World Scientific Research Journal Volume 7 Issue 6, 2021
ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

Effects of Transgenic Soybeans on Reproductive Function in Female Mice

Mingzhu Huang[1], Juan Cai[1], Chunmeng Li[1], Dongyu Yang[1], Jiange Qiu[1], Chunbin Lu[1, a, *] and Biao Liu[2]

[1]Department of Developmental and Regenerative Biology, School of Jinan University, Guangzhou, 510632, China

[2]Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of China, Nanjing, 210042, China

[a]tcblu@jnu.edu.cn

Abstract

The reproductive toxicity of glyphosate-resistant transgenic soybean has not been evaluated in female mice. Thus, in this study the reproductive toxicity potential of transgenic soybeans was assessed. Four-week-old female mice were fed with transgenic soybeans and the non-transgenic near isoline control soybean for 30 and 90 days, respectively. The developmental status of oocytes, apoptosis of zygotes, and the pathologic effect and apoptosis of ovarian tissues were studied to assess the effects of transgenic soybeans on reproductive function in female mice. The development of oocytes in the transgenic diet group was normal. No significant differences were noted in ovarian tissues, number of oocytes, oocyte maturation, and abnormal oocytes between the two groups of mice fed diets containing transgenic soybeans and non-transgenic soybeans for 30 and 90 days. There were no significant differences in pathologic effects and apoptosis of ovaries between mice fed the transgenic and non-transgenic soybeans. In the case of zygote apoptosis, dense nuclei were not observed, indicating no treatment-related side effects on the fertilization process. It is concluded that glyphosate-resistant transgenic soybeans have no reproductive toxicity potential in female mice and transgenic soybeans are as safe as conventional soybeans.

Keywords

Transgenic Soybeans; Oocyte Quality; Ovary Apoptosis; Zygote Apoptosis; Reproductive Toxicity.

1. INTRODUCTION

Genetically-modified organisms (GMOs) have been genetically modified using genetic technology to insert foreign genes with novel agronomic traits. Over the past two decades, an increasing number of transgenic crops have been successfully developed and commercially cultivated for superiority, such as tolerance to herbicides, diseases, and pest resistance. The development of transgenic plant technology has accelerated the pace of genetic improvement of crops. Research and application have rapidly expanded GMOs globally and the products are increasingly used in food and food industries.

Understandably, debates on the potential risk to human health have been ongoing since the commercialization of the first GMO crop, glyphosate-resistant transgenic soybeans, in the 1990s. Although no adverse effects have been associated with the safety of GM foods during the nearly 20-year history of edible GM foods, such as soybeans and corn, it has been shown that

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

YieldGard® MON810 maize, which contains the CaMV 35S promoter, the hsp 70 intron of maize, the cryI(A)b gene, and the NOS terminator, causes a complex recombination by the inserted gene [1]. Public health concern about consuming transgenic crops involves the adverse effects on the body. Until the present, rodents have been the most common animal model for establishing safety based on routine analysis for biochemical processes and growth.

Scientific studies in which rodents were used as the animal model to assess the safety of transgenic crops include acute toxicity and sub-chronic toxicity trials, and nearly all of these studies confirmed that genetically-modified crops have no toxicity or adverse effects. Wang Er-hui et al. [2] designed a 90-day feeding study to evaluate safety with respect to the reproductive system in Wistar rats by measuring body weight, serum chemistry profiles, sperm parameters, and relative organ/body weights. No diet-related significant differences were observed between rats that were fed a diet containing transgenic rice TT51 and the non-transgenic counterpart (MingHui63). Er-hui et al.[2] concluded that transgenic rice TT51 did not appear to exert any adverse effects on the reproductive system in male rats. Wang [3] fed the transgenic soybean, MON87708, and the near-isogenic non-transgenic soybean, A3525, to rats for 90 days; food consumption/utilization, body weight, organ weight, hematologic parameters, serum biochemistry profile, and anatomic pathologic effects were recorded. The results demonstrated no adverse effects of the two soybeans in rats during the experimental period, so the transgenic soybean, MON87708, was deemed safe.

Research involving common transgenic crop toxicity often focuses on nutrition, hematology, pathology, toxicology, genesiology, and serum biochemistry [4,5]. Transgenic crops have not been fully tested to demonstrate absence adverse effects. The reproductive system, especially the female reproductive system, is sensitive and vulnerable to external factors, including diet. Indeed, the reproductive system may be impaired when in contact with hazardous substances while other systems have no untoward effect [6]. Hence, reproductive toxicity is a major indicator used to evaluate the safety of transgenic crops. Nevertheless, the female reproductive toxicity potential of GMOs has not received full attention. Safety assessment of the reproductive system has most often been conducted using male rats as the model [7], while reproductive system experiments involving female mice often focus on the development of offspring [8, 9]. The effect of GM on the female reproductive system is often related to the coefficient and pathologic changes in the uterus, ovaries, and fallopian tubes [10-12]. There are two different conclusions regarding the GM impact on the uterus and ovary. Most researchers have suggested that transgenic crops do not result in pathologic changes in the uterus, ovaries, and vagina [13]; however, Brasil [14] fed weanling female rats with transgenic soybeans for 15 months and reported that the morphology of the uterus and ovaries was altered. Thus, there is no definitive answer about whether transgenic crops have adverse effects on the female reproductive system.

