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Preconception Exposure to Dietary Levels of Genistein Affects Female Reproductive Outcomes

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Abstract

Genistein is a phytoestrogen found in soy and soy-based products. Previously, we found that genistein adversely affected estradiol levels and follicle growth *in vitro*. Proper hormone production and follicle growth are key regulators of normal fertility. Therefore, we hypothesized that genistein adversely affects female fertility and pregnancy outcomes. To test this hypothesis, we dosed sexually mature female CD-1 mice (35 days) with 0, 300, 500, or 1000 ppm genistein for 30, 60, 150, and 240 days. At the end of the dosing periods, we measured mating rate, pregnancy rate, fertility rate, gestation time, parturition time, pup mortality, litter size, average pup weight, and estradiol and progesterone levels. We found that chronic, preconception exposure to genistein affects gestation time, parturition time, litter size, pup weight, and pup mortality. Additionally, genistein exposure for 240 days appears to have a protective effect on fertility rate, but does not affect hormone levels *in vivo*.

Keywords

genistein; preconception exposure; fertility; pregnancy; hormones

1. Introduction

Genistein is an isoflavone phytoestrogen commonly found in plants and plant based products. Genistein is the predominant phytoestrogen in soy (*Glycine max*) and soy-derived products and it accounts for two thirds of soy isoflavone content [1]. Therefore, human and animal exposure to genistein occurs primarily through the consumption of soy and soy-based dietary products [1]. Exposure to genistein can also occur from taking dietary soy supplements [1]. Recent studies indicate that many people consume phytoestrogen dietary

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supplements for the prevention or treatment of a variety of ailments, including cancer, cardiovascular health, sexual dysfunction, and depression [1–5].

Although a few studies indicate that botanical compounds such as genistein may be useful in treating some adverse medical conditions [3, 5], the broader physiological impacts are less understood. Genistein exposure is of particular concern in regards to female health and fertility. This is because genistein, similar to other xenoestrogens, can bind to and signal through estrogen receptors [6]; thereby impacting estrogen signaling pathways and resulting in long-lasting, adverse female health effects. Studies have shown that exposure to environmental estrogens affects female fertility in numerous species [7–9]. Most previous studies have examined the effects of an early life exposure to genistein on female fertility. These studies show that embryonic or neonatal exposures to genistein cause multi-oocyte follicle development, increase atresia in follicles [10], reduce fertility, and cause premature reproductive senescence [11–17] in rodents.

Understanding the impacts of genistein on the developing and pre-pubertal ovary is important; however, it is equally as critical to evaluate the effects of an adult exposure to genistein on female reproductive outcomes. Women are often exposed to genistein in their diet and as potential preventive agents and treatments for a variety of conditions, including cancer, neuronal loss, kidney disease, and menopausal symptoms [3]. Unfortunately, epidemiological studies investigating the effects of botanical chemicals, including genistein, on women's health are scarce. One study suggests that consumption of a soy-based diet is associated with decreased circulating estradiol levels in premenopausal women [18]. Other studies have examined the effects of genistein on cultured human luteinized granulosa cells and found that exposure to genistein inhibits steroidogenesis [19–21].

Interestingly, studies conducted using animal models suggest that genistein has effects on steroidogenesis in isolated and cultured ovarian follicle cells. Specifically, genistein inhibits steroidogenesis or steroidogenic enzymes in cultured rat pre-antral follicles [22], rat granulosa-luteal cells [23], porcine granulosa, theca, or luteal cells [24–27], and mouse antral follicles [28]. Additionally, studies have shown that genistein exposure alters follicle growth [17], induces follicular atresia [29], inhibits oocyte maturation [30], and inhibits antral follicle growth [28] in rodents. Collectively, these studies show that genistein exposure can affect hormone levels and follicle growth, both of which are necessary for normal female fertility [31].

Although previous studies document adverse effects of genistein exposure on key factors of fertility, they did not directly measure the effects of genistein exposure during adulthood on female fertility. Additionally, many of the previous studies examined the effects of a short-term exposure to high doses of genistein. Thus, this study was designed to test the hypothesis that long-term, preconception exposure to dietary levels of genistein during adulthood reduces fertility in female mice. To closely resemble human exposure to genistein, we used doses of genistein that resulted in serum circulating genistein levels similar to those observed in humans after exposure to genistein through the diet.

