Possible Hazards of Soybean Phytoestrogens Ingestion on In-Utero Development of Albino Rats

El-Mahdy, T.O.M.[1]; El-Nahla, S.M.M.[1]; Takahashi, S. [2,3,4]; Basha, W.A. [1,2,3] *
[1]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
[2]Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, Ibaraki, Japan.
[3]Department of Anatomy and Embryology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan.
[4]International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Ibaraki, Japan.
walaaanatomy85@yahoo.com

Abstract: Soy-based diets are commonly used not only by human but also for laboratory and domesticated animals. Recently, there is a great deal of argument surrounding soy foods, mostly due to their phytoestrogens content. No data existed about the potential interactive effects of isoflavone mixture present in soybean on the embryonic growth and development, therefore, this work aimed to investigate the teratogenic effect of dietary phytoestrogens given to the pregnant albino rats on different body organs of the foeti during this critical period of in-utero development. A total 30 pregnant albino rats were divided into three groups (10 rats for each): A control group fed on casein based diet free from soybeans, the second group received low phytoestrogenic diet containing 20% soybeans and the third group was fed on high phytoestrogenic diet containing 30% soybeans. All groups were treated from gestation day (GD) zero through (GD) 20. Dams were sacrificed on GD 20 and foeti were examined grossly and microscopically. High phytoestrogen maternally treated foeti showed clear increase in the resorption ratio with marked decrease in the body weight and head size and evidence of encephalocele, microophthalmia, club-foot with histopathological lesions in cerebral cortex, spinal cord, liver, lung, heart, kidney and adrenal gland. Our entire findings confirmed that exposure to a mixture of phytoestrogens present in soybean during the critical periods of development especially the prenatal period possessed a high risk not only on the animal but also on the human.
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1. Introduction

Soy-based diets are commonly used as food source not only for human being but also for laboratory and domesticated animals. Soy derivatives in many forms can serve as ingredients for diet products, act as an alternative for meat products or are included as food ingredients for technological (bread) or cost-effective reasons. (1). A wide variety of marketed soy-based milks, coffee lighteners, yogurts, ice creams, and other dairy products have emerged, and these products are consumed by both adolescents and adults especially by pregnant women (2). Moreover, soy formula is being fed to increasingly more infants as their sole source of nutrition. Soy commonly found in the diets of domesticated and experimental animals (3,4). Soy meal is the most used and preferred protein source in animal feed worldwide due to its relatively high protein content of 44 to 50 % (5).

Recently, there is a great deal of argument surrounding soy foods, mostly due to their phytoestrogens content especially isoflavones, genistein and diadzien that behave like hormones, although they are generally less potent butlike any hormone, too much or too little can alter hormone-dependent tissue functions (6).

Because of maternal dietary phytoestrogen consumption, human fetuses are exposed to phytoestrogens during in utero development (7). Fetal exposure to phytoestrogens is directly related to the maternal serum circulating level (8). Indeed, genistein and diadzien are detected in amniotic fluid from second trimester pregnancies at levels similar to those observed in adult serum (9), and 10–20-fold higher than average amniotic fluid estradiol levels at that time in pregnancy (7).

Although numerous studies have investigated the potential detrimental effects of soy and/or isoflavones on development but comparisons between these studies are difficult due to the lack of standardization of soy nomenclature, the various formulations (soy proteins, pure isoflavones, etc.), doses and routes of exposure (dietary, injection and gavage) and the differences in time during the gestation stage and duration of exposure. Also, it is difficult to evaluate the effects and elucidate the mechanisms by which phytoestrogens and soy potentially affect growing embryo. All of these variables make it difficult to

compare and evaluate the absence or presence of supposed beneficial or harmful effects of soy and phytoestrogens on developing embryo.

Prior to this study, no data existed in the literature about the potential interactive effects of isoflavone mixture present on soybean on embryonic growth and development. Therefore, this work aimed to investigate the teratogenic effect of dietary phytoestrogens when given to the pregnant dams of albino rat from gestation day 0 to gestation day 20 (just before birth) and studying the gross and microscopic examination of all body systems of the foeti during this critical period of in utero development.

2. Materials and Methods

Experimental animals:

The present work was carried out on sexually mature virgin female albino rats (2 months old, weighing 150 - 160 gm, n = 30) and males of the same species and age (280 gm approximately, n = 10) that were obtained from the laboratory animal house, Faculty of Veterinary Medicine, Suez Canal University, Egypt. All animals were house-caged for two weeks in a 12hs light/dark cycle room with temperature adjusted to 22 ± 2^{o} C before the experiments and the animals had free access to water ad libitum during this adaptation period. The animal care committee of Veterinary Medicine faculty, Suez Canal University, Egypt, approved the protocol of this study. The female rats were then randomly assigned to a control group (n= 10) and two experimental groups (n=10) for each.

