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Neonatal exposure to furan alters the development of reproductive systems in adult male Sprague Dawley rats

Humaira Rehman[a], Imdad Ullah[b], Mehwish David[a], Asad Ullah[a], Sarwat Jahan[a,*]

[a] Reproductive Physiology Laboratory Department of Animal Sciences, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan
[b] Department of Zoology, Abbottabad University of Science and Technology, Havalian, Abbottabad, Pakistan

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ABSTRACT

Furan is a colorless toxic organic compound that is produced during thermal degradation of natural food constituents, and is present in various processed foods such as coffee and processed baby foods. The present study investigated the endocrine disrupting potential of furan in Sprague Dawley male pups. On postnatal day 0 (PND 0), pups were divided into five groups. The control group received subcutaneous injections of corn oil (50 μL), while the treated groups were injected with one of four concentrations of furan (1, 5, 10 and 20 mg kg^{-1} d^{-1} in 50 μL corn oil) from PND 1 to PND 10. Our results reveal significant physiological changes in groups receiving the two highest doses of furan (10 and 20 mg kg^{-1} d^{-1}). Fertility was decreased in high dose groups, as evidenced by lower daily sperm production (DSP) and epididymis sperm counts, and dose-dependent histological alterations in the testes. High dose groups showed significant reductions in plasma concentrations of testosterone, LH and GH, while plasma cortisol and final body weight was increased compared to the control group. .The results suggest that neonatal exposure to high concentrations of furan cause structural and endocrine alterations in male neonatal rats, compromising fertility.

1. Introduction

The recent decades have seen a rise in public attention for the adverse effects of chemicals on human health. Various environmental chemicals have been classified as endocrine disrupting chemicals (EDCs) because of their ability to alter animal physiology by disrupting hormonal levels. Especially developmental stages are vulnerable, since changes can become permanent when animals are exposed to EDCs during organ development (Richter et al., 2007), changing the hormonal profile irreversibly (Cooke et al., 2014; Hinson and Raven, 2006; Lafuente et al., 2003; Liu et al., 2010; Sajjad et al., 2018). Such irreversible and long-lasting changes are termed “organizational effects” (Arnold and Breedlove, 1985; Young, 1964). On the other hand, EDCs may be less harmfull after gonodal development is completed, as exemplified by the observation that men exposed to polychlorinated biphenyls at the age of 20 do not show a bias in the number of sons they conceive (del Rio Gomez et al., 2002).

In human infants, the main pathway for exposure to environmental toxicants is via the diet, either from breastfeeding or formula feeding (Lehmann et al., 2018). It is well documented that environmental toxicants like bisphenol, arsenic, chlorinated dioxins, TCDD, furan, polychlorinated biphenyls, DDT and acrylamide are present in breast milk and infant formula foods (Lehmann et al., 2018; Mojska et al., 2012). In males, exposure to the dioxin TCDD between 1 and 9 years is associated with a reduction in sperm counts and sperm motility (Mocarelli et al., 2007) along with a decrease in Sertoli cell number in adulthood (Sharpe et al., 2003). In female infants, exposure to polybrominated biphenyls via breastmilk has been linked to early menarche (Blanck et al., 2000). In addition to these more well-known EDCs, infants and children may are exposed to heat-induced food toxicants such as furan and acrylamide, which also have endocrine disrupting potential (FDA, 2004; Robin and Clanci, 2007). Furan (C_4H_4O) belongs to a group of dioxins (polychlorinated dibenzo-furans, PCDFs) that are produced during thermal degradation of natural food constituents. Furan is found in many processed foods including jarred and canned foods, coffee and infant formulas (Karacaoğlu and Selmanoğlu, 2010; Lehmann et al., 2018; Pluim et al., 1993).

The effect of developmental exposure to furan has been evaluated only in a handful of studies, despite strong experimental and epidemiological evidence that early life stages are highly sensitive to dioxins (Berga, 2008; Birnbaum and Tuomisto, 2000; Peterson et al., 1993; van den Berg et al., 2017). In addition to increased sensitivity, infants have

* Corresponding author.
E-mail addresses: S_humara23@hotmail.com (H. Rehman), drimdadullah@aust.edu.pk (I. Ullah), mehwish28david@gmail.com (M. David), asadaryaan@gmail.com (A. Ullah), drsarwatjahan@gmail.com (S. Jahan).

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higher intake rates of furan as compared to adults, because they rely solely on breastmilk or formula milk which contain high concentrations of furan. Exposure may even start in utero, since furan readily passes the placental barrier (Van Wijnen et al., 1990).A clinical study reported that 11-week old babies had higher thyroid hormone levels when their mother's breastmilk contained higher than average dioxin and furan concentrations (Pluim et al., 1993). In adult rats, exposure to furan resulted in decreased testosterone levels, decreased sperm counts, impaired spermatogenesis and induced apoptosis in Leydig and germ cells (Cooke et al., 2014; Karacaoğlu and Selmanoğlu, 2010). The present study investigated the effect of neonatal exposure to furan on the reproductive system of male rats. We evaluated sexual maturation, sperm parameters, hormonal profile, and histopathological parameters.

2. Materials and methods

2.1. Animal care and maintenance

Animal treatment and experimental rules were officially permitted by the ethical committee of the Department of Animal Sciences, Quaid-i-Azam University in Islamabad, Pakistan. Sprague-Dawley rats, 8 weeks of age, were obtained from the rodent facility of the same department. Animals were acclimatized to the laboratory for two weeks under the following standard conditions. They were fed with laboratory pelleted chow (Sial Scientific Company, Pakistan) and ad libitum access to tap water. The room temperature was maintained at 22–25 °C, humidity at 50 ± 5%, and the photoperiod was 10 h light and 14 h dark. At 10–11 weeks of age, estrous stage females (n = 30) in breeding cagescontaining a male rat. Vaginal smears were collected on consecutive days until the presence of sperm was confirmed. This day was designated as day 1 of gestation (GD1). Pregnant females were kept in single cages until the birth of pups on gestational day 22 (GD22). The day of birth of the pups was considered as postnatal day 0 (PND0). The number of pups born was recorded, and the sex was determined by measuring the anogenital distance (AGD) using a stereomicroscope. Male and female pups were placed in separate cages.

2.2. Experimental design

On PND0, male pups (n = 50) were distributed into five treatment groups (10 pups per treatment group). The control group received a daily SC injection with corn oil (50 μL) from PND1-10, while treated groups received SC injections with furan (either 1, 5, 10 or 20 mg kg^{-1} d^{-1} in 50 μL corn oil) for ten days. The doses were selected according to previous exposure studies with modifications (Karacaoğlu and Selmanoğlu, 2010; Kim et al., 2004; Rawi et al., 2012). We chose to administer furan using SC injections since neonatal rodents have low liver enzymatic activity, therefore there is no difference between oral and non-oral administration (Chapin et al., 2008; Taylor et al., 2008). Animals were kept in these treatment groups for three months. Their developmental stage was monitored to study the following parameters.

2.3. Determination of body weight

Animals were weighed on PND30, PND45, PND80 and on day of preputial separation (see below). Net weight gain within each group was calculated.

2.4. Evaluation of sexual maturation

As a criterion for sexual maturation, the timing of preputial separation for male rats (beginning on PND 35) was assessed and each pup was weighed when these criteria were achieved.

2.5. Evaluation of fertility

At the end of the experiment, three animals from each treatment group were housed together with an untreated female rat, on two occasions. A vaginal smear from each paired female was collected on the four days following the pairing and the number of spermatozoa on it was counted. For the resulting litter, litter size, morbidity, and mortality were noted. Fertility of each male was calculated using the following formula:

Fertility (%) = {(number of pups in the male's litters)/(total number of pups) *100}

2.6. Animal euthanization

At PND80, male rats (n = 7 per group) were weighed, euthanized using an overdose of diethyl ether, trunk blood was collected and testicular and epididymis organs were sampled, weighed and processed for histological and sperm parameters.

2.7. Analysis of plasma biochemistry

Plasma samples, having been stored at −20 °C, were further analyzed for total cholesterol, triglycerides, HDL and LDL using AMP diagnostic kits (AMEDA labor diagnostic Gmbh, Austria) on a chemistry analyzer according to the manufacturer's instructions.

2.8. Sperm parameters

2.8.1. Sperm motility

To analyze sperm motility, the cauda epididymis was cut with a scissors and placed in 0.5 mL pre-warmed (at 37 °C) phosphate buffer saline (PH 7.3). A drop of nigrosin stain was added to it. 50 μL of this solution was put on a pre-warmed (at 37 °C) glass slide and inspected under a light microscope at 40 × magnification. At least 10 fields were observed, and 100 sperm per sample were analyzed for motility. Each sample was evaluated three times, after which the average sperm motility was used as the total sperm motility for a particular sample (Halvaei et al., 2012a).

