Comparison of the toxic effects of different mycotoxins on porcine and mouse oocyte meiosis

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Background: Aflatoxin B1 (AFB1), Deoxynivalenol (DON), HT-2, ochratoxin A (OTA), Zearalenone (ZEA) are the most common mycotoxins that found in corn-based animal feed which have multiple toxic effects on animals and humans. Previous studies reported that these mycotoxins impaired mammalian oocyte quality. However, the effective concentrations of mycotoxins to animal oocytes were different.

Methods: In this study we aimed to compare the sensitivity of mouse and porcine oocytes to AFB1, DON, HT-2, OTA and ZEA for mycotoxin research. We adopted the polar body extrusion rate of mouse and porcine oocyte as the standard for the effects of mycotoxins on oocyte maturation.

Results and Discussion: Our results showed that 10 μM AFB1 and 1 μM DON significantly affected porcine oocyte maturation compared with 50 μM AFB1 and 2 μM DON on mouse oocytes. However, 10 nM HT-2 significantly affected mouse oocyte maturation compared with 50 nM HT-2 on porcine oocytes. Moreover, 5 μM OTA and 10 μM ZEA significantly affected porcine oocyte maturation compared with 300 μM OTA and 50 μM ZEA on mouse oocytes. In summary, our results showed that porcine oocytes were more sensitive to AFB1, DON, OTA and ZEA than mouse oocytes except HT-2 toxin.
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Running Title: Effects of mycotoxins on oocytes

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Abstract

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Results and Discussion: Our results showed that 10 μM AFB1 and 1 μM DON significantly affected porcine oocyte maturation compared with 50 μM AFB1 and 2 μM DON on mouse oocytes. However, 10 nM HT-2 significantly affected mouse oocyte maturation compared with 50 nM HT-2 on porcine oocytes. Moreover, 5 μM OTA and 10 μM ZEA significantly affected porcine oocyte maturation compared with 300 μM OTA and 50 μM ZEA on mouse oocytes. In summary, our results showed that porcine oocytes were more sensitive to AFB1, DON, OTA and ZEA than mouse oocytes except HT-2 toxin.
**Introduction**

Mycotoxins are secondary metabolites produced by fungi, while the most agriculturally common mycotoxins known today include aflatoxins (AF), Deoxynivalenol (DON), T-2, ochratoxin A (OTA), Zearalenone (ZEA) (Grajewski et al. 2012). These mycotoxins have multiple toxic effects on human and animal health in a very low dose, which draws worldwide attention.

Aflatoxins (AF) are the mycotoxins which widely exist in corn-based animal feed. Considering the toxic potency and carcinogenic action, Aflatoxin B1 is the most important AF (Kew 2013). It causes multiple effects including mitochondrial permeability transition, DNA damage (Shi et al. 2015), oxidative stress (Singh et al. 2015), apoptosis (Peng et al. 2016), the defects of skeletal muscle development in different models (Gunduz & Oznurlu 2014). In porcine oocytes, AFB1 is shown to affect cell cycle and induce oxidative stress (Liu et al. 2015).

Deoxynivalenol (DON) that produced mainly by fungi is a common contaminant of cereals including maize, wheat and barley (Wu et al. 2011). After feeding the domestic animals and poultry with DON-contaminated feed, it will exist in meat, milk and eggs. In animals, it causes organ damage and hepatic lipid accumulation (Pietsch et al. 2014), emesis, anorexia, growth retardation, immunotoxicity as well as impaired reproduction and development competence (Pestka 2010). At cellular and molecular level, DON can induce apoptosis (Li et al. 2014), oxidative stress and genotoxicity (Yang et al. 2014). In addition, previous study showed that DON affected spindle morphology in porcine oocytes (Han et al. 2016).

HT-2 is the major metabolite of T-2 which is one of the type A trichothecene mycotoxins produced by different *Fusarium* species. HT-2 causes a myriad of effects including the inhibition
of protein, DNA and RNA synthesis; oxidative stress (Zhang et al. 2016); reduced reproduction (Zhu et al. 2016) and embryo-fetal toxicity (Wang et al. 2014).

Several fungi including *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* and *Penicillium verrucosum* produce ochratoxin A (OTA). OTA is toxic to domestic animals, and its main target organ is kidney (Grajewski et al. 2012). OTA causes several effects like immunotoxicity (Al-Anati & Petzinger 2006), hepatotoxicity, apoptosis, decrease of cell viability, impairment of mouse oocyte maturation and embryonic development (Huang & Chan 2016).