Mating procedure:

After the adaptation period, a single male with couple of female rats were placed together in the same cage for 24hs. On the next morning, detection of the cervical plug indicated successful mating and this was considered the day zero of gestation.

Treatment:

Soy based-diets were prepared and used for the treatment of the pregnant dams of the experimental groups as first treated group was fed on diet low in soy; the second treated group was fed on diet high in soy while the control group was fed on a casein-based diet (free from soy) as shown in Table (1). The pregnant dams were treated from the day zero to the end of the gestation period

Evaluation of food intake and body weight gain of the mothers during pregnancy:

Once pregnancy was established, daily food intake was recorded. Also, the weights of the pregnant mothers were recorded at day zero and at day 20 of gestation then the body weight gain was calculated for each mother by subtraction of its body weight at gestation day zero from its body weight at gestation day 20. Also, the corrected body weight calculated by subtraction of the weight of the gravid uterus of each mother from its total body weight, this help to determine the actual change in the mother's body weight throughout gestation period.

Uterine contents and ovaries examination:

On the 20th day of gestation, pregnant rats were sacrificed after complete ethyl ether anesthetization and cesarean section was carried out. Ovaries were isolated and the corpora lutea were numbered under a stereomicroscope. The freshly obtained uteri were longitudinally incised and the number of both live and dead foeti in each group were recorded as well as the number of post-implantation resorbed foeti were calculated by subtracting the total number of the corpora lutea in both ovaries from the number of implantation sites.

Foetal preparation:

Each fetus (whether live, dead or resorbed) was taken and examined. The weights of the live fetuses were recorded as well as their CVRL, head length (from the frontal bone to the beginning of the first cervical vertebra) and the head circumference by measuring the diameter of the widest area in the head.

A number of 75 foeti from the control group, 57 foeti from the low phytoestrogenic group and 41 foeti from the high phytoestrogenic group were taken, then prepared for gross and microscopic investigation of some visceral organs (lung, liver, kidney and adrenal gland), heart, brain, spinal cord.

Gross examination:

A number of 40, 30 and 20 foeti form casein-based, low phytoestrogenic and high phytoestrogenic treated groups, respectively were fixed in 10% buffered formalin for one week for examination by the naked eye and dissecting microscope to detect any abnormalities either externally or internally and photographs were taken using a digital camera.

Microscopic examination:

Specimens from the brain, spinal cord, lung, liver, heart, kidney, adrenal gland and metacarpal bones of 35, 27 and 21 foeti form casein- based, low phytoestrogenic and high phytoestrogenic groups, respectively, were subjected to the ordinary histological technique for preparing the paraffin blocks. Cross-sections of 4-6 µm in thickness were obtained and stained with Harris Heamatoxylin and eosin stains.

Statistical analysis

All values were presented as mean±standard error. The statistical differences between the groups were determined by analysis of variance (one way ANOVA) using SPSS® software (Statistical Package for Social science, version 17.01, IIIinois, USA). The statistical significance was set at $P>0.05$ while considered highly significant at $P<0.01$.

Table (1): Composition of the experimental diets*/kg of the diet.

Component	Casein-based diet (Control) (gm)	Low phytoestrogen diet (gm)	High phytoestrogen diet (gm)
Yellow Corn	72.5	35	28
Soybean seeds	----	20	30
Rice	----	27	30
Gluten	10	11	5
Casein	10	----	----
Soybean Oil	----	3	3.5
Corn Oil	3	----	----
Di Calcium Phosphate	1.4	1.3	1.1
Ground lime stone	1.5	1.3	1.1
Common Salt	0.4	0.4	0.4
Lysine	0.3	0.2	0.1
Methionine	0.3	0.3	0.3
Premix**	0.6	0.5	0.5
Total	100	100	100

*The control and two experimental diets were formulated to fulfill all the nutritional requirements of pregnant female rat according to (NRC, 1995) using different sources of protein like casein and corn for control diet while various amounts of soybean seeds with corn as a source of protein in case of low and high phytoestrogenic diets but finally all the diet ingredients in all the experimental diets were balanced.

**Premix produced by Muvco. Supplied per kilogram diet: 12.000 and 2.000 IU of vitamin A and D3 و respectively; 10 g vitamin E, 1 g vitamin K, 0.005 g vitamin B2, 0.0015 vitamin B6, 10 g pantothenic acid, 0.02 niacin, 0.6 gm choline chloride, 0.03g iron, 0.06 g manganese, 0.004 g copper, 0.05 gm zinc, 1 mg vitamin B1, 0.001 mg vitamin B12, 1 mg folic acid, 0.05 mg biotin, 0.3 iodine, 0.1 mg cobalt and 0.01 mg selenium.