2.8.2. Assessment of sperm viability

To assess sperm viability we used the eosin-nigrosin staining test. 25 μl of eosin-nigrosin dye was mixed with semen samples. 15 μl of this mixture was placed on a glass slide and dried at room temperature. Smears were examined under a light microscope (40 × magnification). Living spermatozoa remained unstained (white) whereas dead cells were stained red. The percentage of dead and alive spermatozoa was calculated by counting at least 100 sperm cells (Halvaei et al., 2012b).

2.8.3. Epididymal sperm count (ESC)

In order to determine the epididymal sperm count, the epididymis was cut into three small parts; caput, corpus, and cauda. The sections were minced in 1 ml physiological saline and the homogenate was incubated at 37 °C for 15 min. After incubation, 2–3 drops of nigrosine were added into the homogenate, of which ten μL were placed on a prewarmed slide. A minimum of 10 fields were observed. Spermatozoa were counted in each of the three parts of the epididymis using a light microscope at 40 × magnification (Amin et al., 2012).

2.8.4. Daily sperm production (DSP)

Frozen testicular tissues were thawed at room temperature and the tunica albuginea was removed. The tissue was homogeneized in 5 mL NaCl (0.9%)/Triton X-100 (0.5%) solution using a rotor stator homogenizer (IKA-Werke, Staufen, Germany) for 30 s (Robb et al., 1978). The homogenate was diluted 5 times, transferred to a Neubauer chamber and 19th stage spermatids were counted under a light microscope at 40 × magnification. The average of at least three readings was taken as

the number of spermatids. To obtain the daily sperm production (DSP), the number of spermatids (an average of at least three readings of one sample) was divided by 6.3 (number of days the spermatids remain in seminiferous epithelium).

2.9. Histology

Testis and epididymis tissue samples were fixed in 10% formalin for 48 h. Dehydration was carried out at room temperature using ascending concentrations of alcohol, and shifting to xylene. After clearing, tissues were embedded in paraffin wax and five to seven μm thick sections were cut using a microtome (Thermo Fisher Scientific, UK). Sections were transferred to albumenized slides that were preheated to 37 °C. For complete stretching of tissues and removal of bubbles, slides were incubated overnight at 58 °C. Tissues were rehydrated in descending concentrations of alcohol, stained with hematoxylin-eosin stain and covered with a coverslip. The prepared slides were observed under a Leica microscope (New York, USA) equipped with a digital camera (Canon, Japan) at 40 × magnification. The percentage area covered by seminiferous tubules was calculated using ImageJ software as previously described by (Jensen, 2013).The area percentage was calculated by using the following formula

$$\% \text{ As} = \text{As} \times 100/\text{T}$$

Where As is the area covered by seminiferous tubules, T is the total area of the field. The %age of the mean area was calculated between control and treated groups for comparison.In addition, the number of spermatogonia, spermatocytes and spermatids was counted from 50 seminiferous tubules per animal at 100 × magnification.

2.10. Hormonal analysis

Plasma levels of testosterone, LH, FSH, GH and cortisol were determined by Enzyme-linked immune Sorbent assay (ELISA) kits; for LH, FSH and testosterone (Reddot Biotech Inc. Canada), GH (Crystalchem, USA) and for cortisol (Calbiotech, Inc. USA) according to the instructions in the kit.

2.11. Statistical analysis

The results are stated as mean ± SEM. All statistical analysis was done by using 'lme4' (Bates et al., 2014) and 'easyanova' (Arnhold, 2013) packages for R statistical software version 3.2.5 (R Development Core Team, 2016). The effect of the treatment was analyzed using one-way ANOVAs ('ea1' function of 'easyanova') with each of the measured physiological/histological parameters as a response variable and furan concentration as the dependent categorical variable. Post-hoc differences were computed with the Tukey Honest Significant Differences (the R function 'TukeyHSD'). Plasma hormone concentrations of cortisol, plasma testosterone, LH, FSH and GH were correlated amongst each other using Pearson's correlations. We checked each model's residuals for normality using Shapiro-Wilk tests. The assumption of homogeneity of variances was assessed using Levene's test and additivity was verified using Tukey's test of additivity. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of furan on body weight gain, accessory organ weight and abdominal fat

All groups gained body weight from PND 1 to PND, but weight gain was significantly higher in animals treated with the highest dose of furan (20 mg kg^{-1} d^{-1}) in comparison with the control group (Table 1, P = 0.01). No significant difference was observed among other treated groups (Table 1). Weights of accessory reproductive organs (paired testis, paired epididymis, seminal vesicle, and prostate) were not significantly different among furan treatments (Table 1), although there was a tendency for increased weight in the high dose group. Weight of the abdominal fat pad was significantly higher in the high dose 20 mg$kg^{-1}d^{-1}$treated group (P = 0.02) and the same tendency (although not statistically significant) was observed in the 10 mg kg^{-1} d^{-1} group (P = 0.1,Table 1).

3.2. Effect of furan on plasma biochemical parameters, onset of puberty and fertility

Plasma concentrations of protein (P = 0.06), triglycerides (P = 0.001) and HDL (P = 0.001) were significantly reduced in the high dose group (20 mg kg^{-1} d^{-1}) compared to controls (Table 2). Plasma cholesterol and LDL levels showed significant (P = 0.06 and P = 0.001, resp.) increases in the 5, 10 and 20 mg kg^{-1} d^{-1} dose groups (Table 2). The day of preputial separation was not significantly different among furan treatment groups (P = 0.53; Table 2). We found a non-significant reduction in fertility in the high dose group (P = 0.07, Table 1), and there was also a non-significant dose-dependent reduction in fertility % with increasing furan exposure (P = 0.07, Table 2). No signs of morbidity and mortality were recorded in resultant pups.

3.3. Effect of furan on sperm parameters

A marked reduction in daily sperm production (DSP) was observed in response to furan treatment (Table 3). Compared to the control group, the 10 mg $kg^{-1}d^{-1}$ and 20 mg kg^{-1} d^{-1} treatment groups showed a dose dependent decrease in DSP (P = 0.06 and P = 0.02 respectively, Table 3). Furan treatment was furthermore associated with a significant reduction in sperm viability (P = 0.003) and epididymal sperm count (P = 0.05; Fig. 1). A non-significant tendency for lower sperm motility was observed with increasing furan doses (P = 0.16). Epididymal sperm count (caput, corpus, cauda) was significantly lower among the groups treated with the highest doses (P = 0.09, P = 0.004 and P = 0.04 resp. in 5, 10, 20 mg kg^{-1} d^{-1} groups; Fig. 1).

3.4. Effect of furan after neonatal exposure on hormonal profile

Plasma testosterone concentration was reduced in the 10 (p = 0.06) and 20 mg kg^{-1} d^{-1} (p = 0.001) treated groups compared to the control group (Table 4). Similarly, significant reductions in plasma LH level (P = 0.01) and GH concentrations (P = 0.001) were observed in the groups receiving the two highest furan doses (Table 4). A non-significant reduction in FSH level was observed in the high dose group. Cortisol concentrations were significantly elevated in the two high dose groups (P = 0.001 and P = 0.01 in the 10 and 20 mg kg^{-1} d^{-1} groups; Table 4).

To investigate the relationship between plasma cortisol concentration and plasma testosterone, LH, FSH and GH concentrations, we correlated these hormone concentrations in a pairwise fashion (Table 5, Fig. 2). Results indicated that plasma cortisol concentration is negatively correlated with plasma testosterone (r = −0.385, P = 0.006) and GH (r = −0.5131, P < 0.01), while the relationship between cortisol and LH (r = −0.162, P = 0.261) and cortisol and FSH (r = −0.155, P = 0.281) was not significant. Moreover, testosterone and LH, as well as testosterone and GH correlated positively with each other (r = 0.379, P = 0.007 and r = 0.560, P < 0.001, resp.), while testosterone and FSH were not correlated (r = 0.119, P = 0.411). Finally, a positive correlation appeared between LH and FSH (r = 0.298; P = 0.03; Table 5 and Fig. 2).

3.5. Effect of furan on testicular histology

Analysis of testicular histology indicated a signficant reduction in

Table 1
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 mgkg^{-1}d^{-1}) on body weight gain, accessory organ weight and abdominal fat pad weight in adult male rats.

Parameters	Control	Furan (mgkg^{-1}d^{-1})				Statistics
		1	5	10	20	
Body weight gain (g)	236.16 ± 6.01^{a}	237.1 ± 7.49^{ab}	240.7 ± 4.69^{ab}	238.1 ± 3.57^{b}	243.7 ± 8.87^{b}	P = 0.01 F = 3.83
Paired testis weight (g)	1.73 ± 0.05	1.70 ± 0.14	1.69 ± 0.09	1.71 ± 0.08	1.68 ± 0.06	P = 0.9 F = 0.04
Paired Epididymis weight (g)	0.94 ± 0.12	0.80 ± 0.09	0.76 ± 0.06	0.71 ± 0.10	0.65 ± 0.04	P = 0.60 F = 0.69
Prostate (g)	0.47 ± 0.06	0.46 ± 0.17	0.44 ± 0.19	0.47 ± 0.15	0.44 ± 0.08	P = 0.99 F = 0.01
Seminal Vesicle (g)	0.56 ± 0.06	0.55 ± 0.06	0.50 ± 0.19	0.48 ± 0.12	0.44 ± 0.14	P = 0.8 F = 0.27
Fat Pad (g)	1.24 ± 0.02^{b}	1.34 ± 0.03^{ab}	1.40 ± 0.09^{ab}	1.47 ± 0.11^{ab}	1.59 ± 0.09^{a}	P = 0.03 F = 2.96

Values are presented as Mean ± SE of mean (n = 7). P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared control with other treated groups.