Zearalenone (ZEA) produced by various *Fusarium* species is a contaminant of cereal crops and animal feed. Structurally, ZEA is similar to 17β-estradiol and it will compete with 17b-estradiol for binding to estrogen receptors, which leads to reproductive disorders (Cortinovis et al. 2013; Minervini et al. 2001; Minervini et al. 2006). The genotoxicity of ZEA has also been confirmed since it induces DNA fragmentation, apoptosis and interruption of cell cycle progression (Abid-Essefi et al. 2003). Several studies showed that ZEA reduced porcine oocyte developmental competence (Hou et al. 2014; Hou et al. 2015; Komsky-Elbaz et al. 2018).

Although previous studies reported that several components of mycotoxins impaired mammalian oocyte quality. However, different effective concentrations were reported in the oocytes of different animal models. Although AFB1, DON, HT-2, OTA and ZEA all have their metabolites in vivo, these mycotoxins are all detected in ovary, indicating that besides their metabolites, AFB1, DON, HT-2, OTA and ZEA could directly affect ovary functions. In this study we aimed to compare the sensitivity of mouse and porcine oocytes to AFB1, DON, HT-2, OTA and ZEA, which could provide the basic data for mycotoxin studies in the future. We adopted the
polar body extrusion rate of mouse and porcine oocyte as the standard for the toxic effects of mycotoxins on oocyte maturation, and our results showed that except HT-2, porcine oocytes were generally more sensitive to AFB1, DON, OTA and ZEA.

Materials and Methods

Chemicals and regent

AFB1, DON, HT-2, OTA and ZEA were from J&K Chemical Ltd. (Shanghai, China). TCM-199 was from Gibco (Life Technologies, USA). TCM-199 which contained 75 mg/ml of penicillin, 50 mg/ml of streptomycin, 0.5 mg/ml of FSH, 0.5 mg/ml of LH, 10ng/ml of the epidermal growth factor (EGF), and 0.57 mM cysteine was used for oocyte maturation. M2 and M16 culture medium were from Sigma-Aldrich (Merck).

Oocytes collection and culture

We followed the guidelines of Animal Research Institute Committee of Nanjing Agricultural University to conduct the experiments (SYXK-Su-20170007). Germinal vesicle-intact oocytes of mice that obtained from ovaries of 3- to 5-week old ICR mice were collected in M2 medium and cultured with M16 medium (Sigma Chemical Co., St. Louis, MO) under the paraffin oil. These oocytes of mice were placed at 37°C with 5% CO2 for 12 hours to observe the polar body extrusion.

Ovaries of Duroc pigs were collected from prepubertal gilts purchased at a local slaughterhouse of Feng Yong Food Industry. After slaughter, ovaries were placed in a thermos bottle which contained 0.9% physiological saline and then delivered to our laboratory within 2 hours. The temperature of the thermos bottle was close to 38 °C. Once the ovaries were delivered, they were
washed with sterile saline. We aspirated follicular fluids from 3 to 6 mm antral follicles with a 10 ml disposable syringe and an 18 G needle. Cumulus oocyte complexes (COC₅) with intact and compact cumulus were selected for maturation. These oocytes were placed at 38.5°C with 5% CO₂ for 44 hours to observe the polar body extrusion.

**Toxin treatment**

AFB₁, DON, HT-2, OTA and ZEA were dissolved and stored at 50 mM in DMSO. and then diluted into different concentrations with M199 or M16 maturation medium. The GV oocytes were then cultured with these mycotoxins to analyze the maturation rate by the polar body extrusion index. The same quantity of DMSO was added in the control group.

**Statistical analysis**

Data are presented as means (n=3). The concentration-response curves were made by GraphPad Prism 5. At least three biological replicates were used for each analysis. Each replicate was done by an independent experiment at the different time. Results are given as means ± SEM, and two groups were compared by student t-test. A p-value of < 0.05 was considered significant.

