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Review

# Effects of zearalenone and its derivatives on the synthesis and secretion of mammalian sex steroid hormones: A review

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ABSTRACT

Zearalenone (ZEA), a non-steroidal estrogen mycotoxin produced by several species of *Fusarium* fungi, can be metabolized into many other derivatives by microorganisms, plants, animals and humans. It can affect mammalian reproductive capability by impacting the synthesis and secretion of sex hormones, including testosterone, estradiol and progesterone. This review summarizes the mechanisms in which ZEA and its derivatives disturb the synthesis and secretion of sex steroid hormones. Because of its structural analogy to estrogen, ZEA and its derivatives can exert a variety of estrogen-like effects and engage in estrogen negative feedback regulation, which can result in mediating the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the pituitary gland. ZEA and its derivatives can ultimately reduce the number of Leydig cells and granulosa cells by inducing oxidative stress, endoplasmic reticulum (ER) stress, cell cycle arrest, cell apoptosis, and cell regeneration delay. Additionally, they can disrupt the mitochondrial structure and influence mitochondrial functions through overproduction of reactive oxygen species (ROS) and aberrant autophagy signaling ways. Finally, ZEA and its derivatives can disturb the expressions and activities of the related steroidogenic enzymes through cross talking between membrane and nuclear estrogen receptors.

## 1. Introduction

Zearalenone (ZEA), also known as F-2 mycotoxin, is a non-steroidal estrogen mycotoxin produced by several species of *Fusarium* fungi (Stanciu et al., 2018; Tatay et al., 2016). These fungi can contaminate cereal grains, including maize, wheat, sorghum, barley, and oats, and produce ZEA in the field and farm or during harvest and storage at a low temperature and high humidity (Ali and Degen, 2018; Dellafiora et al., 2017). ZEA are not only dangerous for the public health, but they also deteriorate the marketable quality of the contaminated products, causing tremendous economic losses (Reddy et al., 2018). The incidence and extent of ZEA contamination is strictly related to geographic and climate as well as cultivation, harvesting, stocking, and transport conditions (Miraglia et al., 2009). The international trade of these contaminated cereal commodities may have contributed to the worldwide dispersal of ZEA (Zinedine et al., 2007). Several studies carried out in Europe and in transcontinental countries, reported the high incidence of ZEA in cereals and in animal feeding stuffs (Miraglia et al., 2009). The impact of climate on ZEA occurs through multiple pathways. Changes in air and water temperatures, weather-related changes, and extreme events can shift the seasonal and geographic occurrence of fungi contaminants (Castro-Ibanez et al., 2015; Wu et al., 2011). The world's largest agri-food exporters include countries such as Brazil and Argentina and parts of Asia including China and India are identified as hot spots for impacts of climate change (Ray et al., 2012).

Studies have suggested that there are two main routes for human and animal exposure to ZEA and its derivatives (Bertero et al., 2018; Yang et al., 2018a). The first is consuming cereal grains and their

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**Abbreviations**

| | |
|---|---|
| 17β-HSD | 17 beta-hydroxysteroid dehydrogenase |
| 3β-HSD | 3 beta-hydroxysteroid dehydrogenase |
| 4-PBA | 4-phenylbutyrate |
| AC | adenylate cyclase |
| ASK-1 | apoptosis signal-regulating kinase 1 |
| ATM | ataxia telangiectasia mutated |
| CAT | catalase |
| CDK2 | cyclin dependent kinase 2 |
| CHOP | transcriptional factor C/EBP homologous protein |
| CYP11A1 | cytochrome P450 family 11 subfamily A member 1 |
| Cyp11a1 | cholesterol side cleavage enzyme |
| CYP17 | cytochrome P450 family 17 |
| EDS | ethane dimethane sulfonate |
| EGF | epidermal growth factor |
| ER stress | endoplasmic reticulum stress |
| EREs | estrogen response elements |
| ERO1 | endoplasmic reticulum oxidoreductin 1 |
| ERs | nuclear estrogen receptors |
| FAD | Flavin adenine dinucleotide |
| FAS | Fas cell surface death receptor |
| FASL | FAS ligand |
| FSH | follicle-stimulating hormone |
| GADD45A | growth arrest and DNA damage-inducible protein gadd45 alpha |
| GPR30 | G protein-coupled receptor 30 |
| GPXs | glutathione peroxidases |
| GR | glutathione reductase |
| GSH | Glutathione |
| GSSG | glutathione disulfide |
| GST | glutathione S-transferase |
| HB-EGF | heparin bound epidermal growth factor |
| HCG | human chorionic gonadotropin |
| HSD11B1 | hydroxysteroid 11-beta dehydrogenase 1 |
| IGF-1 | insulin like growth factor 1 |
| JNK | c-Jun amino terminal kinase |
| LH | luteinizing hormone |
| LHX3 | LIM class of homeodomain protein-3 |
| MAPK | mitogen activated protein kinase |
| miRNAs | that microRNAs |
| MMP | mitochondrial membrane potential |
| mtDNA | mitochondrial DNA |
| MTT assay | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay |
| NUR77 | nerve growth factor IB |
| P450SCC | P450 side-chain cleavage enzyme |
| PAC | phosphofurin acidic cluster |
| PDE | cyclic nucleotide phosphodiesterase |
| PI3K | phosphatidylinositol-4,5-bisphosphate 3-kinase |
| PITX-1 | pituitary homeobox-1 |
| PKA | protein kinase A |
| Prxs | peroxiredoxins |
| ROS | reactive oxygen species |
| SF1 | steroidogenic factor 1 |
| SOD | superoxide dismutases |
| StAR | steroidogenic acute regulatory protein |
| TRX1 and TRX2 | thioredoxin isoforms |
| ZAN | Zearalanone |
| ZAN-14-glu | Zearalanone-14-Glucuronide |
| ZAN-16-glu | Zearalanone-16-Glucuronide |
| ZEA | zearalenone |
| ZEA-14-glu | zearalenone-14-Glucuronide |
| ZEA-14-sulfate | Zearalenone-14-sulfate |
| ZEA-16-glu | zearalenone-16-Glucuronide |
| ZEA-16-sulfate | Zearalenone-O-14-sulate |
| ZEA-O-14-glu | zearalenone-O-14-Glucuronide |
| ZEA-O-16-glu | zearalenone-O-16-Glucuronide |
| α-ZAL | α-zearalanol |
| α-ZAL-14-glu | α-zearalanol-14-Glucuronide |
| α-ZAL-16-glu | α-zearalanol-16-Glucuronide |
| α-ZEL | α-zearalenol |
| α-ZEL-14-glu | α-zearalenol-14-Glucuronide |
| α-ZEL-16-glu | α-zearalenol-16-Glucuronide |
| β-ZAL | β-zearalanol |
| β-ZAL-14-glu | β-zearalanol-14-Glucuronide |
| β-ZAL-16-glu | β-zearalanol-16-Glucuronide |
| β-ZEL | β-zearalenol |
| β-ZEL-14-glu | β-zearalenol-14-Glucuronide |
| β-ZEL-16-glu | β-zearalenol-16-Glucuronide |

