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The Effects of Prenatally Administered Phytoestrogens on the Reproductive and Behavioral Development of Long-Evans Rats

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THE EFFECTS OF PRENATALLY ADMINISTERED PHYTOESTROGENS ON THE MORPHOLOGICAL AND BEHAVIORAL DEVELOPMENT OF LONG-EVANS RATS

by

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GRADUATE COMMITTEE APPROVAL

of a master's thesis submitted by

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This master's thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the master's thesis of Crystal Blake in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

THE EFFECTS OF PRENATALLY ADMINISTERED PHYTOESTROGENS ON THE MORPHOLOGICAL AND BEHAVIORAL DEVELOPMENT OF LONG-EVANS RATS

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Masters of Science

Equol is known to be a selective androgen modulator and has the ability to bind and inhibit 5-alpha dihydrotestosterone (5α-DHT). Equol is also a selective estrogen receptor modulator and is able to bind beta estrogen receptors with high affinity. As estrogen receptors are found in the hypothalamus, pituitary, and gonads, prenatally administered equol could affect the morphological and reproductive development of offspring. To test this hypothesis, during gestational days 14 to 20, forty-two pregnant Long-Evans rats were given one of six treatments: 1) no treatment, 2) injection with dimethyl sulfoxide (DMSO), 3) injection with 10 mg/kg equol, 4) injection with 21.0 mg/kg equol, 5) injection with 63.0 mg/kg equol, or 6) injection with 90.0 mg/kg flutamide. At birth the pups were weighed, anogenital distance measured, and sex was determined. Some of the animals were sacrificed and trunk blood collected from both the

mothers and pups. Serum levels of phytoestrogens, estradiol, testosterone, and 5α-DHT levels were determined. Some pups were allowed to grow up to day 29 and were tested on the forced-swim test with the parameters of time mobile, time immobile, swim distance, and average speed measured. The flutamide treated pups had the lowest anogenital distance. The low equol dose animals had the largest anogenital distance. There were no significant differences in 5α-DHT serum levels in the male offspring among the treatments. However in non-injected control female offspring displayed significantly lower 5α-DHT levels than all the other groups. Mothers treated prenatally with equol displayed significantly higher circulating equol levels compared to controls values. Rats injected with 63.0 mg/kg of equol gained the least weight during pregnancy. Their offspring also had the lowest body weights at birth. Male and female offspring displayed similar behaviors in the Porsolt forced-swim test among the treatment groups. The low and high equol groups displayed the least depressive-like behaviors. The offspring from mothers treated with the medium and high equol doses both gained the most weight from birth to day 29. Treating pregnant rats with equol during the last week of gestation does not appear to have any affect on morphological genital development of the offspring.

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Introduction:

In rats the critical development period of the reproductive tract occurs during gestational days 12-21. Administering testosterone or 17β-estradiol during this time will alter the reproductive development of rats [1].

Phytoestrogens are plant-derived, non-steroidal molecules that have structural and functional similarity to 17β-estradiol [2]. Phytoestrogens are present in high concentrations in soy and alfalfa products. Some well-known phytoestrogens are coumestrol from alfalfa and the soy isoflavones genistein, daidzein, and daidzein's intestinal metabolite equol [3]. The increase in consumption of soy products raises concerns about the effects of increased prenatal exposure to phytoestrogens.

Like 17β-estradiol, phytoestrogens have an affinity for estrogen receptors alpha and beta. However, most phytoestrogens have a greater affinity for the beta estrogen receptor than the alpha estrogen receptor [4]. These estrogen receptors are expressed in the hypothalamus, pituitary and gonads, which suggests that the alpha and beta estrogen receptors might be involved in reproduction [5]. Since phytoestrogens have the ability to mimic estradiol activity by binding to estrogen receptors, phytoestrogens could alter the physiologic and morphologic development of prenatal offspring leading to altered animal behaviors as well as abnormal external genitalia development. In the brain, sex hormones also influence learning and memory and the structure of sexually dimorphic regions of the brain [6,7].

Of particular interest is the isoflavone equol, which is a metabolite of daidzein, as it has greater biological potency then other phytoestrogens such as daidzein or genistein alone [8]. Because equol is a selective estrogen receptor modulator it could affect human

and animal growth and development [9]. Equol has a relatively high affinity for the beta estrogen receptor, which is present in both the male and female reproductive tract and in the sexually dimorphic regions of the brain [5].

Normally during pregnancy, alpha-fetoprotein binds the mother's estrogen so that the offspring will not be affected by maternal estrogenic hormone actions. However, equol has the ability to bind to alpha-fetoprotein [10], an action that could affect 17β-estradiol binding to alpha-fetoprotein. It is possible that equol competitively binds both estrogen receptor beta in place of 17β-estradiol and binds alpha-fetoprotein, changing the effect of 17β-estradiol to function normally.

Equol is also a selective androgen modulator, having the ability to bind specifically 5α-dihydrotestosterone (5α-DHT) and inhibit its action [11]. However, 5α-DHT is important in the development of male morphology and behavior. If 5α-DHT's hormone action is altered by prenatal exposure to equol it could potentially alter male external genital development and behavior.

The purpose of this study was to examine the effects of equol by prenatal administration on genital development and offspring behavior at postnatal day 29 as assessed by the Porsolt forced swim test.

Hypothesis:

Prenatal administration of equol will alter male morphology in a dose dependent manner and will also have dose dependent detrimental effects on rat behavior at postnatal day 29.

Methods:

Animals. Forty-two female and ten male Long-Evans rats were obtained from Charles River Laboratories at 55 days old. They were given *ad libitum* access to food and water and placed on a 12 hour light/dark cycle. The diet contained less then 10 ppm of isoflavones called by this laboratory the phyto-free diet. The rats were allowed ten days to adapt to their new surroundings before breeding. After insemination the female body weight was recorded and the time marked as day zero of pregnancy.

The rats were divided into six groups for the injection treatments (see below). The pregnant mothers were weighed on gestational day 6, 12, and 20. Starting at gestational day 14 the rats received daily injections until gestational day 20. When injected, the mothers were carefully wrapped in cloth in an attempt to minimize prenatal stress, increase comfort, and lessen struggle. After delivery, the mothers were again weighed and then sacrificed. Trunk blood was collected as well as abdominal white adipose tissue weight. Brains were collected and frozen immediately on dry ice. Pups were counted, weighed, anogenital distance (AGD) measured, and then sacrificed and trunk blood collected by sex based on AGD parameters. If unable to determine pup sex from AGD then the pup was dissected and the presence of the testes was examined in the pelvic cavity to confirm the sex. The number of pups, sex, weight, and AGD were recorded. Some of the mothers and pups from each group, except the flutamide group, were kept alive and allowed to continue to grow. The pups were weaned at postnatal day 21 and moved into group cages according to gender and treatment. Each treatment group remained on the phyto-free diet.

Diet. Before their arrival, the male and female rats had been fed a diet of 200-ppm isoflavones, called the phyto-200 diet. Upon arrival, the rats were placed on a low phytoestrogen diet known as the phyto-free diet; this diet has about 10 ppm of isoflavones, as determined by HPLC [12, 13].

Mating. After allowing time to adapt to our facilities the males were put into cages that have wiring along the bottom to allow the feces to drop onto cardboard sheeting below the cage. One female was placed into each cage over night and afterwards the cardboard below the cage was examined for a vaginal plug consisting of the ejaculate signifying that the male successfully ejaculated and the female was inseminated. Once a female was inseminated she was removed from the cage and another female was introduced to the male. This was done until the vaginal plugs were found for all of the rats. All of the male rats were sexually active and there were approximately 2 to 4 pregnancies obtained per male among all of the treatment groups.

Injections. On day 14 of pregnancy the mothers from the dimethyl sulfoxide (DMSO), Flutamide (androgen-receptor blocker), and Equol treatment groups were injected with 0.1 cc of their specific treatment. The DMSO group received 0.1 cc DMSO. The Equol 10.5 group received 10.5mg/kg body weight of equol, the Equol 21.0 group received 21.0mg/kg body weight of equol, and the Equol 63.0 group received 63.0mg/kg body weight of equol dissolved in DMSO. The flutamide group received 90.0-mg/kg body weight injection of flutamide dissolved in ethanol. The injection volume was equal to 0.1 cc, and each rat was injected daily for 7 days. The mothers were injected subcutaneously at the nape of the neck after being carefully wrapped in cloth to help the mother be more comfortable and secure to avoid stressing the mother.

Afterward each animal was gently placed back into her cage. All treatments were stopped the day before delivery.

Porsolt Forced-Swim Test. The Porsolt forced-swim test is widely used to determine whether a compound has anti-depressant effects. It tests these anti-depressant parameters by measuring animal mobility and immobility. Depression defined clinically refers to certain pathological behaviors that have psychological, neuroendocrine and somatic symptoms. Unfortunately these same parameters cannot be measured in animals. There are only certain behaviors that are relevant when comparing the action of anti-depressants on humans and animals and the most widely studied behavior is immobility. It has been noted that giving anti-depressants to rats decreases time spent in an immobile, passive-like state [14].

