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The Impact of Dietary Organic and Transgenic Soy on the Reproductive System of Female Adult Rat

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ABSTRACT

The goal of this article was to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile and ovary and uterus morphology. Wistar rats were fed three different diets from weaning until sacrifice at 15 months of age. The three diets were: casein-based diet control group (CG), organic soy-based diet group (OSG), or transgenic soy-based diet group (GMSG). There were no differences in food consumption or in the diet isoflavone components among the groups. Compared with the CG diet, both the OSG and GMSG diets were associated with significant reductions in body weight, serum triglycerides, and cholesterol ($P < 0.05$) (CG = 406 ± 23.1; 104.3 ± 13.2; 119.9 ± 7.3 GMSG = 368 ± 17.6; 60.3 ± 4.6; 83.3 ± 5.7 OSG = 389 ± 23.5; 72.3 ± 12.5; 95.5 ± 8.0, respectively). The volume density of endometrial glandular epithelium was greater in the GMSG group (29.5 ± 7.17, $P < 0.001$) when compared with the CG (18.5 ± 7.4) and OSG (20.3 ± 10.6) groups. The length density of endometrial glandular epithelium was shorter in both GMSG (567.6 ± 41.1) and OSG (514.8 ± 144.5) diets compared with the CG ($P < 0.05$) diet. GMSG also resulted in reduced follicle number and increased corpus luteum number compared to the OSG or CG diets ($P < 0.05$). In summary, both GMSG and OSG diets resulted in decreased body weight and lower serum triglyceride and cholesterol levels, and alterations in uterine and ovarian morphology were also observed. The prolonged use of soy-based diets and their relation to reproductive health warrants further investigation. Anat Rec, 292:587–594, 2009.

Key words: transgenic soy; endometrium; ovary; estradiol; cholesterol

Concerns have recently been raised regarding potential risks with soy protein formulae, in particular regarding their high phytoestrogenic isoflavone content. The main consumers for soy consumption include infants with severe lactose intolerance, glactosemia, dietary protein allergy, and infants of vegetarian parents (Turck, 2007). Soyfood has also been used to improve cardiovascular disease risk factors (Anthony et al., 1996; Bairey et al., 2006; Kohno et al., 2006) and to reduce risk, development, or incidence of breast cancer (Jin and Mac Donald, 2002; Liu et al., 2005).

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TABLE 1. Chemical composition of diets

	CG	GMSG	OSG
Water %	2.61 ± 0.51	2.72 ± 0.06	2.17 ± 0.01
Fat %	7.88 ± 0.12	8.53 ± 0.14	8.17 ± 0.11
Minerals %	1.67 ± 0.01	2.11 ± 0.08	2.14 ± 0.06
Protein %	10.95 ± 0.43	12.96 ± 1.18	11.05 ± 0.31
Fiber %	4.78 ± 0.75	4.88 ± 0.43	5.01 ± 0.36
Carbohydrate %	73.87	69.66	69.45
Calories/100 g	1,522.766 kJ	1,522.766 kJ	1,522.766 kJ

CG, control group; GMSG, genetically modified soy group; OSG, organic soy group. Results are present as mean ± standard deviation of three determinations.

Several studies have demonstrated a relationship between soy consumption and uterine and ovarian morphology and function. The majority report exposure to soy or any soy derivative during neonatal or adult life can cause abnormal estrous cycles, altered ovarian function, early reproductive senescence, subfertility/infertility, and uterotropic effects. These findings lead to questions about the safety of soy-based food consumption by women of reproductive age (Jefferson et al., 2002, 2005, 2006; Michael et al., 2006; Piotrowska et al., 2006; Rachon et al., 2007a,b).

Increased dietary soy consumption has lead to the development of transgenically produced soy to increase production and reduce associated cost (Rott et al., 2004). Transgenic soy is a genetically modified organism to which three foreign genes are added, one of them from a virus and the others from a bacterium found in soil. This modification provides the soy plant with resistance to glyphosate herbicides used to destroy weeds that compete with the crop.