**Results**

**Effects of AFB₁ on mouse and porcine oocyte maturation**

We first examined the effects of AFB₁ on mouse and porcine oocytes. Mouse oocytes were cultured for 12 hours with 10, 50, 100 and 150 μM AFB₁. The average polar body extrusion rate of the control group was 79.94±4.3% (n=142) (Figure 1A). Compared with control oocytes, when oocytes were cultured with 10 μM AFB₁, rate of polar body extrusion showed no significant
difference (72.82±4.83%, n=110, p>0.05). Rates of matured oocytes were significantly decreased when the concentration of AFB1 were increased to 50 μM (55.96±4.77%, n=157, p<0.05), 100 μM (17.86±7.51%, n=154, p<0.05), 150 μM (2.15±3.72%, n=146, p<0.001). We cultured the porcine oocytes for 44 h with 5, 10, 50, 100 μM AFB1. The polar body extrusion rate was 80.72±7.05% (n=217) in the control group of porcine oocytes, which was close to mouse oocytes. However, the rate of polar body extrusion in porcine oocytes was decreased with the increased AFB1 concentration. Porcine oocytes cultured with 5 μM AFB1 and the control oocytes showed no significant difference (68.34±15.39%, n=195 vs 80.72±7.05% n=217, p>0.05). However, the rate of polar body extrusion in porcine oocytes was significantly decreased with 10 μM (53.02±7.12%, n=144, p<0.001), 50 μM (22.95±6.76%, n=192, p<0.01), 100 μM (0±0%, n=147, p<0.01) AFB1 treatment (Figure 1B). We also analyzed the mouse groups and porcine groups at the same concentrations. With the same concentration of AFB1 treatment, the rate of porcine oocyte polar body extrusion was lower than mouse oocytes. As shown in Figure 1C, the rates of the mouse and porcine control groups showed no significant difference (p>0.05), while there were significant differences when the concentrations were 10 μM (p<0.05) and 50 μM (p<0.01), indicating that compared with mouse oocytes, porcine oocytes were more sensitive to AFB1.

**Effects of DON on mouse and porcine oocyte maturation**

We next examined the effects of DON on mouse and porcine oocytes. Mouse oocytes were cultured for 12 hours with 1, 2, 3 and 4 μM DON. Our results showed that DON affected mouse oocyte maturation. The average polar body extrusion rate of the control group was 76.73±3.24% (n=162) (Figure 2A), when mouse oocytes cultured with 1 μM AFB1, there was no significantly
difference between the control group and 1 µM group (76.01±2.35% n=139, p>0.05). While the rate of polar body extrusion in mouse oocytes was significantly decreased with 2 µM DON treatment (44.38±4.87%, n=173, p<0.05), 3 µM DON treatment (16.30±4.00%, n=199, p<0.001), 4 µM DON treatment (2.28±0.69%, n=189, p<0.001), compared with the control group. We cultured the porcine oocytes for 44 h with 0.5, 1, 2, 3 µM DON. The polar body extrusion rate was 72.05±2.6% (n=195) in the control group of porcine oocytes, which was close to mouse oocytes. At the concentration of 0.5 µM, the control group and 0.5 µM treatment group (68.42±4.55% n=159, p>0.05) showed no significant difference. However, the rate of polar body extrusion in porcine oocytes was significantly decreased with 1 µM DON treatment (46.29±3.89%, n=176, p<0.05), 2 µM DON treatment (17.02±4.87%, n=145, p<0.01), 3 µM DON treatment (3.29±1.81%, n=132, p<0.001) (Figure 2B). To compare the sensitivity of mouse and porcine, we also analyze the rate of same concentrations. The rates of control groups were close to each other, while at the concentrations of 1 µM (p<0.001), 2 µM (p<0.01), 3µM (p<0.01), polar body extrusion rates of mouse oocytes and porcine oocytes showed significant difference (Figure 2C), indicating that compared with mouse oocytes, porcine oocytes were more sensitive to DON.