In the Porsolt swim test, the rat is placed in a cylinder containing water deep enough to prevent the rat from touching the bottom with its tail. In this case the animal has no choice but to swim as it is unable to either rest on its tail or climb out. In the swim tests the water depth was 13.5 inches [15].

The rats in our laboratory were placed in the water chambers for 6 minutes. Their behavior was recorded by a video camera located above the cylinders. Swimming speed, distance, time spent both mobile and immobile, and total distance traveled were measured using Any-Maze purchased from the Stoelting Company. Animals that display less depressive-related behavior will swim faster, further, and be more mobile. Animals that display more depressive-related behaviors will spend more time immobile and swim slower.

Phytoestrogens. Blood was collected from both mothers and pups sacrificed after birth. Blood was centrifuged at 1500 g and serum collected and via gas chromatography/mass spectrometry (GC/MS) analyzed for the presence of the phytoestrogens genistein, daidzein, and equol in both the pups and mothers.

Testosterone levels. Mother rats' blood was collected and centrifuged at 1500 g and serum collected and frozen at -20ºC until assayed. Testosterone levels were measured with kits obtained from Diagnostic Systems Laboratories. For the testosterone RIA DSL-4000 kit was used.

DHT levels. Serum from mother and newborn pups were examined for DHT levels using a DHT ELISA kit obtained from Alpha Diagnostic International.

Statistics. All data were first analyzed by one-way analysis of variance. Individual variations between treatment groups were measured using the student t-test. All statistics were run using the Minitab statistical software. $P < 0.05$ was considered significant. All results are presented as MEANS ± SEM in all of the graphs and significant differences are marked.

Results:

Birth. There was no significant difference between litter size, female/male birth ratio, or gestational length among the treatment groups ($p< 0.177$, $p< 0.617$, $p< 0.242$ respectively).

Female Birth Weight. The non-injected control group and flutamide group both weighed significantly more than any other group except each other ($p< 0.0001$). The equol 10.5 group also weighed significantly more than the other two equol groups ($p<0.0001$). (see Figure 1)

Male Birth Weight. The non-injected control group weighed significantly more than all other groups except for flutamide ($p<0.0001$). The equol 63.0 group weighed significantly less than all other groups ($p<0.0001$). The DMSO, equol 10.5, equol 21.0 and flutamide groups were not significantly different from each other. (see Figure 2)

Female AGD. The equol 21.0 group was significantly greater than the non-injected controls, the equol 10.5 group, and the flutamide group ($p<0.0001$). The flutamide group was significantly less than the DMSO controls and the equol 21.0 and 63.0 groups ($p<0.0001$). (see Figure 3)

Male AGD. The male anogenital distances showed significant difference. flutamide-treated males, as expected, had significantly smaller AGD than all other treatment groups ($p<0.0001$). The equol 21.0 group was significantly larger than any other group ($p<0.005$). The other groups were not significantly different from one another. (see Figure 4)

AGD/Body Weight Ratio. When comparing AGD across groups only the male and female flutamide groups' anogenital distances were not significantly different from each other. The ratio of AGD to birth body weight ratio showed that in all the groups the males and females were significantly different from each other except for the flutamide group where the male and female ratios were not significantly different from each other ($p< 0.208$). (see Figure 5)

Phytoestrogen. Maternal postpartum serum levels of equol, genistein, daidzein were obtained from gas chromatography/mass spectrometry (GC/MS). The equol 63.0 groups had the highest level of serum equol ($p<0.0001$). The equol 21.0 group serum level of equol was significantly less than equol 63.0 but significantly greater than all

other groups ($p<0.001$, $p< 0.044$ respectively). The equol 10.5 group had significantly less serum equol than both the equol 63.0 or the equol 21.0 groups ($p< 0.01$) and was significantly greater compared to control values. (see Figure 6)

Maternal rat testosterone levels. The flutamide-treated mother rat serum had the lowest amount of blood testosterone (ie 115 ± 6.4 pg/ml). This group was significantly less than the DMSO group at 319 ± 55 pg/ml and the non-injected controls (411 ± 43 pg/ml) and less than all other equol groups that averaged 188 ± 55 pg/ml. All of the equol groups tended to have less serum testosterone than the non-injected controls and DMSO controls but these differences were not significant.

Maternal DHT levels. The flutamide-treated rats were significantly less then the equol 63.0 rats ($p<0.04$). Flutamide-treated rat serum had lower 5α-DHT levels than all of the groups but aside from the equol 63.0 groups this amount was not significant. (see Figure 7)

Fetal DHT Levels. The non-injected control female fetal DHT levels were significantly lower than all other groups ($p<0.02$). The flutamide fetal DHT levels were the highest but not significantly greater than any group but the non-injected controls ($p<0.14$) (see Figure 8). The male fetal DHT levels were not significantly different from each other ($p<0.685$). The Equol 21.0 group had the highest level but this was not significantly different from any other group. (see figure 9)

Day 29 Forced Swim Test. Females. The non-injected controls, equol 10.5 and equol 63.0 were the most mobile and were significantly more mobile than the DMSO and equol 21.0 groups. Equol 21.0 animals were less mobile than the non-injected controls, equol 10.5 and equol 63.0 animals ($p<0.001$). The DMSO controls were less mobile than

the non-injected controls, equol 10.5 and equol 63.0 animals ($p<0.042$, $p<0.029$, $p<0.017$ respectively). The equol 63.0 animals' total distance was significantly further than both the DMSO controls and equol 21.0 animals ($p<0.041$, $p<0.0001$ respectively). The equol 63.0 animals also swam significantly faster than these groups as well ($p<0.043$, $p<0.0001$). The equol 21.0 animals swam significantly less than all other groups except DMSO controls ($p<0.0001$) and swam significantly slower than all other groups except the DMSO controls ($p<0.001$). (see Figures 10-13)

Males. The equol 63.0 group spent more time mobile than all of the other groups. This group was significantly more mobile than the DMSO control group and the equol 21.0 group ($p<0.010$, $p<0.003$ respectively). The Equol 21.0 group was significantly less mobile than the equol 63.0 and equol 10.5 animals ($p<0.003$, $p<0.035$ respectively). The equol 21.0 group swam the significantly least distance compared to all other groups ($p<0.0001$). The equol 63.0 swam the furthest distance and the average speed was the greatest but both were not quite significantly greater then the other groups. (see Figures 14-17)

Body Weight Changes. From birth until postnatal day 29 the rat pups all gained between 70-100 grams. In the female groups both the equol 21.0 and equol 63.0 groups gained significantly more weight than the non-injected controls, DMSO controls, and equol 10.5 groups ($p< 0.006$, $p<0.001$, $p< 0.022$ respectively). (See Table 1) In the male groups the equol 63.0 group gained significantly more weight than the non-injected controls, DMSO controls, and equol 10.5 group ($p<0.026$). The equol 21.0 group gained significantly more weight than the non-injected controls and equol 10.5 group ($p<0.026$). The equol 21.0 and equol 63.0 groups were not significantly different from each other

($p<0.308$). The non-injected controls, DMSO controls, and equol 10.5 group were not significantly different from one another ($p<0.762$). (see Table 1) The non-injected control males, DMSO control males, equol 10.5 males, equol 21.0 males and equol 63.0 males weighed on average 89.4 grams, 93.9 grams, 91.4 grams, 101.0 grams, and 103.4 grams respectively. The non-injected control females, the DMSO females, the equol 10.5 females, the equol 21.0 females and the 63.0 females weighed 82.4 grams, 79.5 grams, 85.2 grams, 92.2 grams, and 92.4 grams. (see Table 1)

Discussion:

Equol has been reported as a selective androgen modulator with the ability to bind and inhibit the action of 5α-DHT. If a 5α-DHT inhibitor or other androgen blocker is administered during gestational days 14-20 male reproductive development is altered and with female-like genital morphology as measured by the AGD [1,11]. Flutamide, used as a positive control in this experiment is a well-known androgen blocker and is a good example of and agent that causes morphology alteration [16, 17]. When equol was administered during this critical time period there was no change in the genital morphology of the male pups which indicates that 5α-DHT functioned normally. The flutamide treated pups had the smallest AGD, and the equol treated groups, even in the highest doses, did not have significantly shortened AGD. While equol has been reported to bind and inhibit 5α-DHT action [11], it appears that the binding is not sufficient to change normal 5α-DHT action in male Long-Evans rats during this critical time period. In fact the 21.0 mg/kg dose of equol increased the AGD in both males and females. However, when AGD was normalized to body weight there were no significant differences between the treatment groups in either the males of the females.