3. Results

Evaluation of the mothers during pregnancy:

Firstly, there were no mortalities among the mothers of the three examined groups throughout the entire period of the experiment. Also, the means of the calculated total body weight gain of the pregnant females in these three groups throughout the full-term gestation were 42.83±0.073, 44.37±0.988 and 40.03±0.189, respectively that revealed no significant difference between the examined groups (Table 2). On the other hand, there was highly significant increase ($P<0.01$) in the corrected body weight gains in the high phytoestrogenic group as its calculated mean was 16±0.189 gm. compared with 9.66±0.073 gm. and 10.2±0.988 gm. in casein- based and low phytoestrogenic groups, respectively (Table 2). In addition, calculation of the resorption rate revealed highly significant increase ($P<0.01$) among the mothers fed on high phytoestrogenic diet where about 26.50% of total foeti were resorped compared with 4.95% resorption rate in those fed on casein- based diet. On the other side, a slightly non-significant increase of resorption rate (10.30%)in low phytoestrogenic group compared with that in case of the casein based group (4.95%) (Table 3).

Gross examination of the opened gravid uterus from the pregnant dams in both casein-based and low phytoestrogenic treated groups revealed normal size of the foeti and rare cases of resorption (Fig.1 a and b), however, in case of high phytoestrogenic group, there was a marked decrease in the size and the number of the foeti with occurrence of resorption (Fig.1 c.1). There was no incidence of abortion (miscarriage) in all the groups, however, all the foeti of 3 females in the high phytoestrogen treated group when dissected at gestation day 20were very small and absorbed (Fig.1 c.1).

Evaluation of the foeti:

Macromorphometric measurements:

Concerning the mean body weight of the foeti, they were 4.55±0.12, 3.9±0.06 and 2.3±0.06 gm. in casein based, low and high phytoestrogenic groups, respectively that showed clear highly significant decrease in the weight of those within the high phytoestrogenic treated mothers compared with the casein- based group foeti ($P<0.01$). Also, there was a significant decrease in the low phytoestrogenic group foeti ($P<0.05$)(Table 3).

Also, measuring the CVRL, their values were 3.7± 0.058, 3.52±0.072 and 2.8±0.06 cm, respectively that clearly showed highly significant decrease in the high phytoestrogenic group foeti ($P<0.01$) and significant decrease in those of the low phytoestrogenic group ($P<0.05$) compared with the foeti from casein-based group (Table 5).

The head parameter measurements such as head length and circumference were about 1.33±0.038 and 1.8±0.048 cm, respectively in the casein-based group while 1.22±0.025 and 1.7±0.047 cm in the low phytoestrogenic treated group. Moreover, in case of high phytoestrogenic treated group, these measurements were 0.905±0.032 and 1.1±0.039 cm

(Table 3). So, a clear highly significant decrease in the head circumference and head length in high phytoestrogenic group in comparison with the control group. While there was non-significant decrease in the head circumference among the low phytoestrogenic group foeti**,** but showed significant decrease in the head length.

Gross morphological findings:

The foeti from mothers of the casein-based and low phytoestrogenic treated groups showed normal external appearance without obvious anomalies (Fig.1 D, E, G&H). On the other hand, about 80% of the foeti of the high phytoestrogen treated group showing anomalies in the head, eyes, nose and limbs (Fig.1 F). While, very low percentage of malformations were manifested in the casein-based (1.7%) and low phytoestrogenic treated group (3.4%) (Table 3).

Malformations in the high phytoestrogenic group were expressed by various degrees of meningeocoele and encephalocoele, which were sac-like protrusions of the brain and meninges through openings in the skull, which caused by improper closure of the skull sutures during fetal development. It was appeared as protrusion between the forehead and the nose or down the middle of the skull or on the backside of the skull (Fig. 1 F). In addition, there was wide and abnormal nasal bone (Fig.1 I).

Sagittal sections in the head of the foeti from the casein-based and low phytoestrogenic treated groups revealed normal shape of the head (Fig. 2 a.1&2 respectively) while, a clear encephalocele appeared in about 90% the fetuses of the high phytoestrogenic treated group (Fig.2 a.3).

A marked shortening in the nasal bone with increased thickness of the incisive bone in the high phytoestrogenic group foeti (Fig.2 a.3) compared with normal bones in casein -based (Fig.2 a.1) and low phytoestrogenic (Fig.2 a.2) groups was also detected. In addition, about 75% of the foeti of the high phytoestrogenic group showed cleft palate that appeared as abnormal opening in the hard palate (Fig. 2 a.3). In addition, a bilateral microphthalmia clearly appeared in the foeti of the high phytoestrogenic treated group (Fig.1 I) compared with normal eye appearance in both casein and low phytoestrogenic treated groups (Fig.1 G&H). Moreover, obvious club-foot anomaly happened in most of the foeti with the foot appeared to be rotated internally at the hock joint (inversion of the foot) as well as short digits (Fig.1 F&I) compared with normal limbs in both casein based and low phytoestrogenic group **(Fig.1 D-E& G-H).**

Microscopical (Histopathological) findings:

Microscopical examination of the cerebral cortex in the foeti of the casein based diet maternally treated group revealed normal arrangement of the cerebral cortex layers into marginal, cortical, intermediate, subventricular and ventricular layers (Fig.2 b.1&2). While in the low phytoestrogenic group foeti showed nearly the same arrangement but with presence of mild edema that appeared between the neurons especially in the cortical and subventricular layer with moderate thickening in the marginal layer which was about 12 µm in thickness in low phytoestrogenic group compared with 8 µm in the casein- based group (Fig.2 b.1&2). On the other hand, the foeti of the high phytoestrogenic group showed normal layer arrangement, very thin marginal layer (about 5µm in thickness) with severe edema in most of the layers and a clear vacuolar degeneration of the neurons within the sub ventricular layer (Fig.2 b.3).