**Effects of HT-2 on mouse and porcine oocyte maturation**

We next examined the effects of HT-2 on mouse and porcine oocytes. The mouse oocytes were cultured for 12 hours with 10, 20, 30 and 40 nM HT-2. The average polar body extrusion rate of the control group was 73.08±1.67% (n=153) (Figure 3A), while the rate of polar body extrusion in mouse oocytes was significantly decreased with 10 nM HT-2 treatment (43.33±4.93%, n=176, p<0.05), 20 nM HT-2 treatment (33.05±2.18%, n=163, p<0.001), 30 nM HT-2 treatment
The porcine oocytes were cultured for 44 h with 10, 50, 100, 150, 200 and 400 nM HT-2. Similar with the mouse oocytes, our results showed that HT-2 affected porcine oocyte maturation. The polar body extrusion rate was 78.19±2.03% (n=171) in the control group of porcine oocytes, which was close to mouse oocytes. However, except the 10 nM HT-2 treatment (68.67±2.99%, n=163, p>0.05), the rate of polar body extrusion in porcine oocytes was significantly decreased with 50 nM HT-2 treatment (47.4±3.36%, n=156, p<0.01), 100 nM HT-2 treatment (25.50±5.14%, n=164, p<0.01), 150 nM HT-2 treatment (21.22±4.07%, n=188, p<0.01), 200 nM HT-2 treatment (18.02±6.69%, n=175, p<0.01), 400 nM HT-2 treatment (8.22±0.78%, n=162, p<0.001) (Figure 3B). The control groups in mouse and porcine have similar values, however, at the concentration of 10 μM (p<0.01) rates of mouse and porcine were significantly different (Figure 3C). Our results showed that with the same concentration of HT-2 treatment the rate of mouse oocyte polar body extrusion was lower than porcine oocytes, indicating that compared with porcine oocytes mouse oocytes were more sensitive to HT-2.

**Effects of OTA on mouse and porcine oocyte maturation**

We next examined the effects of OTA on mouse and porcine oocytes. Mouse oocytes were cultured for 12 hours with 200, 300, 400 and 600 μM OTA. The average polar body extrusion rate of the control group was 80.23±3.87% (n=169) (Figure 4A), compared with the control group, the concentration of 200 μM OTA showed no significant difference (73.77±2.24% n=143, p>0.05), while the rate of polar body extrusion in mouse oocytes was significantly decreased with 300 μM OTA treatment (49.86±4.29%, n=190, p<0.01), 400 μM OTA treatment (31.23±3.64%, n=145,
p<0.01), 600 μM OTA treatment (2.22±3.85%, n=178, p<0.01). The porcine oocytes were cultured for 44 h with 1, 5, 10, 30 and 100 μM OTA. Similar with the mouse oocytes, our results showed that OTA affected porcine oocyte maturation. The control group and 1 μM OTA group showed no significant difference (80.34±7.95% n=164 vs 65.46±4.09% n=178, p>0.05). However, the rate of polar body extrusion in porcine oocytes was significantly decreased with 5 μM OTA treatment (53.83±0.34%, n=154, p<0.05), 10 μM OTA treatment (22.26±3.14%, n=183, p<0.05), 30 μM OTA treatment (22.19±4.87%, n=140, p<0.01), 100 μM OTA treatment (4.24±3.93%, n=144, p<0.01) (Figure 4B). With the same concentration of OTA treatment, the rate of porcine oocyte polar body extrusion was lower than mouse oocytes (Figure 4C), indicating that compared with mouse oocytes, porcine oocytes were more sensitive to OTA.

**Effects of ZEA on mouse and porcine oocyte maturation**

The last we examined was the effects of ZEA on mouse and porcine oocytes. Mouse oocytes were cultured for 12 hours with 10, 50, 100 and 200 μM ZEA. The average polar body extrusion rate of the control group was 81.29±6.06% (n=155) (Figure 5A), when the concentration was 10 μM, the average rate of MII oocytes was 74.52±4.92% (n=154) which showed no significant difference with the control groups (p>0.05). While the rate of polar body extrusion in mouse oocytes was significantly decreased with 50 μM ZEA treatment (54.35±3.9%, n=128, p<0.05), 100 μM ZEA treatment (26.23±8.00%, n=150, p<0.05), 200 μM ZEA treatment (0.00±0.00%, n=132, p<0.01). For porcine oocytes, the average polar body extrusion rate of the control groups was 77.85±9.51% (n=175), while the 5 μM ZEA groups (60.45±1.65%, n=199, p>0.05) showed no significant differences with the control groups. However, the rate of polar body extrusion in
porcine oocytes was significantly decreased with the 10 μM groups (51.42±2.73%, n=190, p<0.05), 30 μdM ZEA treatment (19.10±4.49%, n=147, p<0.05) and 50 μM ZEA treatment (4.08±4.08%, n=148, p<0.01) (Figure 5B). Our results showed that with the same concentration of ZEA treatment, the rate of porcine oocyte polar body extrusion was lower than mouse oocytes. Control groups of mouse and porcine oocytes showed no significant difference (p>0.05). However, there were significant difference between mouse oocytes and porcine oocytes at 10 μM (p<0.01) and 50 μM (p<0.001) (Figure 5C), indicating that compared with mouse oocytes, porcine oocytes were more sensitive to ZEA.