Safety assessment research on the female reproductive system is currently confronted with two problems: the conclusions are disunited and controversial; and the research is not in depth and detailed. Therefore, serious debate on the reproductive safety evaluation of transgenic crops continues. Therefore, we first examined the change in ovaries when females were fed with a transgenic diet, then we determined oocyte quality and zygote apoptosis to confirm the effects of a transgenic diet on oogenesis and fertilization. Unlike other studies, we also determined the effects of transgenic soybeans on offspring development.

Female reproductive toxicity tests generally concentrate on pathologic changes in the reproductive organs (uterus, ovaries, and fallopian tubes), and offspring number and development, but the most important and sensitive reproductive processes, such as oocyte development, fertilization, and zygote development, have not been reported. Because of the gaps in knowledge, further evaluation of female reproductive system toxicity due to glyphosate-resistant transgenic soybeans in 30 and 90 day feeding experiments were warranted to detect

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

oocyte quality, and ovarian and zygote apoptosis. Therefore, we studied the toxicologic effects of transgenic soybeans on the female reproductive system, including fertility.

2. MATERIAL AND METHODS

2.1. Material

2.1.1 Diet and Mice

Glyphosate-resistant transgenic soybean (GTS40-3-2) was obtained from Monsanto Company (St. Louis, Missouri, American); the non-transgenic near isoline soybean is A5403. The diet was processed by the Center of Medicine Experimental Animal of Guangdong and complied with the national standard. The transgenic diet included glyphosate-resistant transgenic soybeans (GTS40-3-2) and the non-transgenic diet contained non-transgenic soybeans (A5403). Soybean meal accounted for 15% of the diet and was processed by the Guangdong Experimental Animal Center with reference to the experimental animal synthetic feed nutrient composition standard (GB14924.3-2010); the composition of the two diets was otherwise equivalent.

Weanling female Kunming mice were obtained from the Center of Medicine Experimental Animal of Guangdong. The animal feeding procedures complied with laboratory animal feeding standards. After 7 days of acclimation, the mice were housed with a 12h/12h light/dark cycle at 22-24°C room temperature. Animals had free access to diet and water during the experiments. All animals were cared for in accordance with institutional and National Institutes of Health guidelines for humane animal use and approved by the Animal Ethics Committee of Jinan University.

2.1.2 Apparatuses and Reagents

A CO_2 cell incubator, petri dishes, stereoscopic microscope, fluorescence microscope, pregnant mare's serum gonadotropin (PMSG; Ningbo Second Hormone Factory, Ningbo, China), human chorionic gonadotropin (HCG; Ningbo Second Hormone Factory, Shanghai, China), hyaluronidase (Sigma, Germany), Hoechst 33258 (Beyotime Biotechnology, Shanghai, China), and mouse tubal fluid medium (MTF) were used by following the protocol for mouse embryos [15]. All chemicals used were analytical reagent grade.

2.2. Ovarian Pathology and Apoptosis Detection

Twenty female mice were randomly divided into 4 groups (CK_{30d}, GM_{30d}, CK_{90d}, and GM_{90d}). Mice in the CK and GM groups were fed non-transgenic or transgenic diets, respectively. At the 30^{th} and 90^{th} days, the mice were euthanized by cervical dislocation. The ovaries were removed and fixed in Bouin's solution. Ovaries were finally embedded in paraffin after dehydration and transparency, then sectioned at a thickness of 5 μm. A portion of the sections was washed in xylene and graded alcohol, then stained with hematoxylin-eosin (H-E). The remaining sections were washed in xylene, a graded alcohol series, and 0.9% NaCl solution, then stained with Hoechst 33258 for 10 min in the dark. All sections were examined under a microscope.

2.3. Oocyte Development

Twenty female mice were randomly divided into four groups after 7 days of acclimation. The groups were designated as follows: CK_{30d}; CK_{90d}; GM_{30d}; and GM_{90d}. The CK_{30d} and GM_{30d} groups were fed a non-transgenic or transgenic diet, respectively, for 30 days. The CK_{90d} and GM_{90d} groups were fed a non-transgenic or transgenic diet, respectively, for 90 days.

On the 30^{th} and 90^{th} days, the mice were induced to ovulate by intraperitoneal injections of 10 IU of PMSG, and 5 IU of HCG 48 h later. The females were euthanized by cervical dislocation after 16 h. Under sterile conditions, the fallopian tubes were removed surgically and washed in a petri dish with 1 ml of MTF medium, then transferred to a 35-mm petri dish. A sterile needle

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

was used to prick the ampulla and collect cumulus-oocyte complexes (COCs). COCs were transferred to a MTF microdroplet containing 300 IU/ml of hyaluronidase. When the cumulus cells were shed, the oocytes were transferred to aMTF microdroplet without hyaluronidase, then the morphology of the oocytes was determined and the number of oocytes was counted.

