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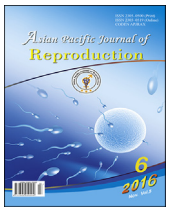

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Hormonal changes and spermatogenesis of male rat puppies born by mothers consuming soybean extract

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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].

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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.

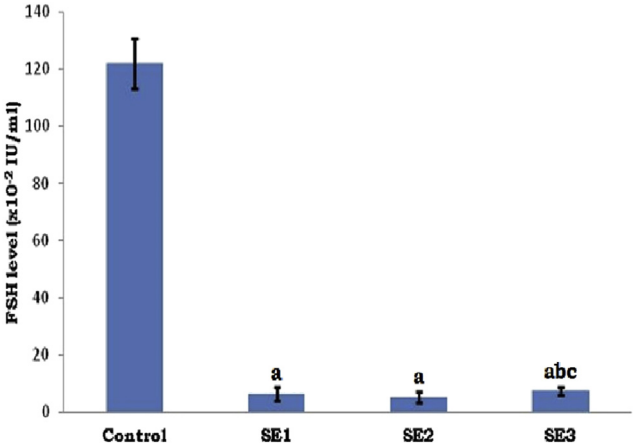

Figure 1. The levels of FSH in the control and treatment groups. SE1 = 69 μg/ml of soybean extract; SE2 = 139 μg/ml of soybean extract and SE3 = 276 μg/ml soybean extract. Data are shown as mean ± standard deviation; [a]: $P < 0.05$ compared with the control group; [b]: $P < 0.05$ compared with SE1;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

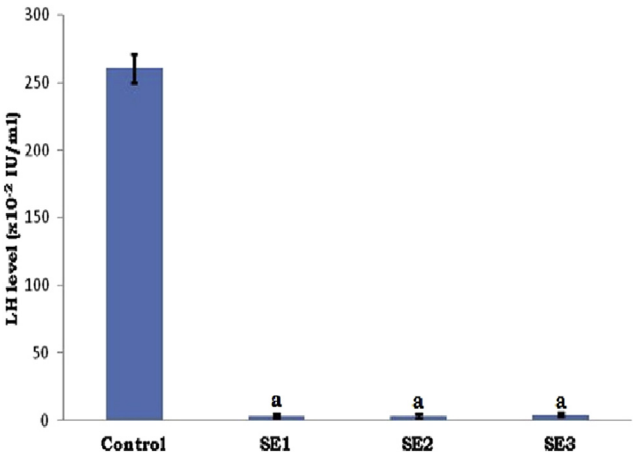

Figure 2. The levels of LH in the control and treatment groups. SE1 = 69 μg/ml of soybean extract; SE2 = 139 μg/ml of soybean extract and SE3 = 276 μg/ml soybean extract. Data are shown as mean ± standard deviation; [a]: $P < 0.05$ compared with the control group; LH: luteinizing hormone; SE: soy extract.