Testicular Functions and Sexual Behavior in Male Japanese Quail after Exposure to Bisphenol A

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ABSTRACT
Recently, with the world progress of industries, several adverse factors such as Endocrine Disrupting Chemicals (EDCs) have appeared. These contaminants have negative devastating effects on the reproductive performance in a large number of domestic and wildlife animal species. Therefore, the objective of this study was to investigate the reproductive and physiological changes due to pre-pubertal exposure of Japanese quail males to Bisphenol A (BPA) as one of EDCs (BPA is a synthetic chemical acts as estrogenic effect). Three weeks old male quails were weekly injected intra-peritoneally (at 3, 4 and 5 weeks old) with BPA at doses of 0, 1, 5, or 10 mg kg\(^{-1}\) b.wt. After 6 weeks exposure of BPA, time of sexual libido, semen characteristics, fertility percent, sexual organs development, histopathology of testes were examined and plasma testosterone concentrations were estimated. The results showed that BPA has adverse and deterioration effects on most of the studied traits. The males received 5 and 10 mg BPA showed significantly delayed time of sexual libido compared with control group. Also, males received 5 mg BPA showed significantly reduced semen volume and cloacal gland area compared with control males. The lowest initial motility and fertility percent (p < 0.05) were detected in 10 mg group while the highest values were obtained in the control group. Males treated with 1 and 10 mg BPA had lower (p = 0.028) foam production than those produced in control males. Plasma concentrations of testosterone were significantly reduced (p<0.000) in all treated groups compared with control group. Histologically, the growth of the testes was negatively affected by exposure to/or over 1 mg kg\(^{-1}\) BPA: Namely, the development of seminiferous tubules and spermatogenesis were severely inhibited compared with control testes. It could be concluded that exposure to estrogenic effects of environmental endocrine disruptors such as BPA before/at puberty lead to malformation of reproductive organs and reduction of reproductive capacity which appears not to regenerate in adult male quail.

Key words: Bisphenol A (BPA), testicular function, sexual behavior, testosterone, Japanese quail

INTRODUCTION
In recent years, numerous adverse effects on reproduction have been observed in a large number of domestic and wildlife animal species (Vos et al., 2000). Most of these effects have been attributed to the influence of certain pollutant chemical substances that are present in the environment (Nilsson, 2000). These contaminant chemicals are called Endocrine Disrupting Chemicals (EDCs) (McLachlan, 2001). Some of EDCs are known to mimic the action of endogenous estrogen and initiate cellular estrogen-dependent processes. Most effects of EDCs on male
reproductive system, including industrial chemicals (xenoestrogens) such as Bisphenol A are mediated through binding to nuclear Estrogen Receptors (ERs) subtypes, ERα and ERβ (Hanafy et al., 2004, 2005). Although, xenoestrogens have recently received increased attention, the risk is not fully understood, data do not fully substantiating yet a causal relationship between exposure and malfunctions. The major concern regarding these synthetic chemicals was with the permanent irreversible damage that could occur due to exposure during critical periods on primary and secondary sex organs development (Hanafy et al., 2006, 2007).

Bisphenol A (BPA), which is one of EDCs is a synthetic monomer widely used to polymerize polycarbonate plastics and resins (Leon-Olea et al., 2014). The environmental accumulation and abundance of BPA has sanctioned the extensive and longstanding exposure of animals and humans (Vandenberg et al., 2013). These chemicals can leach out of plastic products when heated and detectable amounts have been found in food and beverages (Brotons et al., 1995). This transfer effect has been demonstrated previously for BPA under controlled conditions (Hunt et al., 2003). Because plastic products are commonly used in the farms of poultry, BPA or one of its degradation products and metabolites may emerge and be consumed by the growing birds. Although, BPA can bind weakly to both ERs (Escande et al., 2006), it was reported by Steinmetz et al. (1997) that the estrogenic effects of BPA were higher in vivo than expected from in vitro studies. The BPA is a comparatively potent in vivo xenoestrogen in rats, mice and fish (Olsvik et al., 2009; Jones et al., 2011; Jasarevic et al., 2011; Ward and Blum, 2012). Nanjappa et al. (2012) found that exposure of rats to BPA results in decreased production of testosterone and estrogen by male and female gonads, respectively. Similarly, BPA disrupts fertility, spermatogenesis (Peknicova et al., 2002), steroidogenesis (Ahn et al., 2008) and neurobehavioral development in male mouse (Palanza et al., 2008). In birds, upon the injection of BPA and other estrogenic compounds to ova or immature male quail, results in major reprogramming of hepatica yolk precursor genes expression (Ichikawa et al., 2003; Hanafy et al., 2006, 2007). However, the potent effects of BPA on the sexual behavior and spermatogenesis of birds are not up till now well elucidated.