Examination of the histological sections in the spinal cord in the treated groups exhibited normal arrangement of the zones of the spinal cord as an outer zone of white matter and an inner gray matter (Fig.2 c). But in the high phytoestrogenic group of foeti there were marked edema and spacing between the neural cell with clear vacuolar neural degeneration within the gray and white matter (Fig.2 c.3).

Heamatoxylin/eosin-stained heart cross sections showed normal fascicular arrangement of the cardiac myocytes with central nuclei and thin-walled blood vessels in between in in both casein-based and low phytoestrogenic groups of foeti (Fig.2 d.1&2, respectively). On the other hand, there was an evidence of widespread intramuscular cardiac hemorrhage as well as subepicardial bleedings in the high phytoestrogenic group foeti (Fig.2 d.3)

Cross sections from the lung showed normal lung structure and architecture as well as normal inflated alveoli with thin interalveolar septa in both casein-based (Fig.2 e.1) and low phytoestrogenic (Fig.2 e.2) groups. On the other hand, there was an evidence of delayed and immature lung morphogenesis in the high phytoestrogenic group (Fig.2 e.3) as there were hyperplasia of the lung epithelial cells with thickened intercellular septa and marked proliferation of the interstitial stromal cells as well as few and very narrow alveolar air spaces. Also, there was severe interstitial hemorrhage with extravasated red cells.

Examination of the Heamatoxylin and eosin-stained liver cross sections showed preserved normal hepatocyte architecture and arrangement of the hepatocytes in plates with regular configuration of the hepatic sinusoids in both casein-treated (Fig.2 f.1) and low phytoestrogenic groups (Fig.2 f.2). The latter group, also, exhibited mild congestion of the hepatic sinusoids. While in the high phytoestrogenic group of foeti there was severe hemorrhage, congestion and dilatation of the hepatic sinusoids that filled with extravasated red cells (Fig.2 f.3)

Microscopic examination of the kidney of all the groups (Fig.2 g) showed normal kidney structure with an outer cortex and an inner medulla while there was small kidney with clear decrease in the thickness of both regions in the high phytoestrogenic group foeti as its renal cortex measured about 199 µm in thickness while the medulla was 300 µm in thickness (Fig.2 g.3). In case of casein- based diet group the cortex and medulla were 322 µm and 346 µm in thickness, respectively (Fig.2 g.1) while in low phytoestrogenic group, they were 253 µm and 323 µm in thickness for cortex and medulla, respectively (Fig.2 g.2). Also, the interstitial tissue formed of loose connective tissue and vessels was unremarkable in both casein treated and low phytoestrogenic groups (Fig.2 g.1&2, respectively). While in the high phytoestrogenic group there were severe hemorrhage and edema spread in the interstitial tissue in both regions of the kidney (Fig.2 g.3). Moreover, clear obstruction of both the proximal and distal convoluted tubules was clearly appeared in the foeti of this latter group.

The adrenal gland revealed normal size in the foeti of casein- based group (Fig.2 h.1) with normal structure of an outer cortex and an inner medulla as well as an intermediate x-zone and surrounded by connective tissue capsule. While in the foeti of low phytoestrogenic group (Fig.2 h.2) the gland was slightly smaller in size than that in case of the casein - based group, however the microscopic structure was more or less identical to that of the control group. Regarding the adrenal gland of the high phytoestrogenic group foeti (Fig.2 h.3), it was much smaller in size and had greatly thickened capsule and there was an evidence of cystic degeneration as well as focal vacuolar degeneration that mainly affecting the medulla and x-zone and extended to a limited part of the adrenal cortex.

Table (2): Morphometric parameters of the mothers in the three examined groups

Maternal parameter	Casein-based diet group	Low phytoestrogen diet group	High phytoestrogen diet group
Number of examined pregnant females	10	10	10
Number of the aborted females	0	0	2
Body weight (gm) of females at day zero of gestation	155.53±0.057	151.26± 0.13	159.33±0.092
Body weight (gm) of the pregnant females at day 20 of gestation	198.36±0.048	195.63±0.22	199.36±0.370
Body weight gain (gm) at day 20 of gestation	42.83±0.073	44.37±0.988	40.03±0.189
Weight of the gravid uterus (gm) at day 20 of gestation	33.16±0.882	34.16±0.0556	23.91±0.059
Corrected body weight gain (gm) at day 20 of gestation	9.66	10.2*	16.1**

Values are expressed as means ± S.E.M.; *and **denote significant differences from the control value, P <0.05 and P <0.01, respectively (Dunnett).