2. Materials and methods

2.1 Chemicals and diet formulation

Genistein (98% purified determined via HPLC, Botanical Research Center, University of Illinois) was sent to Envigo-Teklad (Madison, WI) to formulate custom diets. We used the AIN-93G, soy and phytoestrogen free diet, as our control and base diet. For the genistein treated diet, we added 300, 500, and 1000 parts per million (ppm) genistein to our base diet. Mice consuming these doses of genistein have been found to have serum genistein levels of 1, 1.8, and 2.6 μM [32]. These serum levels are within the range of those observed after human exposure [1, 33, 34].

2.2 Animals

Female CD-1 (30 days) mice were purchased from Charles River Laboratories (Wilmington, MA) and were allowed to acclimate to the facility prior to dosing. The mice were housed four per cage at the University of Illinois at Urbana Champaign, Veterinary Medicine Animal Facility and were provided food and water *ad libitum*. Temperature was maintained at 22 ± 1 °C and animals were exposed to 12-hour light and dark cycles.

2.3 Study design

Beginning on post-natal day (PND) 35, female CD-1 mice were fed diets containing 0, 300, 500, or 1000 ppm genistein *ad libitum* for 30, 60, 150, or 240 days (Figure 1). All mice and food were weighed and recorded twice a week to ensure all the animals were eating similar amounts of food and that there were no abnormal changes to their weights. Changes in weight and amount of food consumed were analyzed at 30, 60, 150, and 240 days. After each dosing period, 6 mice from each treatment group were euthanized in diestrus (Figure 1). After euthanasia, blood and tissues were collected for analyses as described below. Additionally, after each time point, 6 female mice from each treatment group were paired with an untreated male for breeding (Figure 1). At the time of pairing, all mice were placed on the control diet, and remained on the control diet for the remainder of their time in the study.

2.4 Body weight and food consumption analyses

Body weights and food consumption were measured twice a week for every week throughout the dosing period. Food consumption was calculated by subtracting the amount of food remaining at the end of the week from the amount of food given at the beginning of the week. Food consumption was normalized to the number of animals in each cage. Body weights and food consumption recorded after 30, 60, 150, and 240 days of exposure to genistein were statistically analyzed to examine effects of genistein on body weight and food consumption.

2.5 Organ weight analyses

At the end of each dosing period, 4–6 mice from each treatment group were euthanized in diestrus, and the ovaries, uteri, and livers from all the mice were aseptically removed,

cleaned of fat and interstitial tissues, and weighed. Organ weights were recorded in grams (g).

2.6 Fertility indices and gestation time

To examine the effects of exposure to dietary levels of genistein on female fertility, we analyzed the following factors: 1) mating rate: number of females observed with vaginal sperm plugs / number of breeding pairs $\times$ 100; 2) pregnancy rate: number of females who became pregnant/ number of breeding pairs $\times$ 100; 3) fertility rate: number of females that became pregnant/ number of females plugged $\times$ 100; and 4) gestation time: the length of time between presence of a vaginal sperm plug and the day of birth.

2.7 Pregnancy outcomes

To examine the effects exposure to dietary levels of genistein on pregnancy outcomes, we analyzed the following factors: 1) prolonged parturition: the number of dams that gave birth over a period of time longer than one day; 2) pup mortality: the number of dams that lost any number of pups; and 3) average litter size and average pup weight.

2.8 Hormone levels

To examine the effects of chronic exposure to genistein on sex steroid hormone levels, we euthanized 6 mice from each treatment group after each dosing period and collected sera. We collected sera from all the mice when they were in diestrus to minimize the variability in hormone levels during the estrous cycle. The collected sera were subjected to enzyme linked immunosorbent assays (ELISA) to measure the levels of estradiol and progesterone. ELISA kits were purchased from DRG International Inc. (Springfield, NJ). Assays were run according to the manufacturer's instructions and had intra- and inter-assay coefficients of variability below 10%.

2.9 Statistical analyses

Data were statistically analyzed using SPSS software (IBM, Chicago, IL). A one-way analysis of variance (ANOVA) was used to determine significant changes between groups. If the ANOVA was significant, we used a Dunnett's 2-sided t-test to determine which treatment group was significantly different from the control. Statistical significance was assigned at $p = 0.05$.