Table 2
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 mg kg^{-1} d^{-1}) on biochemical plasma parameters, puberty onset and fertility in adult male rats.

Parameters	Control	Furan (mg kg^{-1}d^{-1})				Statistics
		1	5	10	20	
Protein (mg 0.5g^{-1})	5.51 ± 0.20^{a}	5.46 ± 0.20^{a}	5.47 ± 0.06^{a}	5.16 ± 0.23^{ab}	4.88 ± 0.17^{b}	P = 0.03 F = 2.95
Cholesterol (mg dL^{-1})	40 ± 1.93	43.14 ± 2.66	42 ± 1.99	46.42 ± 1.88	46 ± 1.43	P = 0.06 F = 2.53
Triglyceride (mg dL^{-1}	53.28 ± 1.29^{a}	50.85 ± 1.80^{a}	44.57 ± 0.37^{b}	44.14 ± 1.72^{b}	44.42 ± 1.65^{b}	P = 0.001 F = 12.51
HDL (mg dl^{-1})	73 ± 1.16^{a}	72.14 ± 1.26^{a}	65.57 ± 1.50^{b}	65.71 ± 1.99^{b}	62.42 ± 2.25^{b}	P = 0.001 F = 12.47
LDL (mg dl^{-1})	29.54 ± 1.66^{b}	32.31 ± 1.11^{a}	39.54 ± 2.15^{ab}	34.94 ± 2.09^{b}	40.8 ± 2.33^{a}	P = 0.001 F = 7.75
Day of puberty onset	44.14 ± 0.51	44.42 ± 0.62	44 ± 0.54	43.57 ± 0.37	43.14 ± 0.75	P = 0.53 F = 0.79
No of pups born/female	8 ± 0.38	7.57 ± 0.37	7.85 ± 0.34	8 ± 0.31	6.71 ± 0.48	P = 0.07 F = 0.79
Fertility %	100 ± 4.80	98.24 ± 3.85	97.24 ± 4.69	94.64 ± 4.69	82.76 ± 5.72	P = 0.07 F = 0.79

Values are presented as Mean ± SE of mean (n = 7). P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared control with other treated groups.

the percentage area covered by seminiferous tubules in the two high treatment groups (Table 6; P = 0.01 and P = 0.002 for 10 and 20 mg kg^{-1} d^{-1}, resp.) while the area covered by interstitial spaces increased significant (P = 0.001) compared to the control group. The changes were in the same direction for the other variables measured (tubular and lumen diameter, and epithelial height) but were not statistically significant here (Fig. 3, Table 6).

3.6. Effect of furan on cells in seminiferous tubules

The number of spermatogonia present in the seminiferous tubules of the animals was significantly reduced in the groups exposed to the three

Table 3
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 mg kg^{-1} d^{-1}) on sperm parameters in adult male rats.

	Control	Furan (mg kg^{-1}d^{-1})				Statistics
		1	5	10	20	
DSP (× 10^{6})	3.97 ± 0.23^{a}	3.75 ± 0.77^{a}	2.92 ± 0.311^{ab}	2.55 ± 0.22^{c}	2.37 ± 0.07^{c}	P = 0.01 F = 3.95
Sperm viability %	17.85 ± 1.70^{a}	14.28 ± 1.17^{ab}	13.14 ± 1.37^{ab}	12.71 ± 1.58^{ab}	9.28 ± 0.94^{b}	P = 0.003 F = 5.004
Sperm motility%	80.42 ± 1.92	79.14 ± 2.60	76.85 ± 1.14	74.57 ± 1.48	76.28 ± 1.30	P = 0.16 F = 1.73

Values are presented as Mean ± SE of mean (n = 7). P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared control with other treated groups.

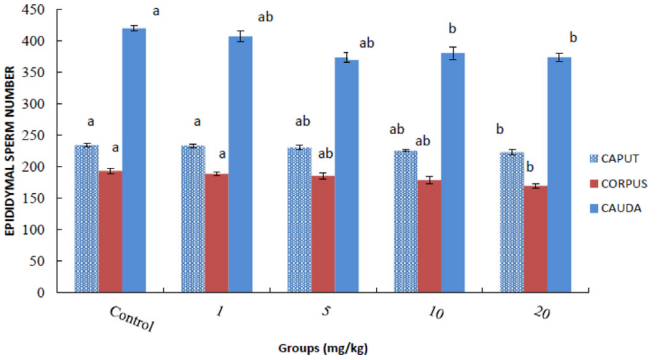

Fig. 1. Effects of neonatal exposure to furan (0, 1, 5, 10, 20 mgkg^{-1}d^{-1}) on epididymal sperm counts (in caput, corpus and cauda parts) in adult male rats. Values represent mean ± SE of mean (n = 7). Letters above bars depict values that are significantly different (P < 0.05) between different doses within the same epididymal part.

highest doses of furan (Table 7; P = 0.003, P = 0.001 & P = 0.001 in 5, 10, 20 mg kg^{-1} d^{-1} doses, resp.). Similarly, a significant (p = 0.001) reduction in a number of spermatocytes was also observed in the two high dose groups (10 mgkg^{-1} and 20 mgkg^{-1}d^{-1}) as compared to control. In addition, the number of spermatids was significantly reduced in the 20 mgkg^{-1}d^{-1} dose group (P = 0.04; Table 7).

3.7. Effect of furan on epididymis histology

No significant morphometric alterations were detected in the histology of epididymis caput tissue of adult rats. There was however a trend for a reduction in tubular and lumen diameters, and increase in epithelial height in the high dose group (Table 8, Fig. 4).

A similar pattern was observed in the cauda epididymis (Table 9, Fig. 5) although here, epithelial height was significantly increased (P = 0.05). No significant difference was recorded in other parameters of epididymis in treated groups when compared to control. No differences between treatment groups were observed for epididymis corpus histology.

4. Discussion

Few animal studies have investigated the possible endocrine disrupting effects of furan. To our knowledge, the current study is the first study that exposed rats to furan at the neonatal stage of life, and estimated the effects on male reproductive system in adulthood. Neonatal male rats were subcutaneously injected with four different furan doses (1, 5, 10 and 20 mgkg^{-1}d^{-1}in 50 µL corn oil), in order to investigate the effects in adult male rats (PND80). We find clear evidence that exposure to the two highest doses of furan (10 and 20 mg kg^{-1} d^{-1}) broadly and consistently affects the development of the male reproductive system, ultimately compromising male fertility in adulthood. The most prominent effects we observed were dose dependent increases in body weight, decreased plasma testosterone, LH and GH, increased cortisol levels, and decreased sperm counts associated with altered testicular and epididymal histology.

Elevated weight gain has been observed in an earlier study where rats were exposed to furan from the weaning period to post-puberty (Karacaoğlu and Selmanoğlu, 2010) and in suckling mice after furan treatment (Blagburn et al., 1998). The mechanism of how furan affects body weight gain is unknown, but one can speculate that it could be the result of a direct effect on nutrition uptake, or an indirect effect via the observed changes in plasma hormones (Mårin et al., 1992). has suggested that cortisol can cause obesity, an idea that has been further developed by later studies (Abraham et al., 2013; FTC, 2004). Also low testosterone levels are associated with adiposity resulting from to metabolic impairments (Fui et al., 2014), while testosterone replacement therapy instigated a reduction in fat mass and prominent increase in fat-free mass in both hypogonadal and normal men (Cox et al., 1999; Evans, 2000; Gulve and Spina, 1995). Rasmussen (2010) reported that GH deficiency is related to increased fat mass and abdominal fat mass accumulation (Rasmussen, 2010). Hence, the increase in weight gain and abdominal fat mass we found in the present study in the group receiving the highest dose (20 mg kg^{-1} d^{-1}) may well have been caused by the elevation of cortisol levels and the decreased level of testosterone, LH and GH.

The relationships we observed between plasma hormone levels furthermore fit well in with earlier studies reporting negative relationships between plasma cortisol vs. testosterone or GH (Wennink et al., 1990). Indeed, many components of the gonadal axis are downregulated by plasma glucocorticoids, either by affecting hypothalamus and pituitary functions, or by affecting the responsiveness of target tissues to gonadal hormones (Borges et al., 1997; Thakore and Dinan, 1994). Increased cortisol secretion has been associated with a decreased production of sex steroids and GH in a multitude of studies (Björntorp, 1995; Burguera et al., 1990; Chen et al., 1997; Liening and Josephs, 2010; Tsigos and Chrousos, 2002; Viau, 2002). High doses of glucocorticoids inhibited testicular Leydig cell function in rats (Bambino and Hsueh, 1981). In humans, exposure to cortisol caused a marked decrease in testosterone production (Cumming et al., 1983). A decrease in the testosterone production in adulthood is a recurrent finding in studies exposing rats to heat-induced food toxicants (El-Akabawy and El-Sherif, 2016; Karacaoğlu and Selmanoğlu, 2010; Salian et al., 2009). Also endocrine disruptors such as PAHs and PCBs, as well as lead and cadmium cause elevated cortisol levels and decreased GH

Table 4
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 mgkg^{-1}d^{-1}) on hormonal profile in adult male rats.