Interestingly, serum 5α-DHT levels were not significantly different in the male offspring. It was expected that the flutamide group at least would have a significant decrease in serum 5α-DHT levels as flutamide is an androgen blocker.

Isoflavones are able to pass through the placenta from maternal to offspring. When a mother is fed a high phytoestrogen diet the offspring about one tenth of the phytoestrogens cross the placenta to the offspring. The dose is even higher when the mother has a phytoestrogen poor diet. The rat placenta is not fully functional until after gestational day 16. Actual exposure to phytoestrogens may occur near the end of gestation [20]. The offspring was exposed to equol but regardless of the dosage no difference was made in AGD.

In this study, behavior was assessed in the young offspring by the Porsolt forced-swim test at day 29 before the females would have started their estrous cycles and making comparisons between the males and females easier to interpret. Females normally have a larger tendency toward depression then males do [18]. In the equol treatment groups the results displayed a U-shape pattern among the equol treatment groups in both the male and female groups. This is not completely understood how at increasing doses the effect should increase at first, then at a higher pharmacological dose the effect would drastically decrease and then at an even higher pharmacological dose the effect would then increase above even the first lower dose. Both the low and high dose animals displayed less depressive-related behaviors meaning that the animals displayed more mobility in the forced-swim test. The medium dose animals displayed the highest depressive related behaviors, also referring to time spent immobile, compared to the low

and high equol doses. It appears that exposing animals to a low dose and pharmacologically high dose both caused less depressive behaviors.

It does appear that the manner of administration of treatments to the animals had an affect. In the female rat swim tests it was noticed that the animals that expressed the least depressive-related behaviors were the low and high dose groups as well as the non-injected control group. These three groups were significantly greater then both the DMSO controls and middle dose group. In the male rat swim tests the low and high doses were not significantly different from either the non-injected controls or the DMSO controls but were significantly greater then the middle dose group. The DMSO group was less then the Equol 21.0 group but this difference was not significant. It appears that administering injections to the pregnant mother rats has a tendency to make the offspring express more depressive-related behaviors. This could be a result of prenatal stress of the injection to the mother as prenatal stress has a tendency to increase likelihood of depressive-related behaviors [19]. It appears that the low and high equol groups can restore animals to the same behavioral levels of the non-injected control rats. Both male and female pups showed similar patterns in this behavioral test but it was much more pronounced in the female.

During pregnancy the mother rats in the high dose group gained significantly less weight then all of the other groups except the non-injected controls. After birth the high dose mothers had the smallest weight though this weight was only significantly different then the DMSO control rats. The medium and high dose female pups had the lowest birth body weight. This was significantly lower then all of the other groups except the DMSO controls pup. In the male groups the high dose pups were significantly less then

all of the other groups. The non-injected controls and flutamide pups weighed the most and this was significant compared to all other groups. The trend in birth weight between the equol treated groups was a decreasing birth weight with increasing dose. This trend was present in both the males and the females.

After the pups were born they were placed on the same phyto-free diet as their mothers. By day 29 the medium and high dose female groups gained significantly more weight then any of the other groups. This was the same with the high dose in the males. The middle dose male pups were significantly greater than the other groups except the DMSO controls (see Figure 19). By day 29 the medium and high dose groups weighed the most and were the largest rats in both sexes. High equol doses appear to lower birth weight. It is not yet understood why these animals would experience the greatest weight gain from birth to day 29.

It is thought that the increase in weight gain from birth could be "catch up" weight. This is weight gain that is experienced by underweight offspring. This "catch up" weight is considered harmful as those who experience it have an increased chance of developing metabolic problems such as obesity, insulin resistance, diabetes as well as increased cardiovascular problems such as hypertension and heart disease [23]. So while phytoestrogens decrease weight gain in both the mother and offspring this could potentially be of a concern during postnatal development.

One limitation in this study included the inability to determine if the behavioral effects on the female rats were due to the DMSO or the prenatal stress of the injection. DMSO is used to help aromatic compounds be absorbed across membranes and is therefore helpful with drug delivery [21]. DMSO has low toxicity [22] and as there was

no change in the males it is assumed that the effect was due to prenatal stress and not the DMSO.

In summary, it appears that prenatally exposing offspring to equol 1) does not alter DHT levels in the male or alter male genital development, 2) the high dose mothers weighed the least before and after birth and their offspring also weighed the least at birth, 3) after birth the high dosed equol treated animals that gained the most weight but displayed the least depressive-like behavior. Injecting the pregnant animals appears to cause prenatal stress on the rats, however, both the low and high equol doses appear to restore depressive-related behaviors to that of the untreated rats.

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Figures:

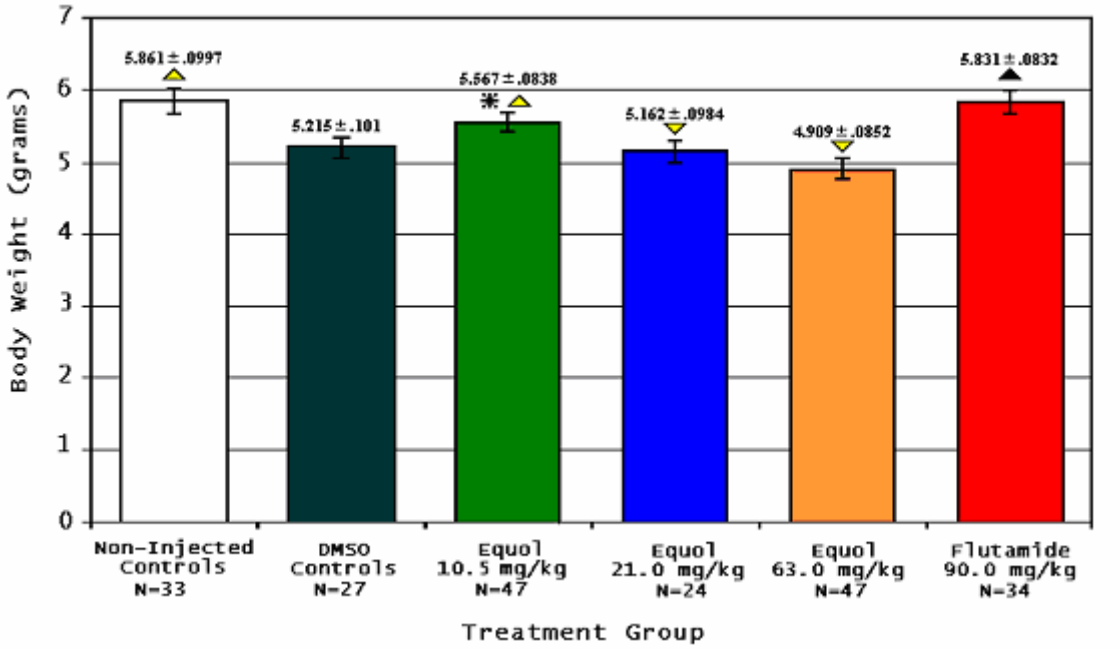

Figure 1: Prenatal Equol Treatment Pups Birth Weight By Treatment Groups (Females). (△ & ▲) Non-injected controls and Flutamide weighed significantly more than all other groups. (✳ △)Equol 10.5 weighed significantly less than non-injected controls and more than Equol 21.0 (▽) and Equol 63.0 (▽).

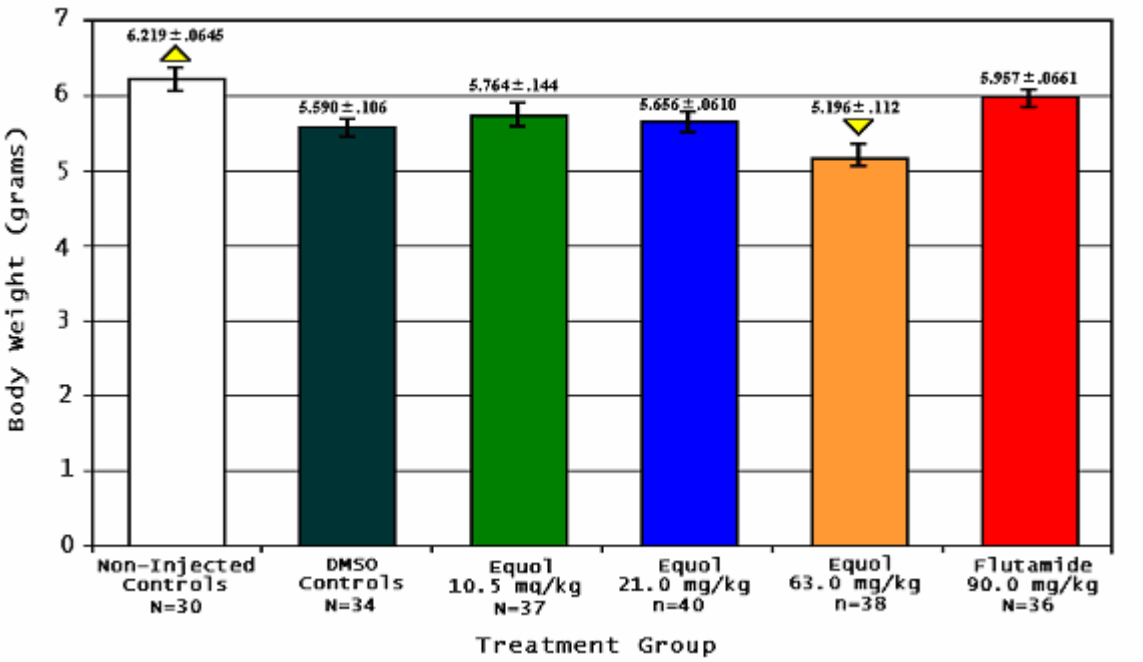

Figure 2: Prenatal Equol Treatment Pups Birth Weight By Treatment Groups (Males). △ Non-injected controls weighed significantly more than all other groups except flutamide and ▽ Equol 63.0 weighed significantly less than all other groups.

