Effects of isoflavone supplementation on endometrial thickness, endometrial hyperplasia, and cancer in ovariectomized cats

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Endometrial hyperplasia, Endometrial thickness, Isoflavone supplementation, Ovariectomized cats.

Summary
Ovariectomy is identified as a standard treatment in different European countries. Isoflavones, as nonsteroidal compounds in plants, are common constituents of soy and soy products. Some available cat diets contain different concentrations of soy products. This study aimed to examine the effects of isoflavone supplementation on endometrial hyperplasia and endometrial thickness in ovariectomized cats. Fifteen neutered adult cats were divided into control, estradiol, and isoflavone groups (five cats per group). Subcutaneous injection of estradiol (0.5 µg) in sesame oil (100 µL) was done for 30 days in estradiol-treated cats. Isoflavone-treated cats ingested a single oral tablet of soy extract for 30 days, while the controls received subcutaneous injections of the vehicle and oral placebo for 30 days. Histopathological findings of hematoxylin and eosin-stained sections revealed a significant difference between the estradiol group and other groups in terms of hyperplastic epithelium and simple hyperplasia. Thickness of myometrium was greater in the estradiol group compared to the isoflavone and control groups. Higher concentrations of estrogen can affect the endometrium and myometrium, while 30-day ingestion of isoflavone didn't have any uterine effect.

Introduction

Elective sterilization of female cats (*Felis catus*) is a common procedure in veterinary practice. Generally, population control, elimination of unwanted behaviors due to hormonal cycling, and prevention of reproductive diseases are among the advantages of sterilization. In sterilization of female cats, only ovaries or ovaries and uterus can be removed (ovariectomy vs. ovariohysterectomy). Although in the United States and Canada, ovariohysterectomy is preferred, in many European countries, ovariectomy has become the standard procedure (Goethem *et al.* 2006). Ovariectomy has potential advantages such as improving the view of the ovarian pedicle, smaller incisions, and lower risk of complications due to surgical uterine manipulation (DeTora and McCarthy 2011).

Isoflavones are nonsteroidal compounds in plants. In particular they are common constituents of soy and soy products (Kurzer and Xu 1997). Isoflavones genistein and daidzein are known to cause changes in food intake, innate and acquired immunity, expression of steroid receptors, and lean body mass of domestic cats (Bell *et al.* 2008, Cave *et al.* 2007a, Cave *et al.* 2007b, Cave *et al.* 2007c), while soy diets lead to changes in the serum thyroid hormone (T4) (White *et al.* 2004).

Genistein and daidzein are the constituents of some available diets for sterile cats. The isoflavone content has been associated with the use of soy products in these diets (Quaas *et al.* 2013). Since the effects of isoflavone supplementation on the endometrium of cats are still unknown, and ovariectomized cats may receive soy-containing diets, we investigated the effects of isoflavone supplementation on endometrial thickness, endometrial hyperplasia, and cancer in ovariectomized cats.

Materials and methods

Animal husbandry

All cats (12-16 months of age, 3.2 ± 0.3 kg in weight) evaluated in this study were from a specific pathogen-free colony. Before the study, the animals were fed a commercial isoflavone-free diet for four weeks; the washout period was determined with respect to the half-life of conjugated genistein (22 hours) (Cave *et al.* 2007d). The animals remained on the same balanced, extruded dry diet (Whiskas with Savory Nuggets; original recipe, Masterfoods USA, Vernon, CA, USA).

The animals' diet consisted of 10% crude fat, 31% (wt/wt) crude protein, 1.8% crude fiber, 8% moisture, and 7% ash, without any soybean or by-products. The animals had free access to fresh food, which was prepared every morning; at the same time, the remaining food was weighed. Once the animals were housed in Semnan University Pet Care Center, they were placed in individual cages with *ad libitum* access to water (unless otherwise stated) at a constant temperature in a 12:12 h light-dark cycle. The animals were used according to the Animal Welfare Act, as well as the Guide for Care and Use of Laboratory Animals (National Research Council 1996). The Animal Use and Care Committee of Semnan University approved this study. Note that this project was ethically approved by ethics committee and the code number for our research was 25875 received from this committee.

