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Embryotoxicity of Transgenic Rice TT51 and Cry1Ab Bt Insecticidal Toxin in Rat Post Implantation Whole Embryo Culture

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Abstract TT51, with a synthetic Cry1Ab/CryAc gene from Bacillus thuringiensis Berliner, was the first genetically modified (GM) plant officially certificated in China. This study was undertaken to investigate potential embryotoxicity of TT51 on rats. Whole embryo culture (WEC) is an effective method for safety evaluation when culture medium, containing substances derived from GM food, is available. Rat embryos explanted from uterus were randomly distributed into 6 groups: Transgenic Rice (TR) group with transgenic rice dietary administration, Parental Rice (PR) group with parental rice dietary administration, Blank Control (BC), Positive control A (PCA) group with Bisphenol A being directly added into the culture medium, Positive control B (PCB) group with Bisphenol A oral administration and Bt protein (BP) group with Bt protein directly added into culture medium. After culturing for 48 h, embryos were scored for growth and differentiation at the endpoint. Embryos in TR group had no significantly lower morphological scores or smaller yolk sac diameter, crown-rump length (CRL) and cranial diameter comparing with those in BC and PR group. Embryos in PCA and PCB group both performed significantly lower developmental parameters and morphological scores than the other three groups. Embryos directly exposed to Bt protein (52 mg/L) exhibited severe morphological anomalies but no significant difference in yolk sac diameter compared with PR, TR and BC groups. In this WEC model, TT51 showed no embryotoxicity to rats although Bt toxin had side effect on embryos when the concentration is equal to the daily intake of Bt protein in TT51 diet. And it also indicated that WEC method can make an option for toxicological assessment of GM food.

Keywords: *transgenic rice, whole embryo culture, embryotoxicity, TT51, Bt protein*

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1. Introduction

Rice (Oryza sativa), a staple food for over 1 billion people in China, is also one of the most widely consumed food crops worldwide [1]. Rice biotechnology has scored tremendous progress since the first genetically modified rice was produced. As insect pests brought about heavy perennial yield losses, especially stem borers, leaf folders and plant hoppers in China [2]. In the middle of 1980 s, the first insect-resistant transgenic plants with genes from the bacterium *Bacillus thuringiensis* (*Bt*) was developed [3]. Since then, Scientists in China had been working on developing safe control tactics for insect pests of rice and dozens of Bt rice genotypes highly resistant to lepidopteron pests have been developed [4,5].

TT51 is an insect-resistant transgenic rice created by micro projectile bombardment with two plasmids, pFHBT1 and pGL2RC7, into the elite Chinese cytoplasmic male sterile (CMS) restorer line, Minghui63 [6]. Fields tests indicated that TT51 could reduce pesticide application and increase rice production efficiency through resistance against major Lepidopteran pests [7]. However, potential unintended effects may have occurred with the insertion of the foreign gene in this transgenic rice. As with all transgenic crops, potential unintended effects due to insertion of the foreign gene are to be evaluated to ensure safety of the crop. Since the application for the commercialization of transgenic rice trials was first filed in 2004, no certificates had yet been given by China's Ministry of Agriculture until 2009 [8]. However, GM rice, as a traditional staple food being commercialized in China, inevitably brings particularly acute debate on this transgenic rice [7].

Safety assessment of GM foods is still dependent on the use of the "substantial equivalence" principle, that "if a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as being safe as the conventional

food" [9]. And 90 day rodent Feeding studies have been recommended to assess the potential adverse effects of transgenic crops [10,11]. However, experimental studies on safety of GM foods were scant and there are still overwhelming doubts about the immature toxicological assessment procedure for GM food [12,13]. Indeed, A few studies have reported obviously harmful effects of GM food. In the study led by Vecchio [14] pregnant Swiss mice and male litters were fed on a standard diet containing 14% GM soybean. By means of immunoelectron microscopy at 2, 5 or 8 months of age, they focused on Sertoli cells, spermatogonia and spermatocytes. Results showed that GM-fed mice of all ages had larger number of perichromatin granules and lower nuclear pore density. Meanwhile, enlargements of smooth endoplasmic reticulum was also observed in Sertoli cells from GM-fed mice. A three-generation study reported minimal histopathological changes in liver and kidney in F3 female offspring of rats fed a Bt maize [15]. Another study revealed a temporary decrease of pre-mRNA transcription and splicing in embryos from mice fed GM soybean; moreover, pre-mRNA maturation seems to be less efficient in embryos from GM-fed mice than in control [16]. Dona and Arvanitoyannis concluded that GM foods appeared to have toxic effect based on most studies summarized, including reproductive effects, and might alter the hematological, biochemical, and immunologic parameters [17]. GM rice, as a traditional staple food being commercialized in China, inevitably brings particular public concerns about the daily exposure for a long period.

