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 μ M AFB1 and 1 μ M DON significantly affected porcine oocyte maturation compared with 50 μ M AFB1 and 2 μ M 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 μ M OTA and 10 μ M ZEA significantly affected porcine oocyte maturation compared with 300 μ M OTA and 50 μ M 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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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 17 β -estradiol and it will compete with 17 β -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 reagent

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 (COCs) 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 ($72.82 \pm 4.83\%$, $n=110$, $p>0.05$). Rates of matured oocytes were significantly decreased when the concentration of AFB1 were increased to $50 \mu\text{M}$ ($55.96 \pm 4.77\%$, $n=157$, $p<0.05$), $100 \mu\text{M}$ ($17.86 \pm 7.51\%$, $n=154$, $p<0.05$), $150 \mu\text{M}$ ($2.15 \pm 3.72\%$, $n=146$, $p<0.001$). We cultured the porcine oocytes for 44 h with 5, 10, 50, $100 \mu\text{M}$ AFB1. The polar body extrusion rate was $80.72 \pm 7.05\%$ ($n=217$) 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 $5 \mu\text{M}$ AFB1 and the control oocytes showed no significant difference ($68.34 \pm 15.39\%$, $n=195$ vs $80.72 \pm 7.05\%$ $n=217$, $p>0.05$). However, the rate of polar body extrusion in porcine oocytes was significantly decreased with $10 \mu\text{M}$ ($53.02 \pm 7.12\%$, $n=144$, $p<0.001$), $50 \mu\text{M}$ ($22.95 \pm 6.76\%$, $n=192$, $p<0.01$), $100 \mu\text{M}$ ($0 \pm 0\%$, $n=147$, $p<0.01$) 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 $10 \mu\text{M}$ ($p<0.05$) and $50 \mu\text{M}$ ($p<0.01$), 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 $4 \mu\text{M}$ DON. Our results showed that DON affected mouse oocyte maturation. The average polar body extrusion rate of the control group was $76.73 \pm 3.24\%$ ($n=162$) (Figure 2A), when mouse oocytes cultured with $1 \mu\text{M}$ AFB1, there was no significantly

difference between the control group and 1 μM group ($76.01 \pm 2.35\%$ $n=139$, $p>0.05$). While the rate of polar body extrusion in mouse oocytes was significantly decreased with 2 μM DON treatment ($44.38 \pm 4.87\%$, $n=173$, $p<0.05$), 3 μM DON treatment ($16.30 \pm 4.00\%$, $n=199$, $p<0.001$), 4 μM DON treatment ($2.28 \pm 0.69\%$, $n=189$, $p<0.001$), 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 $72.05 \pm 2.6\%$ ($n=195$) 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 ($68.42 \pm 4.55\%$ $n=159$, $p>0.05$) showed no significant difference. However, the rate of polar body extrusion in porcine oocytes was significantly decreased with 1 μM DON treatment ($46.29 \pm 3.89\%$, $n=176$, $p<0.05$), 2 μM DON treatment ($17.02 \pm 4.87\%$, $n=145$, $p<0.01$), 3 μM DON treatment ($3.29 \pm 1.81\%$, $n=132$, $p<0.001$) (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 ($p<0.01$), 3 μM ($p<0.01$), 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 $73.08 \pm 1.67\%$ ($n=153$) (Figure 3A), while the rate of polar body extrusion in mouse oocytes was significantly decreased with 10 nM HT-2 treatment ($43.33 \pm 4.93\%$, $n=176$, $p<0.05$), 20 nM HT-2 treatment ($33.05 \pm 2.18\%$, $n=163$, $p<0.001$), 30 nM HT-2 treatment

($7.08 \pm 0.89\%$, $n=150$, $p<0.001$), 40 nM HT-2 treatment ($1.05 \pm 0.10\%$, $n=169$, $p<0.01$). 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 $78.19 \pm 2.03\%$ ($n=171$) in the control group of porcine oocytes, which was close to mouse oocytes. However, except the 10 nM HT-2 treatment ($68.67 \pm 2.99\%$, $n=163$, $p>0.05$), the rate of polar body extrusion in porcine oocytes was significantly decreased with 50 nM HT-2 treatment ($47.4 \pm 3.36\%$, $n=156$, $p<0.01$), 100 nM HT-2 treatment ($25.50 \pm 5.14\%$, $n=164$, $p<0.01$), 150 nM HT-2 treatment ($21.22 \pm 4.07\%$, $n=188$, $p<0.01$), 200 nM HT-2 treatment ($18.02 \pm 6.69\%$, $n=175$, $p<0.01$), 400 nM HT-2 treatment ($8.22 \pm 0.78\%$, $n=162$, $p<0.001$) (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 $80.23 \pm 3.87\%$ ($n=169$) (Figure 4A), compared with the control group, the concentration of 200 μM OTA showed no significant difference ($73.77 \pm 2.24\%$ $n=143$, $p>0.05$), while the rate of polar body extrusion in mouse oocytes was significantly decreased with 300 μM OTA treatment ($49.86 \pm 4.29\%$, $n=190$, $p<0.01$), 400 μM OTA treatment ($31.23 \pm 3.64\%$, $n=145$,