derived products that are contaminated with ZEA or toxigenic fungi (Zheng et al., 2018d). The second is consuming animal-derived food, including meat, eggs, and milk, which was derived from animals that were already contaminated by ZEA or were injected with its derivatives to promote growth (Belhassen et al., 2015; Jeong et al., 2010; Mauro et al., 2018). The adverse effects of ZEA have been widely investigated in human, farm animals, poultry, birds and companion animals such as pig, ovine, bovine, horse, chicken, birds, dogs and cats (Blajet-Kosicka et al., 2014; Minervini and Dell'Aquila, 2008). Among these animals, pigs are the most susceptible species to the toxicity of ZEA, gilts being the most sensitive, and poultry, horse, birds are lower susceptible species (Malekinejad et al., 2006b; Olsen et al., 1987). The oestrogenic syndrome in swine affects primarily the reproductive tract and mammary gland, and in more sensitive young gilts 1–5 ppm of ZEA induce clinical signs such as hyperemia, edematous swelling of the vulva, sometime vaginal prolapse and even rectal prolapse (Devreese et al., 2015). Feeding mature laying hens a diet contaminated with ZEN up to 800 mg/kg did not affect their reproductive performance (Allen et al., 1981). In cow, infertility, reduced milk production and hyperoestrogenism have been associated with ZEA and heifers were fed with ZEA containing diet over three estrous cycles, conception rates decline from 87 to 62% (D'Mello et al., 1999). (A daily intake of 12 mg ZEA for eight weeks during the breeding season did not affect the weekly sperm production, spermatozoal mass motility of spermatozoal morphology of adult ram (Milano et al., 1991). A field outbreak of ZEA mycotoxicosis in horses was associated with corn screenings containing approximately 2.6 mg/kg of ZEA and the concentration of the toxin recorded in naturally contaminated oats does not have a significant effect on the release of reproductive hormones, cycle length or uterine histology in mares (Aurich et al., 2006).

Accumulating evidence has suggested that exposure to ZEA can impair the reproductive capacity and disturb the production and development of sperm and oocytes in humans and animals (Adibnia et al., 2016; Takagi et al., 2008; Yang et al., 2018c; Yousef et al., 2017). Several studies have also shown that ZEA can not only disrupt puberty and the estrous cycle, but also could affect early pregnancy events including fertilization, embryo development, and embryo implantation (Zhao et al., 2014). Futhermore, it was also indicated that human puberty timing can be advanced by ZEA exposure. ZEA and their metabolites have been implicated in a number of incidences involving precocious puberty among young girls in various countries (Mukherjee et al., 2014). ZEA is suspected to be a triggering factor for central precocious puberty development in girls (Massart et al., 2008). ZEA exposure can contribute to the incidences of premature thelarce and central idiopathic precocious puberty in girls and the urinary levels of ZEA in girls with premature thelarche and idiopathic central precocious puberty were increased 2 and 2.8 fold respectively (Asci et al., 2014).

It has been reported that ZEA affects the reproductive capability by influencing the synthesis and secretion of steroid sex hormones, including testosterone, estradiol, and progesterone in males and females. ZEA and its metabolites can act as potential endocrine disruptors by altering the production of hormone such as testosterone (Long et al., 2016; Zhao et al., 2014). After treatment with a single oral dose of ZEA for 14d, the plasma levels of testosterone and estrogen were decreased in healthy mice (Long et al., 2016). Steroid sex hormones are synthesized primarily in the adrenal glands, gonads, and placenta, and play vital roles in regulating sexual differentiation and production, controlling sexual behavior patterns, and maintaining secondary sex characteristics (Behan et al., 2003). Steroid sex hormones include androgens, estrogens and progestogens, of which the most important derivatives are testosterone, estradiol, and progesterone, respectively, in the human and animal body (Erol et al., 2019). Therefore, any toxin that alters the balance of sex hormones can disturb reproductive functions. The fact that ZEA and its derivatives can affect the reproductive capability by influencing the synthesis and secretion of steroid sex hormones raises a question: How do ZEA and its derivatives affect the synthesis and secretion of sex steroid hormones? Thus, the purpose of this article was to discuss and summarize the available mechanisms and current data concerning the synthesis and secretion of steroid sex hormones influenced by ZEA and its derivatives.

## 2. The biotransformation of ZEA in animals and humans

ZEA is synthesized by several *Fusarium* species *F. graminearum* (also known as *Gibberella zeae*), *F. culmorum*, *F. semitectum*, *F. cerealis*, and *F. equiseti*. In recent years, several studies have suggested that the structure of ZEA can be modified by microorganisms and plants via glycosylation, sulfation, and acetylation of trichothecenes (Fig. 1) (Brodehl et al., 2014; Dellafiora et al., 2018; Freire and Sant'Ana, 2018). Fungal strains can not only produce ZEA, but also have the ability to convert ZEA into various metabolites (Table 1) (Brodehl et al., 2014). After consumption, the modified forms of ZEA are completely converted into ZEA by the animal or human intestinal microbiota. Studies have demonstrated that zearalenone-16-O-β-glucoside was almost completely converted into ZEA after incubation with intestinal bacteria in water (Kovalsky Paris et al., 2014). ZEA-14-sulfate, ZEA-14-O-β-glucoside, and ZEA-16-O-β-glucoside were hydrolyzed in gastrointestinal tract of pigs (Binder et al., 2017). After conversion, ZEA is rapidly absorbed and initially metabolized into its derivatives by the intestine and liver (Mauro et al., 2018; Yang et al., 2018b).

