Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net

Original research http://dx.doi.org/10.1016/j.apjr.2016.10.009

# Hormonal changes and spermatogenesis of male rat puppies born by mothers consuming soybean extract

Nurdiana Nurdiana[1*], Elly Mayangsari[1], Bayu Lestari[1], Bambang Setiawan[2]

[1]Department of Pharmacology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia

[2]Research Center for Toxicology, Cancer and Regenerative Medicine, Department of Medical Chemistry and Biochemistry, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia

ARTICLE INFO

*Article history:*
Received 15 Aug 2016
Received in revised form 26 Oct 2016
Accepted 27 Oct 2016
Available online 3 Nov 2016

*Keywords:*
Soybean extract
Reproductive hormones
Male rats
Testis
Spermatogenesis

ABSTRACT

**Objective:** To analyze the effect of administering soybean extracts during pregnancy and breastfeeding on hormonal disturbances and impaired spermatogenesis in male rats born to mothers receiving soybean extract.

**Methods:** Twenty-eight male Wistar rat puppies were divided into four groups: the control group (no treatment) and male rat puppies whose mothers have been given soy extracts of various doses (68.88 μg/ml, 137.76 μg/ml and 275.52 μg/ml). Female rats were given soybean extracts during pregnancy and lactation until the male puppies were one month. Analysis of the levels of LH, FSH and testosterone was performed by ELISA technique. Testicular spermatogenesis was analyzed by histopathology.

**Results:** FSH levels were significantly lower for all three doses of soy extract than those of the control group ($P < 0.05$). FSH levels increased significantly in the group treated with the highest dose of soybean extract relative to those of the lower doses ($P < 0.05$). LH levels were significantly lower for all three doses of soy extract than those of the control group ($P < 0.05$). Testosterone levels were significantly lower for the highest dose of soybean extract relative to those of the control group ($P < 0.05$). Histology of the seminiferous tubules revealed that increasing the soy bean extract dose correlated with increasing constraints to spermatogenesis.

**Conclusion:** Administration of soybean extract from the intrauterine period, during lactation and at the age of two months, to male rats leads to hormonal changes and impaired spermatogenesis.

## 1. Introduction

Endocrine disruptor (ED) is an exogenous substance or mixture capable of altering the function of the endocrine system. EDs have adverse effects on intact organisms and their progeny and sub-populations [1]. Chemical compounds classified as EDs are diverse, such as industrial chemicals, pharmaceutical compounds, cosmetic compounds and heavy metals [2–4]. These compounds can be found in the ecosystem, as contaminants in the food chain, and in the work environment [5].

Phytoestrogens which are abundantly found in soybeans and soybean products, have properties similar to estrogen or act as beneficial anti-estrogens. However, the benefits of phytoestrogens are indirect and inconsistent. Exposure to estrogen compounds, especially in certain periods of life, leads to malignancies and some anomalies in the reproductive system [6]. Structurally, phytoestrogens are similar to endogenous estrogen and have an affinity for estrogen receptors. There are various types of phytoestrogens, including isoflavones, prenylated flavonoids and coumestants. Genistein and daidzein are the most common of these compounds [7]. Previous studies demonstrated that genistein given in the maternal diet during lactation would significantly lead to growth inhibition of male progeny [8].

Impaired spermatogenesis and male infertility occur as a result of the action of ED compounds. ED compounds will disrupt the biosynthesis, the metabolism and the action of hormones [9,10]. Isoflavones may downregulate mRNA expression of follicle-stimulating hormone receptors, inhibin α, INHβB, androgen-binding protein and transferrin in Sertoli cells [11].

*Corresponding author: Nurdiana Nurdiana, Department of Pharmacology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia.
Tel: +62 8123388428
E-mail: farmakodes@gmail.com
Peer review under responsibility of Hainan Medical College.