Modified Cry proteins forming pores in insect cell membranes [18]; Since natural Bt toxins have long been used, their modified counterparts are often compared with them. they account for 39% of edible GM plant worldwide [19]. Modified Bt toxins are truncated, adapted and modified sequences; so that their bioactivity possibly exhibit quite a difference from natural ones. [20]. In the reported in vitro cell culture tests on Bt protein, toxicity of Bt toxins has been observed in mammals at a 50% lethal concentration (LC 50) from 10 to 520 ppb [21,22,23]. Until now, developmental toxicity of Bt toxins has never been tested in embryo culture model. However, serum residues of Cry1Ab protein (a Bt toxin) has been found in pregnant women living in Eastern Townships of Quebec, Canada, where Bt maize have been regularly consumed by humans since 1996 [24].

Whole embryo culture (WEC) is one of the three in vitro embryotoxicity tests validated by the European Centre for the Validation of Alternative Methods (ECVAM) and it appears to be an excellent method to screen chemicals for teratogenic hazard [25]. Compared to in vivo testing, it is cheap and rapid without experimentation on live adult animals. None of the three alternative methods have ever been applied to risk assessment of whole food which not be able to add into the in vitro culture system directly. However, Adverse embryonic outcomes (malformations or embryotoxicity) in the WEC model are directly related to the serum concentration of the compound being tested. Through providing diet or oral administration to rats, serum made from the rats as culture medium indeed receive GM food intervention. If changes or side effects did occur and reflect in serum after consumption of GM food, we can observe embryotoxicity of GM food through this adjusted WEC model.

In vitro tests are frequently recommended as a first step to replace animal models in toxicity studies. In this study, We aimed to test whether TT51 and Bt protein (Cry1Ab) have side effect on rat embryos by applying the adjusted WEC model mentioned above and verify the hypothesis that WEC is a prosperous new and efficient method for risk assessment of GM food

2. Materials and Methods

2.1. Ethics Statement

This study was approved and performed in accordance with the guidelines for animal experiments of the Peking University Health Science Center. And it was also approved by Medical Ethic Committee of Peking University Health Science Center. Animals used in the study were taken to ameliorate suffering by ether anesthetization.

2.2. Animal Housing and Mating

Sprague–Dawley rats, purchased from Experimental Animal Division of Peking University Health Sciences Center, Beijing, China., were housed in the specific pathogen-free animal facility in a climate controlled room with a 12-h on/off light cycle.

Male rats (approximately 8–10 weeks of age and weighing 300 g) were bred and maintained for the preparation of immediately centrifuged serum.

After 2 weeks of acclimatization, nulliparous female rats (approximately 8–10 weeks of age and weighing 180–200 g) were housed together with adult male rats from the same strain and supplier for overnight (one pair per cage). After coitus (termed as gestation day 0) females were housed singly. Tap water and standard diets were provided adlibitum. Animals were monitored daily for general health. Studies were approved and performed in accordance with the guidelines for animal experiments of the Peking University Health Science Center. This study was approved by Medical Ethic Committee of Peking University Health Science Center.

2.3. Chemicals and Feeds

2.3.1. Compound for Positive Control

Bisphenol A (BPA, 97% purity, ACROS) was dissolved and diluted in DMSO (Sino-pharm Chemical Reagent Co. Ltd) with the final 300 mg/ml concentration for oral administration and 80 mg/L for culture medium.

2.3.2. Bt Toxin Preparations

The Cry1Ab toxin is obtained from the natural Bacillus thuringiensis subspecies kurstaki HD-1 strain and expressed as single gene products in Escherichia coli. The 65 kDa activated toxins were isolated by ion exchange HPLC and the pure toxin fractions were desalted and lyophilized and stored at−80°C. Then, Bt protein was diluted in DMSO at 1 mg/ml, and then diluted in ICS.