In Japanese quail, the importance of estrogen for reproductive structures and organization of sexual behavior is well characterized (Schumacher et al., 1989; Ottinger et al., 2001). As a consequence, the effects of a particular EDCs over the life cycle of the animal will diverge enormously. Often, embryonic exposure to estrogenic EDCs will have life long effects due to action of the estrogenic compounds on sexual dimorphism differentiation of brain and organs structures. Furthermore, exposure to the estrogenic chemicals during maturation period has consequences beyond impaired function of the reproductive organs (Halldin, 2005). Therefore, the objective of the present study was to evaluate the after exposure effects of BPA injection of immature male Japanese quail on reproductive organs and sexual development.

MATERIALS AND METHODS
Experimental design and treatments: This experiment was carried out at the Poultry Farm, Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt during winter season, 2014. Hatched quails were raised in a floor brooder, under temperature control and were provided with water and fed ad libitum on commercial quail diet allover experiment duration. At two weeks old, a total of 40 immature male quails, free from apparent clinical ailment were placed in metal cages (120×60 cm, height 50 cm). After one week from acclimatization, three week old, male quails were divided into four groups of 10 birds each. Birds of group 1 received only the vehicle solvent (Corn oil) and served as positive control. The
The volume of Corn oil injected was kept at 0.1 mL per 100 g body weight. The BPA (4, 4'-isopropylidenediphenol) was dissolved in Corn oil and intraperitoneally injected to quail weekly for three consecutive weeks (at 3, 4 and 5 weeks old) at doses of 1, 5, 10 mg kg$^{-1}$ b.wt., for three experimental groups, respectively. All experimental groups were performed in accordance with institutional guidelines concerning animal use.

**Measurements and laboratory analysis**

**Fertility test:** When male reached sexual maturity at 8 weeks old, treated males were housed with equal number of non-treated egg-laying female quails (1:1) for natural mating. After a week of birds adaptation, fertile eggs were collected daily for 6 consecutive days and stored at 18°C and 65% relative humidity till incubation. Eggs were set in the incubator for 8 days at 37.5°C and 60% relative humidity. Fertility was ensured and verified by inspection of the broken open all eggs. Fertility percentage was calculated as ratios of number of fertile eggs to the number of total eggs set in incubator.

**Sexual organs:** At week 12 of male age, live body weight, cloacal gland area and foam production were recorded for all birds. The cloacal gland areas (the longest length×the greatest width) were measured using a digital caliper (Hanafy and Khalil, 2015). The foam produced by each male was collected three times with 15 min time interval in between. Quantitative measurements (weights) on cloacal gland foam production were conducted immediately using an electronic analytical balance.

**Sexual libido, semen collection and semen quality evaluation:** One week before semen collation, treated males were individually housed in battery metal cages (20×30 cm with 30 cm height). A receptive egg-laying female were introduced to the male in the same cage. Time of sexual libido was estimated in terms of reaction time in seconds, from the time of female was placed inside the male’s cage up to the point when the male started mounting. Semen collections were made as follows: when the male mount the female to mating, the male was moved immediately before ejaculation of semen. Each male was gently restrained on the palm of the left hand and foam was squeezed out before semen collection. The lumbar region towards tail was massaged 3-4 times smoothly and applied gentle pressure on either side of the vent using thumb and fore finger. The semen was collected in a micropipette to quantify the volume (Hanafy and Khalil, 2015). To prevent contamination with foam, feaces and watery fluid, cloacal gland was cleaned gently with tissue paper before collection.

Semen characteristics were estimated immediately after collection in each ejaculate such as: semen ejaculates volume and initial motility (%). Ejaculate volume was measured to the nearest 1 μL using a graduated collection micropipette. Sperm motility (%) was estimated microscopically at 400x using a drop of semen with a drop of warm 0.9% saline solution (NaCl) and covered with cover slip on a warm slide.

