Biol Trace Elem Res (2015) 164:106–113
DOI 10.1007/s12011-014-0195-1

Effect of TiO_2 Nanoparticles on the Reproduction of Silkworm

Min Ni · Fanchi Li · Binbin Wang · Kaizun Xu · Hua Zhang · Jingsheng Hu · Jianghai Tian · Weide Shen · Bing Li

Received: 3 November 2014 / Accepted: 24 November 2014 / Published online: 4 December 2014

Abstract Silkworm (*Bombyx mori*) is an important economic insect and the model insect of Lepidoptera. Because of its high fecundity and short reproduction cycle, it has been widely used in reproduction and development research. The high concentrations of titanium dioxide nanoparticles (TiO_2 NPs) show reproductive toxicity, while low concentrations of TiO_2 NPs have been used as feed additive and demonstrated significant biological activities. However, whether the low concentrations of TiO_2 NPs affect the reproduction of *B. mori* has not been reported. In this study, the growth and development of gonad of *B. mori* fed with a low concentration of TiO_2 NPs (5 mg/L) were investigated by assessing egg production and expression of reproduction-related genes. The results showed that the low concentration of TiO_2 NPs resulted in faster development of the ovaries and testes and more gamete differentiation and formation, with an average increase of 51 eggs per insect and 0.34×10^{-4} g per egg after the feeding. The expressions of several reproduction-related genes were upregulated, such as the yolk-development-related genes *Ovo-781* and vitellogenin (*Vg*) were increased by 5.33- and 6.77-folds, respectively. This study shows that TiO_2 NPs feeding at low concentration can enhance the reproduction of *B. mori*, and these results are useful in developing new methods to improve fecundity in *B. mori* and providing new clues for its broad biological applications.

Keywords *Bombyx mori* · Titanium dioxide nanoparticles · Reproductive capacity · Fecundity · Growth and development

Introduction

Silkworm (*Bombyx mori*) belongs to Lepidoptera (Bombycidae) and is an economically important insect. It was domesticated from wild *B. mori* over 5700 years ago [1]. *B. mori* is a complete metamorphosis insect with four stages of development of about 50 days: egg, larva, pupa, and adult. The adult insect lays about 500 eggs per animal and is designated as the model insect of Lepidoptera since 2002 [2]. In 2003, the complete genome of the insect was sequenced, and the sequence information has been widely used in genetics, developmental biology, ecology, toxicology, cell biology, and biochemistry studies [3].

Titanium dioxide nanoparticles (TiO_2 NPs) are widely used as an additive in the food industry, cosmetics, electronics, coatings, medical products, and animal husbandry [4–7]. In animal husbandry, TiO_2 NPs are used to promote animal growth [8] and protein synthesis [9]. Previous studies found that TiO_2 NPs can improve the growth and antioxidation capacity of plants [10, 11] and enhance carbon and nitrogen metabolism and increase the resistance of spinach [11]. In *Drosophila melanogaster*, ingestion of TiO_2 NPs up to a dose of 200 μg/mL during the larval stage did not affect the development or survivorship [12]. Zhang et al. found that feeding *B. mori* with 5 mg/L of TiO_2 NPs resulted in increased weight and leaf-silk conversion, improved activity of the midgut proteases for more efficient nutrient uptake, and increased protein synthesis and metabolism [13]. Previous studies have shown that more than 90 % of *B. mori* proteins were allocated to the silk gland and the ovaries for silking and oviposition [14]. Increased silking has been observed after

Min Ni and Fanchi Li contributed equally to this study.

M. Ni · F. Li · B. Wang · K. Xu · H. Zhang · J. Hu · J. Tian · W. Shen · B. Li (✉)
School of Basic Medicine and Biological Sciences, Soochow University, Suzhou, Jiangsu 215123, People's Republic of China
e-mail: lib@suda.edu.cn

W. Shen
National Engineering Laboratory for Modern Silk, Soochow University, Suzhou, Jiangsu 215123, People's Republic of China

TiO_2 NPs feeding [13]. However, whether the feeding will affect the reproduction activity of *B. mori* remains unclear.

In the present study, gonad development, oviposition, and expression of reproduction-related genes were investigated after feeding *B. mori* with a low concentration of TiO_2 NPs (5 mg/L) to understand the effect of TiO_2 NPs on fecundity.

Materials and Methods

Insect and Chemicals

The larvae of *B. mori* strain (Suju, Minghu) maintained in our laboratory were reared on mulberry leaves under a 12-h light/12-h dark cycle. TiO_2 NPs were prepared via the controlled hydrolysis of titanium tetrabutoxide. The detailed synthesis and characterization of TiO_2 NPs were described in our previous reports [15, 16]. The average particle size of the TiO_2 NPs powder suspended in 0.5 % (*w*/*v*) hydroxypropyl methylcellulose (HPMC) K_4 M solvent after 24-h incubation ranged from 5 to 6 nm. The mean hydrodynamic diameter of TiO_2 NPs in HPMC solvent ranged between 208 and 330 nm (mainly 294 nm), and the zeta potential was 9.28 mV [16].