2.3.3. Plant Materials

Transgenic rice (TT51) and its parental non-transgenic rice (Minghui63) were cultivated in the experimental field of Central China Agricultural University (Wuhan, China) and provided by Chinese Center for Disease Control and Prevention.

2.3.4. Certification and Compositional Analysis of Rice

Samples of TT51 and Minghui63 were evaluated for the presence of the CryAb/CryAc gene using polymerase chain reaction (PCR) following standards of the Ministry of Agriculture of China [26] and the Bt protein was detected with an antibody specific enzyme linked immunosorbent assay (ELISA). TT51 rice was found to be positive for the presence of Cry1Ab/Cry1Ac gene and protein while Minghui63 was found to be negative. Compositional analysis reports of TT51 rice and Minghui63 were provided by the Chinese Center for Disease Control and Prevention (Table 1). Analysis of crude nutrients: protein, fat, fiber, moisture and ash were determined in accordance with Chinese Standard methods [27,28,29,30]. Carbohydrate: Carbohydrate was determined using the following calculation carbohydrate% =100 %- (% protein + % fat + % ash + % moisture) as described by Han et al [31].

Table 1. Proximate concentrations of rice flour in g/ 100 g, (n=2)

Nutrients	TT51		Minghui63		literature data[a]
Protein	8.063	8.066	7.844	7.885	6.1-9.5
Fat	1.013	0.959	1.121	1.104	1.4-5.3
Moisture	11.123	11.121	10.870	10.852	9.1-14.1
Ash	0.4801	0.4766	0.4637	0.4792	0.9-1.5
Total dietary fiber	5.29	5.27	2.65	2.69	0.5-6.8
Carbohydrate	74.03	74.03	77.04	76.98	57-77

[a]Ranges from minimum to maximum reported values

2.3.5. Diet Formulation and Compositional Analysis

Based on the results of the compositional analysis, flours of TT51 and Minghui63 were formulated into rodent diets at concentration of 81.8% by mass. Carbohydrate in TT51 formula was completely derived from transgenic rice. And casein was added into the feeds in order to make equivalent protein content to the AIN-93G as protein stemmed from rice can't meet the formulating requirement. Other macronutrients in formula feeding follow the same principle as protein when nutrients contents from transgenic rice or parental rice are lower than the standard AIN- 93G formula. An additional AIN93-G rice-based diet was included as negative control. All diets were produced following AIN93-G guidelines [32]. All diets were produced by Hua Fu Kang Feed Co. Ltd. (Beijing, China). The composition of all diets is summarized in Table 1, and the nutritional composition of these diets is presented in Table 2.

Table 2. Composition of diets for rats

Ingredient (%)	AIN93-G	TT51	Minghui63
Maize	0.00	81.77	81.77
Casein	20.00	13.57	13.41
Cane sugar	10.00	0.00	0.00
Corn starch	52.95	0.00	0.00
Soybean oil	7	6.10	6.18
Cellulose	5	2.82	0.68
Minerals	3.50	3.50	3.50
Vitamins	1.00	1.00	1.00
Methionine	0.18	0.18	0.18
Choline Choride	0.17	0.17	0.17

Table 3. Nutritional composition of diets

Components (%)	AIN93G	TT51	Minghui63
Crude Protein	20	20	20
Crude Fat	7.0	7.0	7.0
Carbohydrate	62.95	62.95	68.76
Cellulose	5.0	5.0	5.0
Energy, kcal/g	3.77	3.77	4.00

2.4. Experimental Design

2.4.1. Preparation of Immediately Centrifugal Serum (ICS)

Animals for the preparation of ICS were divided into 6 groups as follows (Table 4):

Table 4. interventions given to animals for the preparation of ICS

Group	Diet or Dosage
Blank Control (BC)	Fed by AIN- 93G diet
Transgenic Rice (TR)	Fed by Transgenic rice formulating feeds containing 81.8% genetically modified rice
Parental Rice (PR)	Fed by Parental rice formulating feeds containing 81.8% parental rice
Positive Control A (PCA)	Fed by AIN- 93G diet but BPA was directly added into the corresponding ICS at the concentration of 80mg/L
Positive Control B (PCB)	Orally administered BPA at the dose of 100 mg per kilogram of bodyweight per day (mg/kg·d) for 3 days. On the third day, ICS was prepared 1 hour after administration.
Bt Protein (BP)	Fed by AIN- 93G diet but Bt protein was directly added into the corresponding ICS at the concentration of 52 µg/L

2.4.2. The WEC Procedure

After mating, female rats were housed individually. At day 9.5 of gestation, rat embryos with 2−5 somites were explanted from the uterus and randomly distributed into these 5 groups of ICS listed in Table 1. All details of the protocol are described in the standard operating procedure of the WEC, the Invittox Protocol No. 123 [33]. Three embryos were placed in a sealed culture flask (50 mL) containing 4 mL culture medium. Culture flasks were gassed with increasing oxygen concentrations for three times. After 48 h of culturing at 37°C, the embryos were morphologically examined according to the protocol.