3. Results

3.1 Effect of genistein on body weight over lifetime

We examined the change in body weight of each animal twice a week throughout the dosing periods. We found no significant differences in weight in the genistein treated animals compared to control at any time point (Figure 2).

3.2 Effect of genistein on daily food consumption over lifetime

To determine if animals were eating similar amounts of food during the course of the study, we measured the amount of food consumed twice a week throughout the dosing period. We

found no significant differences in the amount of food consumed by the genistein treated animals at any time point compared to control (Figure 3)

3.3 Effect of genistein on organ wet weights

We did not observe any significant changes in ovarian or uterine weights in genistein treated mice when compared to control at any time point (Table 1; n=4–6). Additionally, we did not observe any significant changes in liver weights after 30, 60, and 150 days of dosing. However, we observed a significant decrease in liver weight after exposure to 500 ppm genistein for 240 days when compared to control (Table 1; n=6, p 0.05).

3.4 Effect of genistein on fertility indices

At the end of each dosing time point, animals were paired with a mate for breeding. Beginning at this point, we measured mating rate, pregnancy rate, fertility rate, gestation time, and the number of dams presenting with prolonged parturition. We found no significant differences in the time to mating in the genistein treated animals when compared to control at any time point (Table 2). Similarly, we did not observe any differences in the mating rate after 30, 60, or 240 days of dosing in the genistein treated animals compared to control (Table 2). However, after 150 days of dosing, the 500 ppm genistein group had a decreased mating rate (only 83% of dams successfully mated with a male) compared to control (100% of dams successfully mated with a male) (Table 2; n=6).

We did not observe any difference in the pregnancy rate after 30 or 60 days of dosing in the genistein treated groups compared to control (Table 2). However, after 150 days of dosing, both the 500 and 1000 ppm groups had a decreased pregnancy rate compared to control. In both genistein treated groups, only 83% of dams became pregnant (Table 2; n=6). After 240 days of dosing, pregnancy rate decreased in the control, 300, and 500 ppm groups, but not in the 1000 ppm group (Table 2; n=6). Specifically, only 67% of the dams in the control group became pregnant, only 50% of the dams in the 300 ppm group became pregnant, and only 83% of the dams in the 500 ppm group became pregnant, whereas 100% of the animals in the 1000 ppm group became pregnant (Table 2; n=6).

We did not observe any differences in the fertility rate in any treatment groups after 30 and 60 days of dosing (Table 2). However, after 150 days of dosing, both the 500 and 1000 ppm groups had decreased fertility rates compared to control (Table 2; n=6). In both groups, only 83% of dams became pregnant after successfully mating with a male. After 240 days of dosing, the control, 300, and 500 ppm groups had reduced fertility. Specifically, 67% of the dams in control group, 50% of the dams in the 300 ppm group, and 83% of the dams in the 500 ppm group were fertile, whereas 100% of the dams in the 1000 ppm group were fertile (Table 2; n=6).

3.5 Effect of genistein on gestation time

Next, we examined gestation time at each time point. After 30 days of dosing, we observed significantly decreased gestation time in the dams treated with 500 and 1000 ppm genistein compared to control (Figure 4; n=6, p 0.05). After 60 days of dosing, we observed an increased gestation time in the 500 ppm genistein treated group compared to control, but this

was of borderline significance (Figure 4; $n=6$, $P=0.08$). We did not observe any differences in the gestation time after 150 days and 240 days of dosing in any of the treatment groups (Figure 4; $n=6$).

3.6 Effect of genistein on parturition and pup mortality

We also examined the number of dams that presented with prolonged parturition at each time point. After 30 and 60 days of dosing, we did not observe any dams having a prolonged parturition. However, after 150 days of dosing, we observed 1 dam in the control group, 4 dams in the 300 ppm group, and 2 dams in 1000 ppm group with prolonged parturition (Table 3; $n=6$). After 240 days of dosing, we did not observe prolonged parturition in the control and 500 ppm groups. However, in the 300 ppm group, 1 out of the 3 dams that became pregnant had a prolonged parturition (Table 3; $n=6$). Additionally, after 240 days of dosing, 1 dam from the 1000 ppm group displayed prolonged parturition (Table 3; $n=6$).

