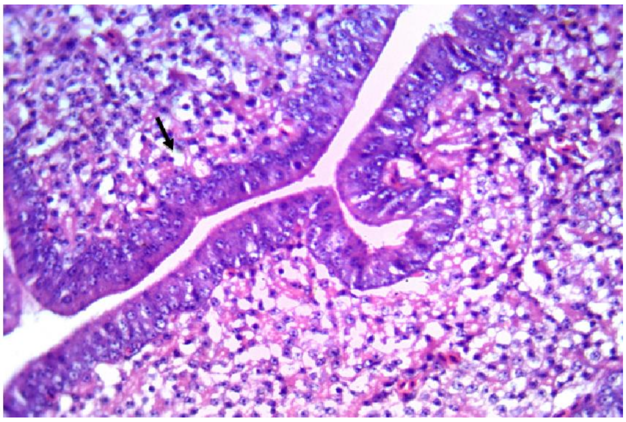

Figure (8): Uterus 10 (mg BPA /kg BW group).

CONCLUSION

The BPA treatment decreased body weight and delayed puberty and initiation of egg production in female quail. Eggs with thinner shells and reduced egg quality were produced by female quail exposed at puberty to BPA supporting the hypothesis that eggshell thickness may be induced during development and maturation by estrogenic pollutants. Since ovary function appeared normal in all birds the severe malformations of the left oviduct are the most likely causes behind the total inhibition of egg production. Results confirm that administration of BPA to female Japanese quail at puberty may represent very sensitive stage to test the estrogenic potency of different chemical compounds.

REFERENCES

Abdelnabi, M. A. and M. A. Ottinger (2003). Hypothalamic indolamines during embryonic development and effects of steroid exposure. General and Comparative Endocrinology, 130: 13-19.

Berg, C, K. Halldin and B. Brunstrom (2001). Effects of bisphenol A and tetrabromobisphenol A on sex organ development in quail and chicken embryos. Environmental Toxicology and Chemistry, 20: 2836-2840.

Duncan, D. B. (1955). Multiple Range and Multiple F-Tests. Biometrics, 11: 1-42.

El-Gawish, R., M. Elshabrawy Ghanem and T. Maeda (2008). Low doses of bisphenol A and o, p'-DDT at early stage of life delayed sexual maturity in female Japanese quail. Livestock Research for Rural Development 20(11): Article #188.

Escande, A., A. Pillon, N. Servant, J. P. Cravedi F. Larrea, P. Muhn, J. C. Nicolas, V. Cavaillès and P. Balaguer (2006). Evaluation of ligand selectivity using reporter cell lines stably expressing estrogen receptor alpha or beta. Biochemical Pharmacology, 71: 1459-1469.

Etches, R. J. (1987). Calcium logistics in the laying hen. Journal of Nutrition, 117: 619–628.

Furuya, M, K. Adachi, S. Kuwahara, K. Ogawa and Y. Tsukamoto (2006). Inhibition of male chick phenotypes and spermatogenesis by bisphenol-A. Life Sciences, 78: 1767-1776.

Halldin, K., C. Berg, A. Bergman, I. Brandt and B. Brunström (2001). Distribution of bisphenol A and tetrabromobisphenol A in quail eggs, embryos and laying birds and studies on reproduction variables in adults following *in ovo* exposure. Archives of Toxicology, 75: 597-603.

Hanafy, A. M., T. Sasanami and M. Mori (2005). Binding of xenoestrogens and phytoestrogens to estrogen receptor β of Japanese quail (Coturnix japonica). Journal of Poultry Science, 42: 238-244.

Hanafy, A. M., T. Sasanami and M. Mori (2006). Effects of diethylstilbestrol and ethinylestradiol on gene transcription of very low-density apolipoprotein II in the liver of Japanese quail, Coturnix japonica. Environmental Toxicology and Chemistry, 25: 1354-1359.

Hanafy, A. M., T. Sasanami and M. Mori (2007). Sensitivity of expression of perivitelline membrane glycoprotein ZP1 mRNA in the liver of Japanese quail (Coturnix japonica) to estrogenic compounds. Comp. Biochemistry and Physiology Part C: Toxicology and Pharmacology, 144: 356-362.