Numerous studies have demonstrated that ZEA is metabolized in the livers of various animal species (Dong et al., 2010). The predominant formation of α-ZEL was formed not only by the liver, but also by the especially gastrointestinal tissues (Dong et al., 2010). In addition to the intestine and liver, other tissues, including the kidney, prostate, hypothalamus, testis, and ovary also have the ability to metabolize ZEA (D'Mello et al., 1999). The highest concentration was observed in small intestine, followed by kidneys, liver, adipose tissue, lung, and serum at both infusion rates. ZEA concentrations in stomach, heart, brain, spleen, muscle, and testes were lower than in serum (Shin et al., 2009). Several studies have indicated that the reduction of ZEA has also been observed in human prostateglands, sow intestinal mucosa and rumen fluid (Seeling et al., 2006). Taken together, ZEA can be modified by microorganisms and plants, which can produce many masked ZEA compounds in food or feed.

The metabolism of ZEA can be divided into two phases: Phase-I metabolism and phase-II metabolism (Zheng et al., 2018a). In phase-I, ZEA was converted to ZAN, α-ZEL, β-ZEL, α-ZAL, and β-ZAL by the enzymes of 3α-hydroxysteroid dehydrogenase (3α-HSD) or 3β–hydroxysteroid dehydrogenase (3β -HSD) family (Malekinejad et al., 2005). In phase-II, the metabolites from phase-I are sulfated and glucuronidated. The glucuronic acid group is supplied by uridine 5′-diphosphate glucuronic acid (UDPGA), which is catalyzed by uridine 5′-diphosphate glucuronosyltransferase (UGT)(Malekinejad et al., 2005). The metabolites, including ZEA-Glucuronide (ZEA-Glu), α-zearalenol-Glucuronide (α-ZEL-Glu), β-zearalenol-Glucuronide (β-ZEL-Glu), Zearalanone-Glucuronide (ZAN-Glu), α-zearalenol -Glucuronide (α-ZAL-Glu), and β-zearalenol-Glucuronide (β-ZAL-Glu), are produced in this phase (Zheng et al., 2018a).

Accumulating evidence has indicated that ZEA showed considerable differences in metabolic process among different animal species. ZEA was preferentially converted into α-ZEL, rather than β-ZEL, in the post-mitochondrial fractions of pigs, sheep and cattle, whereas in chickens and rats more β-ZEL than α-ZEL was produced. Pigs seem to convert ZEA predominantly into α-ZEL, whereas in chickens β-ZEL is the dominant hepatic metabolite (Yang et al., 2017b). It was also indicated that the highest rate of glucuronidation for ZEA, α-ZEL and β-ZEL was found in pigs, compared to the other species, and the lowest rate of glucuronidation was observed in chickens (Malekinejad et al., 2006b). One of the reasons why pigs convert ZEA predominantly into α-ZEL more than other animal species could be that pigs have higher level of 3α-HSD enzyme more than other animals. Another reason could be that ZEA undergoes substantial enterohepatic recirculation in pigs prior to its excretion via feces or urine (Hennig-Pauka et al., 2018). Futhermore, a study from *in vivo* has suggested that reduction, hydroxylation, and glucuronidation were the major metabolic pathways in rats, while reduction and sulfation predominate in chickens (Yang et al., 2017b). Studies have indicated that the estrogenic potency of these metabolites varies, with α-ZEL showing the highest binding affinity to estrogen receptors, followed by the parent ZEA, and the lowest binding affinity to estrogen receptors has been found for β-ZEL (Malekinejad et al., 2006b). The estrogenic activities are different and can be ranked as follows: α-ZEL = α-ZAL > ZAN > ZEA = β-ZAL > β-ZEL in human breast cancer cells *in vitro* (Parveen et al., 2009). The pig liver predominantly forms α-ZEL correlates with the higher sensitivity of pigs to the estrogenic effects of ZEA and the high rate of β-ZEL production found with chicken liver samples corresponded to the low sensitivity of this animal species towards the adverse effects of ZEA. The hepatic conversion of ZEA into α-ZEL has to be considered as a bioactivation reaction, whereas the transformation into β-ZEL can be regarded as a detoxification reaction. Taken together, the individual susceptibility of individual animal species might be explainable by differences in the metabolic conversion of ZEA.

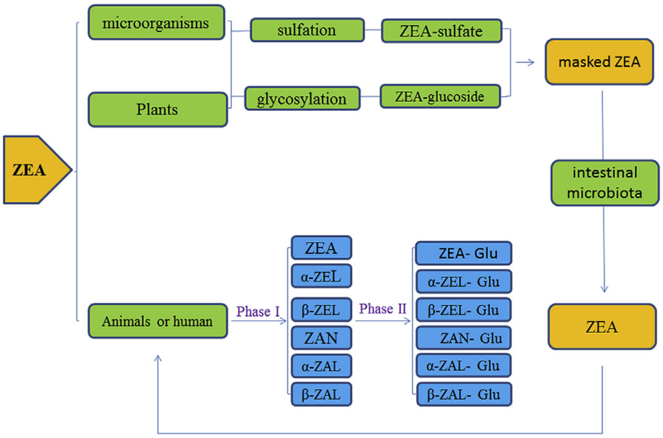
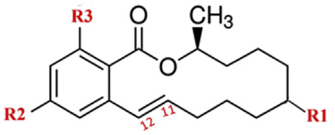


**Fig. 1.** Zearalenone (ZEA) can be modified by microorganisms and plants, and these masked ZEA compounds can be completely converted into ZEA by the animal or human intestinal microbiota. In humans and animals, the metabolism of ZEA can be divided into two phases including Phase-I metabolism and Phase-II metabolism.