At the end of culturing, embryos were evaluated by observing a few endpoints for their growth (crown–rump length, yolk sac diameter and cranial diameter) and differentiation (number of somites and morphological score) with the aid of a dissection stereo-microscope as described in protocol. Briefly, an ocular micrometer was used to measure crown-rump length and a morphological score system was used to evaluate the phenotype of the embryos. Also, the number of somites was counted. Development of the general embryonic shape, differentiation of the neural tube, head, eyes, ears, heart, fore and hind limbs, caudal part of the trunk referred to as tail and the presence of blood in the embryo itself were assessed. Furthermore, the development of the yolk sac was estimated regarding its blood vessels system. Embryos were considered to be viable when a beating heart and blood flow through the yolk sac vessels were observed. Specific dysmorphogenesis of embryos were determined by disproportional development of single organ anlagen in comparison.

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) was used to test differences between groups of continuous variables(crown − rump length, yolk sac diameter and cranial length). Tamhane's T2 test was used as a posteriori test when a difference was found with ANOVA. Morphological scores as categorical variables were analyzed base on the Kruskal−Wallis test. Nemenyi test in non-parametric statistical was performed to achieve multiple comparisons. All analyses were performed by using SPSS software (PASW version 18.0.2, 2009; SPSS Inc, Chicago, IL).All tests of significance were 2-sided and a probability value of <0.05 was considered as significant.

3. Results

3.1. Embryos Development of Rats in TR Group Compared with that in PR and BC Groups

Results showed that yolk sac diameter, crown-rump length and cranial diameter have no significant differences between TR group and PR group, as well as the data of TR group without significant difference compared with BC group ($p > 0.05$, Table 2). No significant difference of morphological scores was observed either. ($p > 0.05$, Table 3).

3.2. Embryos Development of Rats in TR Group Compared with that in PCA and PCB Groups

According to the results of the previous study on embryotoxicity of BPA on rat and mice in our lab(Yong, Long Ding xin, 2003) and published BPA pharmacokinetic data, we chose the concentration of 80 mg/L for PCA group and dosage 100 mg/ (kgbw·d) of oral administration for PCB group. Rat embryos in PCA and PCB group both have significantly lower biometric and morphologic parameters (yolk sac diameter, crown-rump length and cranial diameter) compared with that in TR group ($p < 0.05$, Table 2). There were significant reductions in morphological scores of embryos in PCA group compared with that in TR group ($p < 0.05$, Table 3). Embryos in PCB group had significant reduction in morphological scores of yolk sac circulation, heart, mid brain, fore brain, mandibular process, fore limb, hind limb and somites compared with that in TR group ($p < 0.05$, Table 3). Abnormal development of the second visceral arches and open neural pores and aberrations in the head region of the embryos were observed in two positive control groups as specific dysmorphogenesis induced by BPA (Figure 1, Figure 2).

3.3. Embryos Development of Rats in BP Group Compared with that in BC and TR Groups

Compared with group BC and TR, yolk sac diameter in BP group showed no significant difference ($p < 0.05$), Table 5. However, significant lower crown-rump length and cranial diameter was observed in BP group than both BC and TR groups ($p < 0.05$, Table 5). Embryos treated with Cry1Ab Bt protein exhibited significantly lower morphological scores in all this scoring subjects than those of BC and TR groups ($p < 0.05$ Table 6). Although yolk sac diameter of embryos in BP group showed no difference, Bt protein seriously affected organogenesis and development of neural tube (Figure 1, Figure 2).