2.4. Detection of Zygote Apoptosis

Twenty female mice were divided into 4 groups (CK_{30d}, CK_{90d}, GM_{30d}, and GM_{90d}). On days 30 and 90, the mice were induced to ovulate by intraperitoneal injections of 10 IU of PMSG followed by 5 IU of HCG 48 h later. Each female was caged overnight with one adult male. If the vaginal plug and sperm appeared the following day before 8 am, the mice were considered to have been fertilized. These females were sacrificed by cervical dislocation at 12 pm. The fallopian tubes were removed surgically and placed in MTF medium with hyaluronidase, then a sterile needle was used to prick the ampulla and collect zygotes. When the cumulus cells were shed, the zygotes were transferred to a slide and fixed. The slides were stained with Hoechst 33258 for 10 min in the dark, then examined under a fluorescence microscope.

2.5. Growth and Development of Offspring During Pregnancy and Lactation

Twenty female mice were randomly divided into 4 groups (n=5). Groups A and B were fed a non-transgenic diet for 90 days, and groups C and D were fed a transgenic diet. Each female mouse was caged overnight with one adult male mouse and fed a non-transgenic diet. The females were checked each morning for copulation plugs. If a vaginal plug was present, the mated females were caged separately. Mated females in group A were fed a non-transgenic diet until the end of lactation. Group B was fed a transgenic diet after mating until the end of lactation. Group C was fed a non-transgenic diet instead of mating. Group D was fed a transgenic diet. The number of pups per litter, sex ratio of females-to-males, pup survival ratio, and pup development index (body weight, body length, and tail length) were measured and recorded.

The specific experimental process are shown as follow.

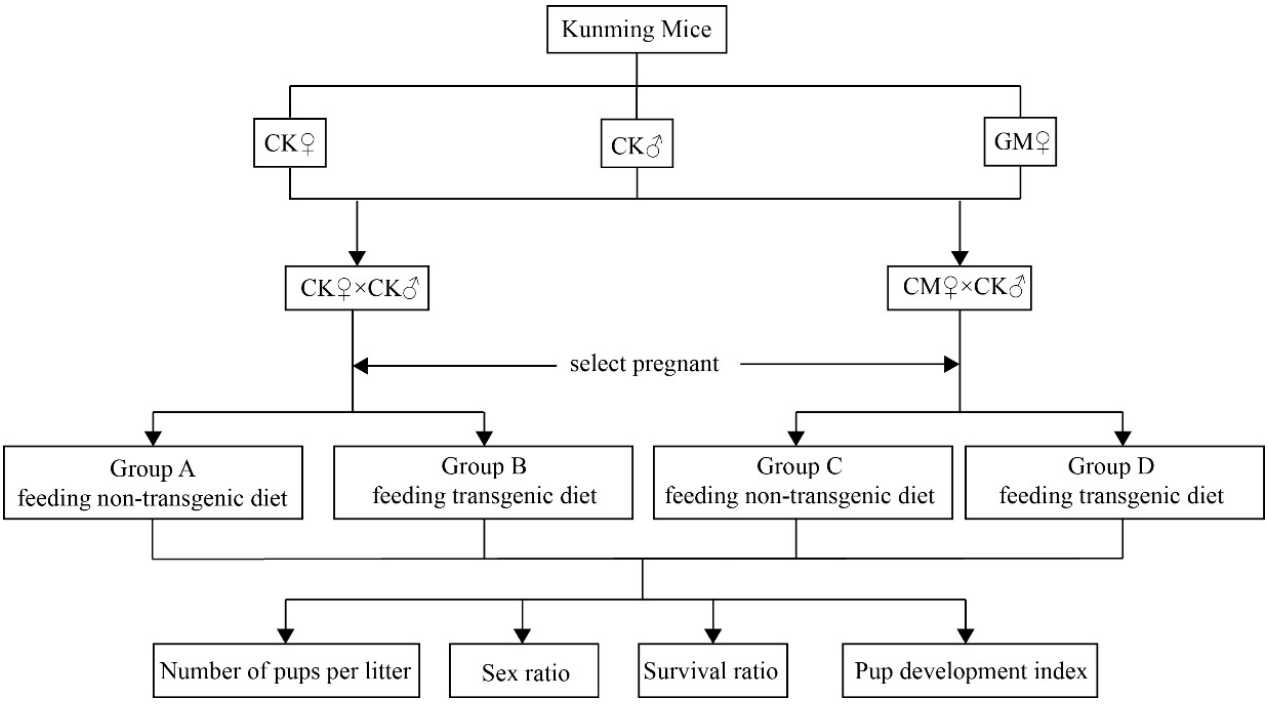

Figure 1. The specific experimental process

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

3. RESULTS

3.1. Effects of Transgenic Soybean on Ovarian Pathology and Ovarian Cell Apoptosis in Female Mice

The ovary slides were stained with H-E, then examined under a microscope. Ovarian pathology results from the 30- and 90-day feeding experiments are presented in Figure 2. Ovarian pathologic analysis was performed in the CK and GM groups; no typical histopathologic observations were present in the GM group. Specifically, the structure of the ovaries was normal, follicles of different sizes were present, and a corpus luteum and atretic follicles were observed deep within the ovary.

