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# Endocrine-Mediated Effects of Genistein on Pups Born to Dams Fed a Phytoestrogen-Enriched Diet

**Kanji Yamasaki***

*Chemicals Assessment and Research Center, Chemicals Evaluation and Research Institute, Tokyo, Japan*

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Dietary phytoestrogens are well known to have potential hormonal effects owing to their interaction with estrogen receptors [1]. Abnormalities in reproductive health due to a high intake of phytoestrogens have been reported in several women [2,3]. Interestingly, genistein–a type of phytoestrogen–is known to have estrogenic properties, and various hormonal effects of this compound have been reported in women [4-8]. In toxicity studies, endocrine-mediated effects have been reported in rat pups of dams treated with genistein during the gestational and/or lactational periods [9-13], but these studies used soy-free or low-phytoestrogen diets. Women ingest dietary phytoestrogens since their infancy, and some of them take genistein as a supplement in their adulthood. Therefore, this study was performed to examine whether endocrine-mediated effects by genistein appeared in pups born to dams maintained on a diet containing phytoestrogens from early stages of their life.

This study was performed under Good Laboratory Practice guidelines, and all animals were cared for in accordance with the principles outlined in the guide for animal experimentation prepared by *the Japanese Association for Laboratory Animal Science*. Thirty Crl:CD (SD) male and sixty female rats at 10 weeks of age were purchased from Charles River Japan, Inc. (Shiga, Japan) and were mated at 12 weeks of age. These animals were given the similar diet used in this study from the infant. Fifty dams were divided into 5 experimental groups consisting of 10 dams per group. The litters were culled according to the OECD guideline for testing of chemicals (no. 415, One-Generation Reproduction Toxicity Study) on Postnatal Day (PND) 4, keeping four males and four females per dam. All pups were weaned at PND 21. Dams were orally gavaged with 0, 3, 10, 30, and 100 mg/kg/day genistein from Gestational Day (GD) 6 to PND 20. Before weaning the pups, each group was randomly assigned to two groups: a group sacrificed at 10 weeks of age and another group used to evaluate the reproductive performance of the offspring (caesarean group). The pups were given a diet contained phytoestrogens throughout the study. Ano-genital distance was measured at PND 4. Vaginal opening was examined for all weaning female rats from PND 21, and preputial separation was examined for all male rats from PND 35 until it occurred. A diet was analyzed, and total phytoestrogen content was 299.8 μg/g diet; daidzin is 117.0 μg/g, daidzein is 48.7 μg/g, genistin is 129.0 μg/g, and genistein is 5.1 μg/g. In the group sacrificed at 10 weeks of age, all pups were examined. Females were killed in the diestrous stage after 10 weeks of age. At the time of necropsy, the following organs were weighed: uterus, ovaries, testes, epididymides, ventral prostate, and seminal vesicles with coagulation gland, brain, liver, adrenals, kidneys, thyroids, and pituitary. The histopathological examination was performed as follows: testes, epididymides, uterus, ovaries and vagina. In the caesarean group, two females and two males per dam in each group were assigned to this group. The estrous cycle was evaluated based on vaginal cytology from 10 weeks of age until mating. The treated females in each group were mated with treated males at 13 weeks of age without brother-sister mating, and numbers of copulated pairs and pregnant females were counted and then the copulation index and conception index were calculated. The pairing days until copulation was also counted. Caesarean sections were performed under anesthesia at GD 20, and numbers of corpora lutea, implantation sites, resorptions, dead fetuses, live fetuses per litter, numbers of males and females, fetus weight per litter and placenta weight per litter were counted and the preimplantation loss, postimplantation loss, dead fetus index and sex ratio were calculated.

All pups of 1 dam in the 30 mg/kg group and of 2 dams in the 100 mg/kg group died after delivery. No significant abnormalities were detected in the reproductive parameters. The body weights of male pups decreased significantly in the 10, 30, and 100 mg/kg groups from PND 28 to 70. No abnormalities were observed in the parameters of ano-genital distance, preputial separation, vaginal opening and estrous cycles. No dose-related organ weight changes were observed in the group sacrificed at 10 weeks of age, and pathological abnormalities were not detected in any group. In the caesarean group, no abnormalities were detected in any group.

The present study was designed to examine the endocrine-mediated effects in pups born to dams given genistein from GD 6 to PND 20, and a diet containing phytoestrogens was given to dams from their weaning and to pups from their weaning to necropsy.

In the diet used in this study, the phytoestrogen content was 299.8 μg/g. The rats were assumed to ingest phytoestrogens at about 6–9 mg/day from the diet (assuming a rat eats a diet of 20–30 g/day). Human intake of phytoestrogens from food is 0.6–15 mg/day in UK and USA [14] and 16–22 mg/day in Japan [15]. The daily intake of genistein in this study was set at 1, 3, 10, and 33 mg (assuming a pregnant rat body weight of 330 g) by the present administered doses of phytoestrogens at 3, 10, 30, and 100 mg/kg/day. The upper limit of phytoestrogens as a supplement for humans is recommended to be 29–30 mg/day according to in the Food Standard Agency and Food Safety Commission, respectively [14,15]. Therefore, the quantities of phytoestrogens and genistein used in this study would be suitable for humans.

Although various endocrine-mediated effects of genistein have been reported in neonatal rodents fed phytoestrogen-free diets [1,16], only a few reports have described the effects on pregnant female rats and their pups. Female SD rats given genistein at concentrations of 0,

***Corresponding author:** Kanji Yamasaki, Chemicals Evaluation and Research Institute, 1-4-25 Kouraku, Bunkyo-ku, Tokyo 112-0004, Japan, Tel: +81-3-5804-6136; Fax: +81-3-5804-6149; E-mail: yamasaki-kanji@ceri.jp

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5, 25, 100, 250, 625, and 1,250 ppm (0, 0.3, 1.7, 6.4, 16, 38, and 72 mg/kg/day) from GD 7 to weaning under a phytoestrogen-free diet led to reduced body weight in dams, and reduced body weights, decreased ventral prostate weight, and histological changes in sex and accessory sex organs in their pups [11,12]. Although the maximum dose in the present study was higher than that of these studies, no abnormal endocrine-mediated findings were detected in the present pups. In addition, prepubertal urogenital abnormalities, smaller male anogenital distances and testis size, and delayed preputial separation were detected in pups born to dams given genistein at doses of 5 and 300 mg/kg/day throughout gestation and lactation under a phytoestrogen-free diet [17]. The difference between our study and these previous studies lay in whether a diet containing phytoestrogens was given to dams from early stages of their life. It was reported that the uterotrophic assay of genistein using pups born to dams given a phytoestrogen-rich diet supplemented with 232 ppm daidzein and 240 ppm genistein was minor [18]. Recently, phytoestrogens have been found to show estrogen agonistic or antagonistic activity depending on whether estradiol is also present [16,19]. When genistein is administered to pregnant rats fed a diet containing phytoestrogens from infancy, the apparent estrogenic effects may not appear in their pups.

In conclusion, we performed an in utero through lactational exposure study of genistein using pups born to dams kept on a diet containing phytoestrogens. Although some level of pup mortality and reduced body weights of male pups were observed, apparent endocrine-mediated effects were not detected in the pups.

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