To date, there has been controversy surrounding the effects of soybean exposure on hormonal disturbances and impaired spermatogenesis in males born to mothers that are exposed ED compounds.

Hence, the purpose of the present study was to analyze hormonal disturbances and impaired spermatogenesis in male rats born to mothers receiving soybean extract during pregnancy and lactation.

## 2. Materials and methods

### 2.1. Laboratory animals

This study used male rats born to female rats that were given soybean extracts during pregnancy and lactation until the age of one month. One-month-old rats were separated from their mothers and given soybean extract using a feeding tube until 2 months of age. The control group did not receive soybean extract during this period. Twenty-eight male Wistar rat puppies were randomly divided into four groups: the control group (no treatment) and the groups treated with various doses of soybean extract (68.88 µg/ml, 137.76 µg/ml and 275.52 µg/ml).

### 2.2. Soybean extraction

An Argomulyo soybean plant was used in the present study from the Indonesian Legumes and Tuber Crops Research Institute Malang of East Java, Indonesia. Dried soybeans were crushed to obtain soybean powder. Soybean powder was macerated in 95% methanol. The procedures for maceration were in accordance with previous studies. Soybean extract was orally administered to pregnant female rats, which was continued until lactation and the puppies were one month of age.

### 2.3. Analysis of hormone levels

Analysis of the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone was performed by enzyme-linked immunosorbent assay (ELISA). Analytical procedures were performed according to the manufacturer's instructions.

### 2.4. Histopathology

Histopathology was performed on isolated testicles from male offspring. The analytical procedures were performed in accordance with previous studies. The quality of spermatogenesis was analyzed based on Jonsen's criteria [12,13].

### 2.5. Ethics

The present study passed the review of the ethics committee of the Medicine Faculty of Brawijaya University Malang, East Java, Indonesia.

### 2.6. Statistical analysis

All data are presented as mean ± SD. Differences in levels among treatment groups were analyzed by analysis of variance (ANOVA) using the SPSS 16.0 statistical package. A $P$-value <0.05 was considered statistically significant.

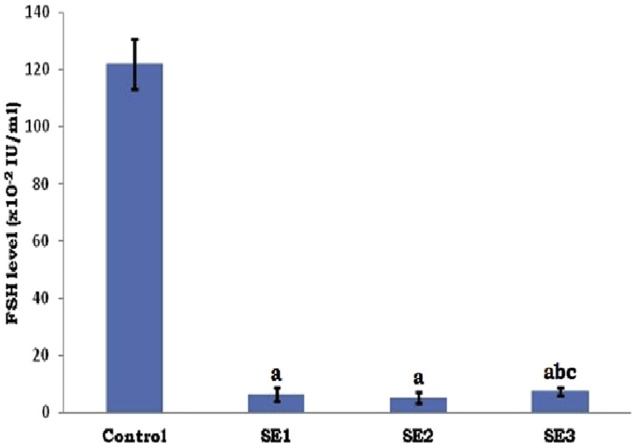


**Figure 1.** The levels of FSH in the control and treatment groups. SE1 = 69 µg/ml of soybean extract; SE2 = 139 µg/ml of soybean extract and SE3 = 276 µg/ml soybean extract. Data are shown as mean ± standard deviation; [a]: $P < 0.05$ compared with the control group; [b]: $P < 0.05$ compared with SE1; [c]: $P < 0.05$ compared with SE2. FSH: follicle-stimulating hormone; SE: soy extract.

## 3. Results

Figure 1 shows the FSH levels in the different treatment groups. FSH levels were significantly lower for all three doses of soybean extract as compared to the control group ($P < 0.05$). FSH levels increased significantly for the group treated with the highest dose of soybean extract relative to the lower doses ($P < 0.05$). There were no significant differences in FSH levels between the doses ($P > 0.05$).

LH levels for the different treatment groups are presented in Figure 2. LH levels were significantly lower for all three doses of soybean extract as compared to the control group ($P < 0.05$). There were no significant differences in LH levels among all the soybean extract treatments ($P > 0.05$).