Surgical neutering

After a 10-hour fasting period, water was withheld from the animals for two hours before inducing anesthesia. Queens were pre-medicated with atropine 0.005 mg/kg body weight (BW), subcutaneously (s.c.). Anaesthesia was induced with ketamine, 10 mg/kg BW, i.v., and diazepam, 0.2 mg/kg BW, intravenously (i.v.), and subsequently they were intubated and maintained on isoflurane (1.5%) in oxygen. Afterwards, bilateral ovariectomy, using standard techniques, was applied. Queens were maintained on lactated Ringer solution for one day and were administered ceftriaxone, 25 mg/kg BW, i.v., q12 h and tramadol, 5 mg/kg BW, per os (p.o.), q12 h for three days. Elizabethan collars were fitted to female cats and removed for suture removal after 10 days. After 30 days, the uteri were sampled from animals under general anesthesia after a hysterectomy.

Experimental design

The animals were divided into control, estradiol, and isoflavone groups (five cats per group) on experimental day 0. On day 1, treatment inititiated. The experimental design was completely randomized and fifteen cats were randomly allocated to three experimental groups. The estradiol group received subcutaneous injections of estradiol (0.5 µg) in sesame oil (100 µl) for 30 days. The isoflavone group ingested an oral soy extract tablet. Food was withheld for 18 hours before oral administration of the soy tablet containing 25 mg of genistein and 19 mg of daidzein (50 mg; Soyagol, Goldaru Pharmaceutical Co., Iran) for 30 days. Note that all cats received control treatments. The estradiol group received oral placebos, the isoflavone group was given vehicle injections, and the control group received oral placebos and subcutaneous vehicle injections. All treatments were performed every morning immediately before the fresh diet.

Uterine samples

Specimens were fixed in 10% nonbuffered formalin, and hematoxylin and eosin was employed to stain 4-µm tissue sections. A single pathologist (blind to regimens) interpreted and classified the results, based on standard criteria (Emons *et al.* 2015).

Histopathological analysis was performed in luminal epithelial and glandular endometrium, while the histometry of myometrial thickness was examined in the horn and body of the uterus in all groups. Histopathological findings in haematoxylin and eosin-stained sections were investigated in eight microscopic fields and captured by a camera, connected to an optic microscope in all samples.

Statistical analysis

Data were introduced in SPSS version 19.0. One-way ANOVA and Tukey's post-hoc tests were applied to examine myometrial thickness, while Wilcoxon signed rank test was performed to compare changes in epithelial hyperplasia, endometrial hyperplasia, and endometrial stromal edema. Post hoc pairwise comparisons were made using Mann-Whitney U test.

Results

A significantly hyperplastic epithelium was found in the estradiol group compared to others ($p < 0.05$, Table I, Figure 1, A-C).

According to the results, there was no atypical, or complex hyperplasia in endometrial glands in the groups, while typical hyperplasia was more frequently found in the estradiol group, compared to others ($p < 0.05$, Table I, Figure 2, A-C). Also, no significant difference was found between the groups in terms of stromal edema in the endometrium ($p > 0.05$, Table I, Figure 3, A-C). Myometrial thickness

was determined in scanned images of all samples across all groups. A significant difference was found between the estradiol group and others ($p < 0.05$, Table I, Figure 4, A-C).

Discussion

Despite of the reported uterine effects related to genistein intake in humans (Unfer *et al.* 2004), rats (Diel *et al.* 2004) and mice (Jefferson *et al.* 2002) so far, to the best of our knowledge, there were no studies testing the effect of genistein on the uterus in cats. Evidences of uterine hyperplasia has been reported following acute, high dose (53 mg/kg/day) (Rimoldi *et al.* 2007) and chronic, low dose (1.6 mg/kg/day) genistein treatments (Retana-Márquez *et al.* 2012). Our findings also indicated that 30 days treatment with 50 mg/day/cat did not determine endometrial hyperplasia, but treatment with estradiol resulted in endometrial hyperplasia. Hyperplasia could lead to cancer development (Salleh *et al.* 2013). Recently, genistein has been shown to promote the development of endometrial cancer in rats where administration of 150 mg/kg/day of this compound orally stimulates excessive epithelial proliferation (Kakehashi *et al.* 2012). The longest randomized clinical trial was conducted to evaluate the endometrial effects of phytoestrogen administration. The results suggested that endometrial hyperplasia increased (Unfer *et al.* 2004), while studies with a shorter duration showed a reduction in endometrial hyperplasia (Hale *et al.* 2001, Murray *et al.* 2003). In this study, we observed that isoflavone ingestion has no significant effects on glandular endometrium or histometry of myometrial thickness, compared to the control group. However, estrogen could affect uterine tissues, causing a significant increase in myometrial thickness. Similarly, Salleh and colleagues (Salleh *et al.* 2013) as well as Möller and colleagues (Möller

Table I. *Different histological classifications of uterine tissues in treated and control groups.*