Table (3): Morphometric parameters of the foeti in the three examined groups

Fetal parameters	Casein-based diet group	Low phytoestrogen diet group	High phytoestrogen diet group
Total number of foeti	75	57	41
Number of the live foeti	75	57	41
Number of the dead foeti	0	0	0
Percentage of malformed foeti	1.7%	3.4%	80%
Number of resorped foeti & (%)	4(4.95%)	7 (10.30%)	15 (26.50%) **
Fetal body weight (gm)	4.55±0.12	3.9±0.06*	2.3±0.06**
Fetal CVRL (cm)	3.7± 0.058	3.52±0.072*	2.8±0.06**
Fetal head length (cm)	1.33±0.038	1.22±0.025*	0.905±0.032**
Fetal head circumference (cm)	1.8±0.048	1.7±0.047	1.1±0.039**

Values are expressed as means ± S.E.M.; *and **denote significant differences from the control value, P <0.05 and P <0.01, respectively (Dunnett).

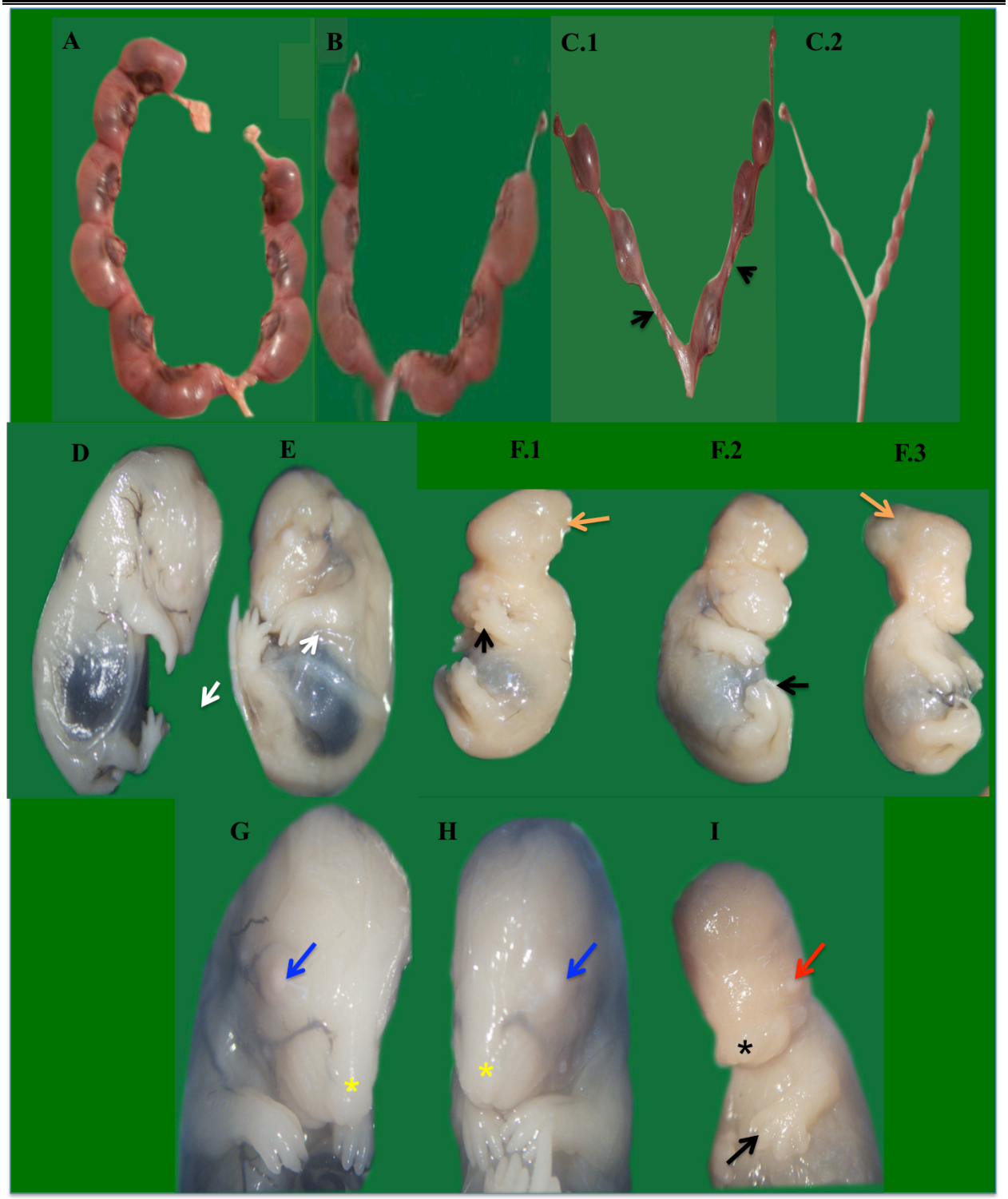

Fig. 1: A photograph (A-c.2) showing the difference in the size of the gravid uterus at gestation day 20. Normal appearance of the uterus and normal size of the foeti in casein- based (A) and low phytoestrogenic (B) treated mothers. Few number of reduced size foeti with clear post implantation resorption (black head arrows) in some dams (C.1), as well as totally absorbed foeti inside the uterus of three others (C.2) among the high phytoestrogenic treated mothers. Little difference in the size between GD 20 fetuses of casein (D&G) and low phytoestrogenic (E&H) groups with normal eyes (blue arrow). A clear decrease in the body size (F&I) with clear microophthalmia (red arrow) with various degrees of encephalocele (yellow arrow) in the high phytoestrogenic maternally treated foeti with presence of a club foot (black arrow). Abnormal and wide nasal bone (black star) in the high phytoestrogenic group of foeti compared with normal appearance in the other two groups (yellow stars).

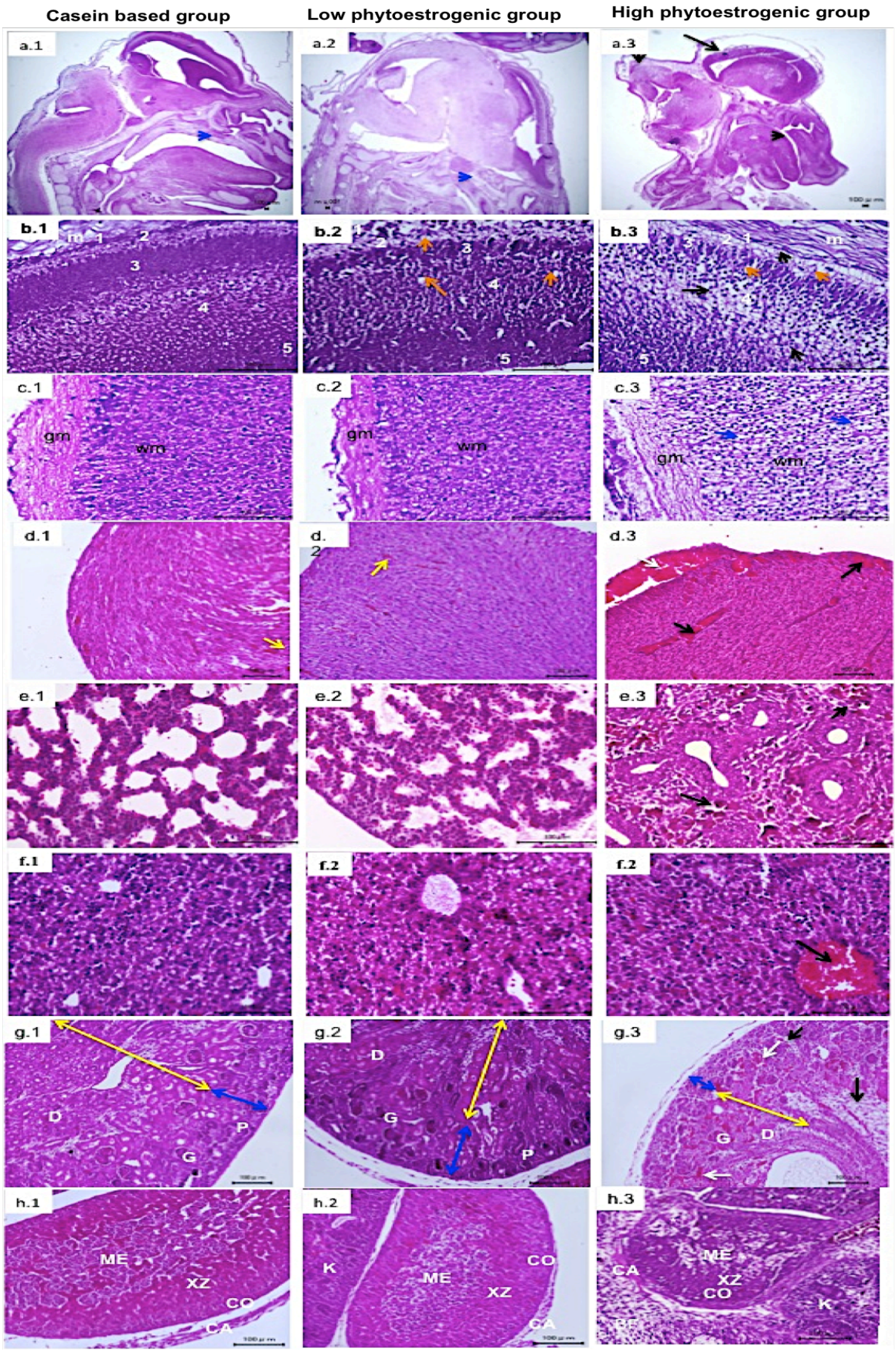
Casein based group
Low phytoestrogenic group
High phytoestrogenic group
a.1
a.2
a.3
b.1
b.2
b.3
c.1
c.2
c.3
d.1
d. 2
d.3
e.1
e.2
e.3
f.1
f.2
f.2
g.1
g.2
g.3
h.1
h.2
h.3
gm
wm
D
G
P
ME
XZ
CO
CA
K