After birth, we also observed the litters to determine if the pups were alive or dead. After 30 days of dosing, 1 dam from the control, 300 ppm, and 1000 ppm groups had some pup mortality (Table 3; $n=6$). After 60 days of dosing, 2 dams from the 500 ppm and 1000 ppm genistein treated groups had some pup mortality (Table 3; $n=6$). After 150 days of exposure, 3 dams in the control group experienced pup mortality (Table 3; $n=6$). Additionally, after 150 days of exposure, 4 dams from the 300 ppm genistein treated group, 1 dam from the 500 ppm genistein treated group, and 1 dam from the 1000 ppm genistein treated group experienced pup mortality (Table 3; $n=5-6$). After 240 days of exposure, 1 dam from the control group, 4 dams from the 500 ppm genistein treated group, and 3 dams from the 1000 ppm genistein treated group experienced some pup mortality (Table 3; $n=3-5$).

3.7 Effect of genistein on litter size and pup weight

After birth, we analyzed average litter size and pup weight. After 30 days of dosing, we did not observe any significant differences in the average litter size or pup weight in any treatment group compared to control (Figure 5; $n=6$). After 60 days of dosing, we observed a significant decrease in litter size in the 500 ppm genistein treated group when compared to control (Figure 5; $n=6$, $p=0.05$). Additionally, after 60 days of dosing, we observed a significant increase in average pup weight in the 500 ppm genistein treated group when compared to control (Figure 5; $n=6$, $p=0.05$). After 150 and 240 days of dosing, we did not observe any significant changes in the average litter size or average pup weight in any of the groups when compared to control (Figure 5; $n=3-6$).

3.8 Effect of genistein on hormone levels

We did not observe any significant changes in serum estradiol levels in any genistein treated groups compared to controls at any time point (Figure 6; $n=4-6$). Additionally, we did not observe any significant changes in serum progesterone levels in any genistein treated groups compared to controls at any time point (Figure 6; $n=4-6$). Similarly, we did not find any significant changes in the ratio of estradiol to progesterone in any of the genistein treated groups when compared to control (Figure 6; $n=4-6$).

4. Discussion

This study is the first to specifically examine the effects of chronic, preconception exposure to dietary levels of genistein on female fertility and pregnancy outcomes. Our results show that exposure to genistein (500 and 1000 ppm) for 30 days significantly decreased gestation time compared to control. Further, exposure to genistein (500 ppm) for 60 days decreased litter size and increased average pup weight. In addition, genistein exposure (300 ppm) for 150 days increased the number of dams with prolonged parturition. Further, genistein exposure (500 ppm) for 240 days decreased liver weight and increased pup mortality. Interestingly, dams exposed to genistein (1000 ppm) for 240 days maintained 100% fertility and pregnancy rates, but exposure to genistein did not affect estradiol and progesterone levels at any doses or time point. Collectively, these results provide new insight on how preconception exposure to dietary levels of genistein affects numerous fertility and pregnancy outcomes.

Previous studies have examined the effects of neonatal genistein exposure on fertility outcomes [11]. One such study has shown that neonatal exposure to environmentally relevant levels of genistein can affect female fertility in mice. Specifically, Jefferson *et al.* found that neonatal exposure to genistein (0.5 and 5 mg/kg/day) significantly decreased the number of dams producing live pups when bred at 6 months. Additionally, they found that neonatal exposure to 50 mg/kg/day genistein decreased the number of dams producing live pups when bred as early as 2 months of age [11]. From these results, the authors concluded that neonatal genistein exposure may cause early reproductive senescence. Similar to Jefferson *et al.*, we observed that genistein exposure altered fertility rates in our study. However, Jefferson *et al.* appeared to have a linear dose dependent decrease in fertility, whereas we observed different effects at different doses of genistein. Specifically, after 240 days of dosing, we observed a natural decline in fertility rate of the control group (67% fertile). In comparison, the 300 ppm group had reduced fertility rate (50%), whereas the 500 and 1000 ppm had greater fertility rates (83% and 100%, respectively) than control. This would indicate that perhaps relatively higher doses of genistein have a protective effect on fertility later in life. The difference between our fertility results and the ones observed by Jefferson *et al.* [11] likely stem from the drastically different exposure windows. However, despite having different results, both our study and the one conducted by Jefferson *et al.* [11] clearly indicate that exposure to environmentally relevant doses of genistein affects fertility outcomes in female mice.