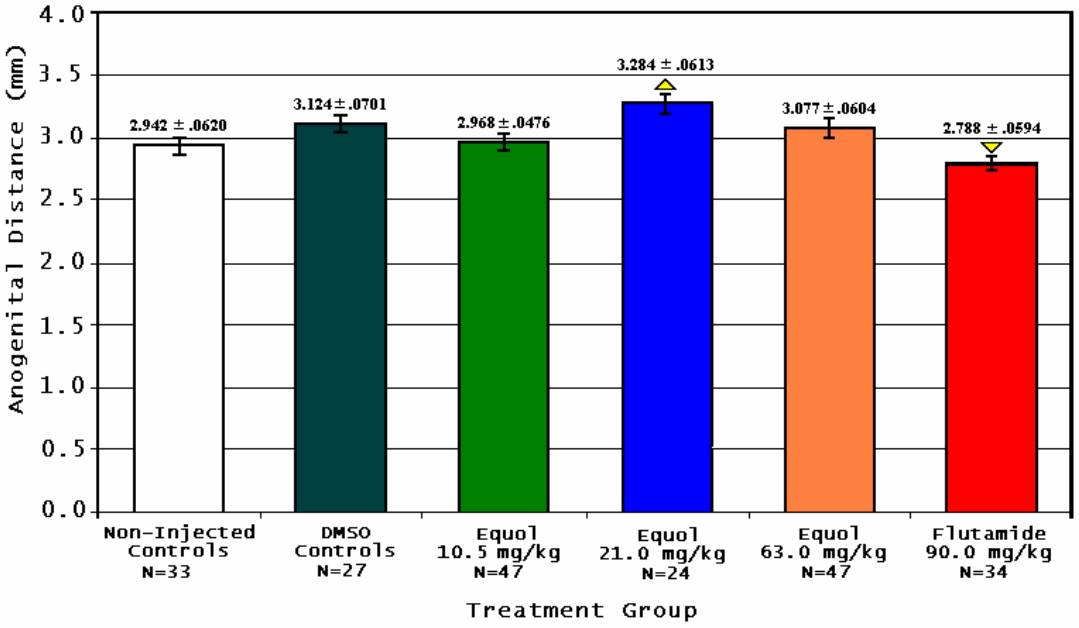

Figure 3: Prenatal Equol Treatment Birth Anogenital Distance (Females). ▲ Equol 21.0 animals AGD was significantly more than the non-injected controls, flutamide, and Equol 10.5. ▼ Flutamide AGD was significantly less than DMSO controls, Equol 21.0 and Equol 63.0.

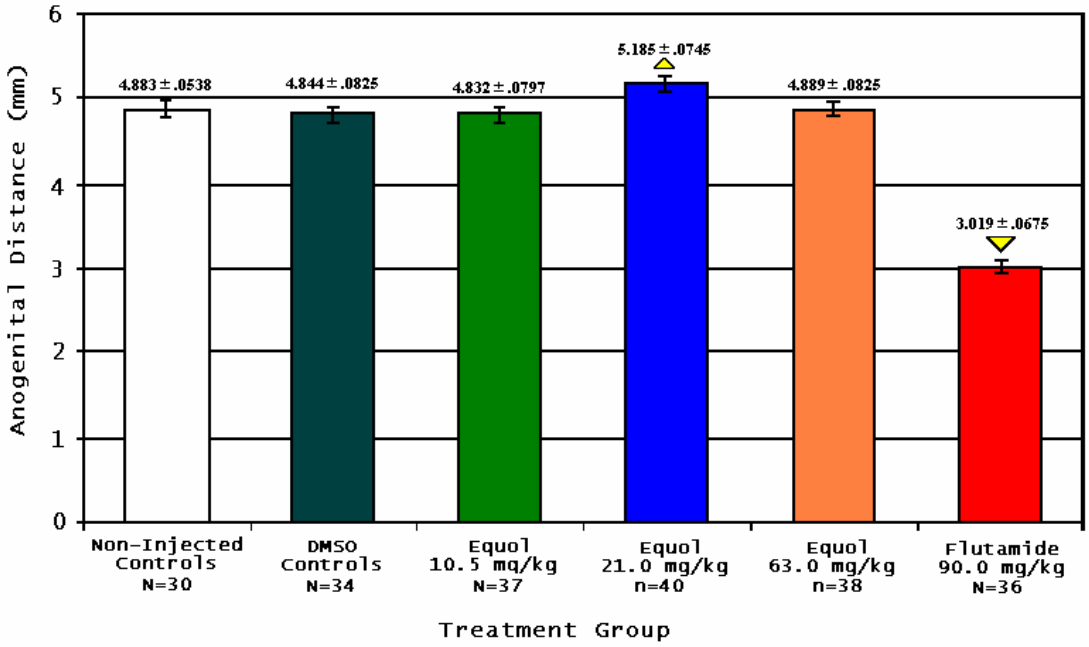

Figure 4: Prenatal Equol Treatment Birth Anogenital Distance (Males). ▼ Flutamide AGD was significantly less than all other groups. ▲ Equol 21.0 AGD was slightly but significantly greater than all other groups.

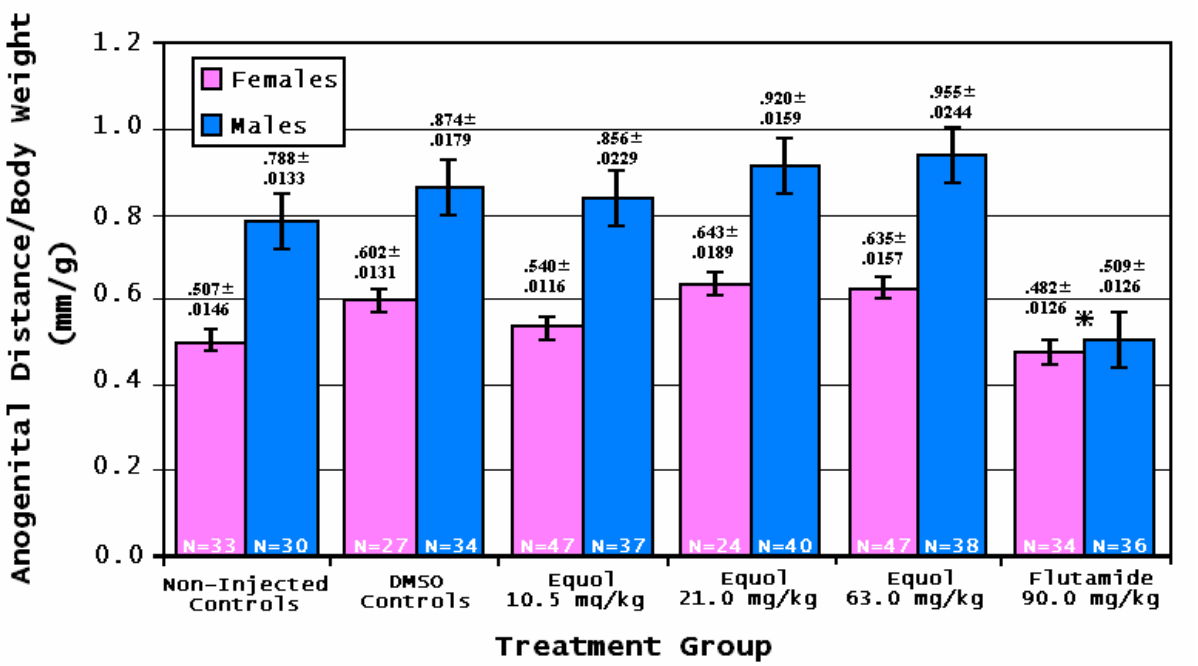

Figure 5: Prenatal Equol Treatment Anogenital Distance/Body Weight Ratio At Birth. * Only the flutamide group had no significant differences in AGD/Body Weight Ratio between males and females.