Hansen, W. M, J. D. Gonzalo and M. C. Sandra (2011). Enzymatic activity in turkey, duck, quail and chicken liver microsomes against four human cytochrome P450 prototype substrates and aflatoxin B. Xenobiotics. 1:1.

Hermansson, A. (2007). Effects on the reproductive system in domestic fowl (*Gallus domesticus*) after embryonic exposure to estrogenic substances. Diss. (sammanfattning/summary) Uppsala: Sveriges lantbruksuniv, Acta Universitatis Agriculturae Sueciae, 20: 1652-6880.

Isola, J. J. (1987). The effect of progesterone on the localization of progesterone receptors in the nuclei of chick oviduct cells. Cell and Tissue Research, 249:317–23.

Jobling, S., D. Casey, T. Rodgers-Gray, J. Oehlmann, U. Schulte-Oehlmann, S. Pawlowski, T. Baunbeck, A. P. Turner and C. R. Tyler (2003). Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquatic toxicology, 65: 205-220.

Lahnsteiner, F, B. Berger, M. Kletzl and T. Weismann (2005). Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, Salmo trutta f. fario. Aquatic Toxicology, 75(3): 213-224.

Magyar, K. (2011). The pharmacology of selegiline. International Review of Neurobiology, 100: 65–84.

Nunez, A. A., K. Kannan, J. P. Giesy, J. Fang and L. G. Clemens (2001). Effects of Bisphenol A on energy balance and accumulation in brown adipose tissue in rats. Chemosphere, (42): 917–922.

Oshima, A, R. Yamashita, K. Nakamura, M. Wada and K. Shibuya (2012). *In ovo* exposure to nonylphenol and bisphenol A resulted in dose-independent feminization of male gonads in japanese quail (*Coturnix japonica*) embryos. Environmental Toxicology and Chemistry, 31: 1091-1097.

Panzica, G. C., E. Mura, E. D. Miceli, M. A. Martini, S. Gotti and C. Viglietti-Panzica (2009). Effects of xenoestrogens on the differentiation of behaviorally relevant neural circuits in higher vertebrates. Annals of the New York Academy of Sciences, 1163: 271-278.

Ruiz-Cortés, Z. T. (2012). Gonadal Sex Steroids: Production, Action, and interactions in Mammals. Chapter 1. "Steroids—From Physiology to Clinical Medicine. Sergej M. Ostojic, editors. 3–42.

SPSS, (2009). SPSS Statistics for Windows, Version 18.0. SPSS Inc., USA.

Tollefsen, K. E., R. Mathisen and J. Stenersen (2002). Estrogen Mimics bind with similar affinity and specificity to the hepatic estrogen receptor in Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss). General and Comparative Endocrinology, 126: 14-22.

Vandenberg, L. N., T. Colborn, T. B. Hayes, J. J. Heindel, D. R. Jr Jacobs, D. H. Lee, J. P. Myers, T. Shioda, A. M. Soto, F. S. vom Saal, W. V. Welshons and R. T. Zoeller (2013). Regulatory decisions on endocrine disrupting chemicals should be based on the principles of endocrinology. Reproductive Toxicology, 38C: 1–15.

Vandenberg, L. N., R. Hauser, M. Marcus, N. Olea and W. V. Welshons (2007). Human exposure to bisphenol A (BPA). Reproductive Toxicology, 24(2): 139-77.

Vandenberg, L. N., M. V. Maffini, C. Sonnenschein, B. S. Rubin and A. M. Soto (2009). Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrinology Reviews, 30: 75-95.

Wend, K., P. Wend and S. A. Krum (2012). Tissue-Specific Effects of Loss of Estrogen during Menopause and Aging. Frontiers in Endocrinology, 3:19.

Yoshimura, T., Y. Suzuki, E. Makino, T. Suzuki, A. Kuroiwa, Y. Matsuda, T. Namikawa and S. Ebihara (2000). Molecular analysis of avian circadian clock genes. Brain research. Molecular brain research, 78: 207–215.

اثر حقن البسيفينول أ في التجويف البطنى على الإنتاج وصفات جودة البيض في السمان اليابانى

وائل محمد احمد عبد الرحيم، احمد محمد حنفى، احمد محمود عبد الغنى، حسن احمد عبد الغفار

قسم الإنتاج الحيواني، كلية الزراعة، جامعة قناة السويس، ٤١٥٢٢ الإسماعيلية، مصر.