Table 5. Developmental characteristic of rat embryos among all the 6 groups

	BC n=18	TR n=19	PR n=17	PCA n=19	PCB n=14	BP n=14
Yolk sac diameter (mm)	5.30±0.73	5.84±0.93	5.93±0.58	3.93±0.62$^{*\#a}$	4.60±0.53$^{*\#}$	5.83±1.05
Crown-rump length (mm)	4.98±0.76	5.19±1.28	4.35±0.45	2.98±0.81$^{*\#a}$	4.17±1.18	3.50±0.52$^{*\#a}$
Cranial diameter (mm)	2.17±0.44	2.20±0.32	2.49±0.31	1.00±0.29$^{*\#a}$	1.84±0.13$^{\#}$	1.66±0.21$^{*\#a}$

Values are means ± SEM *: vs. TR group $p<0.05$; $^{\#}$: vs. PR group $p<0.05$; ^{a}: vs. BC group $p<0.05$

Table 6. Morphological score of embryo organ among all the 6 groups

	BC n=18	TR n=19	PR n=17	PCA n=19	PCB n=14	BP n=14
Yolk sac circulation	3.50±0.61	3.14±0.77	3.76±0.43	1.47±0.51*#a	2.07±0.61*# a	2.50±0.52#a
Allantois	2.83±0.38	2.86±0.36	3.18±0.39	1.00±0.74*# a	2.14±0.77#	1.93±0.99*#a
flexion	3.50±1.34	3.07±1.86	3.35±0.49	0.32±0.48*# a	2.29±1.14	1.50±1.01*#a
Heart	3.61±0.50	3.57±0.64	3.47±0.51	0.95±0.41*# a	2.00±0.78*# a	1.14±0.86*# a
Neural tube	4.00±0.00	4.00±0.00	4.00±0.00	0.53±0.69*# a	3.00±1.04# a	1.43±1.16*#a
Hind brain	3.94±0.23	3.86±0.36	4.00±0.00	0.32±0.58*# a	2.50±1.29# a	1.43±1.16*#a
Mid brain	4.00±0.00	4.00±0.00	4.00±0.00	0.47±0.51*# a	2.64±1.33*# a	1.36±1.28*#a
Fore brain	3.89±0.32	3.93±0.27	4.00±0.00	0.47±0.69*# a	2.64±1.22*# a	1.50±1.29*#a
Auditory system	4.00±0.00	3.71±0.83	4.00±0.00	0.00±0.00*# a	2.50±1.34# a	1.00±1.47*#a
Optics system	3.83±0.38	3.71±0.61	3.94±0.24	0.26±0.45*# a	2.57±1.28#	0.93±1.50*# a
Olfactory system	3.94±0.24	3.79±0.43	4.00±0.00	0.16±0.38*# a	2.50±1.34# a	0.86±1.35*#a
Visceral arch	3.50±0.85	3.00±1.03	3.41±1.00	0.21±0.54*# a	1.57±0.51# a	0.64±1.00*#a
Maxillary process	2.39±0.61	2.50±0.76	3.29±0.47	0.11±0.31*# a	1.57±0.64#	0.36±0.63*#a
Mandibular process	2.72±1.07	3.14±0.66	3.59±0.51	0.11±0.31*# a	1.64±0.63*#	0.50±0.76*#a
Fore limb	3.00±1.13	2.86±1.35	3.65±0.99	0.16±0.37*# a	1.21±.080*# a	0.57±0.65*#a
Hind limb	2.89±0.32	2.71±0.46	3.35±0.61	0.21±0.53*# a	1.50±0.85*# a	0.29±0.61*#a
somites	3.67±0.48	3.50±0.76	4.00±0.00	0.16±0.37*# a	2.07±1.20*# a	0.57±0.65*#a

Values are means±SEM *: vs. TR group $p<0.05$; #: vs. PR group $p<0.05$; a: vs. BC group