On the other hand, organic soy is grown in an ecological manner, without chemical products that would contaminate or modify the product. Organically produced soy, however, results in a significant loss in productivity and increased cost (Magkos et al., 2003).

On the basis of the concern about the use of genetically modified food on health and the lack of data on the effects of transgenic soy on the reproductive female system, the goal of this article was to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile in serum, and ovary and uterus morphology.

MATERIAL AND METHODS

Animals

The handling of the animals was approved by the Animal Care and Use Committee of State University of Rio de Janeiro, which based their analysis on the Guide for the Care and Use of Laboratory Animals (Bayne, 1996), and the study design was approved by the local Ethical Committee for the care and use of laboratory animals.

The biological assay was conducted on 24 female Wistar rats from the Laboratory of Experimental Nutrition (LABNE) of the Department of Nutrition and Dietetics, Nutrition College, Fluminense Federal University. The rats were divided into three groups of eight animals each, which received the experimental diets, as follows: control group (CG) fed a casein-based diet, organic soy group (OSG) fed an organic soy-based diet supplemented with 0.3 g cysteine, and a genetically modified soy group (GMSG) receiving a transgenic soy-based diet. As recommended by the American Institute of Nutrition-93, cystine was added to the OSG diet as a metionine precursor. However, because of concerns about genetic modification, we decided to not make an additional manipulation of the transgenic soy-based diet, so no cysteine was added to this diet.

During the studies, the rats were kept in polypropylene cages, in an environment with controlled temperature at 22°C and a 12-h light/dark period. Water and diets were offered ad libitum. Food consumption and animal weight were recorded daily.

To evaluate the prolonged use of soy by two generations, the animals used in this study were the offspring of parents (preceding generation) who also received the same diet throughout their lives. The animals were fed the above diets exclusively, from weaning until they were 15 months old. At the end of this period, the animals were euthanized under thiopental anesthesia (0.10 mL/100 g body weight), blood collection was made through cardiac puncture, and serum stored at −20°C to determine 17β-estradiol, cholesterol, and triglyceride serum levels. The left ovary and horn of the uterus were carefully removed, weighed, and fragmented according the Ortrip method (Mandarim-de-Lacerda, 2003). The material obtained was fixed in formalin (pH 7.2) and processed following the routine histological procedures for embedment in paraffin. Sections of 5 μm of thickness were stained by the hematoxylin and eosin for the analysis of the integrity of the specimens and exclusion of the samples that had artifacts.

Diets

Transgenic soy was supplied by Jasmine Integral Foods (Curitiba, PR, Brazil) and organic soy was supplied by Bunge Foods (Porto Alegre, RS, Brazil). The soybeans were processed as described in Soares et al. (2005) to minimize the antinutritional factors, and then the beans were used as the protein source for diet preparation. All diets were prepared in the LABNE according to the rules of the Committee on Laboratory Animal Diets, 1979, modified according to the recommendations of the American Institute of Nutrition-93 (Reeves et al., 1993) and the chemical composition are shown in Table 1. The ingredients of the diets were homogenized in an industrial mixer with boiling water. The mass obtained was transformed into tablets, which were dried in a ventilated oven at 60°C for 24 h, properly identified and stored refrigeration until the time for use.

The isoflavone content was determined as described by Klump et al. (2001). Briefly, samples of organic and transgenic soy were extracted at 65°C with methanol-

TABLE 2. Total and individual isoflavone components of diets (mg/g diet)

Groups	Total Isofl (mg/g)	Daidzein (mg/g)	Genistein (mg/g)	Daidzin (mg/g)	Glicitin (mg/g)	Genistin (mg/g)
GMSG	0.396 ± 0.03	0.032 ± 0.003	0.038 ± 0.003	0.063 ± 0.002	0.014 ± 0.002	0.249 ± 0.05
OSG	0.384 ± 0.04	0.030 ± 0.004	0.034 ± 0.002	0.067 ± 0.005	0.018 ± 0.001	0.235 ± 0.06

GMSG, genetically modified soy group; OSG, organic soy group. Data are reported as mean ± standard deviation of eight animals. The CG (control group) did not contain isoflavones.

