İstanbul Üniv. Vet. Fak. Derg.
39 (2), 168-174, 2013
Araştırma Makalesi

J. Fac. Vet. Med. Istanbul Univ.
39 (2), 168-174, 2013
Research Article

Effects of Bisphenol A and Diethylstilbestrol on the Involution of Bursa of Fabricius in the Hens

Funda YİĞİT[1], Abit AKTAŞ[1], Suzan DAĞLIOĞLU[1]*

[1]Department of Histology and Embryology Istanbul University Veterinary Faculty, 34320, Avcilar, Istanbul

**Corresponding Author: Funda YİĞİT Department of Histology and Embryology Istanbul University Veterinary Faculty, 34320, Avcilar, Istanbul*
e-mail: fyigit@istanbul.edu.tr

Geliş Tarihi / Received: 24.02.2012

ABSTRACT

The bursa of Fabricius is a primary lymphoid organ in birds responsible for B lymphocyte maturation. Sex steroid hormones influence regression of the bursa of Fabricius. Bisphenol A (BPA) is an estrogenic chemical that is widely used in the manufacture of plastics and epoxy resins. This experiment was conducted to determine the effects of BPA on the involution of bursa of Fabricius in the adult hens. The synthetic estrogen diethylstilbestrol (DES) was used as a positive control. The test compounds were injected into the yolk of embryonated eggs. Immunohistochemical procedure performed to determine estrogen receptor alpha (ERα) expression in bursa of Fabricius. In high dose BPA (134 µg/g egg) and DES (0.2 µg/g egg) groups, the bursa somatic index, the number and diameter of the follicles in bursa of Fabricius were statistically decreased compared to control and low dose BPA (67 µg/g egg) and DES (0.02 µg/g egg) groups ($P<0.05$). There were no specific immune reactions for ERα in bursa of Fabricius in all groups. These results suggest that embryonic exposure to BPA and DES induced the regression of the bursa of Fabricius in hens.

Key Words: Bisphenol A (BPA), diethylstilbestrol (DES), bursa Fabricius, hen, estrogen receptor alpha (ERα)

ÖZET

TAVUKLARDA BİSFENOL A VE DİETİLSTİLBESTROL'ÜN BURSA FABRİCİUS'UN İNVOLUSYONU ÜZERİNE ETKİSİ

Bursa Fabricius kuşlarda B lenfositlerin olgunlaşmasından sorumlu primer (birincil) lenfoid organdır. Seks steroid hormonları bursa Fabricius'un gerilemesini etkiler. Bisfenol A (BPA), plastik ve epoksi reçine üretiminde yaygın olarak kullanılan bir östrojenik bir kimyasaldır. Bu çalışma, BPA'nın ergin tavuklarda bursa Fabricius'un involusyonu üzerine etkisini belirlemek amacıyla yapıldı. Pozitif kontrol olarak sentetik östrojen olan dietilstilbestrol (DES) kullanıldı. Test bileşikleri embriyolu yumurta sarısının içine enjekte edildi. Bursa Fabricius'da östrojen alfa reseptörü (ÖRα) ekspresyonunu belirlemek için immunohistokimyasal yöntem kullanıldı. Yüksek doz BPA (134 mg/g yumurta) ve DES (0,2 mg/g yumurta) gruplarında, bursa somatik indeks, bursa Fabricius'un follikül sayısı ve çapı, kontrol ve düşük doz BPA (67 µg/g yumurta) ve DES (0,02 µg/g/ yumurta) gruplarına göre istatistiksel olarak azalmıştı ($P<0,05$). Tüm gruplarda bursa Fabricius'da spesifik ÖRα immun reaksiyon yoktu. Bu sonuçlar, embriyonik dönemde BPA ve DES'e maruz kalan tavuklarda bursa Fabricius'un gerilemesini indüklediğini göstermektedir.