	Control	Furan (mg kg^{-1}d^{-1})				Statistics
		1	5	10	20	
Testosterone (ng ml^{-1})	4.45 ± 0.20^{a}	4.28 ± 0.025^{a}	4.13 ± 0.17^{a}	3.83 ± 0.16^{ab}	3.24 ± 0.15^{b}	P = 0.001 F = 8.95
LH (ng ml^{-1})	1.79 ± 0.04^{a}	1.74 ± 0.04^{ab}	1.69 ± 0.03^{ac}	1.63 ± 0.03^{bc}	1.59 ± 0.04^{c}	P = 0.01 F = 3.41
FSH (mlUml^{-1})	1.03 ± 0.09	1.02 ± 0.005	0.96 ± 0.10	0.89 ± 0.08	0.80 ± 0.02	P = 0.1 F = 1.57
GH (ng ml^{-1})	210.7 ± 1.75^{a}	203.6 ± 2.13^{ab}	197.8 ± 2.28^{bc}	190 ± 3.62^{cd}	181.6 ± 3.90^{d}	P = 0.001 F = 15.45
Cortisol (ng ml^{-1})	39.8 ± 0.89^{c}	41.2 ± 0.94^{bc}	44.1 ± 0.88^{ac}	44.4 ± 1.56^{ab}	46.5 ± 1.05^{a}	P = 0.001 F = 5.94

Values are presented as Mean ± SE of mean (n = 7). P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared to control with other treated groups.

Table 5
Summary of the Pearson's correlations between plasma, cortisol, testosterone, LH, FSH and GH in adult male rats neonatally exposed to furan (0, 1, 5, 10, 20 mg kg^{-1} d^{-1}).

Parameters	Correlations				
	Cortisol (ng ml^{-1})	Testosterone (ng ml^{-1})	LH (ng ml^{-1})	FSH (mlUml^{-1})	GH (ng ml^{-1})
Cortisol (ng ml^{-1})	r = 1				
Testosterone (ng ml^{-1})	r = −0.385**	r = 1			
	P = 0.006				
LH (ng ml^{-1})	r = - 0.162	r = 0.379**	r = 1		
	P = 0.261	P = 0.007			
FSH (mlUml^{-1})	r = −0.155	r = 0.119	r = 0.298*	r = 1	
	P = 0.281	P = 0.411	P = 0.036		
GH (ng ml^{-1})	r = −0.513**	r = 0.560**	r = 0.261	r = 0.102	r = 1
	P = 0.000	P = 0.00	P = 0.250	P = 0.482	

Pearson's correlation and sets significant relation; Pearson's correlation is shown with r whenever significant by P -value.

levels in fish and mammals (Sajjad et al., 2018; Tan et al., 2007; Tort et al., 1996; Zimmer et al., 2009). This suggests that the growth of the reproductive organs can be disturbed by reduced testosterone, which in turn could be driven by increased cortisol levels.

We found a consistent decrease in both the quality and number of sperm from males neonatally exposed to the two highest doses of furan. A significant reduction in sperm viability and motility was observed in highest dose treated group, and daily sperm production and epididymis (caput, corpus and cauda) sperm counts were reduced in a dose-dependent manner. These findings may be explained by the lower testosterone and LH concentrations. The elevated cortisol levels may have contributed to the inhibition of spermatogenesis, disturbance of spermiation and impairment of sperm quality (Castranova et al., 2005; Pressman et al., 2018). Also furan exposure at the weaning stage of life resulted in spermatogenic impairment and apoptosis in germ cells and Leydig cells which was associated with lower LH and testosterone levels (Karacaoğlu and Selmanoğlu, 2010).

Histological analysis of the testis showed a significant decline in the seminiferous tubules' diameter, epithelial height and area covered by the seminiferous tubule, associated with exposure to furan, and wider lumen and larger spaces in the interstitium. In the groups receiving the highest doses, prominent multilayered spermatogonia were recorded, while seminiferous tubules lumen was not heavily packed with elongated spermatozoa. These findings are in accordance with earlier reports of apoptosis in germ cells and Leydig cells after furan exposure (Karacaoğlu and Selmanoğlu, 2010). In contrast to the testes, morphometric findings of the epididymis showed few significant alterations. The only change observed was in the caput epididymal diameter,

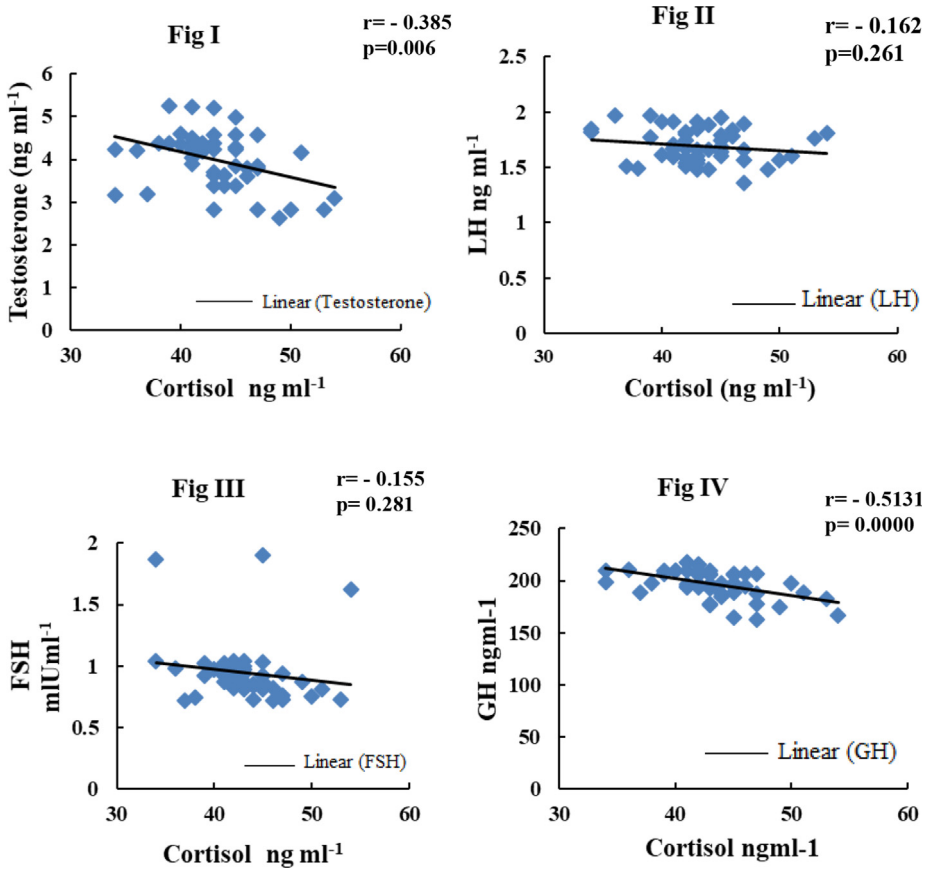

Fig. 2. Plasma testosterone (I), LH (II), FSH(II) and GH (IV) as a function of plasma cortisol concentration in adult male rats neonatally to furan (0, 1, 5, 10, 20 mg$kg^{-1}d^{-1}$). Pearson's correlation coefficient (r) and P values are provided in the top right of each graph.

Table 6
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 mgkg^{-1}d^{-1}) on testicular tissue histopathology in adult male rats.

Parameters	Control	Furan (mg kg^{-1}d^{-1})				Statistics
		1	5	10	20	
Area of seminiferous tubule %	89.18 ± 0.63^{a}	87.05 ± 0.78^{ab}	86.11 ± 0.90^{ab}	84.97 ± 1.26^{b}	84.35 ± 0.94^{b}	P = 0.002 F = 4.25
Area of interstitium %	12.66 ± 0.58^{a}	13.89 ± 0.52^{a}	14.56 ± 0.49^{b}	17.20 ± 0.59^{bc}	17.84 ± 0.56^{c}	P = 0.001 F = 16.18
Seminiferous tubule diameter (μm)	234.26 ± 6.82	227.43 ± 6.73	223 ± 10.49	223.42 ± 7.38	218.88 ± 9.65	P = 0.7 F = 0.49
Epithelial height (μm)	63.23 ± 2.51	60.04 ± 2.87	61.84 ± 2.88	58.11 ± 2.42	58.51 ± 2.56	P = 0.60 F = 0.68

Values are presented as Mean ± SE of mean (n = 7). P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared to control with other treated groups.