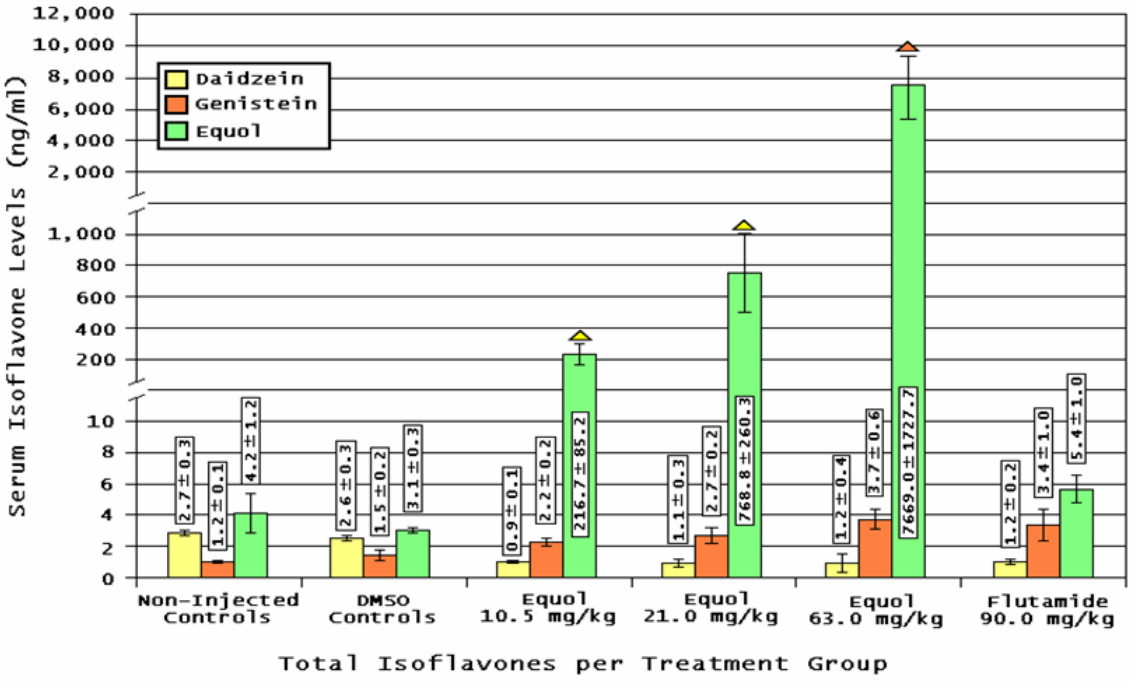

Figure 6: Prenatal Equol Treatment Mother Postnatal Serum Levels of Isoflavones. ▲ Equol 63.0 was significantly greater than all other groups. △ Equol 10.5 and Equol 21.0 were significantly greater than the control groups and flutamide and significantly different from each other.

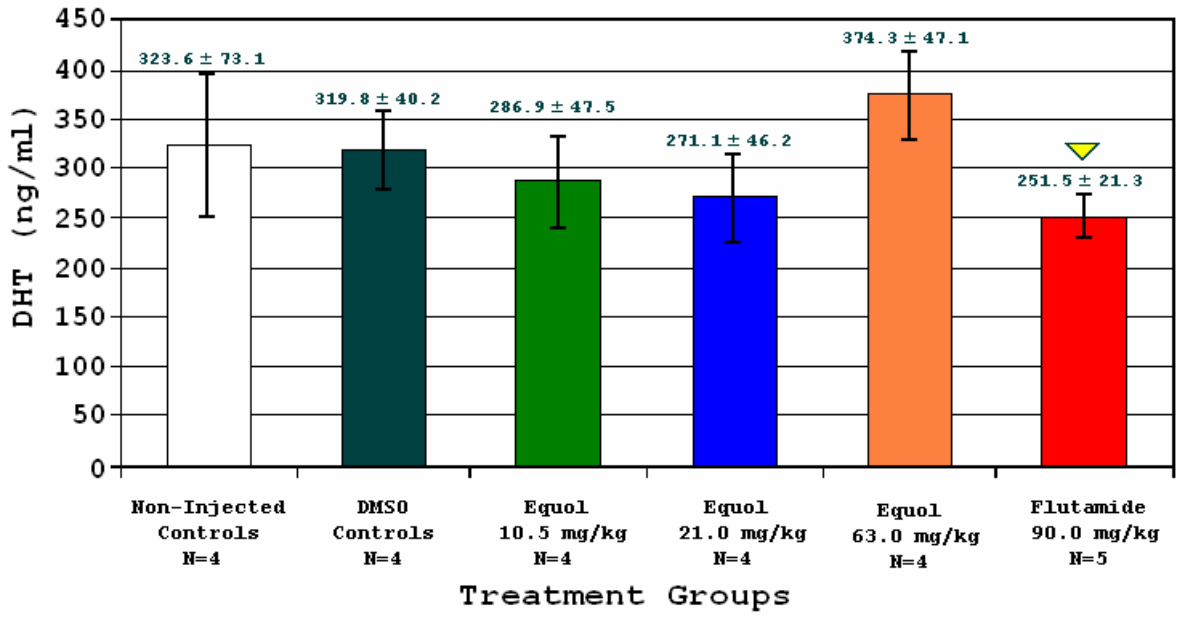

Figure 7: Prenatal Equol Treatment Mother Post Delivery Serum DHT Levels. ▽Flutamide levels were significantly less than Equol 63.0 but otherwise the levels were not significantly different from each other.

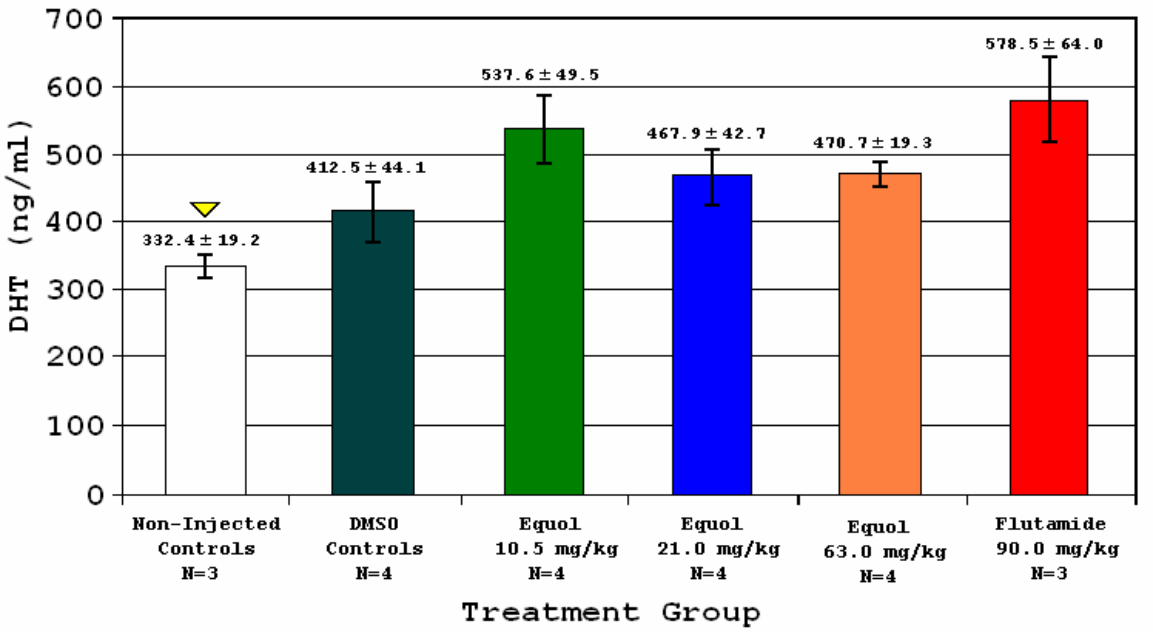

Figure 8: Prenatal Equol Treatment Female DHT Serum Levels. ▽ The non-injected controls had significantly less serum DHT levels than all other groups. The other groups were not significantly different from each other.