**Blood samples:** All treated male quails were weighed at 12 weeks of age and then slaughtered by cutting their jugular vein. Jugular blood samples were collected in heparinized plastic tubes and centrifuged at 3000 rpm for 15 min. Plasma was separated and stored at -20°C until the hormonal assay was conducted. Plasma testosterone level was determined by ELISA kits manufactured by
The sensitivity of the assay was 70 pg mL\(^{-1}\) and the percentage of recovery was 100-105%. The intra- and interassay coefficients of variation for testosterone were 5.54 and 10.5%, respectively.

**Morphology and histopathology of testes:** Immediately after male slaughtered, the abdominal cavity was opened and the right and left testes were removed and weighed separately. To assess BPA induced changes in gonadal development, the left testis was processed for histological analysis. Testes tissues were fixed in 10% formaldehyde fluid and embedded in paraffin wax blocks and then sectioned at 6 µm with the microtome (The Gemmary, Fallbrook, USA). The resultant sections were stained with hematoxylin and eosin using standard procedures. Sections were visualized by PM-10M3 camera (Universal Infinity System, Olympus, Japan). Left testes were assessed for changes in general morphology and stage of spermatogenesis as described by Mather and Wilson (1964).

**Statistical analysis:** Data was analyzed using the General Linear Model (GLM) procedure of SPSS (SPSS., 2009). Differences among means were detected using Duncan’s new multiple test (Duncan, 1955).

**RESULTS**

From Table 1, it seems that administrating levels of BPA at the levels as high as 10 mg has non-significant effects on male's average body and testis weights (left, L or right, R) or percentages of testes relative to whole body weight after 6 weeks from treatment. Therefore, the results indicating BPA as cause of other health effects on body weights or testes weights and ratios were not conclusive. Hence, there is doubt/uncertainty about BPA-related hazards on quail body growth. However and though of the insignificance, the absolute weight differences between the R or L testis in the treated groups were somewhat higher and ranged from 0.13 g in T2 to 0.49 g in T1 versus only 0.05 g in the control group. As for percentages of testis, the absolute “Percentage differences” between the R. and L. testis in the treated groups ranged from 1.65 in T2 to 6.04 in T1 versus only 0.61% in the control group. Therefore, though of approximate symmetry in weights and percentages of L and R-testis in untreated male quails. This asymmetry seems to be fluctuating between the right and left sides of mail quail body.

Moreover, results of this study showed that injection of BPA in intraperitoneal cavity for immature male quail had unfavorable effects on most reproductive and physiological parameters of adult birds (Table 2). Exposure to BPA caused a significant dose-dependent delay time of sexual libido in male quail (p=0.038). The lowest dose (1 mg BPA) caused non-detectable significant effect on time of sexual libido while significant effects were obtained on males receiving >5 mg BPA kg\(^{-1}\). Also, the same trend was recorded in semen volume. At 1 mg, BPA significantly decreased efficiency of sperm production by 20% relative to control males. Furthermore, initial motility of sperm had a tendency to decrease with increased dose of BPA. While the lowest dose (1 mg kg\(^{-1}\)) had significantly decreased motility by 22%, the highest dose (10 mg kg\(^{-1}\)) reduced sperm motility of injected males by 44% compared to control group (Table 2).

Cloacal gland area was also affected by treatment with BPA. Exposure to BPA caused significant disparity in the treated groups vs. the control group (97.7, 87.3 and 95.3 vs. 100%, respectively). As shown in Table 2, injection of 5 mg kg\(^{-1}\) BPA was significantly decreased cloacal gland area compared with 1mg or control groups. Changes in cloacal gland area were similarly with
Table 1: Mean±SE of male quail’s body and testis weight and percentages after 6 weeks of treatment by graded levels of BPA