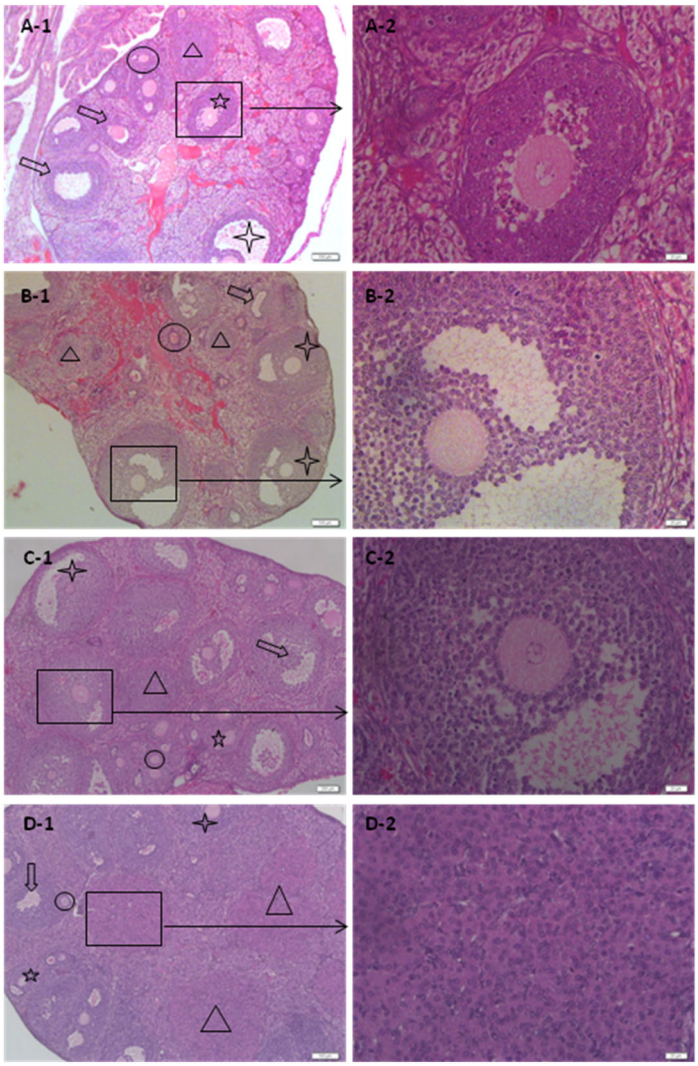

Figure 2. Effects of transgenic soybean on ovary pathology in female mice (H-E staining, 100×, 400×)

Ovary pathology was detected by H-E staining, A:CK_{30d} B:GM_{30d} C:CK_{90d} D:GM_{90d}. 1 (left) showed the result of ovary pathology under 100×, 2 (right) showed the result of black frame of 1 (left) under 400×. Arrow showed atretic follicles, triangle showed corpus luteum, roundness showed primordial pollicles, five-pointed star showed primary follicle, star showed secondary follicles. n=10 per group.

The ovary slides were stained with Hoechst 33258 for 10 min in the dark, then examined under a fluorescence microscope. Ovarian apoptosis results for the 30- and 90-day groups are presented in Figure 3. Under a white light, whether CK or GM group, the ovarian structure was

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

grossly normal, and the number and distribution of follicles, oocytes, and granulosa cells were normal. Under fluorescence, a normal nucleus of ovarian tissue was stained in blue and an abnormal nucleus was stained in white, but no white-stained nuclei were observed (Figure 3). No treatment-related changes were observed between the experimental and control groups.

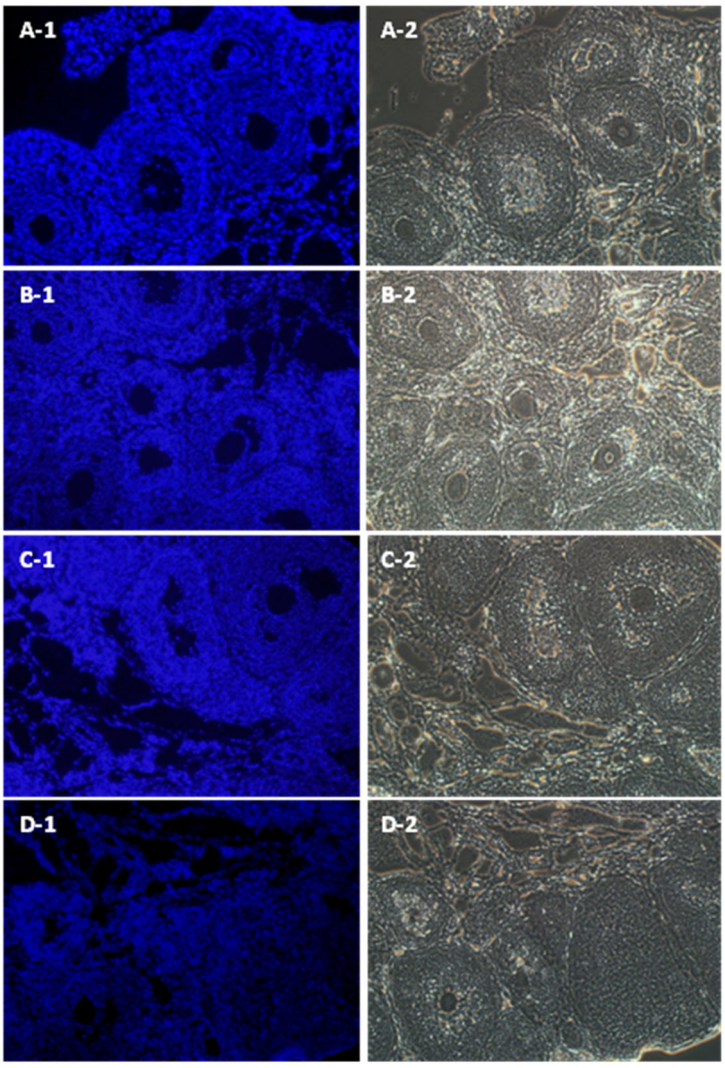

Figure 3. Effects of transgenic soybean on ovary cell apoptosis in female mice (Hoechst 33258 staining, 200×)