Discussion

In present study we used the polar body extrusion as the index for oocyte maturation to compare the sensitivity of mouse and porcine oocytes to AFB1, DON, HT-2, OTA and ZEA. And our results provided the basic database for the mycotoxin on oocyte studies.

Mycotoxins are shown to affect human and animal health from multiple aspects, such as immune system, micro-organisms. And recent years, the toxicity of mycotoxins on reproductive system especially on oocytes and sperms were reported. Our previous work found that when 50 μM AFB1 affected cumulus oocyte complexes growth, especially the polar body extrusion was significantly reduced in porcine oocytes (Liu et al. 2015). However, 10 μM AFB1 significantly increased the proportion of sperm with fragmented DNA in mice (Komsky-Elbaz et al. 2017). This indicated that even in the reproductive system, the sensitivity of different cell types or animal models to the mycotoxins is different. Our results showed that 10 μM AFB1 affected porcine
oocyte maturation instead of 50 μM AFB1 in mouse oocytes.

2 μM DON was shown to effect the formation of the meiotic spindle in porcine oocytes (Hou et al. 2015). While a recent study showed that 10 μM DON affected the morphology of pig ovaries with an ex vivo approach (Gerez et al. 2017). Our recent study also showed that 3 μM DON exposure altered autophagy/apoptosis and epigenetic modifications in porcine oocytes (Han et al. 2016). In present study our results showed that 1 μM DON affected porcine oocyte maturation instead of 2 μM DON in mouse oocytes, which showed similar sensitivity pattern to AFB1. However, HT-2 had different sensitivity pattern compared with AFB1 and DON. HT-2 toxin was shown to affect cytoskeletal dynamics, apoptosis/autophagy, oxidative stress, and epigenetic modifications in mouse oocytes (Zhu et al. 2016). Our results showed that 10 nM HT-2 affected mouse oocyte maturation while the similar results only occurred at 50 nM HT-2 exposure for porcine oocytes. Further study is still needed to explore the toxic effects of HT-2 toxin in different reproductive cell types like cumulus cells and sperm.

Recent study indicated that OTA significantly impaired oocyte maturation, IVF rates and inhibited embryonic development in vitro, because OTA could induce caspase-dependent apoptosis with in vivo model (Huang & Chan 2016). 1-10 μM OTA in the drinking water was adopted in this study. Our results showed that 10 μM OTA affected porcine oocyte maturation instead of 300 μM OTA in mouse oocytes. The big difference for the OTA in mouse oocyte between in vivo and in vitro model needs more study to explain. For ZEA, at the concentration of 30 μM, ZEA was shown to affect porcine oocyte maturation and embryonic development through oxidative stress, autophagy and early apoptosis (Komsky-Elbaz et al. 2018); and for mouse
oocytes, it affected oocyte quality by altering the epigenetic modification levels (Zhu et al. 2014). Our results showed that 10 μM ZEA affected porcine oocyte maturation instead of 50 μM ZEA in mouse oocytes, which showed similar concentration pattern with AFB1 and DON.

Conclusion

In all, our results showed that these five mycotoxins all affected mouse and porcine oocyte quality, however, different sensitivity pattern between mouse and porcine oocytes were found. Generally porcine oocytes were more sensitive to AFB1, DON, OTA, ZEA compared with mouse oocytes except HT-2. Our results provided basic database for the further studies on mammalian oocytes.

Availability of data

All data generated or analyzed in this study are included in this published article.