Figure 3 shows testosterone levels for the different treatment groups. Testosterone levels were significantly lower for the highest dose of soybean extract compared to the control group ($P < 0.05$). There were no significant differences in testosterone levels between the control group and the lower doses ($P > 0.05$).

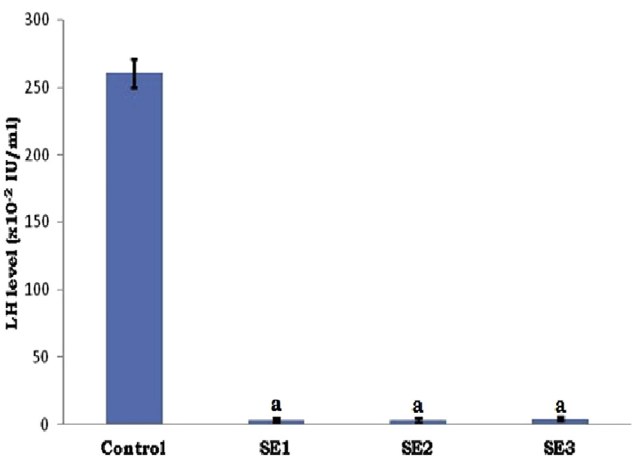


**Figure 2.** The levels of LH in the control and treatment groups. SE1 = 69 µg/ml of soybean extract; SE2 = 139 µg/ml of soybean extract and SE3 = 276 µg/ml soybean extract. Data are shown as mean ± standard deviation; [a]: $P < 0.05$ compared with the control group; LH: luteinizing hormone; SE: soy extract.

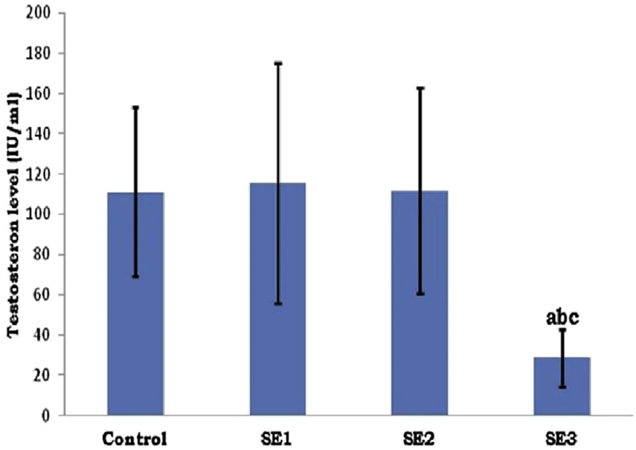


**Figure 3.** Testosterone levels in the control and treatment groups. SE1 = 69 μg/ml of soybean extract; SE2 = 139 μg/ml of soybean extract and SE3 = 276 μg/ml soybean extract. Data are shown as mean ± standard deviation; [a]: $P < 0.05$ compared with the control group; [b]: $P < 0.05$ compared with SE1; [c]: $P < 0.05$ compared with SE2. SE: soy extract.

increase in FSH levels relative to the lower doses ($P < 0.05$). This suggests that administration of soybean extract can alter the binding, release and metabolism of FSH. Previous studies that shown that ED compounds impair endocrine function through altering the bonding, release and metabolism of endogenous hormones [14,15]. Fluctuating levels of FSH indicate an altered feedback mechanism between the testis and hypothalamus. Decreased levels of FSH suggest hypothalamic impairment while increased levels of FSH suggest testicular impairment [16]. This is supported by our histological findings. Meanwhile, the levels of LH were found to be significantly lower for the three groups with soybean extract treatment relative to the untreated control ($P < 0.05$). With the highest dose, there was a trend of increasing LH levels relative to the lower doses, although no significant difference was found.