Method for Suspending TiO_2 NPs

TiO_2 NPs powder was dispersed into 0.5 % (*w*/*v*) HPMC, and the mixture containing TiO_2 NPs was treated with ultrasonication for 30 min and mechanically stirred for 5 min to suspend the compound.

TiO_2 NPs Feeding

Mulberry leave were soaked in 5 mg/L TiO_2 NPs. The soaked leaves were dried naturally at room temperature and used to feed the 5th instar larvae continuously from exuviation till mounting, three times a day. The control larvae were fed with the leaves soaked in water. Female and male *B. mori* in the TiO_2 NPs treatment and control groups were reared separately, and each treatment was repeated three times with 30 larvae.

Sample Preparation and Total RNA Isolation

The larvae were dissected at mature silkworm after being fed with TiO_2 NPs, and the ovaries and testes were collected and stored at −80 °C. Total RNA was extracted from the samples using TRIzol reagent (Takara Bio Inc., Dalian, China) and then treated with DNases to remove the potentially contaminated genomic DNA. The integrity and quality of the RNA were assessed by formaldehyde agarose gel electrophoresis, and the concentration was quantitated spectrophotometrically at 260 and 280 nm.

Histopathological Evaluation

The ovaries and testes were histopathologically examined using the standard laboratory procedures. The samples were embedded in paraffin, sliced to thin sections (5-μm thick), and mounted onto glass slides. After hematoxylin-eosin staining, the sections were evaluated by a histopathologist unaware of the treatments, using a light microscope (Nikon U-III Multi-point Sensor System, Nikon, Tokyo, Japan).

RT-qPCR Analysis

The primers specific for the twelve genes of interest are listed in Table 1. Reverse transcription polymerase chain reaction (RT-qPCR) was performed on a ViiA 7 PCR machine (ABI Applied Biosystems, Foster City, CA, USA) using the SYBR *Premix Ex Taq* kit (Takara Bio Inc., Dalian, China) in a total of reaction of 20 μL under conditions as follows: denaturation at 95 °C for 1 min, followed by 45 cycles of denaturing at 95 °C for 5 s, annealing at 55 °C for 10 s, and extension at 72 °C for 10 s.

Statistical Analysis

All the data were averaged from three independent measurements and expressed as mean±SD. One-way analysis of variance (ANOVA) was carried out to compare the differences of means among the multi-groups. Dunnett's test was performed to compare the data with the control group. Statistical significance for all tests was judged at a probability level of 0.05 and 0.01 ($P<0.05$; $P<0.01$).

Results and Analysis

Effect of TiO_2 NPs Feeding on Gonad Development

In order to investigate the effect of TiO_2 NPs on gonad development, ovaries and testes of mature silkworm were dissected and examined under a microscope. As shown in Fig. 1a, the morphology of the ovaries and testes was normal in control *B. mori* and *B. mori* fed with TiO_2 NPs, but the sizes of the ovaries and testes were larger in TiO_2-NPs-fed *B. mori*. Histopathological observations showed that the animals in the control and treatment groups had differentiating sperm and egg cells with normal morphology and thin wall (Fig. 1b). After the TiO_2 NPs treatment, the ovaries showed denser oocyte differentiation and formation compared with those of the control (Fig. 1b). However, no significant difference was observed in testes (Fig. 1b), indicating that TiO_2 NPs exhibited stronger impact on ovaries. Gonad development is also regulated by endocrine hormones secreted under external

Table 1 Primers of twelve genes selected for RT-qPCR analysis

Gene name	Forward primer	Reverse primer	Primer size (bp)
Actin 3	CGGCTACTCGTTCACTACC	CCGTCGGGAAGTTCGTAAG	147
Ovo	GGACCTTGATTCACCGACAG	CGAACTTTGTTGGGTCGTC	125
Ovo-781	GTCCTGCAGCTGCTGTAAG	CGAGCAAACTCTGCAAACGAAC	137
Ovo-498	GACGAAGGATGTGTGCGTG	AGGCATACGTGTGCTGGAC	119
Otu	GTGACAGAGGAAACGGACAT	ACTTCGGCTTGAGAATTGC	136
Aly	GAATGCCCGCTAGGATCAG	GTCTTGCATTTGGATGCCTAC	148
Vlg	GACCATGATGATAGGGGTCG	CGATAGGTCTCGGAGGATTC	133
Achi-L	GACCACAGGTACAACGCGTAC	CATCAGCTCCGTCCCAAGAAGC	121
Sxl-S	GAGGAACATGGCAAGCAGAAG	CGGAGGTCTGTTACAAACGCC	144
Sxl-L	CACTCCTGTCAGTAGCTGG	GGTAACGAACATCGCGTAAAG	132
Vg	GTCGATATTGCATCCCCATC	CTTGTGCCATCGATAGAACAG	125

stimuli. After TiO_2 NPs treatment, gonadosomatic indexes (GSIs) were significantly higher than in the control (Fig. 2) with a 20.03 % increase in male and 24.89 % increase in female. These results were consistent with the data in Fig. 1 and showed that TiO_2 NPs promote not only the growth of gonad but also the differentiation and formation of eggs in the ovary.