<table>
<thead>
<tr>
<th>BPA doses (mg)</th>
<th>0 (control)</th>
<th>1 (T1)</th>
<th>5 (T2)</th>
<th>10 (T3)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>243.40±16.73</td>
<td>233.40±6.88</td>
<td>228.00±13.30</td>
<td>236.40±6.18</td>
<td>0.822</td>
</tr>
<tr>
<td>Right testis weight (g)</td>
<td>4.10±0.28</td>
<td>3.81±0.30</td>
<td>4.00±0.28</td>
<td>3.59±0.27</td>
<td>0.617</td>
</tr>
<tr>
<td>Left testis weight (g)</td>
<td>4.05±0.28</td>
<td>4.30±0.42</td>
<td>3.87±0.32</td>
<td>3.80±0.46</td>
<td>0.791</td>
</tr>
<tr>
<td>Total testes weight (g)</td>
<td>8.15±0.45</td>
<td>8.11±0.66</td>
<td>7.87±0.58</td>
<td>7.40±0.72</td>
<td>0.814</td>
</tr>
<tr>
<td>Total testes weight (%)</td>
<td>3.37±0.14</td>
<td>3.47±0.24</td>
<td>3.45±0.15</td>
<td>3.10±0.23</td>
<td>0.548</td>
</tr>
<tr>
<td>R testis (/L+R Weights) (%)</td>
<td>50.31</td>
<td>46.98</td>
<td>50.83</td>
<td>48.51</td>
<td>0.064</td>
</tr>
<tr>
<td>L testis (/L+R Weights) (%)</td>
<td>49.69</td>
<td>53.02</td>
<td>49.17</td>
<td>51.35</td>
<td>0.064</td>
</tr>
<tr>
<td>Difference in testis W. (g)</td>
<td>0.05</td>
<td>-0.49</td>
<td>0.13</td>
<td>-0.21</td>
<td>0.064</td>
</tr>
<tr>
<td>Difference in testis (%)</td>
<td>0.61</td>
<td>-6.04</td>
<td>1.65</td>
<td>-2.84</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Means at any row with no common superscript differ significantly (p<0.05) using Duncan’s multiple range test (Duncan, 1955), BPA: Bisphenol A

Table 2: Mean±SE of male quail’s sexual libido, semen volume, sperm motility, cloacal gland area, foam production, fertility percentage and testosterone level after 6 weeks of treatment by graded levels of BPA

<table>
<thead>
<tr>
<th>BPA (mg)</th>
<th>0 (control)</th>
<th>1 (T1)</th>
<th>5 (T2)</th>
<th>10 (T3)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual libido (Sc)</td>
<td>6.56±1.08b</td>
<td>11.00±2.47ab</td>
<td>20.70±5.43a</td>
<td>22.12±2.07a</td>
<td>0.038</td>
</tr>
<tr>
<td>Semen volume (μl)</td>
<td>25.13±1.97a</td>
<td>20.00±0.81ab</td>
<td>17.88±1.56a</td>
<td>19.50±2.89ab</td>
<td>0.047</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>83.13±2.30a</td>
<td>65.00±8.45ab</td>
<td>71.88±5.88a</td>
<td>47.17±10.30b</td>
<td>0.011</td>
</tr>
<tr>
<td>Cloacal gland area (mm²)</td>
<td>581.80±16.36a</td>
<td>568.50±19.42a</td>
<td>508.20±17.31b</td>
<td>544.40±16.32ab</td>
<td>0.048</td>
</tr>
<tr>
<td>Foam production (mg)</td>
<td>61.25±5.17ab</td>
<td>45.62±6.26b</td>
<td>49.83±8.37ab</td>
<td>42.58±5.37ab</td>
<td>0.028</td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>86.55±2.02a</td>
<td>78.60±2.28ab</td>
<td>80.10±2.55ab</td>
<td>74.26±5.71b</td>
<td>0.049</td>
</tr>
<tr>
<td>Testosterone (ng mL⁻¹)</td>
<td>6.52±1.51a</td>
<td>4.15±1.61b</td>
<td>3.45±0.83b</td>
<td>2.38±0.94b</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Means at any row with no common superscript differ significantly (p<0.05) using Duncan’s multiple range test (Duncan, 1955), BPA: Bisphenol A

plasma levels of testosterone. Consistent with its adverse effect on cloacal gland area, birds treated with BPA had the lowest foam production compared to control group. The significant reduction was observed between 1 and 10 mg groups compare with control birds. On the other hand, the control birds were superior in fertility percentage than all treated groups. Injected male with 10 mg kg⁻¹ BPA was significantly decreased fertility compared with control group but in significant with the rest of treatments. In this context, the results showed highly significant (p<0.000) differences among treatments in plasma testosterone levels. Injected BPA led to linearly and significantly reduction in plasma testosterone compared to control group. At lowest BPA concentration (1 mg), a significant reduction of testosterone was observed in plasma of injected males compared with control males.

Control sections showed compartmentalization of germ cells in the seminiferous tubules with spermatozoa visible in normal-sized lumen (Fig. 1a and b). In contrast, a series of structural changes in the left testis occurred results from BPA exposure before/at puberty (Fig. 1c-h). Immature male exposure to BPA caused does dependent manner of testicular structure in adult male quail. When the birds had reached sexual maturity, BPA caused severe alterations of testis structure starting from 1 mg kg⁻¹. In treated males, the seminiferous tubules appear irregularly arranged with variability of their diameters and irregular rounded to oval shapes being crowded with mild edema of the interstitial space. Most of the tubules showed germinal epithelium with thin basement membranes and scattered congested blood vessels in-between tubules. Most of tubules showed empty lumens other showed full lumens with scattered tubules showing hemorrhage. However, several specimens lost the typical lobular structure showing spermatogenic cysts intermingled with free spermatozoa often degenerating into the lumen.