Ovary cell apoptosis was detected by Hoechst 33258 staining, normal cell nucleus were staining with blue and apoptosis cell nucleus were staining with white. A:CK_{30d} B:GM_{30d} C:CK_{90d} D:GM_{90d}. 1 (left) showed normal cell nucleus were staining with blue under UV light, no white cell nucleus were observed. 2 (right) showed ovarian cell structure and distribution under DIC. n=10 per group

3.2. Effects of Transgenic Soybean on Oocyte Quality in Female Mice

In the 30- and 90-day feeding studies, the number of oocytes in each mouse was calculated and oocyte morphology was observed. Oocyte morphology was based on the description by Deng [16]. Table 1 shows the effect of transgenic soybeans on oocyte quality. There was no significant difference between each group with respect to oocyte number after superovulation treatment ($P>0.05$). The oocyte maturation rate was not significantly different in each group

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

(P>0.05). The average abnormal rate of oocytes was 2.86% and no significant difference was detected between each group (P>0.05).

Table 1. Effects of transgenic soybean on oocyte quality in female mice ($\bar{x}$±s)

Group	Number of Oocyte	Maturing rate of Oocyte(%)	Abnormal rate of Oocyte(%)
GM30d	27.00±2.56	12.21(42/344)	1.69(12/356)
CK30d	35.60±11.41	18.94(50/264)	2.22(6/270)
GM90d	32.40±8.71	18.00(54/300)	3.23(10/310)
CK90d	31.00±5.00	15.82(50/316)	2.47(8/324)

n=10 per group

3.3. Effects of Transgenic Soybeans on Zygote Apoptosis

Zygotes were fixed on slides and stained with Hoechst 33258 for 10 min in the dark, then observed under a fluorescence microscope. The zygote apoptosis results for the 30- and 90-day groups are presented in Figure 4. Female pronuclei, male pronuclei, and first polar bodies were observed under a white light. Under fluorescence, female and male pronuclei and first polar bodies were stained in blue, which indicates that those cells were normal. Compared with the CK group, there was no significant apoptosis of zygotes in the GM group.

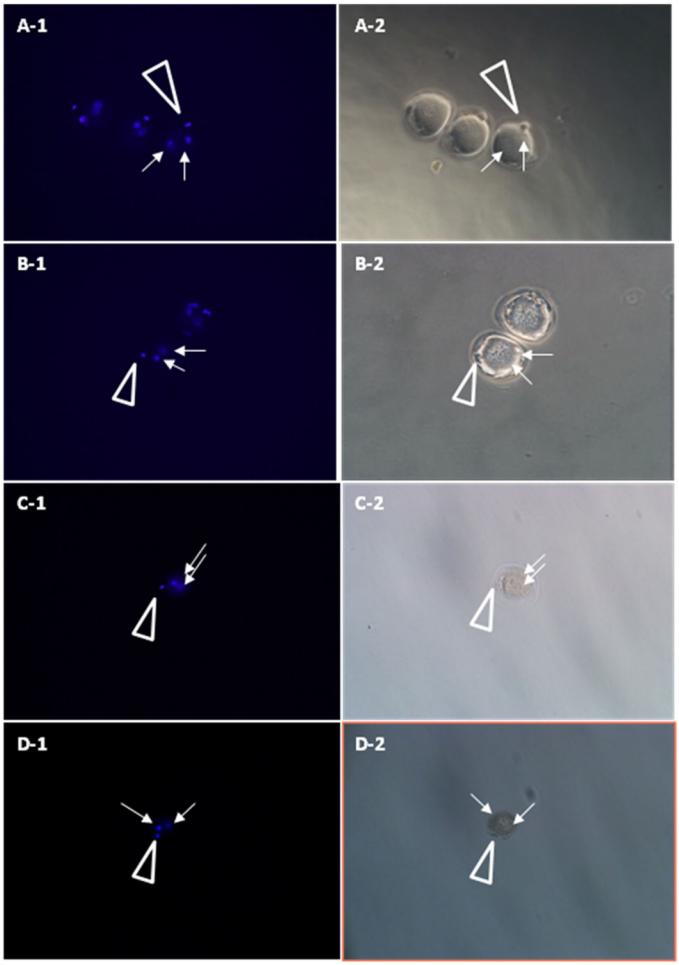

Figure 4. Effects of transgenic soybean on zygote apoptosis in female mice (Hoechst 33258 staining, 200×)