Anahtar Kelimeler: Bisfenol A (BPA), dietilstilbestrol (DES), bursa Fabricius, tavuk, östrojen reseptör alfa (ÖRα)

Introduction

The bursa of Fabricius (BF) is a primary lymphoid organ specifically in birds that is responsible for the maturation of lymphocyte precursor cells into immunologically competent B cells (Fairbrother et al., 2004; Glick et al., 1956). The bursa develops in chick embryo on the 4th day incubation as a dorsal diverticulum of the proctadael region of the cloaca. The wall of the bursa of Fabricius is composed of tunica mucosa, tunica muscularis and tunica serosa. Tunica mucosa is characterized by tall, thick mucosal folds (plicae) filled with polyhedral follicles. Each follicle, composed of lympatic tissue, is divided into a cortex and medulla. The bursa is lined by a pseudostratified columnar epithelium, except at the apex of each follicle, which is covered by an epithelial tuft of simple columnar epithelium (Bacha and Bacha, 2006; Gülmez and Aslan, 1999; Tanyolaç, 1999). It serves as a primary lymphoid organ during early stages of development, but later regresses. This regression is not complete in the Japanese quail, acting as a secondary lymphoid organ. In chickens, the size of bursa increases up to the of 10-12 weeks, and then it starts to involutes, under the influence of sex steroid hormones as a estrogen and androgen (Naukkarinen and Sorvari, 1984; Ylikomi and Touhimaa, 1989). The subepithelial and interfollicular connective tissue increases and follicular cortex narrows. The bursal involution is almost complete by week 24 when the degenerated lymphoid tissue is replaced fibrotic tissue (Norton and Wirain, 1977).

Endocrine disrupting chemicals (EDs), especially estrogenic chemicals, are synthetic or naturally occurring substances that affect the function and development of the endocrine system of animals and humans (Colborn et al., 1993; Lintelmann et al., 2003). The adverse effects of EDs have been reported in the reproductive tissues of mammals and birds (Fry, 1995; Markey et al., 2003). These estrogenic chemicals markedly also affect the immune system indirectly and directly (Ansar, 2000; Quinn et al., 2009; Razia et al., 2005; 2006). Bisphenol A (BPA) is one of these estrogenic chemicals. BPA is a high production volume chemical widely used in manufacturing polycarbonate plastics and epoxy resins used in many industries. It may be found in the consumer products (food and drink packages, baby bottles, dental sealants, lacquers lining coating certain metal products). BPA and its analogs (BPAs) are common pollutants of lakes, rivers and seawater, resulting in chronic exposure by humans and animals (Staples et al., 1998; Yamamoto and Yasuhara, 1999).

Bisphenol A is monomer composed of two unsaturated phenolic rings that resemble diethylstilbestrol (DES). BPA is weakly estrogenic when compared with DES. BPA binds to estrogen receptors (ERs) and mimics estrogenic effects, and therefore may lead to negative health effects. The reaction of BPA with ER is similar to that of estrogen with ER (Kurosawa et al., 2002; Welshons et al., 2003). The recent years, many studies have shown that BPA can induce estrogen-like malformations in the reproductive organs of birds (Berg et al., 2001; Halldin et al., 2001). We recently completed a study on the effects of BPA and DES on the reproductive organs in adult hen (Yigit and Daglioglu, 2010). Studies have reported that BPA administration causes adverse effects on the immune system in mice and rat (Miao et al., 2009; Yoshino et al., 2004). However, there is not enough studies and information on the developmental effects of BPA on immune organs in birds. Therefore, the purpose of this study was to investigate the effects of in ovo administrated BPA on the involution of bursa of Fabricius in the adult hens and also to compare the estrogenic potency of BPA with that of the highly potent synthetic estrogen diethylstilbestrol.

Materials and Methods

Experiment design

Fertilized White Leghorn chicken eggs (Lohmann LSL, Two hundred fifty eggs) were obtained from local breeder flock (Has Tavuk, Bursa). The eggs were incubated at 37.5 ^{o}C and 60% relative humidity and turned every third hour. Bisphenol A (BPA; purity≥99.4%) and Diethylstilbestrol (DES; purity≥99%) were

purchased from Sigma Chemical, MO, USA. The two compounds were dissolved in a mixture of peanut oil, lecithin and propylene glycol emulsion and were injected into yolks at a volume of 100 µl on day 4 incubation (Berg et al., 2001). The doses were determined based on the results of our previous research (Yigit and Daglioglu, 2010). Doses were 67 and 134 µg BPA g/egg and 0.02 and 0.2 µg DESg/egg. After injection, the shell was sealed with paraffin wax and the eggs were returned to the incubator. After hatching, the chicks were provided with food and water ad libitum for 21 weeks. Eight hens were evaluated from each group at 21 weeks of age. The hens were sacrificed by cervical dislocation under the anesthesia of carbon dioxide and weighed. The body weight (g) and bursa weight (g) were recorded for each individual bird, and the bursa somatic index (bursa weight/ body weight) was calculated. The bursa of Fabricius was fixed in 10% neutral buffered formaline for 24 h at room temperature. The paraffin-embedded bursas were sectioned at 5 µm and stained with Mallory's triple staining technique modified by Crossmon for general histological examination (Crossmon, 1937). Histological examination was performed using a light microscope (Nikon Microphoto-FX imaging system). The study was approved by the Ethics Committee of Istanbul University Faculty of Veterinary Medicine (2005/116).