Classification	Estradiol	Isoflavone	Control	P value
Epithelium hyperplasia n/n (%)	5/5 (100)*	0/5 (0)	0/5 (0)	< 0.05
Endometrial gland neoplastic hyperplasia n/n (%)	0/5 (0)	0/5 (0)	0/5 (0)	> 0.05
Endometrial gland atypical hyperplasia n/n (%)	0/5 (0)	0/5 (0)	0/5 (0)	> 0.05
Endometrial gland complex hyperplasia n/n (%)	0/5 (0)	0/5 (0)	0/5 (0)	> 0.05
Endometrial gland simple hyperplasia n/n (%)	5/5 (100)*	1/5 (20)	2/5 (40)	< 0.05
Endometrium stromal edema	5/5 (100)	2/5 (40)	3/5 (60)	> 0.05
Myometrium thickness (mm^2)	16.5*	3	6.4	< 0.05

*Statistically significant ($p < 0.05$).

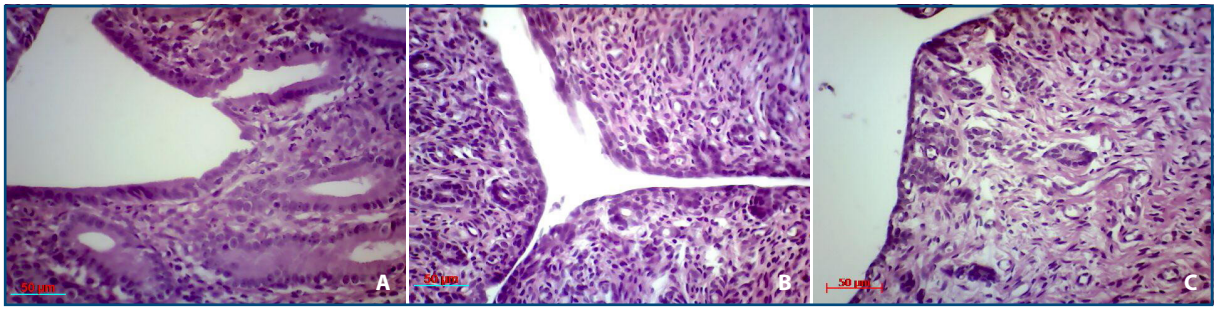

Figure 1. *The epithelium hyperplasia in groups of cats treated with estradiol (**A**), isoflavone (**B**) and placebos (control) (**C**) (Hematoxilin & Eosin staining, 400×).*

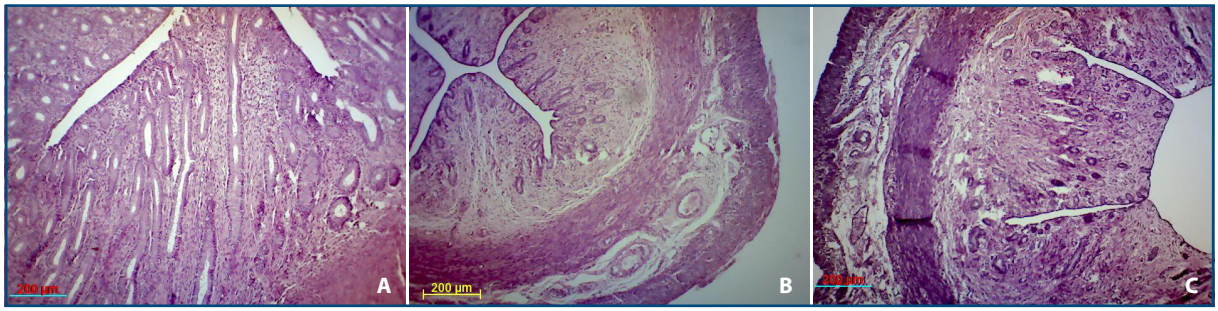

Figure 2. *Endometrial glands simple hyperplasia without any neoplastic, atypical and complex forms in groups of cats treated with estradiol (**A**, Hematoxilin & Eosin staining, 400×), isoflavone (**B**, Hematoxilin & Eosin staining, 100×) and placebos (control) (**C**, Hematoxilin & Eosin staining, 400×).*