TABLE 3. Body, ovary, and uterus weights, ovary and uterus relative weight, cholesterol, triglycerides, and estradiol serum levels

	CG Mean ± SD	GMSG Mean ± SD	OSG Mean ± SD
Body weight (g)	406 ± 23.1[a]	368 ± 17.6[b]	389 ± 23.5[a]
Ovary weight (g)	0.1 ± 0.03[a]	0.1 ± 0.02[a]	0.1 ± 0.02[a]
Ovary relative weight (mg tissue/g body weight)	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]
Uterus weight (g)	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.1[a]
Uterus relative weight (mg tissue/g body weight)	0.1 ± 0.03[a]	0.1 ± 0.03[a]	0.1 ± 0.04[a]
Cholesterol (mg/dL)	119.9 ± 7.3[a]	83.3 ± 5.7[b]	95.5 ± 8.0[b]
Triglycerides (mg/dL)	104.3 ± 13.2[a]	60.3 ± 4.6[b]	72.3 ± 12.5[b]
Estradiol (pg/dL)	149.3 ± 1.0[a]	94.7 ± 15.4[b]	102 ± 6.1[b]

CG, control group; GMSG, genetically modified soy group; OSG, organic soy group.
Values are given as mean ± standard deviation of eight animals. Different superscript letter in the same row means statistically significant differences.

water (80 + 20), saponified with dilute sodium hydroxide solution, and analyzed by reversed phase liquid chromatography, with UV detection at 260 nm. The data were analyzed for individual isoflavone components, subtotals of daidzin, daidzein, glycitin, genistin, and genistein (Table 2).

Stereological Parameters

Sections of 5 μm of thickness were stained with Gomoris' Trichrome (Bradbury and Rae, 1996). The M42 multipurpose test-system was used to quantify the endometrial compartment of the uterus (Mandarim-de-Lacerda, 2003). From each uterus, five different sections were selected from five fragments. Then, five random fields were evaluated from each section at 200× final magnification. Therefore, there were 25 test areas from each uterus. The stereological parameters analyzed were: (1) Volumetric density (Vv) of the glandular epithelium and (2) Length density (Lv) of the endometrial glands (Lv = 2 QA (mm/mm^3), where QA is the number of the glandular profiles in the test area).

Morphologic Classification of Follicles

Sections stained by hematoxylin and eosin (Bradbury and Rae, 1996) were taken at intervals of 50 μm to avoid the same follicle being counted twice. The 5 μm sections were digitized using a video camera coupled to a light microscope with 400× final magnification for primordial follicles and 100× for primary, preantral, antral, and Graafian follicles, and corpus luteum. Follicle types in ovarian cross-sections were defined as follows. Primary follicles comprised an oocyte surrounded by a single layer of cuboidal granulosa cells. Preantral follicles comprised an oocyte surrounded by two or more layers of granulosa cells with no antrum. Antral follicles were distinguished by the presence of an antrum within the granulosa cell layers enclosing the oocyte (Cheng et al., 2002).

Biochemical Analysis

Cholesterol and triglycerides serum concentration were determined by a cholorimetric method (Bioclin, Belo Horizonte, MG, Brazil). 17β-Estradiol serum concentration was determined by radioimunoassay, using a commercial kit (Solid Phase Component System, INC Pharmaceuticals). The sensitivity of the kit was 0.13 pg/dl, and the intra and inter-assay variation coefficients were of 5.5% and 5.3%, respectively.

Statistical Analysis

The data are reported as mean ± standard deviation of eight animals. Statistical significance of experimental observations was determined by ANOVA, followed by Newman Keuls pos + hoc test. The level of significance was set at $P < 0.05$ (Sokal and Rohlf, 1995).

RESULTS

The chemical composition of diets is shown in Table 1. The data related to the total and individual isoflavone components of diets as daidzein, genistein, daidzin, glicitin, and genistin are shown in Table 2. There was no significant difference in the isoflavone components of diets. The food consumption per 100 g of body weight was the same among the groups.