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

Zygote apoptosis was detected by Hoechst 33258 staining. Normal zygotes were staining with blue and apoptotic zygotes were staining with white. A:CK_{30d} B:GM_{30d} C:CK_{90d} D:GM_{90d}. 1 (left) showed normal cell nucleus were staining with blue under UV light, no white cell nucleus were observed. 2 (right) showed ovarian cell structure and distribution under DIC. Arrow showed female pronucleus and male pronucleus, triangle showed first polar body. n=10 per group

3.4. Effects of Transgenic Soybeans on Pregnant and Lactating Mice

Each female mouse was co-housed in a cage with one male mouse and fed a non-transgenic diet. All female mice conceived. The mating behavior of female mice fed a transgenic diet was similar to the non-transgenic diet-treated group, and the pregnancy rate was 100%. Pups were born 19-22 days later; the number of pups per litter was recorded and the pup survival ratios were recorded 4 days after birth. The female-to-male sex ratios were calculated when the pup body was completely covered with hair. As showed in Table 2, the number of pups per litter, survival ratios, and sex ratios were not significantly different between the non-transgenic diet and transgenic diet groups.

Table 2. Effects of transgenic soybean on productive ability in female mice ($\bar{x}$±s)

Group	Number of female mice for experiment	Number of pups per litter	Survival ratios	Sex ratio of females to males
A	5/5	7.60±3.05	100	0.97±0.42
B	5/5	7.80±1.48	100	0.99±0.68
C	5/5	9.00±2.24	100	1.08±0.60
D	5/5	8.60±1.14	100	0.97±0.33

3.5. Effects of Transgenic Soybeans on the Offspring Development Index

Female mice were fed a transgenic diet for 90 days, then housed overnight with male mice fed a non-transgenic diet. One-half of the females were fed with a transgenic diet during lactation, while the other mice were fed a non-transgenic diet until the end of lactation. The results are shown in Table 3. Development of offspring of dams fed a transgenic diet was similar to the offspring of dams fed a non-transgenic diet. The development indices of offspring in the group fed a transgenic diet treatment during lactation were similar to the offspring in the group fed a non-transgenic diet treatment during lactation. There was no statistically significant difference in the development indices between the dams fed a transgenic diet and the dams fed a non-transgenic diet. This finding indicates that there were no untoward effects of the offspring from female mice fed a transgenic diet, whether long-term or during lactation.

Table 3. Effects of transgenic soybean on offspring in development index ($\bar{x}$±s)

Group	Number of total pups	Pup body weight after birth (g)	Pup body length after birth (cm)	Pup tail length after birth (cm)
A	38	1.95±0.18	3.15±0.13	1.10±0.13
B	43	1.91±0.10	3.14±0.13	1.14±0.12
C	45	1.88±0.09	3.18±0.14	1.13±0.12
D	39	1.91±0.17	3.17±0.14	1.11±0.13

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

4. DISCUSSION

Since the first genetically modified crop was developed in 1983, transgenic crops have been grown for 30 years. Transgenic crops have brought great socioeconomic benefits to humans, yet some people think the safety of genetically-modified foods remains a question to be verified. The safety evaluation of transgenic crops is a crucial question and attracts a lot of attention. Most studies have focused on nutrition, allergenicity, and toxicology, but the collective information is still limited [17] many issues have not been addressed. The periodic changes in the ovaries and uterus are strictly regulated by hormones, so the reproductive system is sensitive and vulnerable when exposed to toxins, to which the developing zygote and embryo are particularly sensitive [6]. The normal female reproductive system and fetal development are essential for animal reproduction. In each human ovary, there are approximately 400,000 follicles at birth. These follicles determine the reproductive lifespan of a female, but at puberty approximately one-half of the oocytes remain. Ultimately, about 400 primary follicles will yield mature oocytes. However, exposure to toxins will damage and deplete oocytes, then lead to reduced fertility in females [6, 18]. Hence, the reproductive system is also important for safety assessment of GMOs.

Oocyte competence consists of five levels, as follows: ability to resume meiosis; ability to cleave following fertilization; ability to develop to the blastocyst stage, ability to induce a pregnancy and bring it to term; and ability to develop to term in good health[19]. Hence, oocyte quality is crucial to fertilized egg and fetus development. The data from the current trial showed that there is no significant difference between the 30- and 90-day CK and GM groups with respect to oocyte quality, specific performance in the oocyte number, oocyte maturation, and abnormal oocytes. To summarize, we do not believe transgenic soybeans adversely impact oocyte formation and development in female mice, and oocyte quality was also not affected by transgenic soybeans, thus transgenic soybeans do not adversely affect oocytes in female mice.

As the most important genital organs, the ovary plays a crucial role in the female reproductive system. Female fertility declines when ovarian tissues or cells are damaged, and severe injury can even lead to infertility. Chukwudebe [20] fed rats with transgenic soybean CV127 for 90 days and determined the relative weights of ovaries and pathologic effects in the ovaries between the control and experiment groups. CV127 soybeans was not associated with any histopathologic lesions. Other results of effects of transgenic soybeans on ovarian pathology in transgenic-fed female mice also showed no significant difference between transgenic and non-transgenic groups [21, 22]. In our study, the ovarian tissue structure and cell apoptosis rate in 30- or 90-day feeding experiments were determined; no typical pathologic injuries or ovarian cell apoptosis was detected in the GM groups. The results demonstrated that short- (30 days) and long-term (90 days) feeding experiments had no significant adverse effects on ovarian development.