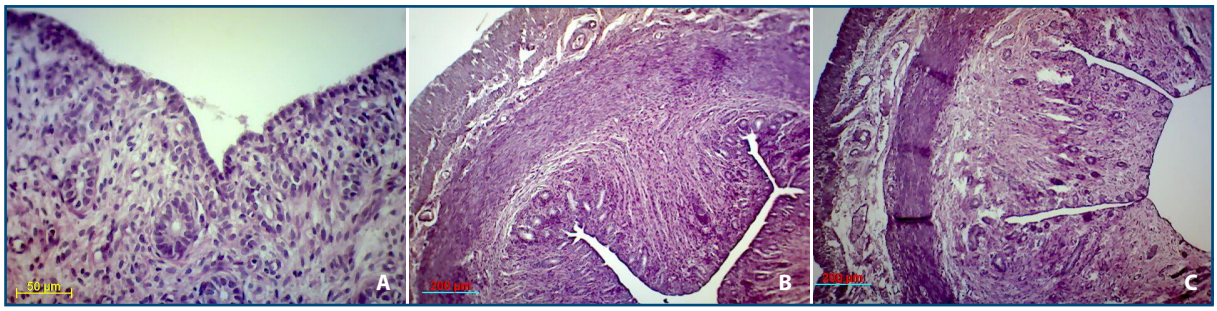

Figure 3. *Stromal edema as an excess liquid in the interstitial (extra cellular) space in endothelium in groups of cats treated with estradiol (***A***), isoflavone (***B***) and placebos (control) (***C***) (Hematoxilin & Eosin staining, 400×).*

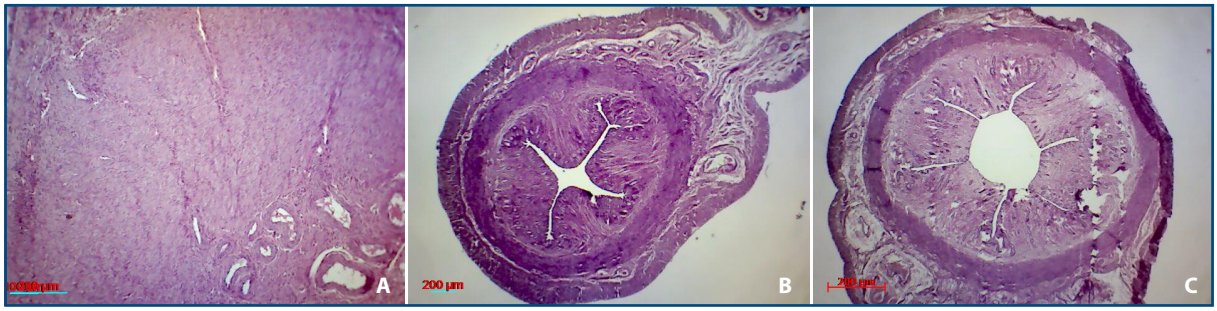

Figure 4. *Myometrium hyperplasia in groups of cats treated with estradiol (***A***), isoflavone (***B***) and placebos (control) (***C***) (Hematoxilin & Eosin staining, 50×).*

et al. 2012) reported that myometrial thickness was increased with genistein. They suggested that genistein stimulates myometrial hypertrophy.

In contrast with long-term studies, our results indicated that longer treatments with isoflavone had no endometrial effects. However, long-term treatment with higher doses of estrogen (estradiol injection) had endometrial and myometrium effects. The cause of difference between our results and the longest trial might be the duration of treatment along with the dose of isoflavone and species. However, since higher concentrations of estrogen affected the endometrium and myometrium in our study, and a previous long-term study confirmed the endometrial effects of isoflavone (Unfer *et al.* 2004), phytoestrogenic supplements can be effective in cats. However, longer experimental studies with isoflavones should be performed.

Conclusions

In conclusion, consumption of 50 mg/day isoflavones in 30 days had not significant effect on uterine tissue in ovarectomized cats, though higher doses or longer intervals should be studied.

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References

Bell K., Ugarte C., Tucker L., Roe W. & Thomas D. 2008. Assessment of reproductive histology and sex steroid receptor expression in the domestic cat (*Felis catus*) following chronic exposure to phytoestrogens. *Reprod Domest Anim*, **43**, 126.

Cave N., Backus R., Marks S. & Klasing K. 2007a. The bioavailability and disposition kinetics of genistein in cats. *J Vet Pharmacol Ther*, **30** (4), 327-335.

Cave N., Backus R., Marks S. & Klasing K. 2007b. Oestradiol and genistein reduce food intake in male and female overweight cats after gonadectomy. *N Z Vet J*, **55** (3), 113-119.

Cave N., Backus R., Marks S. & Klasing K. 2007c. Oestradiol, but not genistein, inhibits the rise in food intake following gonadectomy in cats, but genistein is associated with an increase in lean body mass. *J Anim Physiol Anim Nutr*, **91** (9-10), 400-410.