As shown in Fig. 1d, f and h, the tubules lined by stratified spermatogenic cells showing decrease in number of layers of spermatogenic cells denoting moderate atrophy of germinal
Fig. 1(a-h): Cross section of testes from (a, b) Control males, (c, d) Male exposed to 1 mg BPA, (e, f) Males exposed to 5 mg BPA and (g, h) Males exposed to 10 mg BPA (after 6 weeks from treatments)
DISCUSSION

Results reported herein are a try to throw light on the consequence of exposing animals during critical organizational periods to chemicals pollutants, specially the potential health effects of BPA on the reproductive system of male quails. From Table 2, exposure of quail before/at puberty to BPA, far above the tolerable daily intake or TDI may cause persistent structural and permanent functional changes in the testis of subsequent adult males causing impacts (e.g., reduce, delay) their sexual libido and androgen concentration (i.e., testosterone). In consistence and after exposure, semen volume and sperm motility of sexually matured birds were strikingly affected. In view of that, sexual libido as well as testicular structural abnormalities may perhaps be useful as biomarkers for estrogenic like effects of BPA in domestic birds using quails as an animal model. It is well known that male quail sexual behavior as well as sexual organs are androgen-dependent and develops in response to circulating testosterone (Balthazart et al., 2003). The organizational effects may not become apparent until endogenous hormone concentrations increase during sexual maturation (Guillette and Gunderson, 2001). Estrogenic chemicals cause an irreversibly depressed response to the activating effects of testosterone on sexual behavior in quail males and this effect was not evident until sexual maturity (Halldin, 2005). Hence, any disturbance on rising of sexual hormones production by gonads at puberty can lead to adverse effects on sexual organs developments and sexual behavioral differences.

In the present results, exposure to BPA at puberty severely affected sexual libido manifested later during adulthood lifespan of birds. Similar results have been observed in male Japanese quail exposed in ova (i.e., incubated eggs) to ethynyl estradiol (Halldin et al., 1999). In contrast, in ova treatment with BPA (50, 100 and 200 μg per egg) did not obviously affected the concentrations of circulating testosterone to account for the abnormal behavior in pubertal male quail (Panzica et al., 2005). However, BPA when injected to immature male in the present study showed more activity in reducing circulatory testosterone levels and sexual behavior than in ova injection. Consequently, the permanent effects of the estrogen like compounds on sexual behavioral can be induced by administering during a sensitive developmental stage, such as the period of differentiation or maturation of the reproductive organs (Panzica et al., 2009). Whereas estrogen-induced morphological changes in the testis have been reported to disappear after hatching (Halldin et al., 1999, 2003) while the estrogen-induced effects on the male brain are rather permanent. Generally, it can be suggested that effects of high doses of BPA in pubertal males is expected to be irreversible when the pollutant chemical is existent elongated enough during the period of instigation of sexual behavior (Panzica et al., 2007).

Results also revealed that the cloacal gland area in treated birds with testicular abnormalities was significantly (p<0.048), smaller than that in control males. The decrease in cloacal gland area in the exposed quail may be attributed partially to the reduced testosterone level recorded in the present study (Table 2). Similarly, previous reported studies confirmed that exposure to BPA diminished the weight of testes, the trait that is highly promoted by testosterone in the male
chicken (Furuya et al., 2003). Shit et al. (2010) confirmed the direct relationship between cloacal gland size and testicular activity in quail and substantiated the use of cloacal gland area as an informal straightforward selection marker during the breeding program for detecting sexually active males. Moreover, the correlation coefficient between area of cloacal gland with testicular size, testosterone level and fertility percent was found to be high and ascertained statistically (Biswas et al., 2007). Hence, cloacal gland area provides an external statistic for the quality of androgen status in the male quails during sexual maturation (Ball and Balthazart, 2010).