One of the earliest effects that we observed in our study was the genistein-induced change to the gestation time. We observed that exposure to genistein (500 and 1000 ppm) significantly decreased gestation time after 30 days of exposure (Figure 4; $n=6$, $p=0.05$), and increased gestation time after 60 days of exposure (500 ppm), but this change was of borderline significance (Figure 4; $n=6$, $p=0.08$). To our knowledge, this phenomenon has not been reported in previous studies conducted with neonatal genistein exposure or in studies that examined the effects of adult exposure to other endocrine disruptors. However, a study examining the effects of estrogen receptor (ER) overexpression in mice found similar effects on gestation time. Specifically, 68% of the ER over-expressing mice (3–5 months old) had increased gestation time, whereas none of the control animals had increased gestation times

[35]. Further, the ER over-expressing mice had prolonged labor, with some animals giving birth over the course of three days [35]. Similarly, in our study, we observed a genistein-induced increase in prolonged parturition. Specifically, we observed that 4 out of the 6 animals dosed with genistein (300 ppm) for 150 days took 2–3 days to complete giving birth (Table 3). Due to changes in gestation and parturition times similar to those seen by Davis *et al.*, we speculate that in our study, genistein may be altering the expression of ER in mice treated with genistein for 30, 60, and 150 days. It would be ideal to examine the expression of ER in the ovary and the uterus of genistein treated animals in future studies. Additionally, we observed that the dams that experienced prolonged parturition in the 300 ppm genistein group were the same dams that experienced pup mortality. Anecdotally, we observed that these dams were not able to take the time to clean off their pups and properly nest and nurse because they were in labor for 2–3 days, resulting in their pups not being cleaned, and not receiving warmth or food needed for survival.

In addition to effects on gestation and parturition, we observed a genistein-induced decrease in litter size. After 60 days of dosing, we observed that genistein exposure (500 ppm) significantly decreased the average litter size and increased the average pup weight compared to control (Figure 5; $n=6$, $p < 0.05$). After 150 days of dosing, we again observed a decrease in average litter size in genistein treated dams (500 and 1000 ppm), however, this change was not statistically significant. It is possible that this lack of statistical significance is a result of a small sample size. Most previous studies examining the effects of genistein have not found significant changes in litter size [1], however, Jefferson *et al.* also observed neonatal exposure to genistein caused a decrease in litter size in mice (2–6 months) [11]. It is possible that the observed decrease in litter size is an indicator of early reproductive senescence. Previous studies have shown that the reproductive aging involves gradual changes, including smaller litter size [36, 37], similar to what we observed in our study. The increase in the average pup weight from dams in the 500 ppm group is likely due to the fact that the pups in these smaller litters are able to access more food and therefore grow faster than pups from larger litters.

Another genistein-induced effect that we observed on pregnancy outcomes was an increase in pup mortality. After 240 days of dosing, exposure to genistein increased the presence of pup mortality (500 and 1000 ppm), and increased the severity of it, as indicated by our data that 4 out of 5 dams exposed to 500 ppm genistein lost their entire litters. We observed that the dams in the 500 ppm treatment group had a normal parturition time, and properly groomed and nested their pups after birth. However, by postnatal day 2, 4 out of the 5 dams that gave birth in the 500 ppm group had killed their entire litters. The cause of this infanticide is unknown. However, upon further examination of the pups post mortem, we observed that the pups lacked milk spots, indicating that they were not receiving any milk. Therefore, it is possible that the dams killed their litters to prevent them from starving to death. The exact reason for why the pups were not receiving any milk remains unknown. However, one possibility is that genistein has lasting, pre-conception effects on lactation, thus effecting the ability of the dams to nurse their pups.