Fig. 2: A photograph (a.1-3) showing sagittal section at (GD 20) fetuses of the three treated groups showing clear exencephaly (black arrow), short nasal bone (white arrow) and thick incisive bone (star) together with presence of palatine cleft in the hard palate (black head arrow) in the high phytoestrogenic group foeti compared to normal appearance in the other two groups (blue head arrow). (b.1-2) Showing microscopic cross section of the cerebral cortex with compact and regular arrangement of the neurons into layers, thin- walled vessels and fibrillary meninges (m), marginal layer (1), cortical layer (2), intermediate zone (3), subventricular layer (4), ventricular layer (5), lateral ventricle (ve) with mild edema-spaced neurons (yellow arrows). Severe edema with vacuolar degeneration of neurons (black arrows) and very thin marginal layer appeared in high phytoestrogenic group. (c.1-3) A longitudinal section in the spinal cord showing preserved and normal structure, white matter (white arrow) and gray matter (black arrow) with normal appearance of neural cells, while marked edema and spacing between the neural cells, clear vacuolar neural degeneration in the neural cell bodies of the gray matter and between the neural axons of the white matter (blue arrow) in high phytoestrogenic treated fetuses. (d.1-3) A cross section of the heart showing fascicular arrangement of myocytes with central nuclei. Presence of thin-walled blood vessels (yellow arrow), intramuscular cardiac hemorrhage (black arrow) and subepicardial bleedings (white arrow) in the high phytoestrogenic group. (e.1-3) lung tissue revealing normal inflated alveoli with thin septa in the casein treated and low phytoestrogenic groups while epithelial cell hyperplasia with thickened intercellular septa, narrow alveolar air spaces and extravasated red cells (black arrows) in the high phytoestrogenic group. (f.1-3) A cross section in fetal liver showing preserved architecture of hepatocytes and regular sinusoids while marked dilation, congestion, hemorrhage and extravasated red cells (black arrow) appeared in the high phytoestrogenic group (f.3). (g.1-3) A kidney cross section showing outer cortex (blue arrow) and inner medulla (yellow arrow), glomeruli (G), proximal tubules (P) in the cortex and distal & collecting tubules (D) appearing within the medullary tissue. White arrows indicate severe hemorrhage and black arrow indicates edematous medulla. (h.1-3) Adrenal gland cross section showing normal structure of adrenal gland capsule (CA), cortex (CO), medulla (ME) with X-zone (XZ) between the cortex and medulla. While, thickened capsule with cystic and focal vacuolar degeneration (star) in the high phytoestrogenic group (h.3), Kidney (K) and brown adipose tissue (BR).(H&E stain). Scale bars: 100 μm.

4. Discussion

The present study provided general systemic approach to investigate in details the effect of dietary phytoestrogens during the critical periods of development, especially the prenatal period, using a mixture of phytoestrogens that present in soybean as the total isoflavones. In this connection, there is one study on the effect of phytoestrogens mixture using 26% soybean in pregnant rat diet from day 0 to 21 gestation (10). On the other hand, the previous studies have been performed to study the toxicity effect of only a single compound of phytoestrogens, especially genistein at different dose levels in rats and mice (11, 12, 13, 14,15, 16, 17)

Our experimental data, which were conducted as a screen for detection of the teratogenic potentials of ingestion of the soy phytoestrogens in rats showed no lethal effect on the pregnant mothers as there was no evidence of mortality between the examined groups. However, there was high significant increase in the body weight of dams in the high phytoestrogenic groups, which might be considered a sign of maternal obesity. These data disagreed with (11) and (12) who found that maternal body weight and food consumption in rats were decreased in the high dose treated group. On other hand, (18) in mice and (19) in rats mentioned that there was no estrogenic effect observed in the pregnant dams fed on diet containing high level of genistein.

The current work revealed an increase in the resorption rate of foeti resulting in marked decrease on the pregnancy outcome in the high phytoestrogenic group compared with the other experimental groups. On the other hand, the low phytoestrogenic group showed no adverse effect on the pregnant dams and a non-significant effect on the pregnancy outcome. On the same way, the phytoestrogen treatment just before and during gestation led to a decrease on the pregnancy outcome and an increase in the absorption rate (20,21,22) in women; (23) ingrazing animals, captive cheetahs and California quails; (24) in grazing ewes, (10,12,25) in rats, (14,26,27) in mice as well as (28) in minnows Xenopus and African clawed frog. In contrast, in mice no change in the number of implantation sites after genistein treatment. These inconsistent results among the studies might be due to species difference, dose levels and methods of administration (18).