Cave N.J., Backus R.C., Marks S.L. & Klasing K.C. 2007d. Modulation of innate and acquired immunity by an estrogenic dose of genistein in gonadectomized cats. *Vet Immunol Immunopathol*, **117** (1-2), 42-54.

Diel P., Geis R.B., Caldarelli A., Schmidt S., Leschowsky U.L., Voss A. & Vollmer G. 2004.The differential ability of the phytoestrogen genistein and of estradiol to induce uterine weight and proliferation in the rat is associated with a substance specific modulation of uterine gene expression. *Mol Cell Endocrinol*, **221** (1-2), 21-32.

DeTora M. & McCarthy R.J. 2011. Ovariohysterectomy versus ovariectomy for elective sterilization of female dogs and cats: Is removal of the uterus necessary? *J Am Vet Med Assoc*, **239** (11), 1409-1412.

Emons G., Beckmann M., Schmidt D., Mallmann P. & Uterus commission of the Gynecological Oncology Working Group (AGO). 2015. New WHO classification of endometrial hyperplasias. *Geburtsh Frauenheilk*, **75** (2), 135-136.

Goethem B., Schaefers-Okkens A. & Kirpensteijn J. 2006. Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits of either technique. *Vet Surg*, **35** (2), 136-143.

Hale G.E., Hughes C.L., Robboy S.J., Agarwal S.K. & Bievre M. 2001. A double-blind randomized study on the effects of red clover isoflavones on the endometrium. *Menopause*, **8** (5), 338-346.

Jefferson W.N., Padilla-Banks E., Clark G. & Newbold R.R. 2002. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. *J Chrom B*, **777** (1-2), 179-189.

Kakehashi A., Tago Y., Yoshida M., Sokuza Y., Wei M., Fukushima S. & Wanibuchi H. 2012. Hormonally active doses of isoflavone aglycones promote mammary and endometrial carcinogenesis and alter the molecular tumor environment in Donryu rats. *Toxicol Sci*, **126** (1), 39-51.

Kurzer M.S. & Xu X. 1997. Dietary phytoestrogens. *Ann Rev Nutr*, **17** (1), 353-381.

Murray M.J., Meyer W.R., Lessey B.A., Oi R.H., DeWire R.E. & Fritz M.A. 2003. Soy protein isolate with isoflavones does not prevent estradiol-induced endometrial hyperplasia in postmenopausal women: a pilot trial. *Menopause*, **10** (5), 456-464.

Möller F.J., Ledwig C., Zierau O., Hertrampf T., Degen G.H., Diel P. & Vollmer G. 2012. The rat prepubertal uterine myometrium and not the luminal epithelium is predominantly affected by a chronic dietary genistein exposure. *Arch Toxicol*, **86** (12), 1899-1910.

National Research Council (US). 1996 . Guide for the care and use of laboratory animals Institute for Laboratory Animal Research. National Academies Press, Washington DC.

Quaas A.M., Kono N., Mack W.J., Hodis H.N., Felix J.C., Paulson R.J. & Shoupe D. 2013. The effect of isoflavone soy protein supplementation on endometrial thickness, hyperplasia and endometrial cancer risk in postmenopausal women: a randomized controlled trial. *Menopause*, **20** (8), 840.

Retana-Márquez S., Aguirre F.G., Alcántara M., García-Díaz E., Muñoz-Gutiérrez M., Arteaga-Silva M. & Delgadillo J.A. 2012. Mesquite pod extract modifies the reproductive physiology and behavior of the female rat. *Horm Behav*, **61** (4), 549-558.

Rimoldi G., Christoffel J., Seidlova-Wuttke D., Jarry H. & Wuttke W. 2007. Effects of chronic genistein treatment in mammary gland, uterus, and vagina. *Environ Health Perspect*, **115** (Suppl 1), 62.

Salleh N., Baines D.L., Naftalin R.J. & Milligan S.R. 2005. The hormonal control of uterine luminal fluid secretion and absorption. *J Membrane Biol*, **206** (1), 17-28.

Unfer V., Casini M.L., Costabile L., Mignosa M., Gerli S. & Di Renzo G.C. 2004. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. *Fertil Steril*, **82** (1), 145-148.

White H.L., Freeman L.M., Mahony O., Graham P.A., Hao Q. & Court M.H. 2004. Effect of dietary soy on serum thyroid hormone concentrations in healthy adult cats. *Am J Vet Res*, **65** (5), 586-591.