192 $p<0.01$), 600 μM OTA treatment ($2.22\pm3.85\%$, $n=178$, $p<0.01$). The porcine oocytes were cultured
 193 for 44 h with 1, 5, 10, 30 and 100 μM OTA. Similar with the mouse oocytes, our results showed
 194 that OTA affected porcine oocyte maturation. The control group and 1 μM OTA group showed no
 195 significant difference ($80.34\pm7.95\%$ $n=164$ vs $65.46\pm4.09\%$ $n=178$, $p>0.05$). However, the rate of
 196 polar body extrusion in porcine oocytes was significantly decreased with 5 μM OTA treatment
 197 ($53.83\pm0.34\%$, $n=154$, $p<0.05$), 10 μM OTA treatment ($22.26\pm3.14\%$, $n=183$, $p<0.05$), 30 μM
 198 OTA treatment ($22.19\pm4.87\%$, $n=140$, $p<0.01$), 100 μM OTA treatment ($4.24\pm3.93\%$, $n=144$,
 199 $p<0.01$) (Figure 4B). With the same concentration of OTA treatment, the rate of porcine oocyte
 200 polar body extrusion was lower than mouse oocytes (Figure 4C), indicating that compared with
 201 mouse oocytes, porcine oocytes were more sensitive to OTA.

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

203 The last we examined was the effects of ZEA on mouse and porcine oocytes. Mouse oocytes
 204 were cultured for 12 hours with 10, 50, 100 and 200 μM ZEA. The average polar body extrusion
 205 rate of the control group was $81.29\pm6.06\%$ ($n=155$) (Figure 5A), when the concentration was 10
 206 μM , the average rate of MII oocytes was $74.52\pm4.92\%$ ($n=154$) which showed no significant
 207 difference with the control groups ($p>0.05$). While the rate of polar body extrusion in mouse
 208 oocytes was significantly decreased with 50 μM ZEA treatment ($54.35\pm3.9\%$, $n=128$, $p<0.05$),
 209 100 μM ZEA treatment ($26.23\pm8.00\%$, $n=150$, $p<0.05$), 200 μM ZEA treatment ($0.00\pm0.00\%$,
 210 $n=132$, $p<0.01$). For porcine oocytes, the average polar body extrusion rate of the control groups
 211 was $77.85\pm9.51\%$ ($n=175$), while the 5 μM ZEA groups ($60.45\pm1.65\%$, $n=199$, $p>0.05$) showed
 212 no significant differences with the control groups. However, the rate of polar body extrusion in

porcine oocytes was significantly decreased with the 10 μ M groups ($51.42 \pm 2.73\%$, $n=190$, $p<0.05$), 30 μ M ZEA treatment ($19.10 \pm 4.49\%$, $n=147$, $p<0.05$) and 50 μ M ZEA treatment ($4.08 \pm 4.08\%$, $n=148$, $p<0.01$) (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 μ M ($p<0.01$) and 50 μ M ($p<0.001$) (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 μ M AFB1 affected cumulus oocyte complexes growth, especially the polar body extrusion was significantly reduced in porcine oocytes (Liu et al. 2015). However, 10 μ M 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 μ M AFB1 affected porcine

oocyte maturation instead of 50 μ M AFB1 in mouse oocytes.

2 μ M 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 μ M DON affected the morphology of pig ovaries with an *ex vivo* approach (Gerez et al. 2017). Our recent study also showed that 3 μ M DON exposure altered autophagy/apoptosis and epigenetic modifications in porcine oocytes (Han et al. 2016). In present study our results showed that 1 μ M DON affected porcine oocyte maturation instead of 2 μ M 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 μ M OTA in the drinking water was adopted in this study. Our results showed that 10 μ M OTA affected porcine oocyte maturation instead of 300 μ M 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 μ M, 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 μ M ZEA affected porcine oocyte maturation instead of 50 μ M 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.

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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 μ M AFB1 for 12 h. (B) Rate of polar body extrusion after AFB1 treatment. Porcine oocytes were cultured with 0, 5, 10, 50, 100 μ M 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 μ M. (B) Rate of polar body extrusion after DON treatment. For porcine oocytes, DON concentration was 0, 0.5, 1, 2, 3 μ M. 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 μ M. (B) Rate of polar body extrusion after OTA treatment. For porcine oocyte culture, OTA concentration was 0, 1, 5, 10, 30, 100 μ M. *, 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 μ M ZEA. (B) Rate of polar body extrusion after ZEA treatment. Porcine oocytes were cultured with 0, 5, 10, 30, 50 μ M 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

372 oocytes. The grey line represented rate of mouse polar body extrusion. The black line represented
 373 rate of porcine polar body extrusion. *, significance, $p < 0.05$; **, significance, $p < 0.01$.

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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 μ M AFB1 for 12 h. (B) Rate of polar body extrusion after AFB1 treatment. Porcine oocytes were cultured with 0, 5, 10, 50, 100 μ M 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 μ M. (B) Rate of polar body extrusion after OTA treatment. For porcine oocyte culture, OTA concentration was 0, 1, 5, 10, 30, 100 μ M. *, 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 μ M ZEA. (B) Rate of polar body extrusion after ZEA treatment. Porcine oocytes were cultured with 0, 5, 10, 30, 50 μ M 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, $p < 0.01$.