Apoptosis plays an important role in life activities and is closely related to many life processes. Excessive or hampered apoptosis can affect normal life activities. There are various methods by which to detect apoptosis; in our studies, we chose Hoechst 33258 to detect apoptosis. Unlike DAPI, which utilizes fixed cells, Hoechst 33258 dyes living cells. Fertilized eggs have the capacity to produce any cell type, so it supports the development of the fetus during subsequent phases of development [23]. If the process of zygote development is disturbed, the subsequent development and function will be influenced and changed. With respect to zygote apoptosis, we did not find a significant difference between the CK and GM groups, whether 30 or 90 days, so we suggest that transgenic soybeans would not promote apoptosis of fertilized eggs and parental generation, which fed with transgenic soybean, would not affect the development of fertilized eggs.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

It has been demonstrated that maternal nutritional status has a significant influence on embryonic and fetal development. Some nutrients may have a modulatory effect on the expression of development toxicity, which can result in abnormal development of the embryo and fetus [24]. Brake and Evenson [25] studied the effects of transgenic soybeans on fetal, postnatal, pubertal, and adult testicular development in mice. The results showed that transgenic soybeans had no effect on macromolecular synthesis or cell growth and differentiations of testes, and there were no differences in litter size and body weight between the transgenic diet and control groups. In our study transgenic soybeans had no effect on the number of pups per litter, survival ratio, female-to-male sex ratio, pup body weight, pup body length, and pup tail length between groups C and D; similar findings were demonstrated between groups A and B. Our results showed that transgenic soybeans did not affect the mating and reproductive capacity of female mice, and did not affect the development of pups during gestation and lactation.

The reproductive system is sensitive and vulnerable. Reproductive health is crucial to human development, therefore the effect of transgenic crops on the human reproductive system has attracted the attention of the researchers and public. In the past few years the main research effort of our group has focused on the effect of glyphosate-resistant transgenic soybeans on reproductive system in mice. The parental generation were fed with transgenic soybeans for 120 days and no significant pathologic changes were observed in the testis and sperm. The results indicated that feeding long-term with transgenic soybeans did not have a negative influence on the murine reproductive system [26]. The first filial generation was fed with transgenic soybeans for 30, 60, 90, and 120 days and there were no significant differences between the experiment and control groups in sperm quality and testicular function, thus suggesting that transgenic soybeans have no genetic additive effects on mice [27]. Subsequently, we further studied the impact on the testis and sperm function, and the results showed that transgenic soybeans did not have an adverse impact on apoptosis of testicular cells, percentage of testis cell populations, and development and function of sperm [28, 29]. For female mice, after 30- or 90-d feeding experiments, there were no significant differences in the estrous cycle, fertilization, and development of embryos between the experimental and control groups, so transgenic soybeans did not have potential reproductive toxicity on female mice [30].

5. CONCLUSION

In the current study, the totality of results demonstrated that GM soybeans had no adverse effects on oocyte quality, ovarian pathology and apoptosis, and zygote apoptosis in female mice in the 30- or 90-d feeding experiments, and transgenic soybeans had no potential reproductive toxicity on the female reproductive system.

ACKNOWLEDGMENTS

These author's contributed equally to this work. The authors acknowledge support from the National Major Special Projects for Genetically Modified Organisms.

REFERENCES

[1] ROSATI A, PATRIZIA B, SANTARLASCI A, et al. Characterisation of 3′ transgene insertion site and derived mRNAs in MON810 YieldGard® maize[J]. Plant Molecular Biology, 2008, 67(3): 271-281.

[2] WANG Er-hui, YU Zhou, HU Jing, et al. Effects of 90-day feeding of transgenic Bt rice TT51 on the reproductive system in male rats[J]. Food and Chemical Toxicology, 2013, 62: 390-396.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

[3] WANG Xiao-yun, HE Xiao-yun, ZOU Shi-yin, et al. A subchronic feeding study of dicamba-tolerant soybean with the dmo gene in Sprague–Dawley rats[J]. Regulatory Toxicology and Pharmacology, 2016, 77: 134-142.

[4] DOMINGO J L. Safety assessment of GM plants: An updated review of the scientific literature[J]. Food and Chemical Toxicology, 2016, 95: 12-18.

[5] VENDÔMOIS J D, CELLIER D, VÉLOT C, et al. Debate on GMOs health risks after statistical findings in regulatory tests[J]. INT J BIOL SCI, 2010, 6(6): 590-598.

[6] THOMAS M A, THOMAS J A. Toxic responses of the reproducive system[J]. Casarett & Doull'S Toxicology, 2001, 4: 547-581.

[7] HU Yi-chun, QIN Zhuo, GONG Zhao-long, et al. Three-generation reproduction toxicity study of genetically modified rice with insect resistant genes[J]. Food and Chemical Toxicology, 2017, 99: 190-198.

[8] DOLAYCHUK O P, ROSTYSLAV S F, KOVALCHUK I I. Immunological and reproductive functions of the female rats fed by natural and genetically modified soybeans[J]. International Journal of Physiology and Pathophysiology, 2014, 5(1): 49-55.