**Table 1**
The chemical structures of ZEA and its main derivatives.



| | R1 | R2 | R3 | 11,12 |
|---|---|---|---|---|
| ZEA | = O | -OH | -OH | double bond |
| ZEA-14-sulfate | = O | -sulfate | -OH | double bond |
| ZEA-14-glu | = O | -glucoside | -OH | double bond |
| ZEA-O-14-glu | = O | -O-glucoside | -OH | double bond |
| ZEA-16-sulfate | = O | -OH | -glucoside | double bond |
| ZEA-16-glu | = O | -OH | -glucoside | double bond |
| ZEA-O-16-glu | = O | -OH | -O-glucoside | double bond |
| α-ZEL | (R)-OH | -OH | -OH | double bond |
| α-ZEL-14-glu | (R)-OH | -glucoside | -OH | double bond |
| α-ZEL-16-glu | (R)-OH | -OH | -glucoside | double bond |
| β-ZEL | (S)-OH | -OH | -OH | double bond |
| β-ZEL-14-glu | (S)-OH | -glucoside | -OH | double bond |
| β-ZEL-16-glu | (S)-OH | -OH | -glucoside | double bond |
| ZAN | = O | -OH | -OH | single bond |
| ZAN-14-glu | = O | -glucoside | -OH | single bond |
| ZAN-16-glu | = O | -OH | -glucoside | single bond |
| α-ZAL | (R)-OH | -OH | -OH | single bond |
| α-ZAL-14-glu | (R)-OH | -glucoside | -OH | single bond |
| α-ZAL-16-glu | (R)-OH | -OH | -glucoside | single bond |
| β-ZAL | (S)-OH | -OH | -OH | single bond |
| β-ZAL-14-glu | (S)-OH | -glucoside | -OH | single bond |
| β-ZAL-16-glu | (S)-OH | -OH | -glucoside | single bond |

## 3. ZEA and its derivatives can disturb the synthesis and secretion of steroid sex hormones

ZEA and its derivatives can disturb the synthesis and secretion of steroid sex hormones, including testosterone, estradiol, and progesterone. Several studies have demonstrated that ZEA can affect the production of testosterone *in vitro* and *in vivo* (Table 2) (Gao et al., 2018; Yang et al., 2007a). The *in vivo* data suggested that ZEA could reduce the level of testosterone in mice serum after treatment by intraperitoneal injection in a dose-dependent manner (Yang et al., 2007a). Feeding pregnant rats with a ZEA-contaminated diet (0, 5, 10 and 20 mg/kg) during the whole gestation period caused a decrease in the concentrations of testosterone in the serum of F1 adult male rats (Gao et al., 2018). The concentrations of testosterone in plasma were decreased during the consumption of ZEA in boars fed 40 ppm ZEA from 14 to 18 weeks of age (Berger et al., 1981). *In vitro* studies also indicated that the biosynthesis of testosterone was inhibited by ZEA via crosstalk between the orphan nuclear receptor Nur77 and estrogen receptor signaling in mouse Leydig cells (Liu et al., 2014). ZEA and α-ZOL could inhibit the synthesis of human chorionic gonadotropin (hCG)-induced androgen production in both primary culture of Leydig cells and Leydig cell lines *in vitro* (Gao et al., 2018; Li et al., 2014).

Furthermore, many studies have indicated that ZEA and its derivatives could disturb the production of estradiol (Belli et al., 2010; Wang et al., 2010). When 75-day-old female pigs were treated with diets supplemented 0.5 or 2.0 mg/kg ZEA for 21 days, the level of estradiol was decreased at day 21 (Wang et al., 2010). Pregnant female SD rats were ZEA by gavage once a day at doses of 0, 1, 2, 4, or 8 mg/kg on gestation days, and the results suggested that the levels of estradiol were decreased in the 2, 4, and 8 mg/kg groups (Belli et al., 2010). Feeding the pregnant rats with ZEA (0, 5, 10, and 20 mg/kg feed) on gestational days 0–21, caused decreases in the levels of estrogen in a dose-dependent manner in both maternal and F1 adult rats (Gao et al., 2017). 75-day-old female pigs were treated the diets supplemented with 0, 0.5, or 2.0 mg/kg ZEA for 21 days, and the results indicated that the level of estradiol increased at days 7 and 14, but decreased at day 2 (Wang et al., 2010). After treatment with a single oral administration of ZEA for 14 d, the plasma levels of testosterone and estrogen were decreased in healthy male Kunming mice (Long et al., 2016). ZEA had a biphasic effect on insulin like growth factor 1 (IGF-1)-induced estradiol

**Table 2**
ZEA can disturb the synthesis and secretion of sex steroid hormones, including testosterone, estradiol, and progesterone.

| Mycotoxin | Exposure Dose | Exposure Period | Animal or cell type | Effects | Ref |
|---|---|---|---|---|---|
| ZEA, α-ZEL | 25, 50 and 75 mg/kg daily | 7 days | male mouse | testosterone↓ | Yang et al. (2007a) |
| ZEA | 5, 10 and 20 mg/kg | during the whole gestation period | pregnant rats | testosterone↓<br>estradiol↓ | Gao et al. (2018) |
| ZEA | 40 μg/kg | 14–18 weeks of age | boars | testosterone↓ | Berger et al. (1981) |
| ZEA, α-ZEL | 10-4-10-8 M | 24 h | mouse primary Leydig cells | testosterone↓ | Yang et al. (2007b) |
| ZEA | 1, 5, 10 and 20 μg/mL | 24 h | mouse primary Leydig cells | testosterone↓ | Liu et al. (2014) |
| ZEA | 5 μM | 24 h | mouse Leydig cells line | steroidogenesis↓ | Li et al. (2014) |
| ZEA | 150 and 300 ng/testis | 21 d | rats | testosterone↓ | Zhou et al. (2018) |
| ZEA, α-ZEL, β-ZEL | 100 mM | 48 h | H295R cells | testosterone<br>estradiol↓<br>progesterone↓ | Frizzell et al. (2011) |
| ZEA | 0.5 or 2.0 mg/kg | 21 d | female pigs | estradiol↑ | Wang et al. (2010) |
| ZEA | 1, 2, 4 or 8 mg/kg | gestation days 6-19d | Pregnant female SD rats | estradiol↓ | Collins et al. (2006) |
| ZEA | 5, 10 and 20 mg/kg | during the whole gestation period | Pregnant female SD rats | estradiol↓ | Gao et al. (2017) |
| ZEA | 0.5or 2.0 mg/kg | 21 d | 75-day-old female pigs | estradiol(7-14d)↓<br>estradiol(14-21d)↓ | Wang et al. (2010) |
| ZEA | 0–3000 ng/mL | 1 d | porcine granulosa cells | larger doses estradiol↓ | Ranzenigo et al. (2008) |
| ZEA | 40 mg/kg | 14 d | Kunming mice | testosterone↓<br>estrogen↓ | Long et al. (2016) |
| ZEA | 108 mg | postmating 2–15 d | swine | Progesterone↓ | Long and Diekman (1986) |
| ZEA | 2, 4 and 8 mg/kg | gestation days 6-19d | Pregnant female SD rats | Progesterone↓ | Collins et al. (2006) |
| ZEA | 1.1, 2.0 and 3.2 mg/kg | 18 d | pigs | Progesterone↓ | Chen et al. (2015b) |
| ZEA | 50–75 μg/kg (b.w) | 42d | female Beagle | Progesterone↓ | Gajecka et al. (2013) |
| ZEA | 5 -100 Mm | 24 h | MA-10 mouse | Progesterone↓ | Savard et al. (2016) |

production, increasing the production of estradiol at smaller doses and inhibiting it at larger doses (Ranzenigo et al., 2008).