Analysis of bursa of Fabricius

Stereo Investigator (Leica Microsystems) software was used for determining the numbers and diameters of follicles in the sections taken from bursa of Fabricius. For this purpose mean follicle number was evaluated by counting 25 areas, selected in a systematic-random manner, within the whole surface of sections taken from every animal. The area of the unbiased counting frame was 1 mm^2 (1000x1000 µm), the step size was 4 mm^2 (2000x2000 µm) and in every section the mean follicle number was determined in 0.25 cm^2 area. For the determination of follicle diameters, the diameters of the follicles contacting to the right top corner of the counting frame was measured and the mean follicle diameters in every group calculated (Piskin et al., 2009).

Immunohistochemistry

The immunostaining was performed on 5 µm sections of bursa, using the streptavidin-biotin-peroxidase technique. The sections were incubated with anti-human estrogen receptor monoclonal antibody for 24h at 4°C (NCL-ER-LH2, 1:50 dilution, Novocastra Laboratories, UK) which has been reported to croos-reactivity with chicken ERα (Sheng and Ronald, 2000). The immunoreactions were visualized by incubating with 3'3 diaminobenzidine chromogen solution (DAB, Zymed, USA). The slides were counterstained with Mayer's hematoxylin. In the negative controls the estrogen receptor was replaced with phosphate buffered saline (PBS). Hen uterus was used as a positive control for ERα.

Statistical Analysis

Statistical Analysis was performed by one-way analysis of variance (ANOVA), followed by a Duncan test using the SPSS 11.0 programs.

Results

As shown in Table 1, no significant difference in the body weight after treatments with either BPA or DES at any of the doses was observed($P>0.05$). Bursa somatic index was decreased in high dose BPA and DES groups, and the difference was statistically significant ($P<0.05$), in comparasion with the control and the other experimental groups (Table 1). The number and diameter of the follicles were decreased significantly in high dose BPA and DES groups compared to control and low dose BPA and DES groups ($P<0.05$) (Table 1).

In the control group, lymphoid cells were located more intensively in the lymphoid follicles of bursa (Figure 1). In high dose BPA and predominantly in high dose DES groups a prominent decline was observed in the lymphocyte intensity in centrum of lymphoid follicles in the bursa compared to the control and low dose BPA and DES groups. Lymphoid cells were depleted in some follicles. In

addition, within the bursal plicae an increased visibility of the stroma was noted (Figure 1).

ERα immunostaining in bursa of Fabricius was not observed in all groups. Immunostaining was observed in uterus tissues of adult hens which were used as a positive control for staining.

Table 1. Body weight (g), bursa somatic index (bursa weight/body weight), follicular number and diameter (µm) the bursa of Fabricius of the hens in all groups.

Tablo 1. Tüm gruplardaki tavukların, vücut ağırlığı (g), bursa vücut ağırlığı indeksi (bursa ağırlığı/vücut ağırlığı), bursa Fabricius'un follikül çapı (µm) ve sayısı.

Groups	Body weight (g)	Bursa somatic index	Bursal follicle measures	
			Follicular number	Follicular diameters (µm)
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0.02 µg DES/g egg	1281±49.396	0.00228±0.000136 [a]	387.8±8.32[a]	394.1±19.90[a]
0.2 µg DES/g egg	1320±40.190	0.00158±0.000051[b]	357.6±6.69[b]	318.2±7.87[b]
67 µg BPA/g egg	1302±40.766	0.00221±0.000086 [a]	393.1±10.72[a]	404.4±15.95[a]
134 µg BPA/g egg	1295±72.916	0.00175±0.000126 [b]	365.6±10.50[b]	327.3±11.80[b]
Control	1311±54.985	0.00245±0.000065[a]	398.8±8.95[a]	415.6±6.65[a]

[a,b]: The differences between the means of groups with different superscripts (in the same columns) are significant (P<0.05).