Effects of TiO_2 NPs Feeding on Fecundity

In order to study the effect of TiO_2 NPs feeding on fecundity, the size of pupae, ovipositor of virgin moth, and oviposition were compared between the *B. mori* fed with TiO_2 NPs and those without TiO_2 NPs feeding (Fig. 3). As shown in Fig. 3a, the 3-day-old pupae had normal morphology and color in the control and treated groups. However, they were slightly larger after feeding with TiO_2 NPs with body weight increases of 0.23 and 0.17 g for female and male pupae, respectively. These data are consistent with the data in Fig. 1, indicating that TiO_2 NPs treatment increased the absorption and accumulation of nutrients in *B. mori*. The ovipositors of virgin moths are shown in Fig. 3b. As shown, there were more densely distributed eggs in the ovipositors of TiO_2-NPs-fed animals than those of the control, suggesting that TiO_2 NPs feeding is beneficial for egg formation in the adult stage. In order to determine the cumulative effect of TiO_2 NPs feeding on oviposition, fecundity was investigated (Table 2) and 51 more eggs per insect and 0.34×10^{-4} g more mass per egg were found after TiO_2 NPs feeding (Table 2). However, fertilization rates were not different between the two groups ($P>0.05$). The morphology of the eggs is shown in Fig. 3c. The density of eggs was higher in insects fed with TiO_2 NPs than in the control. These data suggested that TiO_2 NPs not only increase the nutrient accumulation and transformation during the reproductive development but also improve the oviposition ability in *B. mori*.

Effects of TiO_2 NPs Feeding on Expression of Gonad Development-Related Genes

To investigate the effects of TiO_2 NPs on transcription and expression of gonad development-related genes, the transcript levels of these genes in the ovaries and testes were determined by the RT-qPCR method using *actin 3* as the housekeeping gene (Fig. 4). The TiO_2-NPs-induced upregulated genes in the ovaries are shown in Fig. 4a, including vitellogenin (*Vg*), *Ovo*, *Ovo-781*, *Ovo-498*, *Otu*, *Sxl-S*, and *Sxl-L*, with 6.77-, 2.00-, 5.33-, 2.99-, 2.43-, 1.73-, and 2.30-folds increase, respectively. *Vg*, *Ovo*, *Ovo-781*, *Ovo-498*, and *Otu* are related to nutrition metabolism in ovarian development [17, 18]. The upregulation of these genes following TiO_2 NPs feeding indicates that the compound can increase nutrient accumulation in the ovary for the development of eggs. *Sxl-S* and *Sxl-L* are the self-splicing regulatory genes, and their upregulation is an indication that TiO_2 NPs facilitate the self-modification of female transcription factors. The TiO_2-NPs-induced upregulated genes in the testes are shown in Fig. 4b, including *Achi-L*, *Aly*, and *Vlg* with 3.55-, 5.78-, and 2.36-folds increase, respectively. The three genes are related to energy metabolism and gamete differentiation in the testis, and their upregulation suggests that TiO_2 NPs treatment can provide sufficient energy for better gamete formation [19]. The above results show that TiO_2 NPs can upregulate the transcription of genes related to reproductive development of gonads and could be responsible for the reproductive ability of *B. mori*.

Discussion

Effects of TiO_2 NPs on the Biological Environment

As a new additive, TiO_2 NPs are widely used in bleaching, food additive, cosmetics, air purification fields, and biological