We also examined the effects of chronic exposure to dietary levels of genistein on steroid hormone production. We found that genistein exposure did not affect estradiol and

progesterone levels at any time points (Figure 6). Contrary to our results, numerous *in vitro* studies conducted on adult ovarian cells show that exposure to genistein reduces estradiol biosynthesis and alters progesterone production [19, 27, 28, 38, 39]. One major difference between our study and the previous *in vitro* studies is the dose selection. All previous *in vitro* studies directly exposed the ovarian cells to doses of genistein that range from 30–100 μ M. In contrast, the doses of genistein in our study (300, 500, 1000 ppm) result in serum genistein levels of 1, 1.8, and 2.6 μ M, respectively [32]. It is very likely that the doses used in our study are too low to have any direct effects on hormone production. Another possibility is that the doses from our study are having subtle effects on hormone production, which could not be detected with the sample size in our study.

Collectively, this study provides new information on the effects of long term, preconception exposure to dietary levels of genistein on female fertility and pregnancy outcomes. Dosing animals through their diet for longer time periods allowed us to more closely mimic human and animal exposure to genistein. The results from this study indicate that even at doses obtainable through dietary exposure, genistein is capable of having effects on female fertility. Although exposure to genistein was stopped at the time of mating, the preconception exposure can impact pregnancy outcomes such as gestation time, parturition time, litter size, pup weight, pup mortality, and maternal behavior.

Interestingly, many of the genistein induced effects on fertility and pregnancy outcomes occurred at the lower two doses of genistein (300 and 500 ppm), but not as the highest dose of genistein (1000 ppm). Further, genistein exposure at 500 ppm, but not 1000 ppm significantly decreased liver weight at 240 days. Although the reasons for the non-linear dose responses with genistein are unclear, it is possible that genistein is acting like an endocrine disrupting chemical in that it exerts non-monotonic dose responses. Several studies indicate that many endocrine disrupting chemicals exert non-monotonic dose responses, leading to less effects at higher doses compared to lower doses [40,41].

Future studies should be conducted to examine the underlying mechanism by which genistein imparts these effects on pregnancy outcomes, and to examine whether exposure to genistein (1000 ppm) maintains the protective effect on fertility. Many women are exposed to genistein and other phytoestrogen-based botanicals as part of their regular diet or as therapeutics for reproductive and non-reproductive diseases, but their effects remain under studied. This study and future ones are critical for better understanding the broader physiologically effects of genistein.

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Highlights

- Genistein exposure for 30 days decreases gestation time
- Genistein exposure for 60 days decreases litter size and increases pup weight
- Genistein exposure for 150 days increases parturition time
- Genistein exposure for 240 days increases infanticide
- Genistein exposure does not affect estradiol and progesterone levels *in vivo*

Figure 1. Study design

Beginning at PND 35, female CD-1 mice were fed a phytoestrogen free base diet containing 0, 300, 500, or 1000 ppm genistein for 30, 60, 150, or 240 consecutive days. After each time point, 6 mice from each treatment group were paired with a male for breeding tests. Additionally, 6 mice were euthanized, and their sera, ovaries, uteri, and livers were collected for analysis.

Figure 2. Effect of genistein on body weight over lifetime

Body weights of all mice were analyzed after 0, 30, 60, 150, and 240 days of dosing. This graph represents the mean $\pm$ SEM of the weights of mice in each treatment group (0 days: n=48; 30 days: n=48; 60 days: n=35–36; 150 days: n=23–24; 240 days: n=10–12).

Figure 3. Effect of genistein on daily food consumption over lifetime

Food consumption was analyzed after 30, 60, 150, and 240 days of dosing. This graph represent the mean $\pm$ SEM of amount of food consumed daily, per mouse (0 days: n=48; 30 days: n=48; 60 days: n=35–36; 150 days: n=23–24; 240 days: n=10–12).

Figure 4. Effect of genistein on gestation time

During each breeding test, we measured gestation time. This graph represents the mean $\pm$ SEM of the length of gestation. Asterisks (*) indicate a significant difference between the control group and genistein treated groups ($n=3-5$; $p < 0.05$). The arrow (^) indicates a difference between the control group and the 500 ppm genistein treated group that is borderline significant ($n=5$; $p=0.08$). Note, $n=2$ for 1000 ppm treated group after 60 days of dosing, therefore no statistical analyses were performed using this group.