Discussion

None of doses BPA and DES induced any significant changes in the body weight of hen compared to the control group. Furuya et al. (2006) reported that oral administration of BPA ranging from 2 µg to 200 mg/kg for 16 wk had no effect on the body growth of roosters. Yoshimura et al. (2000) found that adult male and female quails, which were oral administration of 0.01 and 0.1 mg/l DES for 58 days, showed no influence on the body weights. The findings and our results indicate that the administration of BPA and DES during incubation and posthatching period does not affect the body weight increase of chicks. The current study showed that embryonic exposure high dose BPA and DES were reduced in the number and diamater of follicles and bursa somatic index in bursa of Fabricius of the hens. Gildersleeve et al. (1985) found that 0.9-125 µg DES/egg injected on day 1 of incubation had not fully developed of the bursa of Fabricius in chicks. Recently it was reported that BPA injected on day 9 of incubation at a dose of 250 µg/egg caused a reduction the index of bursa of Fabricius and the number of the follicles of the chick embryos (Dongmei et al., 2008). Al-Afaleq and Homedia (1998) 50% reduction in bursal weihgt occurring in chicks that were exposed to 1 µg/kg estradiol in feed for 50d. Kondo et al. (2004) also reported that the weight of the bursa of Fabricius significantly decreased after treatment with estrogen on the 14th day of embryogenesis in chick. Razia et al. (2006) showed that embryonic exposure to 17β-estradiol and nonylphenol (weak estrogenic compound) in Japanese quail resulted in disapperarance of lymphoid cells from the lymphoid follicles in the bursa.

A recent study has demostrated that embryonic exposure to 50 and 500 µg/egg estradiol in quail resulted in follicle size and numbers in hatchling bursas were reduced. Adult bursas were significantly larger than controls. However, this study bursal histology was not examined in adults (Ouinn et al., 2009).

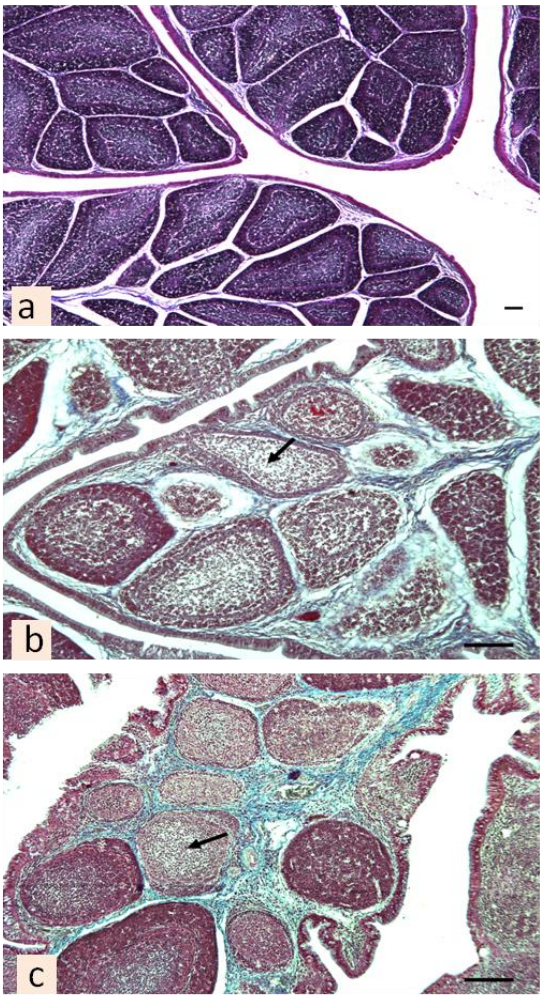

Figure 1. Effects of BPA and DES treatments on the histology of the bursa of Fabricius; (a) Control, (b) High dose BPA group (134 µg/g), (c) High dose DES group (0.2 µg/g). Showing lymphocyte depletion (arrow) in the lymphoid follicles after treatments with BPA and DES. Triple staining. Bar in **a-c** 100 µm.