Additionally, it was also suggested that ZEA and its derivatives could perturb the production of progesterone (Chen et al., 2015b; Savard et al., 2016). *In vivo* studies have suggested that ZEA could reduce the serum level of progesterone in pigs fed a diet supplemented with ZEA (0, 1.1, 2.0, or 3.2 mg/kg) for 18d (Chen et al., 2015b). ZEA caused a dramatic effect on the non-physiological concentrations of progesterone in female Beagles fed with 50–75 µg ZEA/kg for 45 days (Gajecka et al., 2013). Other *in vivo* studies demonstrated that ZEA could alter the FSH-induced estradiol and progesterone production in porcine granulosa cells (Ranzenigo et al., 2008). ZEA could decrease progesterone secretion from the MA-10 mouse Leydig cell line after forskolin activation *in vitro* (Savard et al., 2016). Taken together, these results showed ZEA can disturb the synthesis and secretion of steroid sex hormones, including testosterone, estradiol, and progesterone.

## 4. The molecular mechanisms by which ZEA and its derivatives affect the synthesis and secretion of steroid sex hormones

### *4.1. ZEA and its derivatives disturb the expressions of FSH and LH in the pituitary gland*

In the majority of mammals, steroidogenesis occur via the two cell/two gonadotropin model in which androgens are synthesized from cholesterol by LH responsive theca cells, followed by conversion to estrogens in FSH-stimulated granulosa cells (Duydu et al., 2018; Zhang et al., 2017). The pituitary gonadotropins, including FSH and LH, are vital hormones for activating and controlling the synthesis and transport of sex hormones from the gonads into the blood (He et al., 2018a). Diminished secretion of LH or FSH can result in decreased activity of the Leydig cells and granulosa cells, which can affect the synthesis and secretion of steroid sex hormones (Diaz-Ramos et al., 2018). There is increasing evidence that ZEA inhibits the synthesis and secretion of FSH and LH in different animals. ZEA caused reproductive defects and inhibited the synthesis and secretion of FSH and LH in pigs, rats, ewes, and monkeys (Elsasser et al., 1983; He et al., 2018b; Hobson et al., 1977). ZEA could decrease the serum level of FSH and LH in pigs after treatment with 0, 1.1, 2.0, or 3.2 mg/kg ZEA for 18 d (Chen et al., 2015b). After feeding pregnant rats with a ZEA-contaminated (0, 5, 10, and 20 mg/kg) diet during the whole gestation period, the concentrations of LH in the serum of F1 adult male rats decreased (Gao et al., 2018). When ovariectomized gilts were given 1 mg ZEA/kg body weight, the serum concentrations of both LH and FSH were reduced within 6 h (Diekman et al., 1989). In ewes, α-ZAL treatment depressed plasma LH concentrations within 4 h of treatment (Elsasser et al., 1983). In addition, *in vitro* studies have demonstrated that ZEA could inhibit the synthesis and secretion of FSH in the pig pituitary glands (Kolesarova et al., 2017). ZEA and its metabolites decreased the synthesis and secretion of FSH in pigs through the non-classical estrogen membrane receptor G protein-coupled receptor 30 (GPR30) *in vitro* (He et al., 2018a). ZEA inhibited the synthesis and secretion in pigs and led to the reproductive defects through the expression of microRNA miR-7 (He et al., 2018b).