أجريت هذه الدراسة خلال فصل الشتاء ٢٠١٥، في مزرعة الدواجن، قسم الإنتاج الحيواني، كلية الزراعة، جامعة قناة السويس، الإسماعيلية، مصر، ٤٠ أنثى سمان يابانى عمر ثلاث أسابيع غير بالغة تم تقسيمها إلى أربعة مجموعات ١٠ طائر في كل منها وتم حقنها بجرعات مختلفة من البيسيفينول أ. تم إذابة البسيفينول أ في زيت الذرة و حقن في التجويف البطنى للسمان أسبوعيا لمدة ثلاث أسابيع متعاقبة (في ٣ و ٤ و ٥ أسابيع من العمر). مجاميع التجربة تمت معاملتها بهذا المحلول (٠ و٥ و١٠ ملجرام بسيفينول أ /كجم وزن جسم). خلال فترة التجربة تم جمع البيض من كل مجموعة، صفات إنتاج البيض وجودة البيض تم قياسها. تم وزن كل الطيور عند ١٤ أسبوع من العمر وبعد ذلك ذبحت. أظهرت النتائج أن تعرض إناث السمان قبل أو في سن البلوغ للبسيفينول أ تؤخر بداية إنتاج البيض. بينما العمر عند إنتاج أول بيضة في إناث الكنترول تم تحديدها عند ٤٧ يوم من العمر، الإناث المعاملة بالبسيفينول أ كانت الأبطأ في بداية إنتاج البيض. وبالتالي الإناث المعاملة بالبسيفينول أ كانت الأبطأ للوصول إلى ٥٠% من إنتاج البيض النهائي لهم. معدل إنتاج البيض في الكنترول كان غير معنوي وأعلى من معدل إنتاج البيض في كل المجموعات المعاملة بالبسيفينول أ، باستثناء المجموعة المعاملة بـ ١٠ (ملجرام بسيفينول أ /كجم وزن جسم). الإناث المعاملة بالبسيفينول أ أظهرت انخفاض شديد في جودة البيض مقارنة بالكنترول حتى مع الجرعة الأدنى (١ ملجرام بسيفينول أ /كجم وزن جسم). سمك القشرة و وحدة الجودة الداخلية للبيض انخفضت خطيا، ولكن مؤشر شكل البيض ازداد بشدة خطيا مع الزيادة فى جرعة البسيفينول أ مقارنة بمجموعة الكنترول. عدد الحويصلات المبيضية الأقل و طول قناة المبيض الأقل تم تحديدها في مجموعة (١٠ ملجرام بسيفينول أ /كجم وزن جسم)، بينما القيم الأعلى شوهدت في مجموعة (٥ ملجرام بسيفينول أ /كجم وزن جسم). كل تركيزات البسيفينول أ المختبرة أثرت بشدة على إنتاج البيض وجودة البيض في إناث السمان مما يدل على أن اقل تركيز مؤثر كان في مجموعة (١٠ ملجرام بسيفينول أ /كجم وزن جسم). زيادة كبيرة في وزن الإناث والأعضاء التناسلية بعد المعاملة بالبسيفينول أ تم ملاحظتها، مثل الإناث في مرحلة ما قبل التبويض في كل مستويات البسيفينول أ المختبرة، وافترض أن البيسيفينول لم يؤثر على عملية تخليق البويضة ولكن اثر على عملية التبويض، القيمة الأعلى في وزن الجسم والأعضاء التناسلية كانت في إناث مجموعة (٥ ملجرام بسيفينول أ /كجم وزن جسم)، تركيزات الاستراديول ازدادت في المجموعات المعاملة بالبيسيفينول أ. الخلاصة أن المعاملة بالبيسيفينول أ قللت وزن الجسم وأخرت البلوغ و بداية إنتاج البيض في إناث السمان. تأثير التعرض إلى البسيفينول أ نسبى إلى الجرعة ويختلف معتمدا على وقت التعرض.

الكلمات المفتاحية: البسيفينول أ، إنتاج البيض، جودة البيض، الاستراديول، السمان اليابانى.