Interestingly, testosterone level showed an increase after treatment with 68.8 μg/ml and 137.76 μg/ml soybean extract, although these treatments were not statistically different from the control group. Testosterone levels were drastically reduced after treatment with 275.52 μg/ml soybean extract. This indicates that, with the highest dose, there was impaired testicular function. Previous studies have demonstrated that genistein has a significant role in steroidogenesis in the adrenal glands and testes of rats and lowers testosterone levels. Meanwhile, the levels of FSH remain unchanged and the levels of LH tend to increase [17].

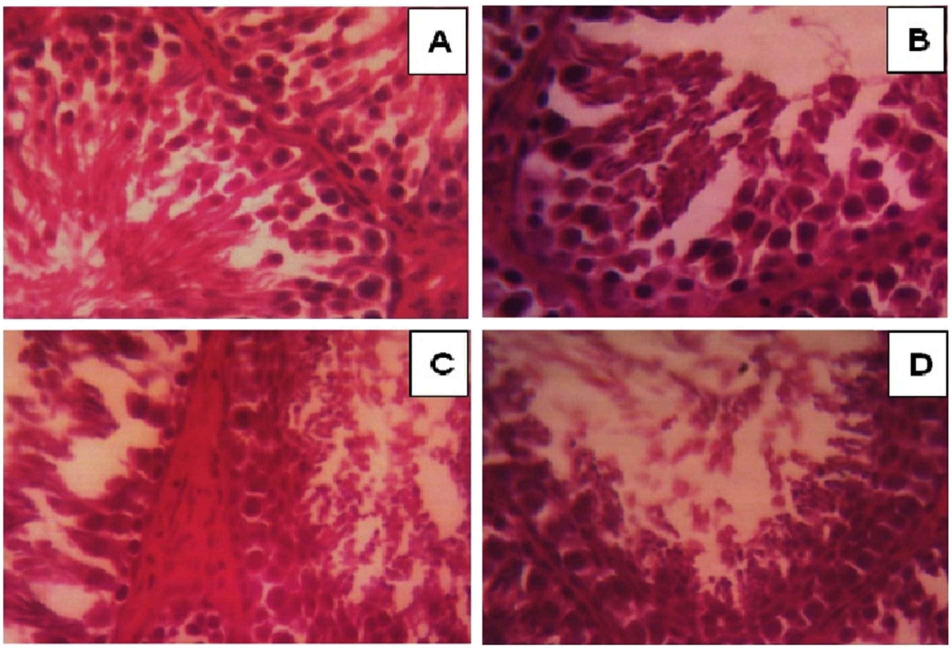


**Figure 4.** Histology of the seminiferous tubules of male rats in the control group (A) and groups treated with the first dose of soy extract (B), the second dose (C), and the third dose (D) (Magnification ×400).

In the control group, there were extremely light spermatogenesis disorder, many advanced spermatids, and the disorganization of the epithelium. On the first-dose group, there were less than five spermatozoa in each tubule and a few advanced spermatids. In the second and third doses groups, there were advanced spermatozoa and spermatid, but only early spermatids were found. At the third dose group, there were only spermatocytes. Hematoxylin eosin staining, magnification of 400 times (Figure 4).

## 4. Discussion

In the present study, FSH levels decreased significantly for all three doses of soybean extract relative to the control group ($P < 0.05$). With the highest dose, there was a significant

With regard to the histology of the seminiferous tubules, it was found that the higher the dose of extract then more severe the constraints to spermatogenesis. This finding is consistent with previous studies that have shown that genistein treatment delays spermatogenesis in male rats and humans [18,19].

In conclusion, administration of soybean extract from the intrauterine period, during lactation and at the age of two months to male rats will lead to hormonal changes and impaired spermatogenesis.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

## Acknowledgements

We thank the laboratory assistants at the Laboratory of Pharmacology of Medicine Faculty of Brawijaya University Malang of East Java, Indonesia. This study was funded by a grant from the Educational Development Fund of Medicine Faculty of Brawijaya University Malang of East Java, Indonesia.