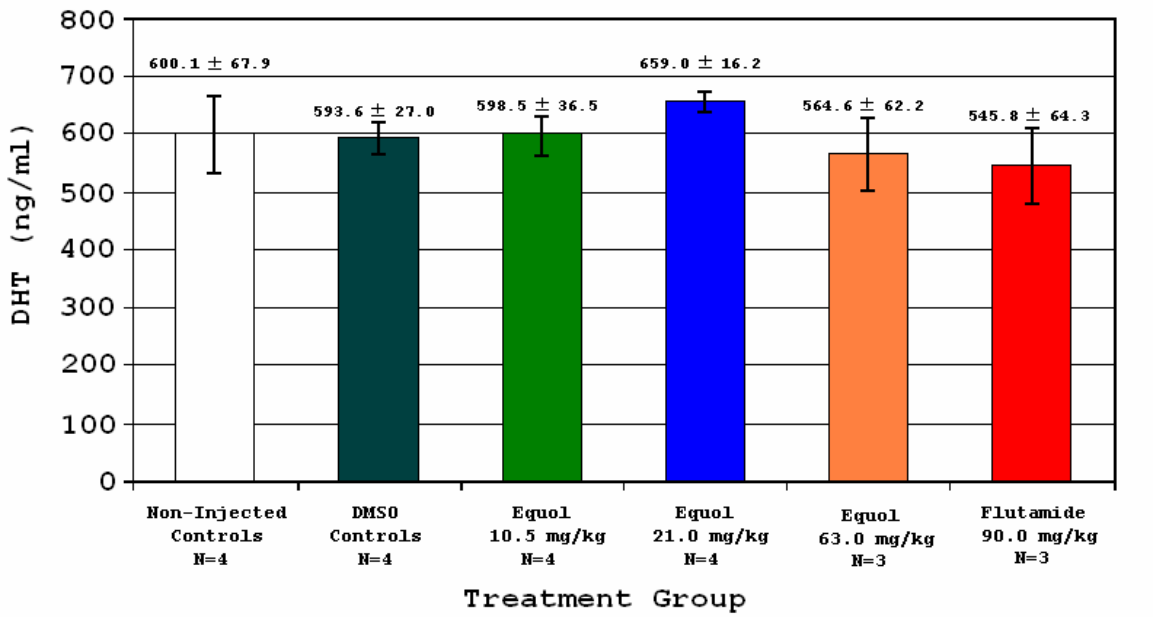

Figure 9: Prenatal Equol Treatment Male DHT Serum Levels. There were no significant differences in serum DHT levels between the males.

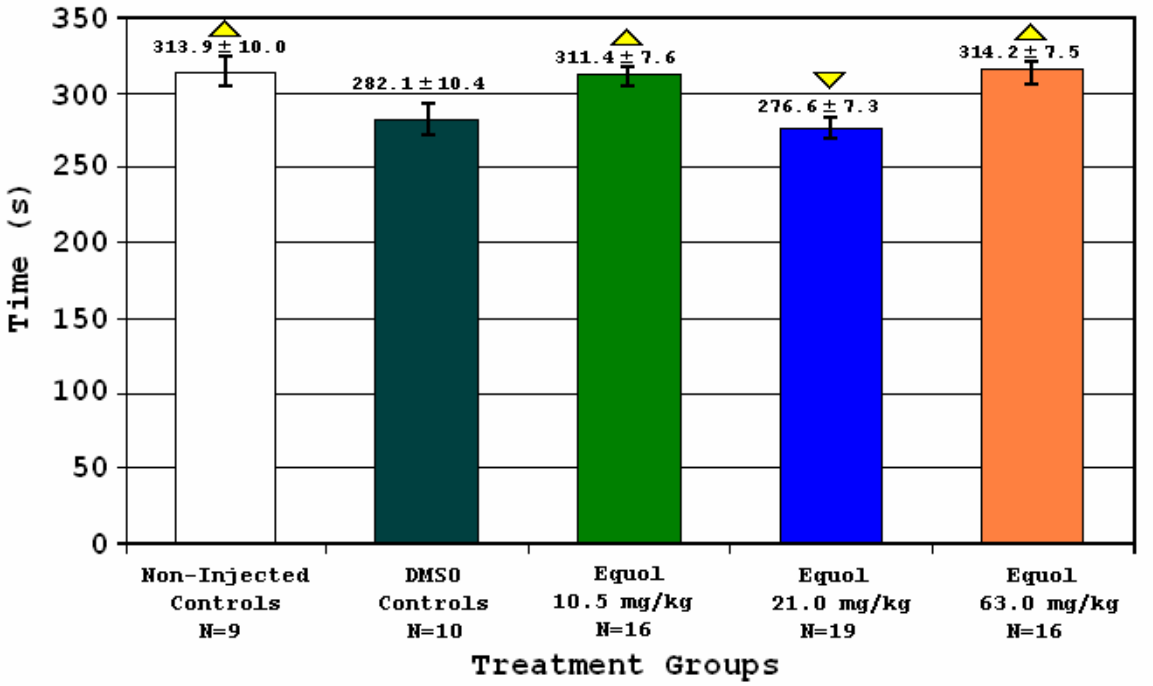

Figure 10: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Time Spent Mobile (Females). ▲ The non-injected controls, Equol 10.5 and Equol 63.0 were significantly more mobile than the DMSO controls. ▼ The Equol 21.0 was significantly less mobile than the non-injected controls and Equol 63.0 animals.

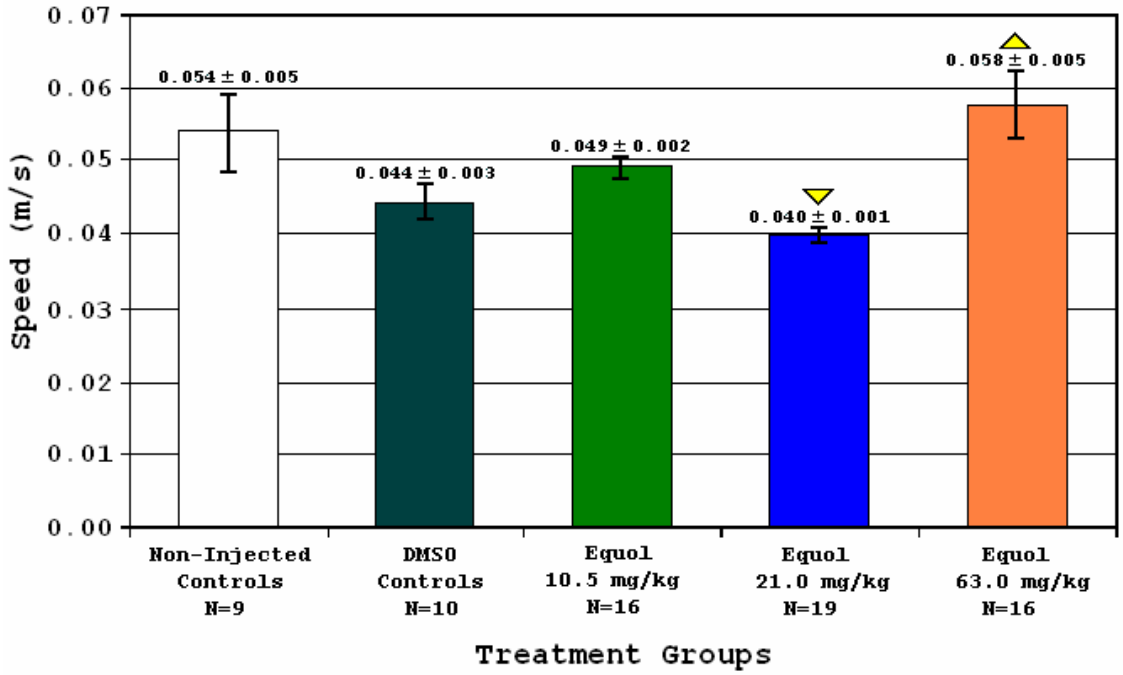

Figure 11: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Average Speed (Females). △ The Equol 63.0 animals swam significantly faster than the DMSO controls and Equol 21.0 animals. ▽ The Equol 21.0 animals swam significantly slower than all of the other animals except the DMSO controls.