[9] ZHOU Xing-hua, DONG Ying, ZHAO Yan-sheng, et al. A three Generation reproduction study with Sprague–Dawley rats consuming high-amylose transgenic rice[J]. Food and Chemical Toxicology, 2014, 74: 20-27.

[10] LAURA M A, MALLEY B L, SUSAN A B. Subchronic feeding study with genetically modified stacked trait lepidopteran and coleopteran resistant (DAS-Ø15Ø7-1xDAS-59122-7) maize grain in Sprague-Dawley rats[J]. Food and Chemical Toxicology, 2009, 47(7): 1512-1520.

[11] WANG Er-hui, ZHOU Yu, JING Hu, et al. A two-generation reproduction study with transgenic Bt rice TT51 in Wistar rats[J]. Food and Chemical Toxicology, 2014, 65: 312-320.

[12] RASMUSSEN M A, CUTLER S A, WILHELMS K, et al. Effects of BT (bacillus thuringiensis) corn on reproductive performance in adult laying HENS[J]. INT J Poult SCI, 2007, 6: 169-171.

[13] APPENZELLER L M, MUNLEY S M, HOBAN D, et.al. Subchronic feeding study of herbicide–tolerant soybean DP-356Ø43-5 in Sprague–Dawley rats[J]. Food and Chemical Toxicology, 2008, 46: 2201-2213.

[14] FLA´VIA BITTENCOURT BRASIL, LAVI´NIA LEAL SOARES, FARIA T S, et al. The impact of dietary organic and transgenic soy on the reproductive system of female adult rat[J]. The Anatomical Record, 2009, 292: 587-594.

[15] NAGY A, GERTSENSTEIN M, VINTERSTEN K. Manipulating the mouse embryo: a laboratory manual(third edition)[M]. New YORK: Cold Spring Harbor Laboratory Press, 2005.

[16] DENG Xiao-hui. Technology and color Atlas of reproductive medical science[M]. Jinan: Shandong Science and Technology Press, 2004.

[17] DOMINGO J L. Safety assessment of GM plants: An updated review of the scientific literature[J]. Food and Chemical Toxicology, 2016, 95: 12-18.

[18] ARMENTI A E, ZAMA A M, PASSANTINO L, et al. Developmental methoxychlor exposure affects multiple reproductive parameters and ovarian folliculogenesis and gene expression in adult rats[J]. Toxicology and Applied Pharmacology, 2008, 233(2): 286-296.

[19] MARC-ANDRE´ S, FRANCOIS R, PATRICK B, et al. Contribution of the oocyte to embryo quality[J]. Theriogenology, 2006, 65(1): 126-136.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202106_7(6).0044

[20] CHUKWUDEBE A, PRIVALLE L, REED A, et al. Health and nutritional status of Wistar rats following subchronic exposure to CV127 soybeans[J]. Food & Chemical Toxicology an International Journal Published for the British Industrial Biological Research Associat, 2012, 50(3/4): 956-971.

[21] SONG Huan, HE Xiao-yun, ZOU Shi-ying, et al. A 90-day subchronic feeding study of genetically modified rice expressing Cry1Ab protein in Sprague–Dawley rats[J]. Transgenic Research, 2015, 24(2): 295-306.

[22] YANG Qing-qing, HE Xiao-yun, WU Hong-yu, et al. Subchronic feeding study of high-free-lysine transgenic rice in Spragus-Dawley rats[J]. Food and Chemical Toxicology, 2017, 105: 214-222.

[23] SAIZ N, PLUSA B. Early cell fate decisions in the mouse embryo[J]. Reproduction, 2013, 145(3): R65-R80.

[24] ANTONIO GARCIA M T, HERRERA D A, PINEDA P J. Hematological effects of Arsenic in rats after subchronical exposure during pregnancy and lactation_ The protective role of antioxidants[J]. Experimental and Toxicology Pathology, 2013, 65(5): 609-614.

[25] BRAKE D G, EVENSON D P. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development[J]. Food and Chemical Toxicology, 2004, 42(1): 29-36.

[26] LU Chun-bin, YANG Dong-yu, GAO Chen, et al. Safety assessment of reproductive system in male fed with genetically modified soybeans[J]. Journal of Yangzhou University(Agricultural and Life Science Edition), 2012, 33(1): 23-27.

[27] LU Chun-bin, ZHOU Wen, LIU Biao. Effects of transgenic soybean on reproductive system in male mice[J]. Soybean Science, 2013, 32(1): 119-123.

[28] LU Chun-bin, CAI Juan, QI Qing-lin, et al. Effects of glyphosate-resistant transgenic soybean on male mice testis[J]. Journal of Jinan University(Natural Science & Medicine Edition), 2014, 35(3): 245-249.

[29] LU Chun-bin, QI Qing-lin, ZHOU Wen, et al. Effect of glyphosate-resistant tranegenic soybean on micronucleus of sperm cells and acrosomal reaction rate in male mice[J]. Science and Technology of Food Industry, 2014, 35(11): 350-354.

[30] LU Chun-bin, ZHENG Jian-xin, CAI Juan, et al. Effects of genetically modified soybean on embryonic development and the ability of fertilization in female mice[J]. Soybean Science, 2014, 33(4): 478-582.