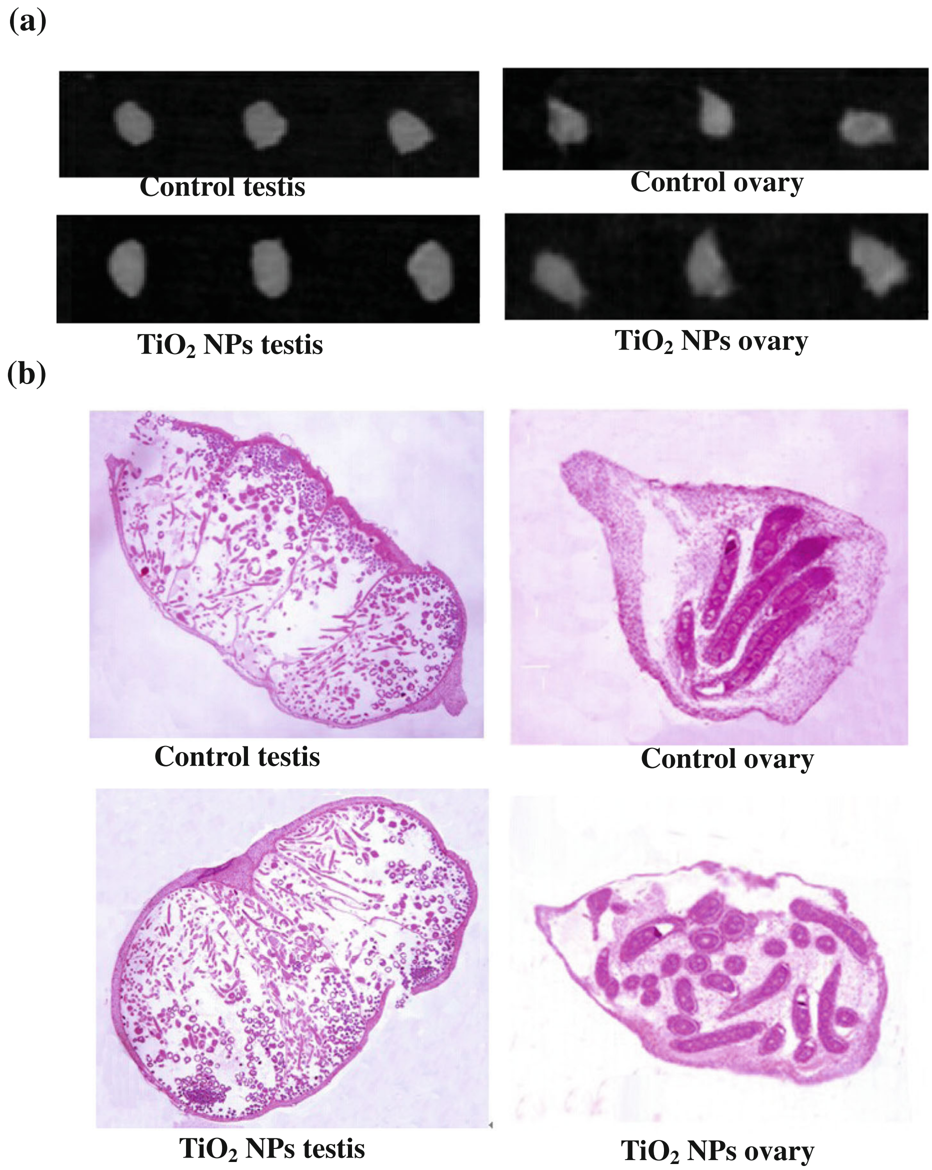

Fig. 1 Effects of TiO_2 NPs on the gonadal development (**a**) and histophysiological examination of the gonad of mature silkworm (**b**)

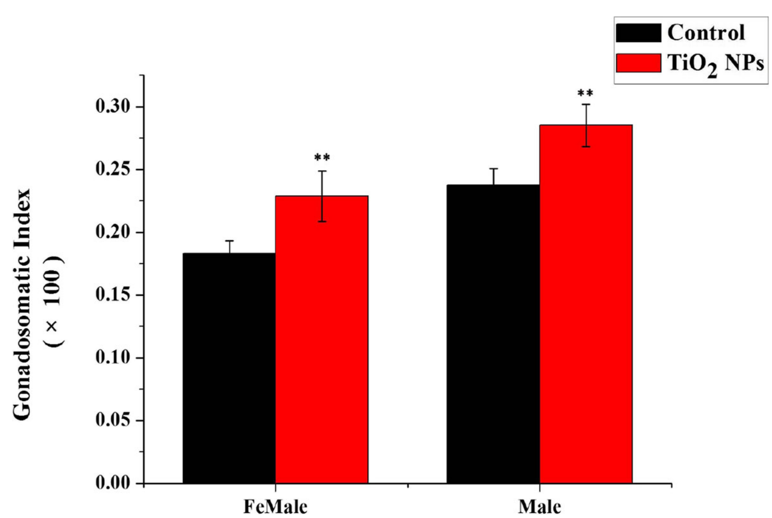

Fig. 2 Effect of TiO_2 NPs on the gonadosomatic index of mature silkworm. Gonadosomatic index (GSI=gonad wet weight (g)/total body wet weight (g)×100) of mature silkworm (n=10). Each group was analyzed in triplicate, and the results are shown as mean ±standard error. Significant differences between groups were analyzed using one-way ANOVA and Tukey's multiple comparison test. *Asterisks* (**) indicate significant differences compared to the control values (p<0.01)