Table 3 shows the body and organs weights, cholesterol, triglycerides, and estradiol serum levels of all

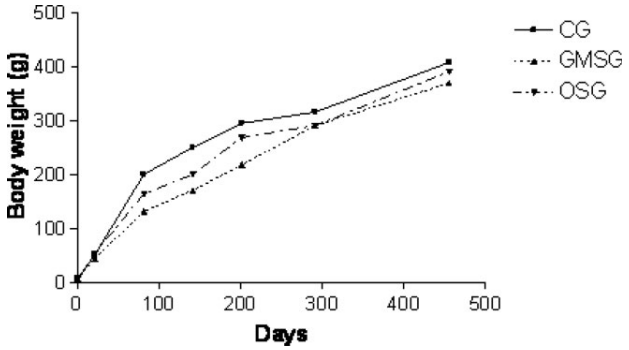

Fig. 1. Body weight from birth to adult age of the control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG).

groups. Both OSG and GMSG groups had lower body weight when compared with CG, but this reduction was significant only in the GMSG ($P < 0.05$). There was no significant difference in the ovary or uterus absolute and relative weights (mg of tissue/g body weight) for the GMSG and OSG compared with the CG. Both GMSG and OSG groups demonstrated lower serum level of estradiol $P < 0.01$) than the CG. In relation to lipid profile, both GMSG and OSG groups demonstrated lower triglyceryde ($P < 0.05$) and cholesterol (CG vs. OSG = $P < 0.05$; CG vs. GMSG = $P < 0.01$) serum levels than the CG.

Body weight history from birth until adult age is shown in Fig. 1. Figure 2 shows the number of different classes of follicles and corpora lutea in the three examined groups. The number of primordial, primary,