References

Abid-Essefi S, Baudrimont I, Hassen W, Ouanes Z, Mobio TA, Anane R, Creppy EE, and Bacha H. 2003. DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and Caco-2 cells: prevention by Vitamin E. Toxicology 192:237-248. 10.1016/s0300-483x(03)00329-9

Al-Anati L, and Petzinger E. 2006. Immunotoxic activity of ochratoxin A. J Vet Pharmacol Ther 29:79-90. 10.1111/j.1365-2885.2006.00718.x

Cortinovis C, Pizzo F, Spicer LJ, and Caloni F. 2013. Fusarium mycotoxins: effects on reproductive function in domestic animals--a review. Theriogenology 80:557-564. 10.1016/j.theriogenology.2013.06.018

Gerez JR, Desto SS, and Bracarense A. 2017. Deoxynivalenol induces toxic effects in the ovaries of pigs: An ex vivo approach. Theriogenology 90:94-100. 10.1016/j.theriogenology.2016.10.023

Grajewski J, Blajet-Kosicka A, Twaruzek M, and Kosicki R. 2012. Occurrence of mycotoxins in Polish animal feed in years 2006-2009. J Anim Physiol Anim Nutr (Berl) 96:870-877. 10.1111/j.1439-0396.2012.01280.x

Gunduz N, and Oznurlu Y. 2014. Adverse effects of aflatoxin B1 on skeletal muscle development in broiler chickens. Br Poult Sci 55:684-692. 10.1080/00071668.2014.949621

Han J, Wang QC, Zhu CC, Liu J, Zhang Y, Cui XS, Kim NH, and Sun SC. 2016. Deoxynivalenol exposure induces autophagy/apoptosis and epigenetic modification changes during porcine oocyte maturation. Toxicol Appl
Hou YJ, Xiong B, Zheng WJ, Duan X, Cui XS, Kim NH, Wang Q, Xu YX, and Sun SC. 2014. Oocyte quality in mice is affected by a mycotoxin-contaminated diet. *Environ Mol Mutagen* 55:354-362. 10.1002/em.21833

Hou YJ, Zhu CC, Xu YX, Cui XS, Kim NH, and Sun SC. 2015. Zearalenone exposure affects mouse oocyte meiotic maturation and granulosa cell proliferation. *Environ Toxicol* 30:1226-1233. 10.1002/tox.21995

Huang FJ, and Chan WH. 2016. Effects of ochratoxin a on mouse oocyte maturation and fertilization, and apoptosis during fetal development. *Environ Toxicol* 31:724-735. 10.1002/tox.22085

Kew MC. 2013. Aflatoxins as a cause of hepatocellular carcinoma. *J Gastrointestin Liver Dis* 22:305-310.

Komsky-Elbaz A, Saktsier M, and Roth Z. 2017. Aflatoxin B1 impairs sperm quality and fertilization competence. *Toxicology* 393:42-50. 10.1016/j.tox.2017.11.007

Komsky-Elbaz A, Saktsier M, and Roth Z. 2018. Aflatoxin B1 impairs sperm quality and fertilization competence. *Toxicology* 393:42-50. 10.1016/j.tox.2017.11.007

Li D, Ma H, Ye Y, Ji C, Tang X, Ouyang D, Chen J, Li Y, and Ma Y. 2014. Deoxynivalenol induces apoptosis in mouse thymic epithelial cells through mitochondria-mediated pathway. *Environ Toxicol Pharmacol* 38:163-171. 10.1016/j.etap.2014.05.015

Liu J, Wang QC, Han J, Xiong B, and Sun SC. 2015. Aflatoxin B1 is toxic to porcine oocyte maturation. *Mutagenesis* 30:527-535. 10.1093/mutage/gev015

Minervini F, Dell'Aquila ME, Maritato F, Minoia P, and Visconti A. 2001. Toxic effects of the mycotoxin zearalenone and its derivatives on in vitro maturation of bovine oocytes and 17 beta-estradiol levels in mural granulosa cell cultures. *Toxicol In Vitro* 15:489-495.

Minervini F, Giannoccaro A, Fornelli F, Dell'Aquila ME, Minoia P, and Visconti A. 2006. Influence of mycotoxin zearalenone and its derivatives (alpha and beta zearalenol) on apoptosis and proliferation of cultured granulosa cells from equine ovaries. *Reprod Biol Endocrinol* 4:62. 10.1186/1477-7827-4-62

Peng X, Chen K, Chen J, Fang J, Cui H, Zuo Z, Deng J, Chen Z, Geng Y, and Lai W. 2016. Aflatoxin B1 affects apoptosis and expression of Bax, Bel-2, and Caspase-3 in thymus and bursa of fabricius in broiler chickens. *Environ Toxicol* 31:1113-1120. 10.1002/tox.22120

Pestka JJ. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Arch Toxicol* 84:663-679. 10.1007/s00204-010-0579-8