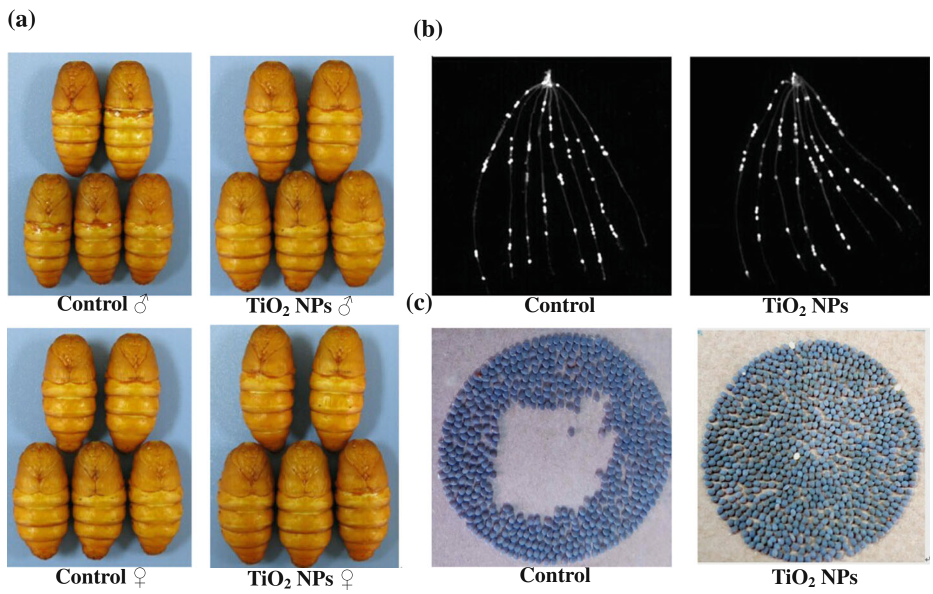

Fig. 3 Effects of TiO_2 NPs on the growth of 3-day-old pupae (**a**), the ovipositor of virgin moth (**b**), and the oviposition of *B. mori* (**c**)

research [20–24]. The safety and efficacy of TiO_2 NPs in various biological environments have been extensively studied. In recent years, TiO_2 NPs have been investigated through whole body exposure, dermal exposure, gastric lavage, inhalation, and feeding using animal models in efforts to define their impacts on various biological and physiological metabolisms for potential biological applications [25–27]. In fact, the different impacts of the compound are resulted from different organ concentration of TiO_2 NPs and different effects on the biological processes. High concentration of TiO_2 NPs is toxic to the reproductivity of CD-1 mice and the fruit fly, *Drosophila* [28]. Low concentration of TiO_2 NPs is not biologically toxic, and instead, it can promote the growth and differentiation of vertebrate cells. Fabian et al. found that TiO_2 NPs at the dose of 5 mg/kg body weight is not toxic to tissue of male Wistar rats [29], which is consistent with what was reported by Umbreit et al. [30]. In addition, a trace amount of TiO_2 NPs incorporated into orthopedic materials is shown to promote the self-repair and regeneration of bones [31]. In cell engineering, a TiO_2-doped glass-microsphere-fortified surface is found to be a suitable surface for cell adhesion and growth [32].

Feeding of *B. mori* with TiO_2 NPs is found to increase the metabolism of proteins and carbohydrates to meet the energy demand for growth and development. It also increases the antioxidation capacity and resistance to pesticide and virus damage [13, 33, 34]. Previous reports have demonstrated that TiO_2 NPs feeding can increase the amount of silk production and improve the silk quality. In this study, for the first time, we found that TiO_2 NPs feeding can promote the growth and development of the gonad (Figs. 1 and 2), increase differentiation and formation of eggs (Fig. 1b), and improve the ability to oviposition (Fig. 3 and Table 2). These findings provide a new clue for the use of low concentration of TiO_2 NPs to improve the reproductive capacity of organisms.

Possible Mechanism Underlying the Effect on Reproductive Ability

Vitellogenin (*Vg*) is a complex precursor of yolk protein. It is involved in energy reserves in embryonic development and is synthesized in the liver of vertebrates, which is an equivalent of fat body in insects [35, 36]. The *Vg* gene is an excellent biomarker for reproductivity and is widely used in determination of the reproductive ability in insects with rich yolk proteins in egg [37]. Previous reports have indicated that the development of *B. mori* ovary is regulated by nutrient accumulation and energy metabolism [37]. Xu et al. pointed out that the development of *B. mori* ovary is associated with the transfer of yolk proteins from the fat body to the ovary. 4-NP (4-nonylphenol) a known endocrine-disrupting chemical is a persistent environmental contaminant. When exposed to 4-NP, the expression of the *Vg* gene was downregulated, leading to the reduced translocation of yolk proteins and abnormal

Table 2 Effects of TiO_2 NPs on the oviposition of *B. mori*

	Egg number (grain)	Egg weight (g)	Unfertilized egg (grain)
Control	569±8 %	6.35×10^{-4}±0.01 %	7±1 %
TiO_2 NPs	620±7 %	6.49×10^{-4}±0.01 %	5±0 %