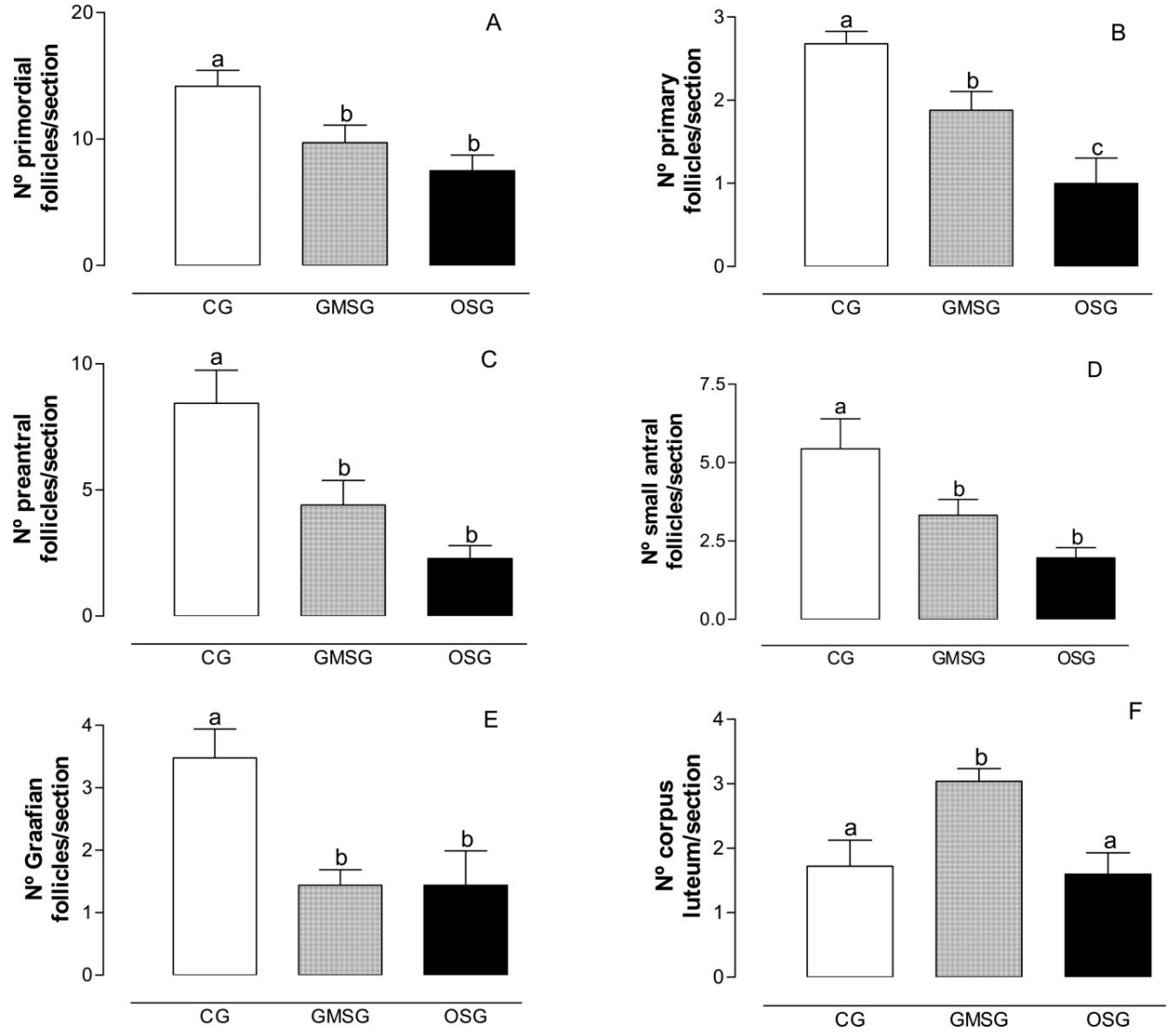

Fig. 2. The number of primordial (**a**), primary (**b**), preantral (**c**), small antral (**d**), graafian (**e**) follicles, and corpus luteum (**f**) in control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG). Values are given as mean ± standard deviation of 8 animals. Different superscript letter means statistical significant differences.

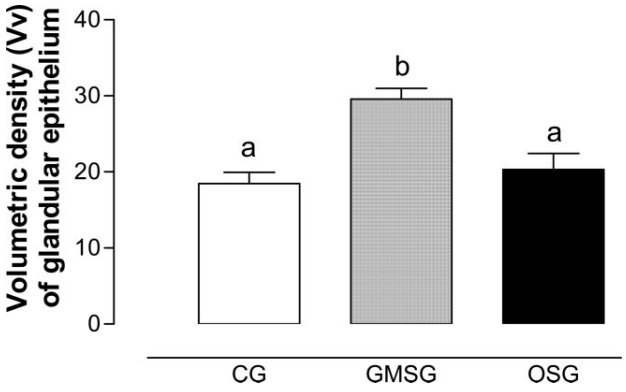

Fig. 3. Volumetric density of glandular epithelium of the control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG). Values are given as mean ± standard deviation of eight animals. Different superscript letter means statistical significant differences.

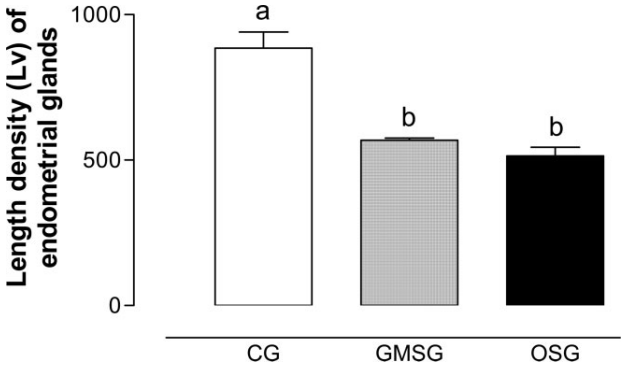

Fig. 4. Length density of endometrial glands of the control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG). Values are given as mean ± standard deviation of eight animals. Different superscript letter means statistical significant differences.

preantral, small antral, and Graafian follicles was significantly reduced in both GMSG ($P < 0.05$) and OSG ($P < 0.01$) groups when compared with the CG. The number of corpus luteum was significantly ($P < 0.05$) increased only in the GMSG when compared with the CG. The number of primary follicles and corpus luteum was significantly different ($P < 0.05$) between the GMSG and OSG groups.