Pietsch C, Schulz C, Rovira P, Kloas W, and Burkhardt-Holm P. 2014. Organ damage and hepatic lipid accumulation in carp (Cyprinus carpio L.) after feed-borne exposure to the mycotoxin, deoxynivalenol (DON). *Toxins (Basel)* 6:756-778. 10.3390/toxins6020756

Shi D, Liao S, Guo S, Li H, Yang M, and Tang Z. 2015. Protective effects of selenium on aflatoxin B1-induced mitochondrial permeability transition, DNA damage, and histological alterations in duckling liver. *Biol Trace Elem Res* 163:162-168. 10.1007/s12011-014-0189-z

Singh KB, Maurya BK, and Trigun SK. 2015. Activation of oxidative stress and inflammatory factors could account for histopathological progression of aflatoxin-B1 induced hepatocarcinogenesis in rat. *Mol Cell Biochem* 401:185-196. 10.1007/s11010-014-2306-x

Wang X, Wang W, Cheng G, Huang L, Chen D, Tao Y, Pan Y, Hao H, Wu Q, Wan D, Liu Z, Wang Y, and Yuan Z. 2014. High risk of embryo-fetal toxicity: placental transfer of T-2 toxin and its major metabolite HT-2 toxin in BeWo cells. *Toxicol Sci* 137:168-178. 10.1093/toxsci/kft233

Wu Q, Lohrey L, Cramer B, Yuan Z, and Humpf HU. 2011. Impact of physicochemical parameters on the...
decomposition of deoxynivalenol during extrusion cooking of wheat grits. *J Agric Food Chem* 59:12480-12485. 10.1021/jf2038604

Yang W, Yu M, Fu J, Bao W, Wang D, Hao L, Yao P, Nussler AK, Yan H, and Liu L. 2014. Deoxynivalenol induced oxidative stress and genotoxicity in human peripheral blood lymphocytes. *Food Chem Toxicol* 64:383-396. 10.1016/j.fct.2013.12.012

Zhang Y, Han J, Zhu CC, Tang F, Cui XS, Kim NH, and Sun SC. 2016. Exposure to HT-2 toxin causes oxidative stress induced apoptosis/autophagy in porcine oocytes. *Sci Rep* 6:33904. 10.1038/srep33904

Zhu CC, Hou YJ, Han J, Cui XS, Kim NH, and Sun SC. 2014. Zearalenone exposure affects epigenetic modifications of mouse eggs. *Mutagenesis* 29:489-495. 10.1093/mutage/geu033

Zhu CC, Zhang Y, Duan X, Han J, and Sun SC. 2016. Toxic effects of HT-2 toxin on mouse oocytes and its possible mechanisms. *Arch Toxicol* 90:1495-1505. 10.1007/s00204-015-1560-3

**Figure legend**

**Figure 1** The effects of AFB1 on the oocyte maturation. (A) Rate of polar body extrusion after AFB1 treatment. Mouse oocytes were cultured with 0, 10, 50, 100, 150 μM AFB1 for 12 h. (B) Rate of polar body extrusion after AFB1 treatment. Porcine oocytes were cultured with 0, 5, 10, 50, 100 μM AFB1 for 44 h. Both polar body extrusion rates of mouse and porcine oocytes were reduced with the increasing concentration. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01; ***, significance, p<0.001.

**Figure 2** The effects of DON on the oocyte maturation. (A) Rate of polar body extrusion after DON treatment. For mouse oocyte culture, the DON concentration was 0, 1, 2, 3, 4 μM. (B) Rate of polar body extrusion after DON treatment. For porcine oocytes, DON concentration was 0, 0.5, 1, 2, 3 μM. The reduction of polar body extrusion was dose-dependent. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate
of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01; ***, significance, p<0.001.

**Figure 3** The effects of HT-2 on the oocyte maturation. (A) Rate of polar body extrusion after HT-2 treatment. Mouse oocytes were cultured with HT-2 at the concentration of 0, 10, 20, 30, 40 nM. (B) Rate of polar body extrusion after HT-2 treatment. For porcine oocytes culture, HT-2 was 0, 10, 50, 100, 150, 200, 400 nM. The rate of polar body extrusion was decreased when the concentration of HT-2 was increasing. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01; ***, significance, p<0.001.