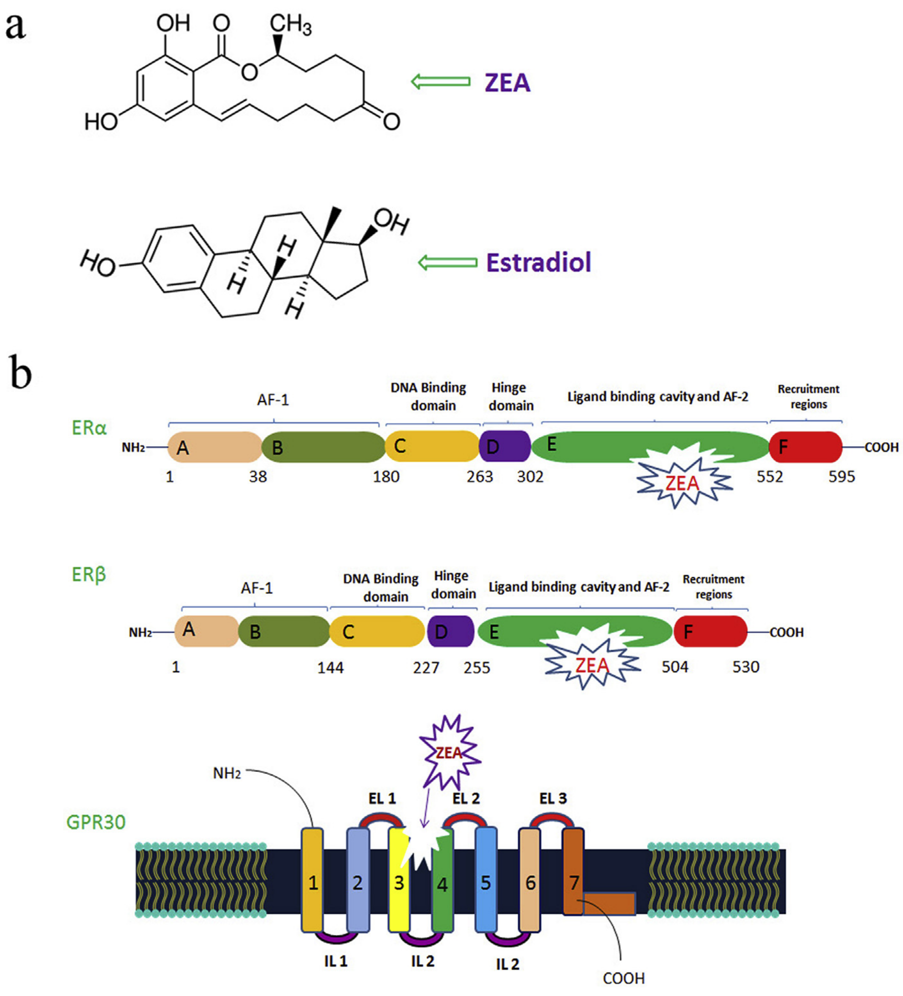


**Fig. 2.** (a) The structures of Zearalenone (ZEA) and estradiol. (b) The Primary Structure of estrogen receptor (ER)α, ERβ, and G protein-coupled receptor 30 (GPR30). ERα and ERβ have five different functional areas, termed A/B, C, D, E and F. Domain A/B contains the activation function-1 (AF-1) domain. Domain C contains the DNA binding domain. Domain D contains the hinge domain, which can link with domain C and E. Domain E contains the activation function 2 (AF-2) domain. Domain F contains the recruitment regions. GPR30 is a 7-transmembrane domains G-coupled protein receptor that has estrogenic activity and shares little homology with the classical ERs. GPR30 consists of an N-terminal domain, seven transmembrane domains, and a C-terminal domain. GPCRs are characterized by an N-terminal domain, followed by 7-transmembrane (7-TM) α-helices (TM-1 to TM-7) connected by three intracellular loops (IL) and three extracellular loops (EL), and finally a C-terminus.

### *4.2. The molecular mechanism by which ZEA and its derivatives disturb the expressions of FSH and LH in the pituitary gland*

#### *4.2.1. ZEA and its derivatives exert the estrogen-like effects and engage in the estrogen negative feedback to regulate the expression of FSH and LH*

ZEA is a selective modulator of estrogen receptors (SERM) and an endocrine disruptor chemical (EDC) because of it disturbs the hormonal balance in animals and humans (Kowalska et al., 2016, 2018). ZEA and its metabolites (Fig. 2) have sufficient structural similarity to endogenous estrogen such that they can bind to estrogen receptors and exert the estrogen-like effects (Dellafiora et al., 2017; Katzenellenbogen and Korach, 1997; Tatay et al., 2018). The estrogenic activities of ZEA and its metabolites are different and can be ranked as follows: α-ZEL = α-ZAL > ZAN > ZEA = β-ZAL > β-ZEL in human breast cancer cells *in vitro* (Parveen et al., 2009). Several studies have indicated that pigs are the most sensitive species to the α-ZEL metabolite, while β-ZOL is the prevalent metabolite in ruminants and poultry, and α-ZOL was reported as the prevalent metabolite in humans (Malekinejad et al., 2006b; Pillay et al., 2002; Videmann et al., 2008). The *in vitro* and *in vivo* suggest that ZEA and its metabolites have multiple estrogenic toxic activities (Kunishige et al., 2017; Tatay et al., 2018). Exposure to ZEA and its derivatives through the diet showed had an endocrine effect, as manifested by their ability to modulate the production of testosterone, progesterone, and estradiol (Frizzell et al., 2011). Thus, ZEA and its metabolites exert the estrogen-like effects and can engage in the estrogen negative feedback regulation to affect hormone biosynthesis, including the production FSH and LH (see Fig. 3).

#### *4.2.2. ZEA and its derivatives affected the production of FSH and LH in the pituitary gland through the GPR30- Gα -AC/cAMP-PKA-ERK-LHX3-miR-7-Fos pathway*

ZEA and its metabolites exert estrogen-like effects that can allow them to engage in feedback effects mediating the production of FSH and LH in the pituitary gland. To exert these important feedback effects from gonad to the pituitary, estradiol binds estrogen receptors, which are distributed in the plasma membrane, nucleus, and mitochondria (Sanchez-Criado et al., 2012). The mechanisms of estrogenic actions can be separated into three main pathways, including genomic, non-genomic, and mitochondrial pathways. Accumulating evidence suggests that ZEA engages in the estrogen negative feedback only through membrane receptor GPR30 and not through nuclear-localized estrogen receptors α or β. Studies have shown that ZEA and its metabolites decreased the synthesis and secretion of FSH and LH through the non-classical estrogen membrane receptor GPR30 in pig and bovine pituitary glands (He et al., 2018a; Nakamura and Kadokawa, 2015). Application of the GPR30 antagonist, G36, reversed the ZEA-mediated inhibition of the secretion of LH from bovine anterior pituitary cells *in vitro* (Nakamura and Kadokawa, 2015).

However, compared with estradiol, what downstream signaling pathway is involved after ZEA binds to GPR30 in the pituitary? Several studies have shown that ZEA can bind membrane ERs and disrupt the balance of ACs and PDEs, which can affect the balance of cAMP synthesis and degradation (Lee et al., 2012). After treatment with ZEA, the level of cAMP was altered, but the gene expressions of the *LHα*, *LHβ*, or *FSHβ* subunits did not change in cattle anterior pituitary cells (Nakamura et al., 2015). Furthermore, *in vitro* studies have indicated that ZEA and its metabolites can influence the phosphorylation levels of ERK and the p38 MAPK signaling pathway in female pig anterior pituitary cells (He et al., 2018a). It was reported that the ERK and p38MAPK signaling pathways are involved in α-ZOL's effects on FSH synthesis in pig pituitary glands (He et al., 2018a; Kolesarova et al., 2017). Additionally, estrogen binds to GPR30, resulting in the phosphorylation of transcription factors, including steroidogenic factor 1 (SF1), pituitary homeobox-1 (PITX-1), nuclear factor Y, activator protein-1, and the LIM class of homeodomain protein-3 (LHX3). Several studies have suggested that LHX3, a downstream molecule in the ERK and p38MAPK pathways, might be involved in regulating the effects of α-ZOL on the synthesis and secretion of FSH in pig pituitary glands (Blaustein, 2010). Recently, it was also reported that microRNAs (miRNAs) can influencing the synthesis and secretion of pituitary hormones. ZEA caused reproductive defects by enhancing the expression of miR-7, which subsequently inhibited the synthesis and secretion of FSH in the pig pituitary gland (Han et al., 2017). The *in vitro* and *in vivo* data suggest that the effects of ZEA rely on GPR30 and miR-7 functions by targeting the *FOS* gene (He et al., 2018b). Taken together, the evidence from current studies suggests that ZEA and its derivatives disturb the expressions of FSH and LH through the GPR30-Gα-AC/cAMP-PKA-ERK-LHX3-miR-7-FOS pathways in the pituitary gland.