The morphometric analysis of the uterus showed that volumetric density of epithelium was significantly higher in the GMSG compared with both CG ($P < 0.01$) and OSG ($P < 0.05$) (Fig. 3). Both soy groups presented a significant reduction in the length density of endometrial glands ($P < 0.01$) when compared with the CG (Fig. 4).

Figure 5 show histological sections of ovary (A, B, and C) in CG, GMSG and OSG groups, respectively. Primary follicles consist of an oocyte surrounded by a single layer of cuboidal granulosa cells. The preantral follicles present a central oocyte surrounded by several layers of granulosa cells and bounded by thecal cells, which form a fibrous theca externa and an inner theca interna with no antrum. In antral follicles, fluid appeared between the granulosa cells, and the drops coalesced to form follicular fluid within the follicular antrum. In Graafian follicles, the follicular antrum is clearly developed, leaving the oocyte surrounded by a distinct and denser layer of granulosa cells, the cumulus oophorus. The corpus luteum is formed by luteal cells and abundant capillaries.

Figure 6 show histological sections of uterus (A, B, and C) in CG, GMSG, and OSG groups, respectively. The endometrial and glandular epithelium, stroma, and myometrium can be observed in the photomicrographs.

DISCUSSION

Soyfood has been reported to have beneficial effects including improving the lipid profile (Simons et al., 2000; Dent et al., 2001; Gardner et al., 2001; Kang et al., 2002; Engelman et al., 2005; Ho et al., 2007), bone metabolism (Marini et al., 2007; Ma et al., 2007), cancer development (Jin and MacDonald, 2002; Liu et al., 2005), without having any effects on the uterus and ovary in postmenopausal women or menopausal animals models (Bahr et al., 2005; Castillo et al., 2006; Kaari et al., 2006; Wood et al., 2006; Marini et al., 2007). However, if soyfood is used for neonatal, young, or adult animals at a reproductive age, it can cause adverse effects related to the reproductive organs (Jefferson et al., 2002, 2005, 2006; Michael et al., 2006; Piotrowska et al., 2006; Rachon et al., 2007a,b). On the basis of the soy consumption increment, this study was designed to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile and ovary and uterus morphology.

Although the use of genetically modified food is still questionable, there is no evidence that genetic modification through biotechnology will impose immediate significant risks as food allergen sources beyond that of our daily dietary intake of foods from crop plants (Helm, 2003) or beyond other methodologies widely accepted in the food industry (Lack, 2002). Also, there is no evidence suggesting that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material (Jennings et al., 2003; Sharma et al., 2006). The data presented here show that the effects of organic and transgenic soy consumption were very similar, except those observed in the corpora lutea and volumetric density of glandular epithelium.

In agreement with the literature, both organic and transgenic soy reduced the body weight (Demonty et al., 2002; Rachon et al., 2007a) and estradiol serum levels (Lu et al., 1996; Nagata et al., 1997; Duncan et al., 1999; Wood et al., 2007). Both soy treatments also improved the lipid profile by reducing cholesterol and triglycerides serum levels (Simons et al., 2000; Dent et al., 2001; Gardner et al., 2001; Kang et al., 2002; Engelman et al., 2005; Ho et al., 2007). Probably the reduction in estradiol serum levels reflects the capacity of isoflavones to bind the estrogen receptor and blocking the actions of endogenous estrogens (Lissin and Cooke, 2000). The alterations presented here were more marked in the transgenic group, which showed the lowest body weight and cholesterol and triglycerides levels.