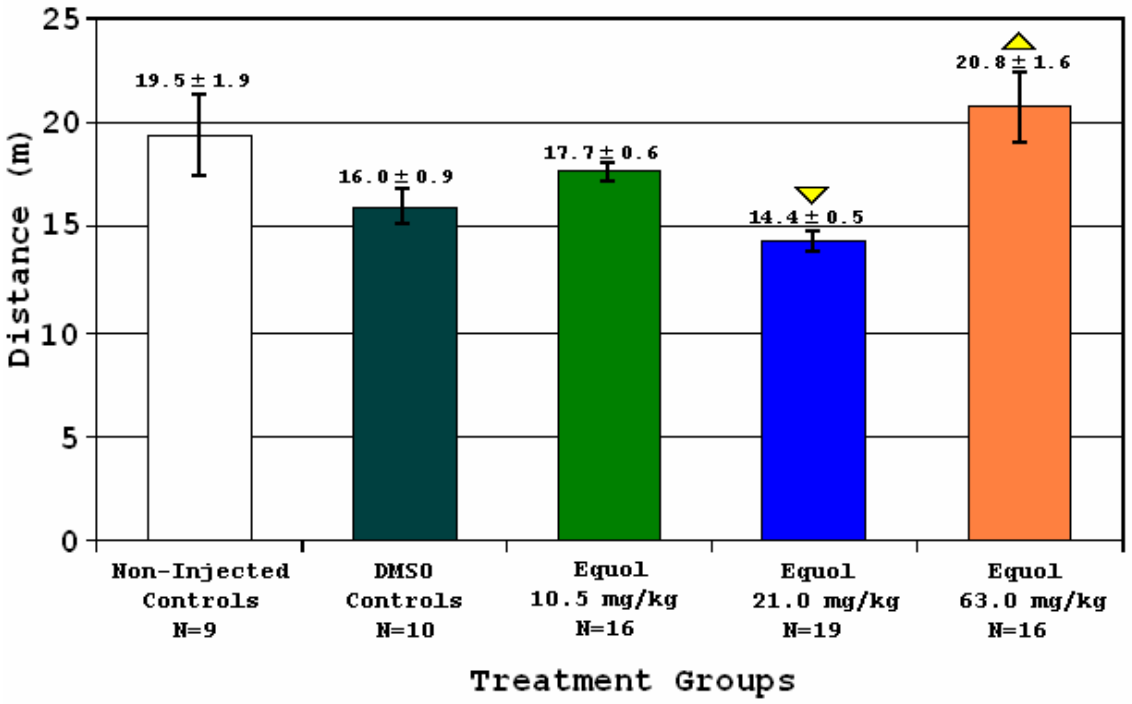

Figure 12: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Total Distance (Females). △ The Equol 63.0 animals swam significantly further than the DMSO controls and Equol 21.0 animals. ▽ The Equol 21.0 animals swam significantly less distance than all of the other animals except the DMSO controls.

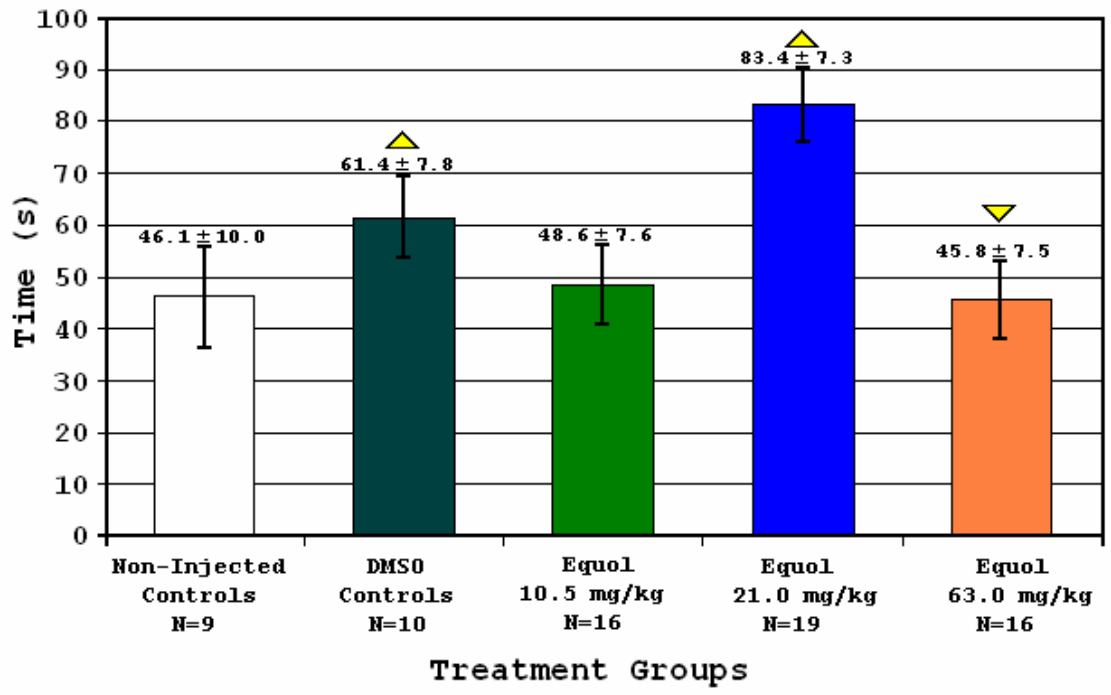

Figure 13: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Time Spent Immobile (Females). ▽ The Equol 63.0 females were significantly less immobile than the DMSO controls and Equol 21.0. △ The Equol 21.0 and DMSO animals were significantly more mobile than Equol 10.5 and Equol 63.0 animals.

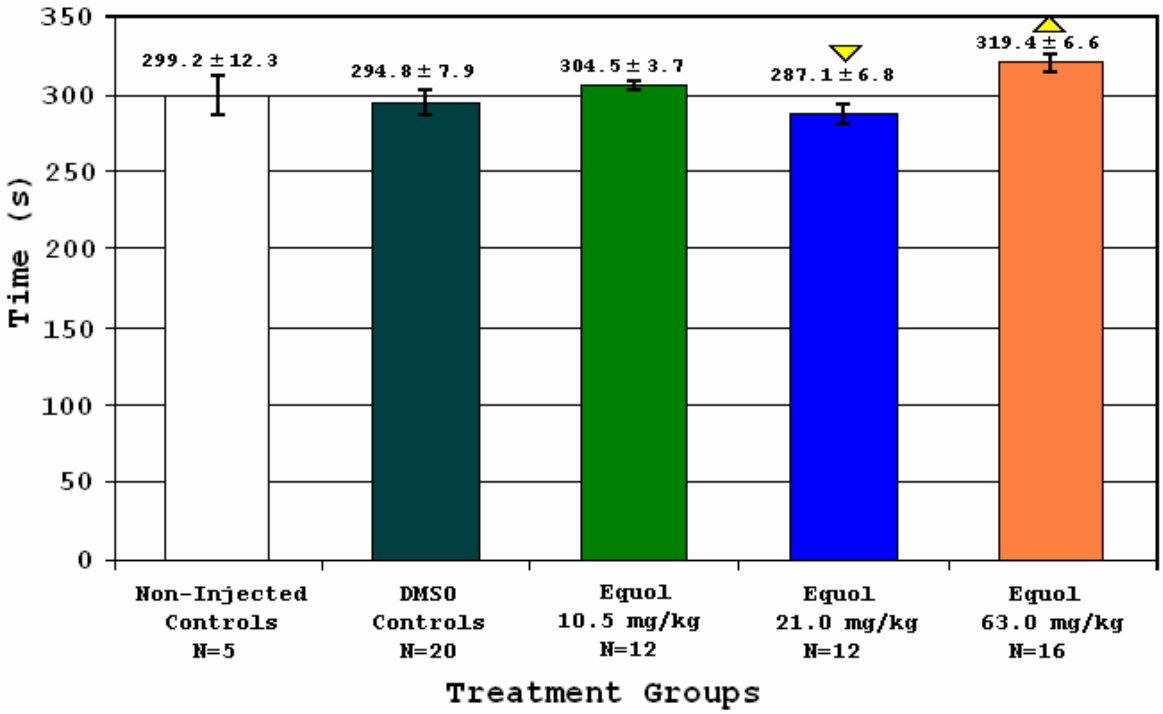

Figure 14: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Time Spent Mobile (Males). △ The Equol 63.0 groups spent significantly more time mobile than the DMSO controls and the Equol 21.0 rats. ▽ The Equol 21.0 rats also were significantly less mobile than the Equol 10.5 rats.

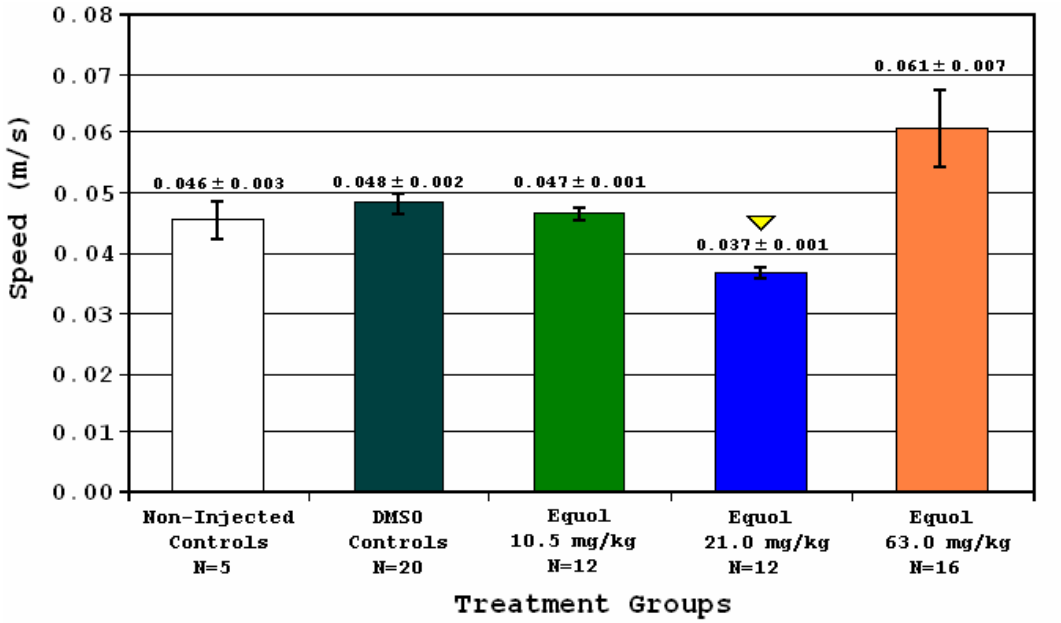

Figure 15: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Average Speed (Males). ▽The Equol 21.0 rats swam significantly slower than all of the other groups.