### *4.3. ZEA and its derivatives can alter the cell function and reduce cell numbers of Leydig cells and granulosa cells*

Leydig cells play a crucial role in synthesizing testosterone, and contribute about 95% of the circulatory androgen level (Yang et al., 2007b). The production of testosterone depends not only on the functions of Leydig cells, but also on their numbers (Zhou et al., 2018). In Leydig cells, LH stimulates the production of testosterone. LH can trigger the generation of cAMP from ATP and activate several signaling pathways, leading to the phosphorylation of steroidogenic acute regulatory protein (StAR), which mediates cholesterol transfer into mitochondria for the first enzymatic conversion of cholesterol into pregnenolone (Jana et al., 2008). Thus, any toxin that alters the function and viability of Leydig cells might influence the synthesis and secretion testosterone (Akingbemi, 2005; Eze et al., 2018). Several studies have indicated that ZEA can inhibit viability and cause cell death in Leydig

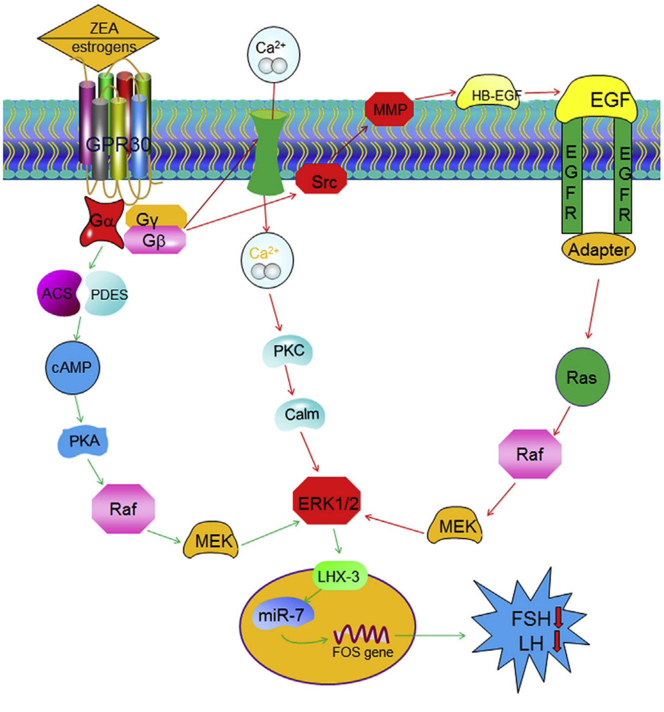


**Fig. 3.** The downstream signaling pathway after Zearalenone (ZEA) and estrogens bind to G protein-coupled receptor 30 (GPR30) in the pituitary gland. ZEA mediated the expressions of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the pituitary gland through the GPR30- Gα-AC/cAMP-PKA-ERK-LHX3-miR-7-FOS pathways (green arrow). Estrogens mediate the expression levels of FSH and LH in the pituitary gland through three main pathways including the GPR30-Gβγ-Src-MMP-pro-HBEGF-EGF-EGFR-Ras-Raf-MEK-ERK pathway, the GPR30-Gβγ-$Ca^{2+}$- PKC-calm-ERK pathway, and the GPR30-Gα -AC/cAMP-PKA-ERK-LHX3-miR-7-FOS pathways (both green and red arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cells (Wang et al., 2014a; Zhou et al., 2018). After treatment with ZEA (2.5, 5, 10, and 20 μg/mL) for 12 h, the growth of rat Leydig cells was inhibited, and the rate of apoptosis increased in a dose-dependent manner (Wang et al., 2014a). ZEA induced cell death of primary mouse Leydig cells via apoptosis, which was mediated by endoplasmic reticulum stress and mitochondrial pathways (Lin et al., 2015). Treatment with ZEA reduced testosterone biosynthesis in mouse Leydig cells *in vivo* and *in vitro* because of the downregulation of the transcription of certain steroidogenic enzymes (Yang et al., 2007a, 2007b).

The major function of granulosa cells is to produce sex steroids. During the follicular phase of the menstrual cycle, FSH stimulates granulosa cells from the anterior pituitary to convert androgens into estradiol via aromatase. In addition, after ovulation the granulosa cells turn into the granulosa lutein cells, which can produce progesterone (Garzo and Dorrington, 1984). Thus, the function and viability of granulosa cells are vitally important for the synthesis and secretion of the sex steroids including, estradiol and progesterone. Several studies have indicated that ZEA can alter the function, inhibit viability, and cause cell death in granulosa cells. *In vitro* studies demonstrated that ZEA could alter the function and genomic stability of porcine granulosa cells (Zhang et al., 2017). The growth of porcine granulosa cells was dramatically decreased and the rate of apoptosis increased after exposure to 10 and 30 μM ZEA for 24 or 72 h (Liu et al., 2018). ZEA and its metabolites impaired cell proliferation, steroid production, and gene expression in bovine small-follicle granulosa cells *in vitro* (Pizzo et al., 2016). After treatment with ZEA, the cell proliferation of mouse granulosa cells was reduced in a dose-dependent manner, as determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and terminal deoxynulceotidyl transferase nick-end-labeling (TUNEL) apoptosis analysis (Hou et al., 2015). Zearalenone induces apoptosis and necrosis in porcine granulosa cells via the caspase-3- and caspase-9-dependent mitochondrial signaling pathway (Zhu et al., 2012).