Also, corroborating previous results (Jefferson et al., 2002, 2005, 2006; Michael et al., 2006; Piotrowska et al., 2006; Rachon et al., 2007a,b), both transgenic and

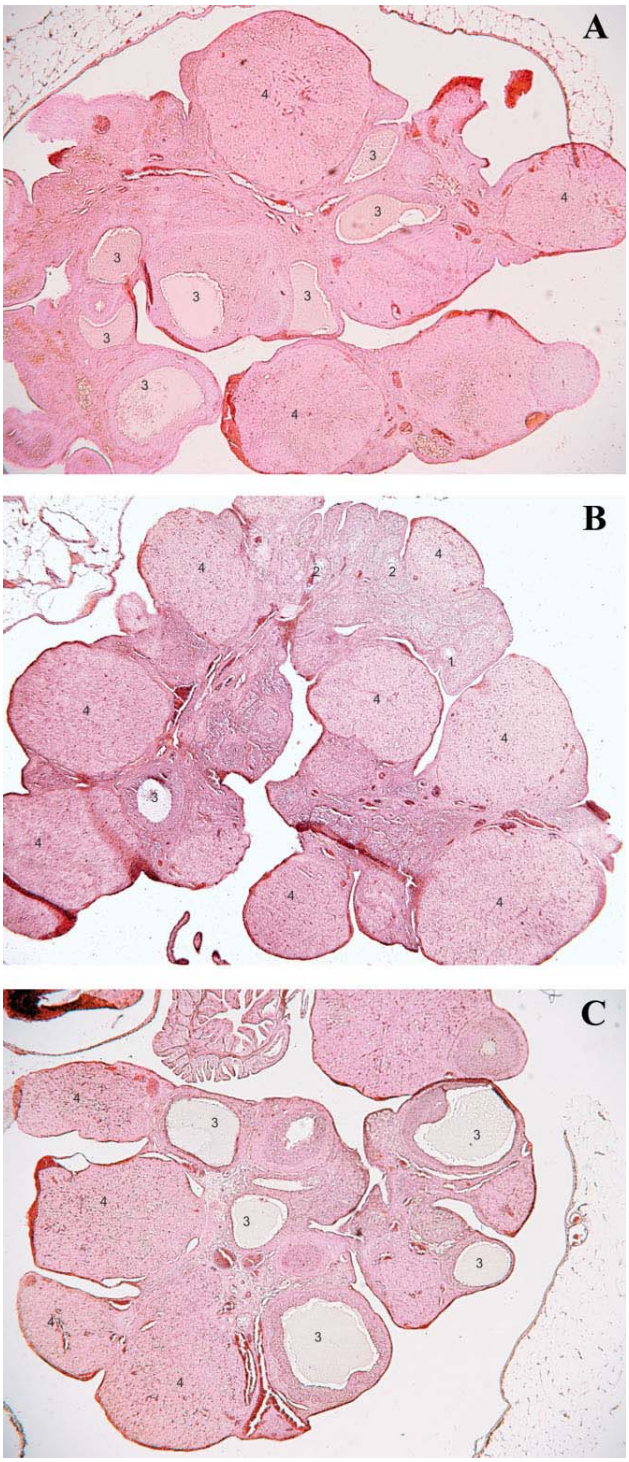

Fig. 5. (**A**) Photomicrograph showing ovary of control group (CG). 3, Graafian follicles; 4, corpus luteum. Magnification ×40. (**B**) Photomicrograph showing ovary of genetically modified soy group (GMSG). 1: preantral follicles; 2: antral follicles; 3: Graafian follicles; 4: corpus luteum. Magnification ×40. (**C**) Photomicrograph showing ovary of organic soy group (OSG). 3: Graafian follicles; 4: corpus luteum. Magnification ×40.