**Figure 4** The effects of OTA on the oocyte maturation. (A) Rate of polar body extrusion after OTA treatment. For mouse oocyte culture, OTA concentration was 0, 200, 300, 400, 600 μM. (B) Rate of polar body extrusion after OTA treatment. For porcine oocyte culture, OTA concentration was 0, 1, 5, 10, 30, 100 μM. *, significance, p<0.05; **, significance, p<0.01. (C) The comparison for the effects of different concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion.

**Figure 5** The effects of ZEA on the oocyte maturation. (A) Rate of polar body extrusion after ZEA treatment. Mouse oocytes were cultured with 0, 10, 50, 100, 200 μM ZEA. (B) Rate of polar body extrusion after ZEA treatment. Porcine oocytes were cultured with 0, 5, 10, 30, 50 μM ZEA. Both mouse and porcine polar body extrusion rate were reducing when ZEA concentration was increasing. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes.
oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01.
Figure 1

The effects of AFB1 on the oocyte maturation

(A) Rate of polar body extrusion after AFB1 treatment. Mouse oocytes were cultured with 0, 10, 50, 100, 150 μM AFB1 for 12 h. (B) Rate of polar body extrusion after AFB1 treatment. Porcine oocytes were cultured with 0, 5, 10, 50, 100 μM AFB1 for 44 h. Both polar body extrusion rates of mouse and porcine oocytes were reduced with the increasing concentration. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01; ***, significance, p<0.001.
A

**MOUSE**

B

**PIG**

C

Rate of polarbody extrusion (%)

Control: 10μM, 50μM, 100μM, 150μM

AFB1 concentration

Rate of polarbody extrusion (%)

Control: 5μM, 10μM, 50μM, 100μM

AFB1 concentration

Rate of polarbody extrusion (%)

MOUSE

PIG

AFB1 concentration/μM

0 5 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150

* p < 0.05

** p < 0.01

*** p < 0.001
Figure 2

The effects of DON on the oocyte maturation

(A) Rate of polar body extrusion after DON treatment. For mouse oocyte culture, the DON concentration was 0, 1, 2, 3, 4 μM. (B) Rate of polar body extrusion after DON treatment. For porcine oocytes, DON concentration was 0, 0.5, 1, 2, 3 μM. The reduction of polar body extrusion was dose-dependent. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01; ***, significance, p<0.001.
Figure 3

The effects of HT-2 on the oocyte maturation

(A) Rate of polar body extrusion after HT-2 treatment. Mouse oocytes were cultured with HT-2 at the concentration of 0, 10, 20, 30, 40 nM. (B) Rate of polar body extrusion after HT-2 treatment. For porcine oocytes culture, HT-2 was 0, 10, 50, 100, 150, 200, 400 nM. The rate of polar body extrusion was decreased when the concentration of HT-2 was increasing. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01; ***, significance, p<0.001.
Figure 4

The effects of OTA on the oocyte maturation

(A) Rate of polar body extrusion after OTA treatment. For mouse oocyte culture, OTA concentration was 0, 200, 300, 400, 600 μM. (B) Rate of polar body extrusion after OTA treatment. For porcine oocyte culture, OTA concentration was 0, 1, 5, 10, 30, 100 μM. *, significance, p<0.05; **, significance, p<0.01. (C) The comparison for the effects of different concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion.
Figure 5

The effects of ZEA on the oocyte maturation.

(A) Rate of polar body extrusion after ZEA treatment. Mouse oocytes were cultured with 0, 10, 50, 100, 200 μM ZEA. (B) Rate of polar body extrusion after ZEA treatment. Porcine oocytes were cultured with 0, 5, 10, 30, 50 μM ZEA. Both mouse and porcine polar body extrusion rate were reducing when ZEA concentration was increasing. (C) The statistical analysis for the effects of same concentration on mouse and porcine oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented rate of porcine polar body extrusion. *, significance, p<0.05; **, significance, p<0.01.
A) MOUSE

B) PIG

C) Graph showing the rate of polar body extrusion vs. ZEA concentration for MOUSE and PIG.

- **MOUSE**
  - Control: 80%
  - 10 μM: 60%
  - 50 μM: 40%
  - 100 μM: 30%
  - 200 μM: 20%

- **PIG**
  - Control: 80%
  - 5 μM: 60%
  - 10 μM: 40%
  - 50 μM: 20%
  - 100 μM: 10%

- Graph for MOUSE and PIG:
  - MOUSE: **,** **,**
  - PIG: **,** **,** **,**