Figure 5. Effects of genistein on litter size and pup weight

After birth, we measured the litter size and pup weights from the dams in each treatment group. These graphs represent the mean $\pm$ SEM of the litter size (Figure 5A) and pup weight (Figure 5B). The asterisks (*) indicate a significant difference between the control group and the genistein treated groups ($n=3-6$; $p < 0.05$).

Figure 6. Effect of genistein on hormone levels

After each dosing period, we measured serum estradiol, and progesterone levels.

Additionally, we calculated the ratio of estradiol to progesterone. These graphs represent the means $\pm$ SEM of estradiol levels (Figure 6A), progesterone levels (Figure 6B), and the ratio of estradiol to progesterone (Figure 6C). 30 days: n=5–6, 60 days: n=6, 150 days: n=5–6, 240 days: n=4–6.

Table 1

Effect of genistein on organ wet weights

	30 days	60 days	150 days	240 days
Ovary weight				
Control	0.016 ± 0.002	0.019 ± 0.002	0.024 ± 0.002	0.027 ± 0.005
300 ppm	0.015 ± 0.002	0.016 ± 0.002	0.023 ± 0.002	0.021 ± 0.004
500 ppm	0.015 ± 0.001	0.017 ± 0.001	0.023 ± 0.005	0.021 ± 0.003
1000 ppm	0.016 ± 0.001	0.020 ± 0.002	0.018 ± 0.003	0.020 ± 0.002
Uterine weight				
Control	0.095 ± 0.007	0.100 ± 0.008	0.137 ± 0.016	0.175 ± 0.029
300 ppm	0.085 ± 0.009	0.118 ± 0.013	0.159 ± 0.072	0.134 ± 0.020
500 ppm	0.118 ± 0.018	0.100 ± 0.007	0.097 ± 0.005	0.184 ± 0.028
1000 ppm	0.121 ± 0.021	0.112 ± 0.086	0.133 ± 0.085	0.188 ± 0.052
Liver weight				
Control	1.10 ± 0.04	1.38 ± 0.05	1.49 ± 0.13	1.78 ± 0.05
300 ppm	1.07 ± 0.05	1.20 ± 0.07	1.50 ± 0.08	1.61 ± 0.06
500 ppm	1.09 ± 0.05	1.32 ± 0.04	1.20 ± 0.13	1.44 ± 0.11
1000 ppm	1.16 ± 0.07	1.33 ± 0.10	1.36 ± 0.02	1.69 ± 0.11

Table 2

Effect of genistein on fertility indices

30 Days	# Breeding Pairs	Mating rate	Pregnancy Rate	Fertility Rate
Control	6	100	100	100
300 ppm	6	100	100	100
500 ppm	6	100	100	100
1000 ppm	6	100	100	100

60 Days	# Breeding Pairs	Mating rate	Pregnancy Rate	Fertility Rate
Control	6	100	100	100
300 ppm	6	100	100	100
500 ppm	6	100	100	100
1000 ppm	6	100	100	100

150 Days	# Breeding Pairs	Mating rate	Pregnancy Rate	Fertility Rate
Control	6	100	100	100
300 ppm	6	100	100	100
500 ppm	6	83	83	83
1000 ppm	6	100	83	83

240 Days	# Breeding Pairs	Mating rate	Pregnancy Rate	Fertility Rate
Control	6	100	67	67
300 ppm	6	100	50	50
500 ppm	6	100	83	83
1000 ppm	6	100	100	100

Table 3

Effect of genistein on parturition and pup mortality

30 Days	# Dams	Prolonged Parturition	Pup Mortality
Control	6	0/6	1/6
300 ppm	6	0/6	1/6
500 ppm	6	0/6	0/6
1000 ppm	6	0/6	1/6

60 Days	# Dams	Prolonged Parturition	Pup Mortality
Control	6	0/6	0/6
300 ppm	6	0/6	0/6
500 ppm	6	0/6	2–6
1000 ppm	6	0/6	2–6

150 Days	# Dams	Prolonged Parturition	Pup Mortality
Control	6	1/6	3–6
300 ppm	6	4–6	4–6
500 ppm	5	0/6	1/5
1000 ppm	5	2–6	1/5

240 Days	# Dams	Prolonged Parturition	# Pup Mortality
Control	4	0/4	1/3
300 ppm	3	1/3	0/3
500 ppm	5	0/5	4/5#
1000 ppm	6	1/6	3/5