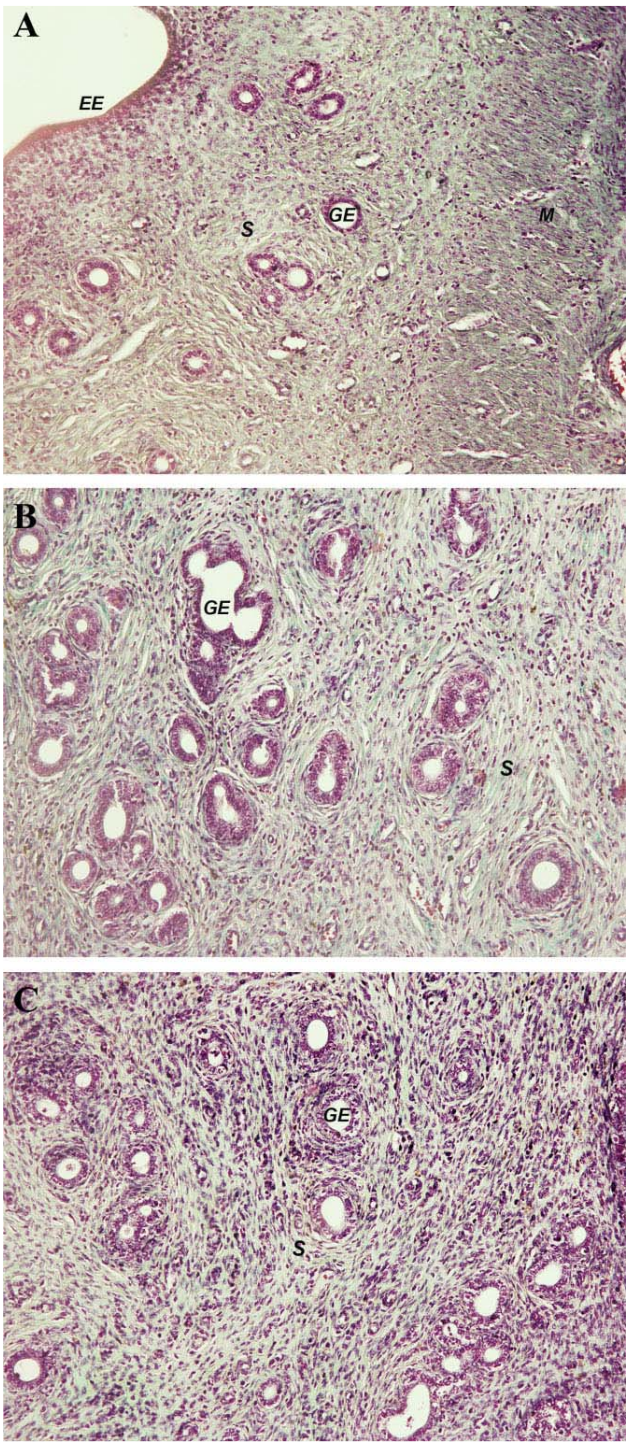

Fig. 6. (**A**) Photomicrograph showing uterus of control group (CG). EE, endometrial epithelium; GE, glandular epithelium; S, stroma; M, myometrium. Magnification ×100. (**B**) Photomicrograph showing uterus of genetically modified soy group (GMSG) GE, glandular epithelium; S, stroma. Magnification ×100. (**C**) Photomicrograph showing uterus of organic soy group (OSG). GE, glandular epithelium; S, stroma. Magnification ×100.

organic soy had an adverse effect on some parameters of the uterus and ovary morphology. The number of the growing follicles was significantly reduced in both soy-treated groups, in spite of normal corpus luteum number in the organic group. In relation to the uterus, both soy-treated groups exhibited a reduction in the length density of the glands, while the volumetric density of the epithelium was unaltered in the organic group. These data suggest that the transgenic and organic soy may have specific effects in the reproductive system.

The isoflavone content of both transgenic and organic soy was evaluated and no significant difference in the individual components or in food consumption was found. So, at this moment we may assume that the differences related to the transgenic and organic soy are not related to isoflavone, but it can probably be related to the small differences in fat, sugar and especially protein or amino acids diet content among the three groups.

In summary, both transgenic and organic-derived soy diets improved the lipid profile and reduced body weight; however, alterations in uterine and ovarian morphology were also found in animals with prolonged exposure to these diets. The prolonged use of soy-based diets and their relation to reproductive health warrants further investigation.

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