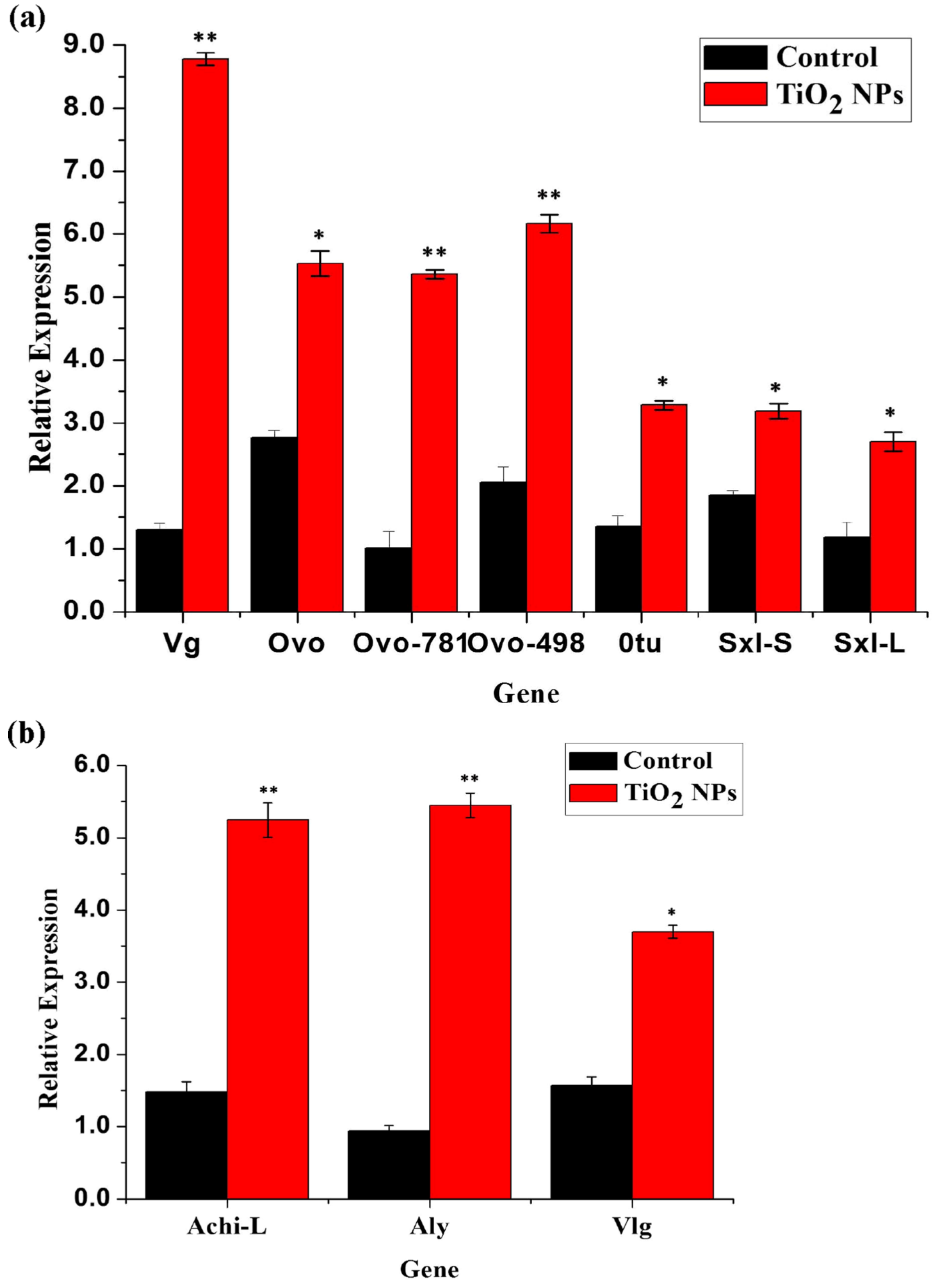

Fig. 4 Analysis of transcriptional levels of the gonad development genes in the ovary (**a**) and testis (**b**) of mature silkworm after TiO_2 NPs feeding. All gene expressions were normalized with *actin 3*. Each test sample contained five individuals and each sample was run in triplicate. *Asterisks* indicate significant differences compared to the control values (*p<0.05, **p<0.01)

development of egg and sperm cells in gonad and subsequently reduced gonadosomatic index, fecundity, and hatching rate [37]. The expression level of the *Vg* gene in the ovary was 6.77-fold more in the TiO_2-NPs-fed animals than in the control (Fig. 4a), suggesting that the compound may promote the development and growth of reproductive gland and improve the fecundity in *B. mori*.

The *Ovo* and *Otu* genes are closely associated with ovarian development. As a transcriptional factor, *Ovo* is involved in energy metabolism. Depletion of *Ovo* expression by RNA interference did not change the fertilization rate greatly in *B. mori* eggs with a slight reduction in the hatching rate and remarkable reduction in egg production by about 15 % [17, 18]. Data in Fig. 4a show that the expressions of *Ovo*, *Ovo-781*, *Ovo-498*, and *Otu* in the ovary of TiO_2-NPs-fed animals were 2.00-, 5.33-, 2.99-, and 2.43-folds that of the control, respectively, indicating that these genes work together with *Vg* to regulate nutrient accumulation in the ovary to provide adequate nutrition for the development of the egg. *Sxl* has an important role in the regulation of sex differentiation in *B. mori*. The female-specific Sxl^+ mRNA encodes a full-length Sxl^+ protein that functions to autoregulate the splicing of its own pre-mRNA, as well as the pre-mRNA of the tra^+

gene. The alternative slicing of the *Sxl* gene is important in regulating the development of the ovary [38]. Data in Fig. 4a show that the expressions of two subtypes of *Sxl-S* and *Sxl-L* of the *Sxl* gene in the ovary of TiO_2-NPs-fed animals increased by 1.73- and 2.30-folds, respectively, comparing with those in the control. These data and results in Fig. 1b all show that upregulation of the *Sxl* gene after TiO_2 NPs feeding promotes the alternative splicing and is conducive to the differentiation and formation of eggs.