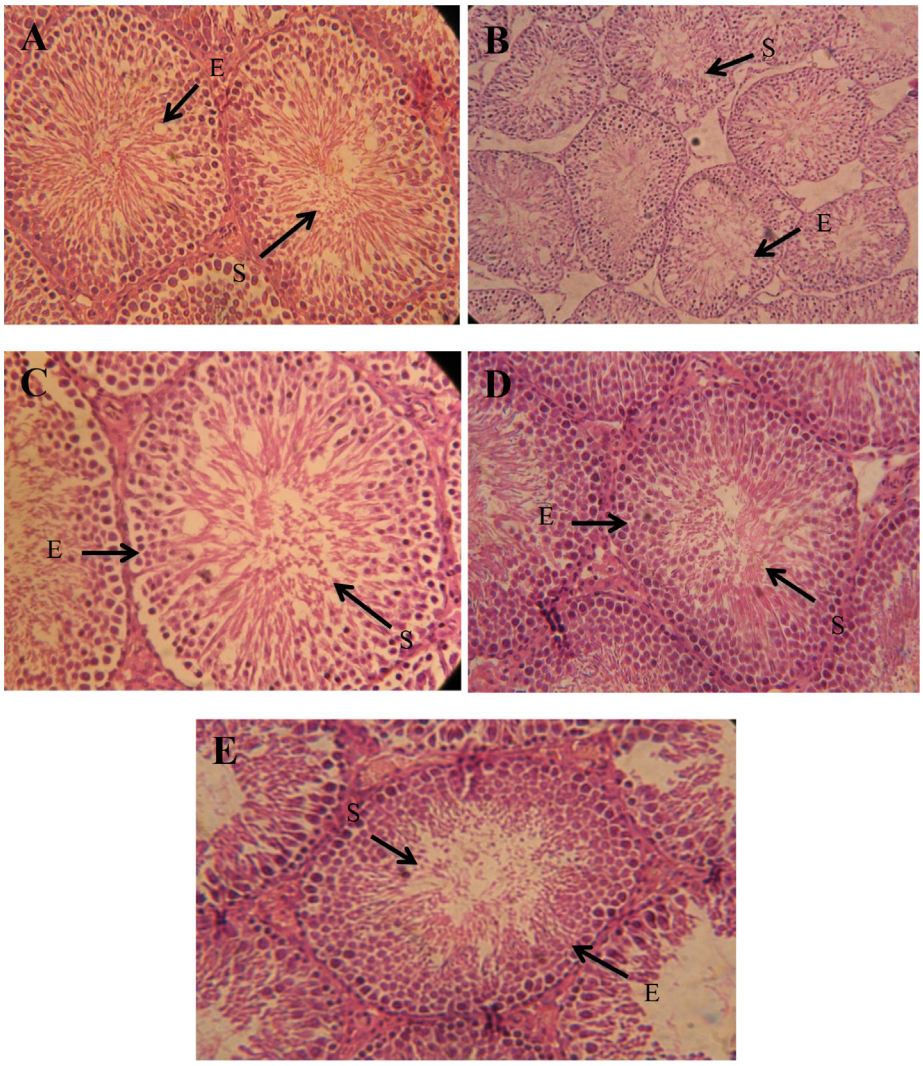

Fig. 3. Photomicrograph (40 × magnification) of seminiferous tubules (hematoxylin-eosin stain) of adult male rats exposed to different doses of furan at neonatal stage. (A) control showing regularly arranged tubules, lumen filled with spermatids with normal germ cells; (B) 1 mg kg^{-1} d^{-1} showing normal spermatogenesis with lumen filled with mature spermatozoa; (C) 5 mg kg^{-1} d^{-1} displays normal morphology with a slightly widened lumen; (D) 10 mg kg^{-1} d^{-1} group showing increased lumen diameter and normal epithelial height; (E) 20 mg kg^{-1} d^{-1} showing a thin degenerated epithelium with empty lumen. Letters indicate spermatozoa (S), and epithelium (E).

which showed a dose-dependent reduction.

We observed increases in plasma cholesterol and LDL while triglyceride (TG) and HDL were decreased in the groups receiving the two highest doses of furan, findings that are in line with the observed increases in body mass and abdominal fat. The increased production of fatty acids leads to a reduction in plasma HDL and elevation in plasma cholesterol concentrations, which can ultimately result in liver dysfunction. Lipoproteins (HDL and LDL) are therefore considered sensitive biomarkers of liver function (Rawi et al., 2012). TG/HDL ratio is clinically used for the detection of apparently healthy individuals with cardiovascular or metabolic impairments (Murguía-Romero et al., 2013). The lowest levels of HDL are detected in patients with fasting chylomicronæmia and in hypertriglyceridemic subjects, suggesting an inverse relationship between the metabolism of triglyceride and HDL (Schaefer et al., 1978). However, the present study detected a decrease in both tryglyceride and HDL associated with furan treatment, which could be explained by the observation that plasma lipoprotein levels often remain low during the early stages of development (Robinson and

Table 7
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 mgkg^{-1}d^{-1}) on different types of cells in seminiferous tubules of testes in adult male rats.

Cells number	Control	Furan (mg kg^{-1}d^{-1})				Statistics
		1	5	10	20	
Spermatogonia (n)	61.4 ± 1.023^{a}	58.6 ± 0.75^{ab}	56.6 ± 0.80b^{c}	55.64 ± 0.98^{bc}	53.08 ± 1.06^{c}	P = 0.001 F = 11.21
Spermatocytes (n)	81.08 ± 1.165^{a}	78.2 ± 1.50^{ab}	77.48 ± 1.137^{ab}	77.12 ± 0.83^{ab}	75.58 ± 1.06^{b}	P = 0.01 F = 3.03
Spermatids (n)	257.52 ± 2.14	255.84 ± 2.24	253.56 ± 2.34	253.32 ± 1.92	248.8 ± 2.39	P = 0.06 F = 2.20

Values are presented as Mean ± SE of mean (n = 7). P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared control with other treated groups.

Seakins, 1963). An alterative explanation for the observed changes in plasma lipoproteins stems from the observation that a number of liver toxins have been reported to inhibit the release of hepatic triglyceride into the bloodstream. Acrylamide, for example, also causes changes in lipoproteins in the same direction (Rawi et al., 2012).This would explain the observed low plasma triglyceride concentration and could also have led to a the buildup of triglyceride in the liver (Robinson, 1973), which has been associated with liver damage and fibrosis in obese mice (Yamaguchi et al., 2007).A third possible explanation for the observed elevated plasma LDL levels could be the overactivation of LDL receptors, as first proposed by Selmanoğllu et al. (2012). In the latter study male rats were exposed to furan, which also resulted in elevated plasma LDL levels. LDL receptors enable cholesterol to enter normal body cells. Once attached to LDL receptors on the hepatocytes, LDLs release their cholesterol and triglycerides. Elevated cholesterol in the cells suppresses the formation of new LDL receptors, resulting in decreased transport of LDL cholesterol into the cells (Elaine, 2009). Excess levels of free cholesterol inhibit cholesterol and LDL receptor synthesis, thus reducing LDL uptake which promotes cholesterol storage. Restricting the LDL uptake and non-functioning of its receptors enhance the serum cholesterol levels (Linda, 2012).

Also the reduction in plasma total protein levels can be regarded as evidence for a reduction in liver function, as previously reported by (Alam et al., 2017; Karakilcik et al., 2004).About 6%–7% of the blood plasma proteins, including albumin, fibrinogen and prothrombin are synthesized in the liver (Osserman and Takatsuki, 1963). According to previous studies, hypoalbuminemia is associated with advanced chronic liver diseases (Koneri et al., 2008). Liver damage could occur via peroxidation of polyunsaturated fatty acids which are detrimental to cellular homeostasis, through the formation of aldehydes. Aldehydes impair nucleotide and protein synthesis, increase production of the pro-inflammatory cytokines TNF-α, and promote an influx of inflammatory cells into the liver, leading to collagen deposition, hepatic injury and inflammatory response (Esterbauer et al., 1991; Yamauchi et al., 2003).

Importantly, we observed a dose-dependent decline in fertility (number of pups conceived) in male rats neonatally exposed to furan. This finding can be adequately explained by the lower testosterone levels that arguably resulted in lower sperm numbers and quality, which were produced by testes showing clear histological abnormalities. We find no evidence that furan exposure caused a delay in the onset of puberty (as determined from preputial separation), which has been observed for ethynylestradiol, among other EDCs (Yoshimura et al., 2005).These results are in accordance with previous studies in which genistein exposure showed no effects on the onset of puberty, but decline in the number of pups produced (Nagao et al., 2001).

5. Conclusion

We find clear evidence that exposure to the two highest doses of furan (10 and 20 mg kg^{-1} d^{-1}) broadly and consistently affects the development of the male reproductive system, which ultimately lead to a reduction in fertility. Our results are in line with previous studies on furan exposure at the weaning stage, and highlight that developmental exposure to furan can cause organizational effects on the development of male reproductive systems. Considering that furan is detected in infant food and baby milk formulas at concentrations in the ng L^{-1} range, this study provides important preclinical data on the minimal furan dose at which endocrine disruption can occur. Our results also warrant investigation of the reproductive system of female rats, as well as a screening for other endocrine effects unrelated to reproductive systems.