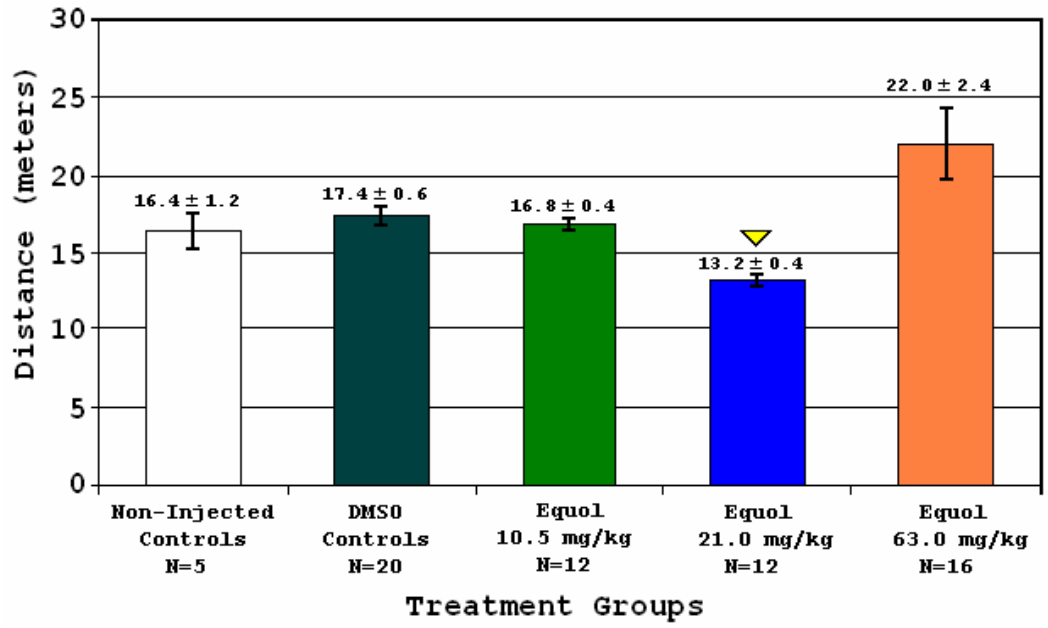

Figure 16: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Total Distance (Males). ▽The Equol 21.0 rats swam a significantly less distance than all of the other groups.

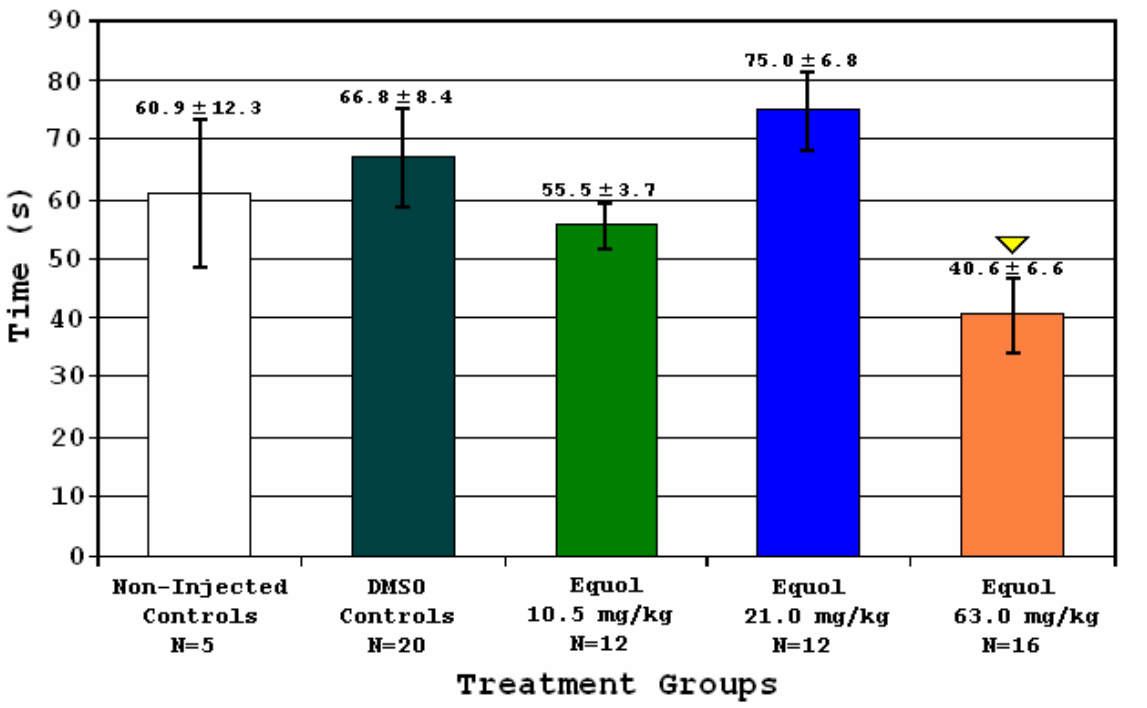

Figure 17: Prenatal Equol Treatment 29 Day Porsolt Forced Swim Test Time Spent Immobile (Males). ▽ The Equol 63.0 males were significantly less immobile than the DMSO controls and the Equol 21.0 group.

Females	Non-Inject n=33	DMSO n=27	Equol 10.5 n=47	Equol 21.0 n=24	Equol 63.0 n=47
Birth Weight	5.86 ± 0.098	5.22 ± 0.101	5.57 ± 0.084	5.16 ± 0.098 *	4.91 ± 0.086 **
	Non-Inject n=33	DMSO n=27	Equol 10.5 n=47	Equol 21.0 n=24	Equol 63.0 n=47
Birth to Day 29	76.5 ± 1.3	74.3 ± 1.3	79.6 ± 1.6	87.0 ± 0.4 #	87.5 ± 0.6 #
Day 29 Body Weight	82.4 ± 1.3	79.5 ± 1.3	85.2 ± 1.6	92.2 ± 0.4 #	92.4 ± 0.5 #
Males	Non-Inject n=30	DMSO n=34	Equol 10.5 n=37	Equol 21.0 n=40	Equol 63.0 n=38
Birth Weight	6.22 ± 0.065	5.59 ± 0.106	5.76 ± 0.144	5.66 ± 0.061	5.20 ± 0.112**
	Non-Inject n=30	DMSO n=34	Equol 10.5 n=37	Equol 21.0 n=40	Equol 63.0 n=38
Birth to Day 29	83.14 ± 0.7	88.2 ± 0.9	85.8 ± 1.6	95.4 ± 0.4 #*	98.2 ± 0.8 #
Day 29 Body Weight	89.4 ± 0.67	93.9 ± 0.96	91.4 ± 1.56	101.0 ± 0.37	103.4 ± 0.80 #

Table 1: Weight Gain from Birth to Postnatal Day 29. * Significantly less then non-injected controls. ** Significantly less than non-injected controls and DMSO controls. # Significantly greater than non-injected controls and DMSO controls. #* Significantly greater than non-injected controls.

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Conference Presentation

Positive Benefits of Consuming Soy-Derived Isoflavones on Body Weight Gain, Adipose Tissue Deposition and Preliminary Cardiovascular Parameters Examined in an Ovariectomized Rat Model, Poster presented at Experimental Biology Annual Meeting 2007, Washington D.C. April, 2007

Abstracts

K. Fabick, C. Blake, J.P. Porter, K.D.R. Setchell, E.D. Lephart. 2007. **Positive Benefits of Consuming Soy-Derived Isoflavones on Body Weight Gain, Adipose Tissue Deposition and Preliminary Cardiovascular Parameters Examined in an Ovariectomized Rat Model.** *Annual Mtg. Experimental Biology* 2007, Washington D.C., Apr. 28-May 2, 2007.

C. Blake, K. Fabick, E.D. Lephart. 2007. **Long-Evans Newborn Male Rats, Prenatally-Treated with Phytoestrogen Display Variations in Body Weight and External Genital Development**. *Annual Mtg. American Association of Pharmaceutical Scientist*, San Diego, CA, Nov. 11-15, 2007.

Publication

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