In the testes of TiO_2-NPs-fed *B. mori*, *Achi-L*, *Aly*, and *Vlg* were 3.55-, 5.78-, and 2.36-folds higher than those of the control, respectively (Fig. 4b), while the differentiation and formation of sperm cells were not different between the two treatments (Fig. 1b), nor the fertilization rate (Table 2), although the testis GSI was higher in TiO_2-NPs-fed *B. mori* than in the control (Fig. 2). These results indicate that increased expressions of *Achi-L*, *Aly*, and *Vlg* following TiO_2 NPs are related to the growth of the testis, but not related to the differentiation and formation of germ cells. More studies are needed to define the sensitivity of ovary and testis to TiO_2 NPs.

The Significance to Improve the Reproductivity of *B. mori*

B. mori is an important social insect. The number and quality of the eggs are important for the silk industry [3, 37]. Our work shows that TiO_2 NPs can increase the amount of eggs and improve the reproductivity of the *B. mori*. This suggests that as a *B. mori* feed additive, TiO_2 NPs can increase the raw cocoon production [14] and also the parent silkworm rearing. These findings broaden the application of TiO_2 NPs as additive in silk production.

In summary, our work has demonstrated that TiO_2 NPs feeding upregulate the expression of genes related to reproductive systems and promote the development and growth of the reproductive gland in *B. mori*. It consequently facilitates the differentiation and formation of gametes in the ovary and increases the amount of produced eggs and fecundity in the insect.

Acknowledgments This work was supported by the National High Technology Research and Development Program of China (863 Program) (Grant No. 2013AA102507), the State Key Laboratory of Silkworm Genome Biology, the Transformation Project of Agriculture Scientific and Technological Achievements (2013GB2C100180), the projects sponsored by the National Cocoons Silk Development Funds in 2014, the Priority Academic Program Development of Jiangsu Higher Education Institutions, the Doctoral Fund of Ministry of Education of the People's Republic of China (20113201110008), the China Agriculture Research System (CARS-22-ZJ0305), and the Science & Technology support Program of Suzhou (ZXS2012005, SYN201406).

References

1. Tanaka K, Uda Y, Ono Y, Nakagawa T, Suwa M, Yamaoka R, Touhara K (2009) Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. Curr Biol 19:881–890
2. Truman JW, Riddiford LM (1999) The origins of insect metamorphosis. Nature 401:447–452
3. International Silkworm Genome Consortium (2008) The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. Insect Biochem Molec 38:1036–1045
4. Cheng D, Yang J, Zhao Y (2004) Antibacterial materials of silver nanoparticles application in medical appliances and appliances for daily use. Chin Med Equip J 4:26–32
5. Cohen MS, Stern JM, Vanni AJ, Kelley RS, Baumgart E, Field D, Summerhayes IC (2007) In vitro analysis of a nanocrystalline silver-coated surgical mesh. Surg Infect 8:397–404
6. YeonáLee H, KunáPark H, MiáLee Y, BumáPark S (2007) A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. Chem Commun 28: 2959–2961
7. Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH (2007) Functional finishing of cotton fabrics using silver nanoparticles. J Nanosci Nanotechno 7:1893–1897
8. Chaudhry Q, Castle L (2011) Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries. Trends Food Sci Tech 22:595–603
9. Liang SS, Makamba H, Huang SY, Chen SH (2006) Nano-titanium dioxide composites for the enrichment of phosphopeptides. J Chromatogr A 1116:38–45
10. Hong FS, Yang F, Liu C, Gao Q, Wan ZG, Gu FG, Wu C, Ma ZN, Zhou J, Yang P (2005) Influences of nano-TiO_2 on the chloroplast aging of spinach under light. Biol Trace Elem Res 104:249–260
11. Zheng L, Hong FS, Lu SP, Liu C (2005) Effect of nano-TiO_2 on strength of naturally and growth aged seeds of spinach. Biol Trace Elem Res 104:83–91
12. Posgai R, Cipolla-McCulloch CB, Murphy KR, Hussain SM, Rowe JJ, Nielsen MG (2011) Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: size, coatings and antioxidants matter. Chemosphere 85:34–42
13. Zhang H, Ni M, Li FC, Xu KZ, Wang BB, Hong FS, Li B (2014) Effects of feeding silkworm with nanoparticulate anatase TiO_2 (TiO_2 NPs) on its feed efficiency. Biol Trace Elem Res 159:224–232
14. Fan JG, Tao WZ (1988) Differences of the protein distribution rate of different silkworm varieties and correlate measurement. J Anhui Agr Sci 4:38 http://xuewen.cnki.net/CJFDAHNY198804019.html
15. Yang P, Lu C, Hua NP, Du YK (2002) Titanium dioxide nanoparticles co-doped with Fe^{3+} and Eu^{3+} ions for photocatalysis. Mater Lett 57: 794–801
16. Hu RP, Zheng L, Zhang T, Gao GD, Cui YL, Cheng Z, Cheng J, Hong MM, Tang M, Hong FS (2011) Molecular mechanism of hippocampal apoptosis of mice following exposure to titanium dioxide nanoparticles. J Hazard Mater 191:32–40
17. Oliver B, Perrimon N, Mahowald AP (1987) The ovo locus is required for sex-specific germ line maintenance in *Drosophila*. Genes Dev 1:913–923
18. Pauli D, Oliver B, Mahowald AP (1993) The role of the ovarian tumor locus in *Drosophila melanogaster* germ line sex determination. Development 119:123–134
19. Ayyar S, Jiang J, Collu A, White-Cooper H, White RA (2003) *Drosophila* TGIF is essential for developmentally regulated transcription in spermatogenesis. Development 130:2841–2852
20. Fisher J, Egerton T (2001) Kirk-Othmer encyclopedia of chemical technology. Wiley, New York