Table 8
Effect of neonatal subcutaneous exposure to furan (0, 1, 5, 10, 20 mgkg^{-1}d^{-1}) on caput epididymis tissue histopathology in adult male rats.

Parameters	Control	Furan (mg kg^{-1}d^{-1})				Statistics
		1	5	10	20	
Tubule diameter (μm)	354.4 ± 8.33	350.08 ± 7.26	347.28 ± 8.12	339.76 ± 9.83	343.9 ± 10.04	P = 0.80 F = 0.40
Lumen diameter (μm)	255.28 ± 7.83	252.14 ± 7.27	253.36 ± 8.05	246.6 ± 9.31	237.84 ± 6.32	P = 0.51 F = 0.81
Epithelial heights (μm)	31.40 ± 0.96	29.00 ± 0.90	29.47 ± 1.02	28.20 ± 0.76	29.16 ± 0.82	P = 0.14 F = 1.73
Epithelium % age	39.25 ± 1.20	36.25 ± 1.13	36.84 ± 1.28	35.25 ± 0.95	36.46 ± 1.03	P = 0.143 F = 1.73
Lumen% age	60.74 ± 1.20^{b}	63.74 ± 1.13^{ab}	63.15 ± 1.28^{a}	64.74 ± 0.95^{ab}	63.53 ± 1.03^{ab}	P = 0.03 F = 2.62

Values are presented as Mean ± SE of mean (n = 7).P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different (P < 0.05) in the rows compared to control with other treated groups.

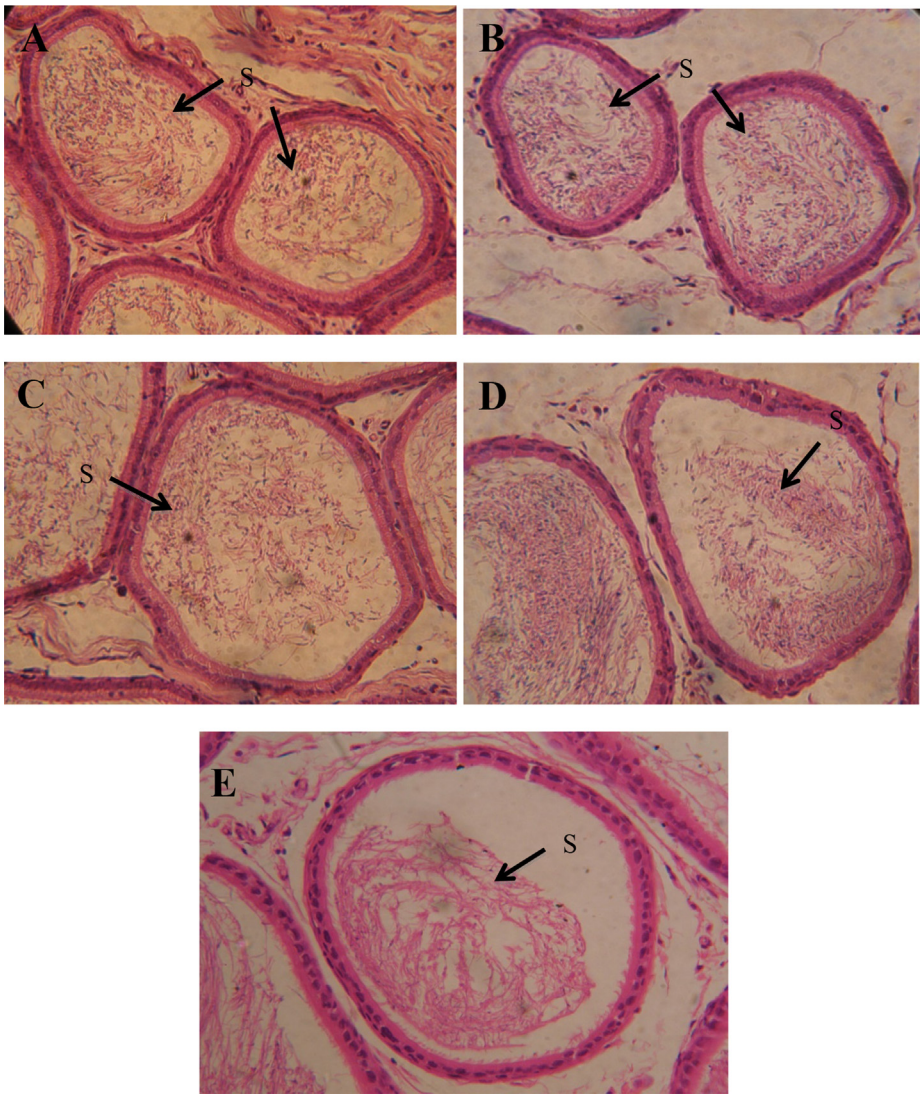

Fig. 4. Photomicrograph (40X magnification) of caput epididymis region (hematoxylin-eosin stain) of adult male rats exposed to different doses of furan at neonatal stage. (H&E, 40X) from: (A) Control group; with normal morphology of caput epididymal cells having thin pseudostratified epithelium lumen heavily filled with spermatozoa, (B) 1 $mgkg^{-1}d^{-1}$group; with thin epithelium-lined with stereocilia, (C) 5 $mgkg^{-1}d^{-1}$group; showing normal spermatozoa in lumen (D) 10 $mgkg^{-1}d^{-1}$group; showing increase in pseudostratified epithelium and decrease in concentration of spermatozoa, (E) 20 $mgkg^{-1}d^{-1}$group; Slight reduction in spermatozoa. Letters indicate spermatozoa (S), and epithelium (E).

Declaration of interest

All the authors who have a major contribution in this research articles have no declarations of interest.

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Table 9
Effects of neonatal subcutaneously exposure to furan (0, 1, 5, 10, 20 $mgkg^{-1}d^{-1}$) on caudal epididymis tissue histopathology in adult male rats.

Parameters	Control	Furan (mg $kg^{-1}d^{-1}$)				Statistics
		1	5	10	20	
Tubule diameter (μm)	454.48 ± 7.13	453.22 ± 8.91	449.24 ± 9.30	449.42 ± 10.09	445.7 ± 10.54	P = 0.9 F = 0.14
Lumen diameter (μm)	415.2 ± 10.99	417.7 ± 8.24	413.3 ± 7.83	409.14 ± 9.57	404.98 ± 11.96	P = 0.90 F = 0.26
Epithelial heights (μm)	27 ± 1.29^{a}	25.03 ± 1.27^{ab}	25.96 ± 1.40^{ab}	23.14 ± 1.69^{ab}	22.03 ± 1.29^{b}	P = 0.05 F = 2.31
Epithelium % age	34.14 ± 1.61^{a}	31.29 ± 1.58^{ab}	32.45 ± 1.75^{ab}	28.93 ± 2.12^{ab}	27.54 ± 1.61^{b}	P = 0.05 F = 2.35
Lumen% age	65.85 ± 1.61^{b}	68.70 ± 1.58^{ab}	67.54 ± 1.75^{ab}	71.06 ± 2.12^{ab}	72.45 ± 1.61^{a}	P = 0.05 F = 2.30

Values are presented as Mean ± SE of mean (n = 7).P and F value in the rows from ANOVA with complete randomized designs followed by Tukey's post hoc shows a pairwise comparison of control with furan treated groups. Mean with different superscript are significantly different ($P < 0.05$) in the rows compared to control with other treated groups.