## References

[1] Slama R, Bourguignon JP, Demeneix B, Ivell R, Panzica G, Kortenkamp A, et al. Scientific issues relevant to setting regulatory criteria to identify endocrine disrupting substances in the european union. *Environ Health Perspect* 2016; **124**(10): 1497-1503.

[2] Iavicoli I, Fontana L, Bergamaschi A. The effects of metals as endocrine disruptors. *J Toxicol Environ Health B* 2009; **12**: 206-223.

[3] De Coster S, van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. *J Environ Public Health* 2012; **2012**:713696.

[4] Casals-Casas C, Desvergne B. Endocrine disruptors: from endocrine to metabolic disruption. *Annu Rev Physiol* 2011; **73**: 135-162.

[5] Baccarelli A, Pesatori AC, Bertazzi PA. Occupational and environmental agents as endocrine disruptors: experimental and human evidence. *J Endocrinol Invest* 2000; **23**: 771-781.

[6] Bar-El DS, Reifen R. Soy as an endocrine disruptor: cause for caution? *J Pediatr Endocrinol Metab* 2010; **23**(9): 855-861.

[7] Khani B, Mehrabian F, Khalesi E, Eshraghi A. Effect of soy phytoestrogen on metabolic and hormonal disturbance of women with polycystic ovary syndrome. *J Res Med Sci* 2011; **16**(3): 297-302.

[8] Ball ER, Caniglia MK, Wilcox JL, Overton KA, Burr MJ, Wolfe BD, et al. Effects of genistein in the maternal diet on reproductive development and spatial learning in male rats. *Horm Behav* 2010; **57**(3): 313-322.

[9] Andersson AM, Jørgensen N, Main KM, Toppari J, Rajpert-De Meyts E, Leffers H, et al. Adverse trends in male reproductive health: we may have reached a crucial 'tipping point'. *Intern J Androl* 2008; **31**: 74-80.

[10] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev* 2009; **30**(4): 293-342.

[11] Dejiao Y, Yanfeng Z, Linxi L, Hua X, Jie H, Yun L. Potential detrimental effect of soy isoflavones on testis sertoli cells. *J Cent South Univ (Med Sci)* 2014; **39**(6): 598-604.

[12] Johnsen SG. Testicular biopsy score count- a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones* 1970; **1**: 2-25.

[13] Ishihara M, Itoh M, Miyamoto K, Suna S, Takeuchi Y, Takekana I, et al. Spermatogenic disturbance induced by di(29ethylexyl) phthalate is significantly prevented by treatment with antioxidant vitamin in the rat. *Intern J Androl* 2000; **23**: 85-94.

[14] Baskin LS, Himes K, Colborn T. Hypospadias and endocrine disruption: is there a connection? *Environ Health Perspect* 2001; **109**: 1175-1183.

[15] Colborn T, Saal FSv, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 1993; **101**: 378-384.

[16] Bateman HL, Patisaul HB. Disrupted female reproductive physiology following neonatal exposure to phytoestrogens or estrogen specific ligands is associated with decreased GnRH activation and kisspeptin fiber density in the hypothalamus. *Neurotoxicology* 2008; **29**: 988-997.

[17] Ohno S, Nakajima Y, Inoue K, Nakazawa H, Nakajin S. Genistein administration decreases serum corticosterone and testosterone levels in rats. *Life Sci* 2003; **74**(6): 733-742.

[18] Delclos KB, Bucci TJ, Lomax LG, Latendresse JR, Warbritton A, Weis CC, et al. Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. *Reprod Toxicol* 2001; **15**: 647-663.

[19] Goodin S, Shen F, Shih WJ. Clinical and biological activity of soy protein powder supplementation in healthy male volunteers. *J Cancer Epidemiol Biomarkers Prev* 2007; **16**: 829-833.