A detailed histopathological examination (Fig. 1) showed that immature male exposure to BPA causes a number of histological abnormalities in various parts of the adult quail testis. Results exhibited various degrees of testicular degeneration that may be consistent with the decreased rates of spermatogenesis in the BPA-treated groups (Fig. 1). In adult quails early treated/injected with BPA, there was substantial reduction in spermatogenesis accompanied with a decrease of spermatozoa in seminiferous tubules. Similar adverse effects were observed when adult mice and rats were treated for 6 days with 20 or 200 mg kg\(^{-1}\) (Toyama et al., 2004).

The effects of BPA reported herein seem to reflect the estrogenic effects on the testis of quail. These results suggested that levels of circulating testosterone in the treated birds were reduced as a direct consequence to the signs of local toxic effects of BPA on testis. However, the present study is one of the earliest reports on doses of the BPA effective enough to cause spermatogenesis degeneration in quails, since all used concentrations of BPA in this study (1, 5 and 10 mg kg\(^{-1}\)) caused sort of and variable degrees of alterations of testis structure and inhibitions of semen production, suggesting that the abnormalities caused by BPA treatments gave harmful and catastrophic effects on sexual activities.

Most, if not all, stimulatory effects of estrogenic chemicals are mediated through binding to ERs, ligand-dependent transcription factor (Evans et al., 1987). In a previous study, hepatic expression of yolk precursor proteins mRNA could be induced in male Japanese quail by in ova injection and three weeks old administration of xenoestrogens (Hanafy et al., 2006, 2007). The existence of ERs in the testis could be involved in the deleterious effects of BPA on testicular development and spermatogenesis (Peknicova et al., 2002). The present results clearly showed that in quails, juvenile exposed to a relatively low dose of BPA causes damage to the motility of sperm as well as reduction of the ejaculate volume, ensuing in subsequent reduced fertility in adulthood. Similar results have been observed in adult male chickens subjected to (0.002-200 mg kg\(^{-1}\)) BPA at 2 weeks of age (Furuya et al., 2006). In contrast, in ova administrations to 200 μg g\(^{-1}\) egg BPA, did not disturb sexual behavior, testis weight or plasma testosterone levels in adult male quail (Berg et al., 2001). This difference in sensitivity to exogenous estrogen between ages of animal may be attributed to the number of ERs represented in target organs during exposing period. The major development of ERs occurred after hatching that accounts for two-thirds of the receptors ultimately expressed in the adult (Evans et al., 1987). However, there are several investigations have established that xenoestrogens are rather weak estrogens with a 5,000-10,000-fold lower binding affinity to ER than synthetic estrogens (Hanafy et al., 2004, 2005). Moreover, a recent study has shown that not all effects of BPA are mediated by the classical nuclear ERs. Non-genomic cell-signaling systems involve serial activation of kinases via ligand binding to cell membrane receptors at very low concentrations (Welshons et al., 2006). Furthermore, xenoestrogens do not bind to the sex hormone binding globulin what may explain their large biological effect even in low concentrations (Slowikowska-Hilczer, 2006).
Moreover, the present results showed that the Leydig cells and the Sertoli cells were sparse in the testis of treated male. Alterations of Leydig cell functions can lead to adverse effects on testosterone synthesis, testicular functions and spermatogenesis process (Senger, 1999). Plasma testosterone levels in present experiment were significantly decreased in treated groups (p<0.000), which may indicate an evidence of decreased steroidogenesis of the testes and impairment and dysfunction of Leydig cells. Similar findings have been reported in rodents (Nanjappa et al., 2012; Savchuk et al., 2013). Also, BPA was found to increase Sertoli cell number per organ but not when expressed as per gram testis of male rat offspring (Wistuba et al., 2001). In contrast, female intrauterine administration of this xenoestrogen resulted in minor increases in Sertoli cell numbers and had no qualitative effect on spermatogenesis of resultant male rat (Wistuba et al., 2003). It is also documented that several EDCs reduces plasma level of various steroid hormone either by decreasing concentration of LH and FSH or by inhibition of process of steroidogenesis in gonads (Evans et al., 2004). These findings, however, provides additional insights into the relationship between time of administration and the effects of estrogenic compounds on oviparous species.

CONCLUSION
In conclusion, results of this study recorded that the lowest dose of BPA (1 mg kg\(^{-1}\) b.wt.) decreased the sexual libido, semen characteristics and fertility percent in most birds analyzed. Results may suggest that exposure to BPA in young birds adversely affects the reproduction of adult male quail and these impaired effects persistent. Accordingly, current study highlights the importance of exposing animals during maturation of the reproductive organs when studying effects of chemicals on the reproductive system.

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