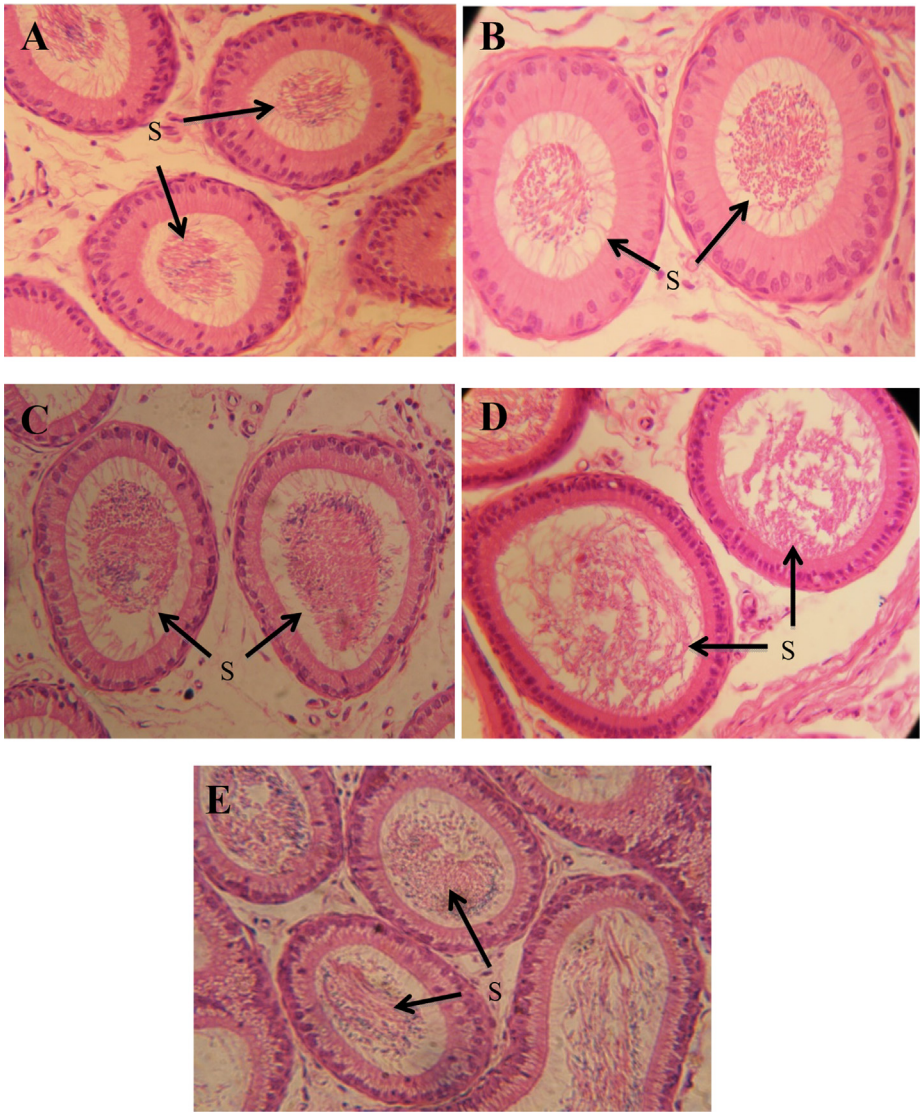

Fig. 5. Photomicrograph (40 × magnification) of cauda epididymis region (hematoxylin-eosin stain) of adult male rats exposed to different doses of furan at neonatal stage. (A) Control; exhibiting normal morphology of cauda epididymis; lumen filled with mature sperms, (B) 1 $mgkg^{-1}d^{-1}$ group; showing normal morphology like control. (C) 5 $mgkg^{-1}d^{-1}$group; showing slight changes in morphology of tubules, surrounded by stroma. (D) 10 $mgkg^{-1}d^{-1}$group; lumen has little number of spermatozoa. (E) 20 $mgkg^{-1}d^{-1}$group; showing a further increase in epithelial height and little lumen sperm concentration.Letters indicate spermatozoa (S), and epithelium (E).

Sweden for language and grammar check of the research article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.020.

References

Abraham, S., Rubino, D., Sinaii, N., Ramsey, S., Nieman, L., 2013. Cortisol, obesity, and the metabolic syndrome: a cross-sectional study of obese subjects and review of the literature. Obesity 21, E105–E117.
Alam, R.T., Zeid, E.H.A., Imam, T.S., 2017. Protective role of quercetin against hematotoxic and immunotoxic effects of furan in rats. Environ. Sci. Pollut. Control Ser. 24, 3780–3789.
Amin, A., Abraham, C., Hamza, A.A., Abdalla, Z.A., Al-Shamsi, S.B., Harethi, S.S., Daoud, S., 2012. A standardized extract of Ginkgo biloba neutralizes cisplatin-mediated reproductive toxicity in rats. BioMed Res. Int. 1–11 2012.
Arnhold, E., 2013. Package in the R environment for analysis of variance and complementary analyses. Braz. J. Vet. Res. Anim. Sci. 50, 488–492.
Arnold, A.P., Breedlove, S.M., 1985. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. Horm. Behav. 19, 469–498.
Bambino, T.H., Hsueh, A.J., 1981. Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis in vivo and in vitro. Endocrinology 108, 2142–2148.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1, 1–23.
Berga, S.L., 2008. Stress and reproduction: a tale of false dichotomy? Endocrinology 149, 867–868.
Birnbaum, L.S., Tuomisto, J., 2000. Non-carcinogenic effects of TCDD in animals. Food Addit. Contam. 17, 275–288.
Björntorp, P., 1995. Endocrine abnormalities of obesity. Metabolism-Clinical and Experimental 44, 21–23.
Blagburn, B.L., Drain, K.L., Land, T.M., Moore, P.H., Kinard, R.G., Lindsay, D.S., Kumar, A., Shi, J., Boykin, D.W., Tidwell, R.R., 1998. Dicationic furans inhibit development of Cryptosporidium parvum in HSD/ICR suckling Swiss mice. J. Parasitol. 851–856.
Blanck, H.M., Marcus, M., Tolbert, P.E., Rubin, C., Henderson, A.K., Hertzberg, V.S., Zhang, R.H., Cameron, L., 2000. Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. Epidemiology 641–647.
Borges, M.H.S., DiNinno, F.B., Lengyel, A.M.J., 1997. Different effects of growth hormone releasing peptide (GHRP-6) and GH-releasing hormone on GH release in endogenous and exogenous hypercortisolism. Clinical endocrinology 46, 713–718.
Burguera, B., Muruais, C., Peñalva, A., Dieguez, C., Casanueva, F.F., 1990. Dual and selective actions of glucocorticoids upon basal and stimulated growth hormone release in man. Neuroendocrinology 51, 51–58.
Castranova, D.A., King, W., Woods III, L.C., 2005. The effects of stress on androgen production, spermiation response and sperm quality in high and low cortisol responsive domesticated male striped bass. Aquaculture 246, 413–422.
Chapin, R.E., Adams, J., Boekelheide, K., Gray Jr., L.E., Hayward, S.W., Lees, P.S., McIntyre, B.S., Portier, K.M., Schnorr, T.M., Selevan, S.G., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res. Part B Dev. Reproductive Toxicol. 83, 157–395.
Chen, S.-y., Wang, J., Yu, G.-q., Liu, W., Pearce, D., 1997. Androgen and glucocorticoid receptor Heterodimer formation a possible mechanism for MUTUAL inhibition OF TRANSCRIPTIONAL activity. J. Biol. Chem. 272, 14087–14092.
Cooke, G.M., Taylor, M., Bourque, C., Curran, I., Gurofsky, S., Gill, S., 2014. Effects of furan on male rat reproduction parameters in a 90-day gavage study. Reprod. Toxicol. 46, 85–90.
Cox, J.H., Cortright, R.N., Dohm, G.L., Houmard, J.A., 1999. Effect of aging on response