21. Kaida T, Kobayashi K, Adachi M, Suzuki F (2004) Optical characteristics of titanium oxide interference film and the film laminated with oxides and their applications for cosmetics. J Cosmet Sci 55: 219–220
22. Esterkin CR, Negro AC, Alfano OM, Cassano AE (2005) Air pollution remediation in a fixed bed photocatalytic reactor coated with TiO_2. AIChE J 51:2298–2310
23. Choi H, Stathatos E, Dionysiou DD (2006) Sol–gel preparation of mesoporous photocatalytic TiO_2 films and TiO_2/Al_2O_3 composite membranes for environmental applications. Appl Catal B-Environ 63:60–67
24. Li FC, Gu ZY, Wang BB, Xie Y, Ma L, Xu KZ, Ni M, Zhang H, Shen WD, Li B (2014) Effects of the biosynthesis and signaling pathway of ecdysterone on silkworm (*Bombyx mori*) following exposure to titanium dioxide nanoparticles. J Chem Ecol 40:913–922
25. Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI (2004) Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 77:347–357
26. Grassian VH, O'Shaughnessy PT, Adamcakova-Dodd A, Pettibone JM, Thorne PS (2007) Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Environ Health Persp 115:397–402
27. Wang JX, Zhou GQ, Chen CY, Yu HW, Wang TC, Ma YM, Jia G, Gao YX, Li B, Sun J, Li YF, Jiao F, Zhao YL, Chai ZF (2007) Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol lett 168:176–185
28. Philbrook NA, Winn LM, Afrooz ARMN, Saleh NB, Walker VK (2011) The effect of TiO_2 and Ag nanoparticles on reproduction and development of *Drosophila melanogaster* and CD-1 mice. Toxicol Appl Pharm 257:429–436
29. Fabian E, Landsiedel R, Ma-Hock L, Wiench K, Wohlleben W, Van Ravenzwaay B (2008) Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. Arch Toxicol 82:151–157
30. Umbreit T, Weaver JL, Miller TJ, Zhang J, Shah R, Stratmeyer ME, Tomazic-Jezic V (2007) Toxicology of titanium dioxide (TiO_2) nanoparticles: 1. Characterization and tissue distribution in subcutaneously and intravenously injected mice. Toxicologist 96:287
31. Donos N, Retzepi M, Wall I, Hamlet S, Ivanovski S (2011) In vivo gene expression profile of guided bone regeneration associated with a microrough titanium surface. Clin Oral Implan Res 22:390–398
32. Guedes JC, Park JH, Lakhkar NJ, Kim HW, Knowles JC, Wall IB (2013) TiO_2-doped phosphate glass microcarriers: a stable bioactive substrate for expansion of adherent mammalian cells. J Biomater Appl 28:3–11
33. Li B, Hu RP, Cheng Z, Cheng J, Xie Y, Gui SX, Sun QQ, Sang XZ, Gong XL, Cui YL, Shen WD, Hong FS (2012) Titanium dioxide nanoparticles relieve biochemical dysfunctions of fifth-instar larvae of silkworms following exposure to phoxim insecticide. Chemosphere 89:609–614
34. Su JJ, Li B, Cheng S, Zhu Z, Sang XZ, Gui SX, Xie Y, Sun QQ, Cheng Z, Cheng J, Hu RP, Shen WD, Xia QY, Zhao P, Hong FS (2013) Phoxim-induced damages of *Bombyx mori* larval midgut and titanium dioxide nanoparticles protective role under phoxim-induced toxicity. Environ Toxicol. doi:10.1002/tox.21866
35. Richard DS, Watkins NL, Serafin RB, Gilbert LI (1998) Ecdysteroids regulate yolk protein uptake by *Drosophila melanogaster* oocytes. J Insect Physiol 44:637–644
36. Matozzo V, Marin MG (2005) Can 4-nonylphenol induce vitellogenin-like proteins in the clam *Tapes philippinarum*? Environ Res 97:43–49
37. Yuan HX, Xu X, Sima YH, Xu SQ (2013) Reproductive toxicity effects of 4-nonylphenol with known endocrine disrupting effects and induction of vitellogenin gene expression in silkworm, *Bombyx mori*. Chemosphere 93:263–268
38. Oliver B, Kim YJ, Baker BS (1993) Sex-lethal, master and slave: a hierarchy of germ-line sex determination in *Drosophila*. Development 119:897–908