Regarding the full-term foeti assessment, it was recorded here a marked decrease in many foetal parameters such as body weight and CVRL in a dose dependent manner in the low and high phytoestrogenic groups compared with the control group, these data agreed with those of (10,12,13,14,25) in rats; (29,30,31,32) in rodents On the other hand, high phytoestrogen treatment of pregnant mice resulted in non-significant effect on the litter size (18).

Also, the foeti of the high phytoestrogenic groups showed a clear decrease in the head parameters, including head length and circumference, in comparison with those of the control and low treated groups. These results suggested that the high phytoestrogenic diet might have an adverse impact on the skull and brain development.

Isoflavones are transferred across the placenta in both rodents and humans to accumulate in the fetus so they suggested that most of organ and systems in the fetus are highly sensitive to phytoestrogens and finally might interfere with the organizational role of estrogen in the developing brain, reproductive system and non-reproductive organs (33,34,35,36). In this connection, it was observed obvious gross and microscopic deformities in the head, eyes, nose and limbs in about 80% of the foeti of the high phytoestrogenic treated group in the current study. These findings coincided with those mentioned by (10,11,12) in the same subject.

On the other hand, there was no evidence of teratogenic events on the effect of phytoestrogen genistein exposure that might be due to short period of treatment together with species difference (15,17,19) in rats as well as (16) in mice.

Meanwhile, the studies along the gestation period in rats did not record any anomalies (4,14,31,32,37). In our point of view, this might be because all of these studies involved the natural delivery and did not do any detailed visceral or skeletal examination just before parturition, which was important for proper detection of any teratogenic effect.

Our data showed clear central nervous system microscopic deformities in the high phytoestrogenic treated group where most of the foeti showed dysmorphology in the brain, cerebral cortex and the spinal cord including severe edema and clear vacuolar degeneration of the neurons in addition to a clear encephalocele. Also, short nasal bone and thickened incisive bone together with cleft palate were observed, the results that were not documented by any of the previous authors in their studies. On the other side, the anomalies of the brain and skull were supported by small head parameters in this group compared with casein-based and low phytoestrogenic diet treated groups.

Also, evidence of widespread intramuscular cardiac hemorrhage as well as subepicardial bleedings were noticed in the high phytoestrogenic group of foeti indicating the harmful impact of high dose of phytoestrogens in the diet of their mothers.

Moreover, occurrence of delayed and immature lung morphogenesis, hyperplasia of the lung epithelial cells with thickened intercellular septa and marked proliferation of the interstitial stromal cells in the high phytoestrogenic treated group confirmed what stated by (10,11) also in rats.

In addition, microophthalmia as well as severe hepatic hemorrhage, congestion and dilatation of the hepatic sinusoids that noticed in the high phytoestrogenic group of foeti agreed with the findings of (10).

A clear decrease in the thickness of both renal cortex and medulla, with severe hemorrhage and edema in the interstitial tissue in both regions as well as clear obstruction of the proximal and distal convoluted tubules appeared in high phytoestrogenic group of foeti, which undoubtly affected the normal renal function. However, (10) recorded a unilateral or bilateral absence of kidney and ureter in the high phytoestrogen rat foeti that not detected in the present work.

Concerning the effect of phytoestrogens on the adrenal gland, it was observed here small adrenal gland with greatly thickened capsule together with focal vacuolar cystic degeneration mainly affecting the medulla and x-zone and extended to a limited part of the cortex in the foeti of the high phytoestrogenic treated group.

All the previously mentioned results confirmed the negative impact on fetal growth and development when the dietary phytoestrogens were given to the pregnant rats throughout the whole period of pregnancy.

In addition, it was clear that there was no obvious difference between the low phytoestrogenic and casein-based diets given to the pregnant rats which indicated that phytoestrogens ingestion are critical at certain limit and below this level they have no or slight adverse effect on the development of the embryo and fetus.

In our point of view, there were many severe pathological lesions either grossly or microscopically that recorded firstly owing to the use of a mixture of phytoestrogens, however, most of the previous studies investigated the effect of one ingredient of the phytoestrogen extracts alone and studied the effect during a short period of gestation as well together with dose and species differences.

In conclusion, our entire findings confirmed that exposure to a mixture of phytoestrogens present in soybean during the critical periods of development especially the prenatal period possessed a high risk not only on the animal but also on the human being.

Corresponding author:
Walaa Abdelwahab Abdelghany Basha (Basha, W.A)
E-mail: walaaanatomy85@yahoo.com
Mailing address:
1-1-1 Tennodai, Tsukuba, Ibaraki, Japan
Laboratory Animal Resource Center
University of Tsukuba

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