to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity. J. Appl. Physiol. 86, 2019–2025.
Cumming, D., Quigley, M., Yen, S., 1983. Acute suppression of circulating testosterone levels by cortisol in men. J. Clin. Endocrinol. Metab. 57, 671–673.
del Rio Gomez, I., Marshall, T., Tsai, P., Shao, Y.-S., Guo, Y.L., 2002. Number of boys born to men exposed to polychlorinated byphenyls. The Lancet 360, 143–144.
El-Akabawy, G., El-Sherif, N.M., 2016. Protective role of garlic oil against oxidative damage induced by furan exposure from weaning through adulthood in adult rat testis. Acta Histochem. 118, 456–463.
Elaine, 2009. Problems with Lipid Metabolism Pharmacology a Handbook for Complementary Healthcare Professionals.
Esterbauer, H., Schaur, R.J., Zollner, H., 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biol. Med. 11, 81–128.
Evans, W.J., 2000. Vitamin E, vitamin C, and exercise–. Am. J. Clin. Nutr. 72, 647S–652S.
FDA, U, 2004. Furan in Food,Thermal.
FTC, 2004. Argets Products Claiming to affect the stress hormone cortisol. Available from: www.ftc.gov/opa/2004/10/windowrock.htm.
Fui, M.N.T., Dupuis, P., Grossmann, M., 2014. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J. Androl. 16, 223.
Gulve, E.A., Spina, R.J., 1995. Effect of 7-10 days of cycle ergometer exercise on skeletal muscle GLUT-4 protein content. J. Appl. Physiol. 79, 1562–1566.
Halvaei, I., Roodsari, H.R.S., Harat, Z.N., 2012a. Acute Effects of Ruta graveolens L. on sperm parameters and DNA integrity in rats. J. Reproduction Infertil. 13, 33.
Halvaei, I., Sadeghipour Roodsari, H.R., Naghibi Harat, Z., 2012b. Acute effects of Ruta graveolens L. on sperm parameters and DNA integrity in rats. J. Reproduction Infertil. 13, 33–38.
Hinson, J., Raven, P., 2006. Effects of endocrine-disrupting chemicals on adrenal function. Best Pract. Res. Clin. Endocrinol. Metabol. 20, 111–120.
Jensen, E.C., 2013. Quantitative analysis of histological staining and fluorescence using ImageJ. Anat. Rec. 296, 378–381.
Karacaoğlu, E., Selmanoğlu, G., 2010. Effects of heat-induced food contaminant furan on reproductive system of male rats from weaning through postpuberty. Food Chem. Toxicol. 48, 1293–1301.
Karakilcik, A.Z., Zerin, M., Arslan, O., Nazligul, Y., Vural, H., 2004. Effects of vitamin C and E on liver enzymes and biochemical parameters of rabbits exposed to aflatoxin B1. Vet. Hum. Toxicol. 46, 190–192.
Kim, H.S., Kim, T.S., Shin, J.-H., Moon, H.J., Kang, I.H., Kim, I.Y., Oh, J.Y., Han, S.Y., 2004. Neonatal exposure to di (n-butyl) phthalate (DBP) alters male reproductive-tract development. J. Toxicol. Environ. Health, Part A 67, 2045–2060.
Koneri, R., Balaraman, R., Firdous, K.M., Kumar, M., 2008. Hepatoprotective effects of Momordica Cymbalaria Fenzl. against carbon tetrachloride induced hepatic injury in rats. Pharmacologyonline 1, 365–374.
Lafuente, A., Cano, P., Esquifino, A.I., 2003. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals 16, 243–250.
Lehmann, G.M., LaKind, J.S., Davis, M.H., Hines, E.P., Marchitti, S.A., Alcala, C., Lorber, M., 2018. Environmental chemicals in breast milk and formula: exposure and risk assessment implications. Environ. Health Perspect. 126, 096001.
Liening, S.H., Josephs, R.A., 2010. It is not just about testosterone: physiological mediators and moderators of testosterone's behavioral effects. Social and Personality Psychology Compass 4, 982–994.
Linda, 2012. Cardiopulmonary Disorders-9 Elsevier's Integrated Review Genetics, second ed. pp. 159–176.
Liu, C., Zhang, X., Deng, J., Hecker, M., Al-Khedhairy, A., Giesy, J.P., Zhou, B., 2010. Effects of prochloraz or propylthiouracil on the cross-talk between the HPG, HPA, and HPT axes in zebrafish. Environ. Sci. Technol. 45, 769–775.
Mårin, P., Darin, N., Amemiya, T., Andersson, B., Jern, S., Björntorp, P., 1992. Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metabolism 41, 882–886.
Mocarelli, P., Gerthoux, P.M., Patterson Jr., D.G., Milani, S., Limonta, G., Bertona, M., Signorini, S., Tramacere, P., Colombo, L., Crespi, C., 2007. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ. Health Perspect. 116, 70–77.
Mojska, H., Gielecińska, I., Stoś, K., 2012. Determination of acrylamide level in commercial baby foods and an assessment of infant dietary exposure. Food Chem. Toxicol. 50, 2722–2728.
Murguía-Romero, M., Jiménez-Flores, J.R., Sigrist-Flores, S.C., Espinoza-Camacho, M.A., Jiménez-Morales, M., Piña, E., Méndez-Cruz, A.R., Villalobos-Molina, R., Reaven, G.M., 2013. Plasma triglyceride/high-density lipoprotein cholesterol ratio, insulin resistance, and cardio-metabolic risk in young men and women. J. Lipid Res., M040584 jlr.
Nagao, T., Yoshimura, S., Saito, Y., Nakagomi, M., Usumi, K., Ono, H., 2001. Reproductive effects in male and female rats of neonatal exposure to genistein. Reprod. Toxicol. 15, 399–411.
Osserman, E.F., Takatsuki, K., 1963. The plasma proteins in liver disease. Med. Clin. N. Am. 47, 679–710.
Peterson, R.E., Theobald, H.M., Kimmel, G.L., 1993. Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. Crit. Rev. Toxicol. 23, 283–335.
Pluim, H.J., De Vijlder, J., Olie, K., Kok, J.H., Vulsma, T., van Tijn, D.A., van der Slikke, J.W., Koppe, J.G., 1993. Effects of pre-and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environ. Health Perspect. 101, 504.
Pressman, A., Hernandez, A., Sikka, S.C., 2018. Lifestyle Stress and its Impact on Male Reproductive Health, Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. Elsevier, pp. 73–83.
R Development Core Team, 2016. R: a Language and Environment for StatisticalComputing. R Foundation for Statistical Computing, Vienna.
Rasmussen, M.H., 2010. Obesity, growth hormone and weight loss. Mol. Cell. Endocrinol. 316, 147–153.
Rawi, S.M., Marie, M.-A.S., Fahmy, S.R., El-Abied, S.A., 2012. Hazardous effects of acrylamide on immature male and female rats. African J. Pharm. Pharmacol. 6, 1367–1386.
Richter, C.A., Birnbaum, L.S., Farabollini, F., Newbold, R.R., Rubin, B.S., Talsness, C.E., Vandenbergh, J.G., Walser-Kuntz, D.R., vom Saal, F.S., 2007. In vivo effects of bisphenol A in laboratory rodent studies. Reprod. Toxicol. 24, 199–224.
Robb, G., Amann, R., Killian, G., 1978. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. Reproduction 54, 103–107.
Robin, L.P., Clanci, S., 2007. Acrylamide, furan, and the FDA. Food Safety Magazine 13, 17–21.
Robinson, D., 1973. Plasma triglyceride metabolism. J. Clin. Pathol. Suppl. 5, 5.
Robinson, D., Seakins, A., 1963. Reduced plasma lipoprotein production as a factor in the development of fatty livers. Biochemical Problems of Lipids 86 (3), 401–440.
Sajjad, S., Malik, H., Saeed, L., Chaudhary, A., 2018. Changes in growth hormone and cortisol profile due to lead induced toxicity in Labeo rohita. Turk. J. Fish. Aquat. Sci. 18, 921–926.
Salian, S., Doshi, T., Vanage, G., 2009. Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. Toxicology 265, 56–67.
Schaefer, E., Anderson, D., Brewer Jr., H., Levy, R., Danner, R., Blackwelder, W., 1978. Plasma-triglycerides in regulation of HDL-cholesterol levels. The Lancet 312, 391–393.
Selmanoğlu, G., Karacaoğlu, E., Kılıç, A., Koçkaya, E.A., Akay, M.T., 2012. Toxicity of food contaminant furan on liver and kidney of growing male rats. Environ. Toxicol. 27, 613–622.
Sharpe, R.M., McKinnell, C., Kivlin, C., Fisher, J.S., 2003. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction 125, 769–784.
Tan, D.X., Manchester, L.C., Terron, M.P., Flores, L.J., Reiter, R.J., 2007. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J. Pineal Res. 42, 28–42.
Taylor, J.A., Welshons, W.V., vom Saal, F.S., 2008. No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24 h after administration in neonatal female mice. Reprod. Toxicol. 25, 169–176.
Thakore, J.H., Dinan, T.G., 1994. Growth hormone secretion: the role of glucocorticoids. Life Sci. 55, 1083–1099.
Tort, L., Kargacin, B., Torres, P., Giralt, M., Hidalgo, J., 1996. The effect of cadmium exposure and stress on plasma cortisol, metallothionein levels and oxidative status in rainbow trout (Oncorhynchus mykiss) liver. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 114, 29–34.
Tsigos, C., Chrousos, G.P., 2002. Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. J. Psychosom. Res. 53, 865–871.
van den Berg, M., Kypke, K., Kotz, A., Tritscher, A., Lee, S.Y., Magulova, K., Fiedler, H., Malisch, R., 2017. WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit–risk evaluation of breastfeeding. Arch. Toxicol. 91, 83–96.
Van Wijnen, J., Van Bavel, B., Lindström, G., Koppe, J., Olie, K., 1990. Placental transport of PCDDs and PCDFs in humans. Organohalogen Compd. 1, 47–50.
Viau, V., 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and-adrenal axes. J. Neuroendocrinol. 14, 506–513.
Wennink, J.M., Delemarre-van de Waal, H.A., Schoemaker, R., Blaauw, G., van den Braken, C., Schoemaker, J., 1990. Growth hormone secretion patterns in relation to LH and testosterone secretion throughout normal male puberty. Eur. J. Endocrinol. 123, 263–270.
Yamaguchi, K., Yang, L., McCall, S., Huang, J., Yu, X.X., Pandey, S.K., Bhanot, S., Monia, B.P., Li, Y.X., Diehl, A.M., 2007. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 45, 1366–1374.
Yamauchi, T., Kamon, J., Waki, H., Imai, Y., Shimozawa, N., Hioki, K., Uchida, S., Ito, Y., Takakuwa, K., Matsui, J., 2003. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J. Biol. Chem. 278, 2461–2468.
Yoshimura, S., Yamaguchi, H., Konno, K., Ohsawa, N., Noguchi, S., Chisaka, A., 2005. Observation of preputial separation is a useful tool for evaluating endocrine active chemicals. J. Toxicol. Pathol. 18, 141–157.
Young, W.C., 1964. The Hormones and Behavior, Comparative Biochemistry V7. Elsevier, pp. 203–251.
Zimmer, K.E., Gutleb, A.C., Lyche, J.L., Dahl, E., Oskam, I.C., Krogenæs, A., Skaare, J.U., Ropstad, E., 2009. Altered stress-induced cortisol levels in goats exposed to polychlorinated biphenyls (PCB 126 and PCB 153) during fetal and postnatal development. J. Toxicol. Environ. Health, Part A 72, 164–172.