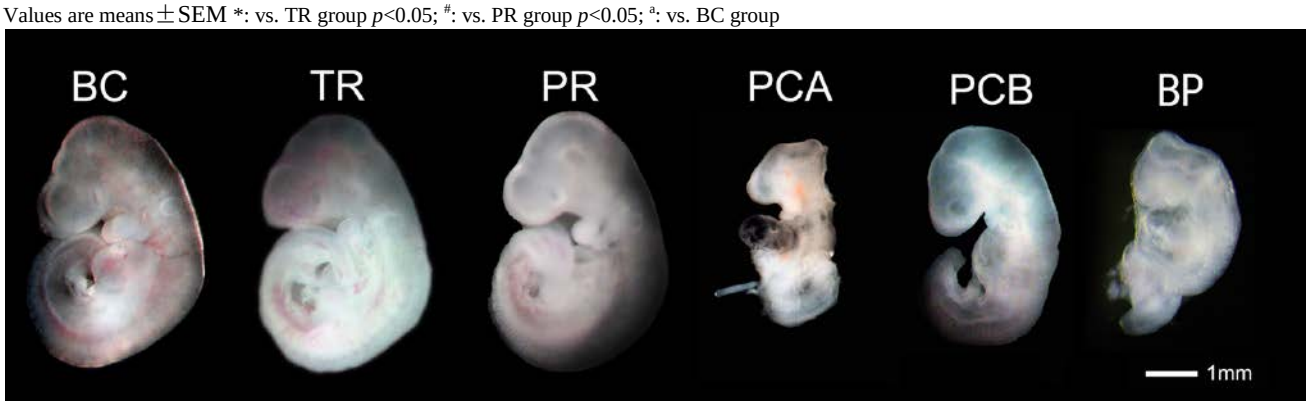

Figure 1. morphological appearances of representative embryos in 6 groups exposed to culture mediums with TR, PR,BPA and Bt protein through different routes of administration

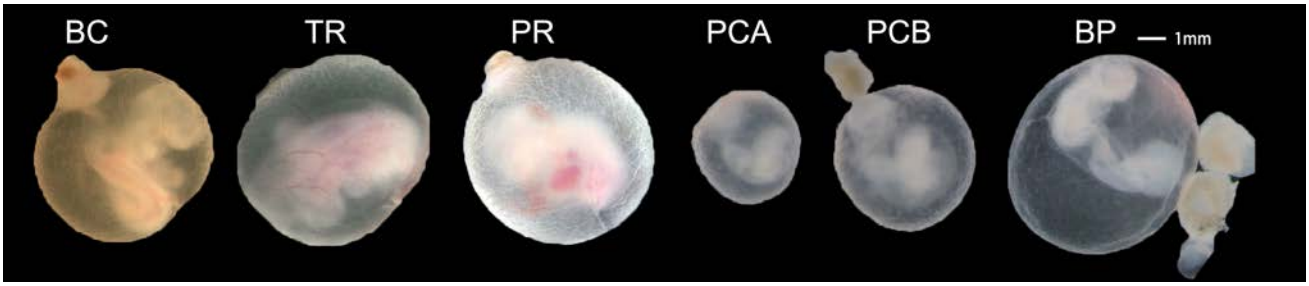

Figure 2. Representative image of rat embryos in 6 groups with yolk sac after being exposed to culture mediums with TR, PR, BPA and Bt protein through different routes of administration

4. Discussion

In recent years, safety of transgenic crops has been a controversial topic. Although previous scientific evidences indicate that GM crops are similar with their conventional counterparts, the general publics still have reasonable doubt whether these transgenic crops cause adverse effects [31]. Some scientists think that transgenic crops have not been adequately tested to be proved no harm to health [34,35].

In our study, TT51 rice and its parental was added into formulating feeds by mass. Carbohydrate in TT51 diet is totally provided by transgenic rice. And we also make sure nutrient composition of TT51 diet and Minghui63 diet has no difference from standard AIN-93G formula. Male rats fed for preparation of ICS, had taken corresponding formulating feeds for 3 days. ICS, derived from male rats fed by the transgenic rice formulation, was used to culture embryos. Bioactive materials originating from diet usually appear in ICS. If they have side effect on development of embryo, embryo with 2−5 somites explanting into the serum will have abnormal morphological development. Compared with paralleled parental rice feeds and blank control diet, transgenic rice did not significantly change the development of rat embryos both in objective measurement of yolk sac diameter, crown-rump length and cranial diameter and morphological scores, which possibly indicated dietary intake of transgenic rice has no embryotoxicity.