Şekil 1. BPA ve DES uygulamasının bursa Fabricius'un histolojisi üzerine etkileri; (a) Kontrol, (b) Yüksek doz BPA grubu (134 µg/g), (c) Yüksek doz DES grubu (0.2 µg/g). BPA ve DES uygulamasından sonra lenfoid foliküllerde lenfosit hücre boşalması ok ile gösterilmiştir. Üçlü boyama. Bar **a-c** 100 µm.

The actions of estrogenic compounds have been proposed to be mediated by the Estrogen receptors (ER) and modulate estrogen-sensitive gene expression (Welshons et al., 2003). Bursal estrogen receptors have been found in chick embryos (Shin et al., 2005) and in the immature (1 to 17 week old) chicks (Sullivan and Wira, 1979). The presence of ER in the bursa suggests that estrogen and estrogenic active chemicals treatment of chick embryos affects development and function of the bursa of Fabricius. Thus, in the present study, we examined the effects of bisphenol A on ERα protein in the bursa of adult hens using immunohistochemistry. ERα Immunostaining was observed in the uterus of adult hens (positive control tissue for ER). The bursa of Fabricius exhibited no immunostaining for ER in all groups.

Effects of BPA and DES on hen bursal size is consistent with previous reports Dongmei et al. (2008), Quinn et al. (2009), Razia et al. (2005 and 2006) of estrogen-like effects on early stages of development. Considering these findings and our results suggest that injection of BPA and DES into the yolk of chick eggs after an initial incubation for 4 days induced the involution of bursa of Fabricius in the hens. The result of our experiment can provide useful references for further studies on the effects of endocrine disrupting chemicals and estrogen on the immune organs in the birds.

Acknowledgements

The present study was supported by Research Found of Istanbul University. Project No: UDP-7861.

REFERENCES

Al-Afaleq, A.I., Homeida, A.M., 1998. Effects of low doses of oestradiol, testosterone, and dihydrotestosterone on the immune response of broiler chicks. Immunopharmacology and Immunotoxicology 20, 315-327.

Ansar, A.S., 2000. The immune system as a potential target for environmental estrogens (endocrine disrupters): A new emerging field. Toxicology 150 (1-3), 191-206.

Bacha, W.J., Bacha, L.M., 2006. Color atlas of veterinary histology. 2nd edition. Blackwell Publishing Ltd, USA.

Berg, C., Halldin, K., Brunstrom, B., 2001. Effects of bisphenol A and tetrabromobisphenol A on sex organ development in quail and chicken embryos. Environmental Toxicology 20, 2826-2840.

Colborn, T., Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives 101, 378-384.

Crossmon, G., 1937. A modification of Mallory's connective tissue stain with a discussion of the principles involved. The Anatomical Record 69, 33-38.

Dongmei, L., Ruiping, S., Junzhen, J.I.A., Lili, C., Jiande, W., Tianlong, L., Yanxin, H., Qiong, W., 2008. Effect of bisphenol-a on histomorphology of central immune organ of chick embryos. Science Technology Review 26 (6), 42-49.

Fairbrother, A., Smits, J., Grasman, K.A., 2004. Avian immunotoxicology. Journal of Toxicology and Environmental Health B 7, 105-137.

Fry, D.M., 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environmental Health Perspectives 103, 165-171.

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

Gildersleeve, R.P., Tilson, H.A., Mitchell, C.L., 1985. Injection of diethylstilbestrol on the first day of incubation affects morphology of sex glands and reproductive behavior of Japanese quail. Teratology 131 (1), 101-109.

Glick, B., Chang, T.S., Jaap, R.G., 1956. The bursa of Fabricius and antibody production. Poultry Science 35, 224-225.

Gülmez, N., Aslan, Ş., 1999. Histological and histometrical investigations on bursa of Fabricius and Thymus of Native Geese. Turkish Journal of Veterinary and Animal Science 23 (2), 163-171.

Halldin, K., Berg, C., Bergman, A., Brandt, I., Brunström, B., 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 (10), 597-603.

Kondo, Y., Goto, C., Abe, A., 2004. Effects of estrogen treatment during the embryogenic period on chick antibody production. The Journal of Poultry Science 41, 85-93.

Kurosawa, T., Hiroi, H., Tsutsumi, O., Ishikawa, T., Osuga, Y., Fujiwara, T., Inoue, S., Muramatsu, M., Momoeda, M., Taketeni, T., 2002. The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. Endocrine Journal 49 (4), 465-471.