As the commercialization of GM food is unstoppable in China, billions of people and wildlife could be exposed to modified Bt toxins. Limited studies have discussed about effects of Bt toxin. For natural Bt toxins, their mechanisms of bioactivity and insect resistance are not fully understood [36], and the metabolism of Bt proteins

in mammals is also unknown [20,37]. Thus understanding their potential side effects is crucial. Based on the assumption that adults rats (weight about 400 g) take transgenic rice as the main energy resource, each animal will eat 40 g rice per day on average following the daily intake calculation: (10 g/100 g bodyweight·d) × body weight. Moreover, hulled TT51 rice was detected with ELISA for its expressed Bt protein level of 1300±13 ng/g. Thus, adult rats feeding on TT51 are daily exposed to no more than 52 μg (1300 ng/g × 40 g). So we chose the dose of 52 μg/ml for whole embryo culture in BP group. Compared with blank diet and transgenic rice diet, direct Bt protein intervention in ICS did show side effects on development of rat embryos, especially on organ formation. Although we have observed positive outcome with Bt protein, TT51 diet did not show any side effect in the WEC model. Obviously, digestion process can make a reduction in vivo for Bt protein expressed in TT51. Bt protein exposure during consumption can appear relatively low to refrain from side effects. And However, the bioaccumulation in tissues or long-term effects, has to be taken seriously as Bt residues were reported to be tested in maternal serum [24]. In vitro tests focus on embryotoxicity of Bt protein have not been included in the procedure for GM crops commercialization such as MON810 [38]. Thus, our results are raising new questions about the safety and risk assessment of these toxins and the Bt crops in general. Bisphenol A, an environmental estrogenic endocrine disruptor, has been widely acknowledged as teratogen and embryotoxic [39].In our previous research, colleagues in our lab did observe that BPA at the concentration of 80 mg/L delayed growth of embryos and induced various embryo defects in WEC model [40]. Rats for preparation of ICS in PCB group were orally administered BPA for 3 days to examine whether WEC could be an effective model for safety evaluation of GM rice. Most of the previous pharmacokinetic studies about blood BPA concentration following oral gavage chose relatively low dosing level(10 or 60 mg/kg) but ICS prepared 1 hour after administration at low dosage didn't show any effects on rat embryos in the pre-experiment. That suggested us to choose a higher oral gavage dosage in PCB group in order to observe the potential embryotoxicity induced by BPA exposure through diets which also simulate the situation of staple food for people with higher consumption. After a series of pre-experiment, we chose the dose of 100 mg/kg·d for oral administration of group PCB among 100, 200 and 300 mg/ (kgbw·d). According to the results, PCA and PCB group both showed consistent effect on embryo development, although there were still differences between the two groups in organogenesis. Multiple comparisons between TR group and positive control groups suggested that dietary intake of transgenic rice didn't have the teratogenic effect at least in the serum level after ingestion of diet mainly composed of transgenic rice.

It is important to consider the limitations of current study. First, transgenic rice was given to animals only for 3 days. But those people who live on rice as staple food usually eat rice for lifetime-long. Small changes due to intake of transgenic rice should be cumulated for a long period to be observed. Further study should include more extensive WEC test with longer diets exposure. The conclusion that TT51 has no embryotoxicity was based on a screening test. But comprehensive evaluation of safety still need a multi-generation model or life-long feeding study to simulate what will happen in human being after decades. Second, according to BPA's pharmacokinetics data we prepared serum of PCB group 1 hour after dosing 100 mg/ (kgbw·d) BPA on the third day, but the concentration of bioactive BPA in the serum did not be accurately detected. So when we got the result that directly-added BPA had significantly stronger embryotoxicity than orally-administered BPA did, the serum with uncertain concentration of BPA in PCB group may explain. Third, design of experiment was established under the hypothesis that toxicity of transgenic rice can be reflected in the serum after intake and digestion by animals. Further study on embryotoxic effect of transgenic rice need to confirm whether Bt toxin appear in the serum and lead to corresponding side effect after a longer consumption period.

Our study made a necessary supplement to previous studies concerning safety of transgenic food and effective method to test its embryotoxicity. We documented that modified Cry1Ab Bt protein can exert toxicity, at least under certain in vitro conditions. It was the first time that WEC had been introduced into safety evaluation of GM food. As a recognized in vitro screening test for embryotoxicity, WEC usually requires chemicals which can be added directly into culture medium so that it was hardly applied to evaluation of whole food. This study designed formulating feeds for rats and then derived the serum of fed rats to culture embryo in order to make transgenic rice appropriate for WEC. The results of this study may present useful suggestion for risk assessment of food and feed derived from genetically engineered plants.

5. Conclusion

Dietary intake of transgenic rice TT51 has no embryotoxicity on rat embryos in WEC model. Howerver, modified Cry1Ab Bt protein had a significant side effect on the development of embryos while it was directly added into the culture medium in WEC.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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