Lintelmann, J., Katayama, A., Kurıhara, N., Shore, L., Wenzel, A., 2003. Endocrine disruptors in the environment. Pure and Applied Chemistry 75, 631-681.

Markey, C.M., Rubin, B.S., Soto, A.M., Sonnenschein, C., 2003. Endocrine disruptors from wingspread to environmental developmental biology. The Journal of Steroid Biochemistry and Molecular Biology 83, 244-253.

Miao, S., Kou, Z., Gao, Z., 2009. Effect of bisphenol A on the structure of organs and the expression of some cytokines in rats. Journal of Hygiene Research 38 (1), 11-14.

Naukkarinen, A., Sorvari, T.E., 1984. Involution of the chicken bursa of Fabricius: A light microscopic study with special reference to transport of colloidal carbon in the involuting bursa. Journal of Leukocyte Biology 35 (3), 281-290.

Norton, J.M., Wirain, C.R., 1977. Dose-related effects of the sex hormones and cortisol on the growth of the bursa of Fabricius in chick embryos. Journal of Steroid Biochemistry 8 (9), 985-987.

Piskin, A., Kaplan, S., Aktaş, A., Ayyildiz, M., Raimondo, S., Aliç, T., Bozkurt, H.H., Geuna, S., 2009. Platelet gel does not improve peripheral nerve regeneration: An electro-physiological, stereological, and electron mic-roscopic study. Microsurgery 29 (2), 144-53.

Quinn, M.J., McKernan, M., Lavoie, E.T., Ottinger, M.A., 2009. Effects of estradiol on the development of the bursa of Fabricius in Japanese quail. The Journal of Experimental Zoology Part A. Ecological Genetics and Physiology 311 (2), 91-95.

Razia, S., Maegawa, Y., Tamotsu, S., Oishi, T., 2006. Histological changes in immune and endocrine organs of quail embryos: exposure to estrogen and nonylphenol. Ecotoxicology and Environmental Safety 65, 364-371.

Razia, S., Soda, K., Yasuda, K., Tamotsu, S., Oishi, T., 2005. Effects of estrogen (17 beta-estradiol) and p-nonylphenol on the development of immune organs in male Japanese quail. Environmental Sciences 12, 99-110.

Sheng, C., Ronald, S.G., 2000. Expression of estrogen receptors in the dorsal root ganglia of the chick. Brain Research 882, 236-240.

Shin, Y.H., Takagi, F., Sugita, S., Han, S., Tsuji, T., Abe, A., Kondo, Y., 2005. Evidence for estrogen receptor expression in the bursal epithelial cells of chicks. Animal Science Journal 76, 255-259.

Staples, C.A., Dorn, P.B., Klecka, G.M., O'Block, S.T., Haris, L.R., 1998. A review of the environmental fate, effects and exposures of bisphenol a. Chemosphere 36, 2149-2173.

Sullivan, D.A., Wira, C., 1979. Sex hormone and glucocorticoid receptors in the bursa of Fabricius of immature chicks. The Journal of Immunology 122, 2617-2623.

Tanyolaç, A., 1999. Özel Histoloji. Yorum Basım Yayın Sanayi Şirketi, Ankara.

Welshons, W.V., Thayer, K.A., Judy, B.M., Taylor, J.A., Curran, E.M., vomSaal F.S., 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environmental Health Perspectives 111, 994-1006.

Yamamoto, T., Yasuhara, A., 1999. Quantities of bisphenol A leached from plastic waste samples. Chemosphere 38, 2569-2576.

Yigit, F., Daglioglu, S., 2010. Histological changes in the uterus of the hens after embryonic exposure to bisphenol A and diethylstilbestrol. Protoplasma 247 (1-2), 57-63.

Ylikomi, T., Tuohimaa, P., 1989. Sex steroid sensitivity of developing bursa of Fabricius. International Journal of Developmental Biology 33, 135-140.

Yoshino, S., Yamaki, K., Li, X., Sai, T., Yanagisawa, R., Takano, H., Taneda, S., Hayashi, H., Mori, Y., 2004. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. Immunology 112, 489-495.

Yoshimura, Y., Tamura, Y., Nishikori, M., Okamoto, T., 2000. Effects of diethyl-stilbestrol intake during growing phase on the reproductive organs in Japanese Quail (Coturnix japonica). The Journal of Poultry Science 37, 323-333.