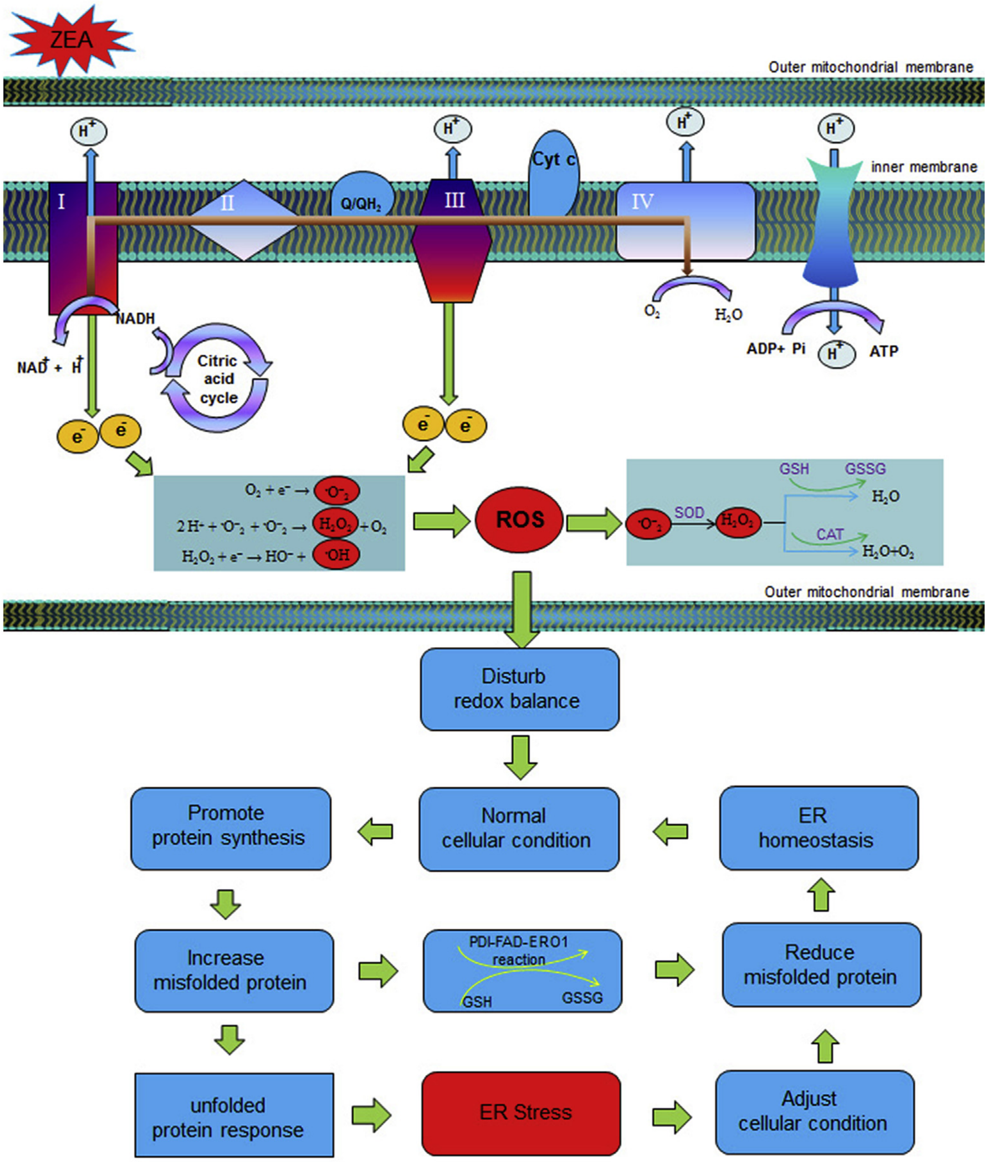


**Fig. 4.** The mechanisms of Zearalenone (ZEA) induced the production of reactive oxygen species (ROS). In the mitochondria, electrons move from an electron donor (nicotinamide adenine dinucleotide-hydrogen (NADH)) to a terminal electron acceptor ($O_2$) through the respiratory chain. ZEA could induce the overproduction of ROS and cause oxidative stress by stimulating the leakage of electrons from proton pump I and III, and destroying the antioxidant defense systems in mitochondria. The overproduction ROS could disturb the redox balance, which could increase the number of misfolded proteins and cause endoplasmic reticulum (ER) stress.

### *4.4. The molecular mechanisms by which ZEA and its derivatives alter the cell function and numbers of Leydig cells and granulosa cells*

#### *4.4.1. ZEA and its derivatives can disturb the cell functions by causing oxidative stress*

The toxicity of ZEA and its metabolites is caused by not only the previously mentioned estrogenic effects, but also by other mechanisms, such as oxidative stress and DNA damage (Tatay et al., 2016; Wu et al., 2014). It was reported that ZEA could generate reactive oxygen species (ROS) and induce oxidative stress, which resulted in DNA damage during the formation of its metabolites (Fig. 4) (Minervini and Dell'Aquila, 2008; So et al., 2014; Tatay et al., 2017). The toxicity of ZEA and α- and β-ZOL has been linked to the generation of oxidative stress in different cells (Lu et al., 2013b;Ouanes-Ben Othmen et al., 2008). ROS comprise three major types: $H_2O_2$, $\cdot OH$, and $\cdot O^{2-}$ (Mates et al., 2012). The major producers of ROS are NADPH oxidase complexes, which are distributed in cell membranes, mitochondria, peroxisomes, and the endoplasmic reticulum (Han et al., 2001; Muller, 2000). ZEA and its metabolites could increase ROS levels and cause the oxidative stress through the mitochondrial pathway (Ben Salem et al., 2016). In mitochondria, electrons move from an electron donor (NADH or QH2) to a terminal electron acceptor ($O_2$) via a series of redox reactions in the respiratory chain, which are coupled to the creation of a proton gradient across the mitochondrial inner membrane through proton pumps I, III, and IV. The resulting transmembrane proton gradient is used to generate ATP via ATP synthase (Mailloux, 2015).

Electrons leak out from the respiratory chain during the process of electron transport to the electron acceptor and bind to oxygen, which is considered to be the main source of ROS (Sinha et al., 2013). To combat the increasing of ROS and protect cells from oxidative stress and damage, cells have their own antioxidant machineries, consisting of several Phase II detoxification enzymes including superoxide dismutases (SOD), glutathione peroxidases (GPXs), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), peroxiredoxins (Prxs), glutaredoxin, thioredoxin isoforms (TRX1 and TRX2), non-enzyme glutathione (GSH), and glutathione disulfide (GSSG). When the balance between the antioxidant defense and ROS production is disrupted, cells are likely to be damaged by oxidative stress (Qin et al., 2015). Several studies have shown that ZEA and its metabolites can stimulate the leakage of electrons and increase the level of free radicals. After treatment with α-ZOL and β-ZOL, the level of mitochondrial anion superoxide increased from 2.4% to 59.1% and 61.31%, respectively, in HEK293 cells (Ben Salem et al., 2016). In addition, ZEA could disturb the cell antioxidant machinery and affect the levels and activities of detoxification enzymes including GSH, SOD, MDA, GPXx, and CAT in different cell types (Tatay et al., 2017; Zheng et al., 2018b). Taken

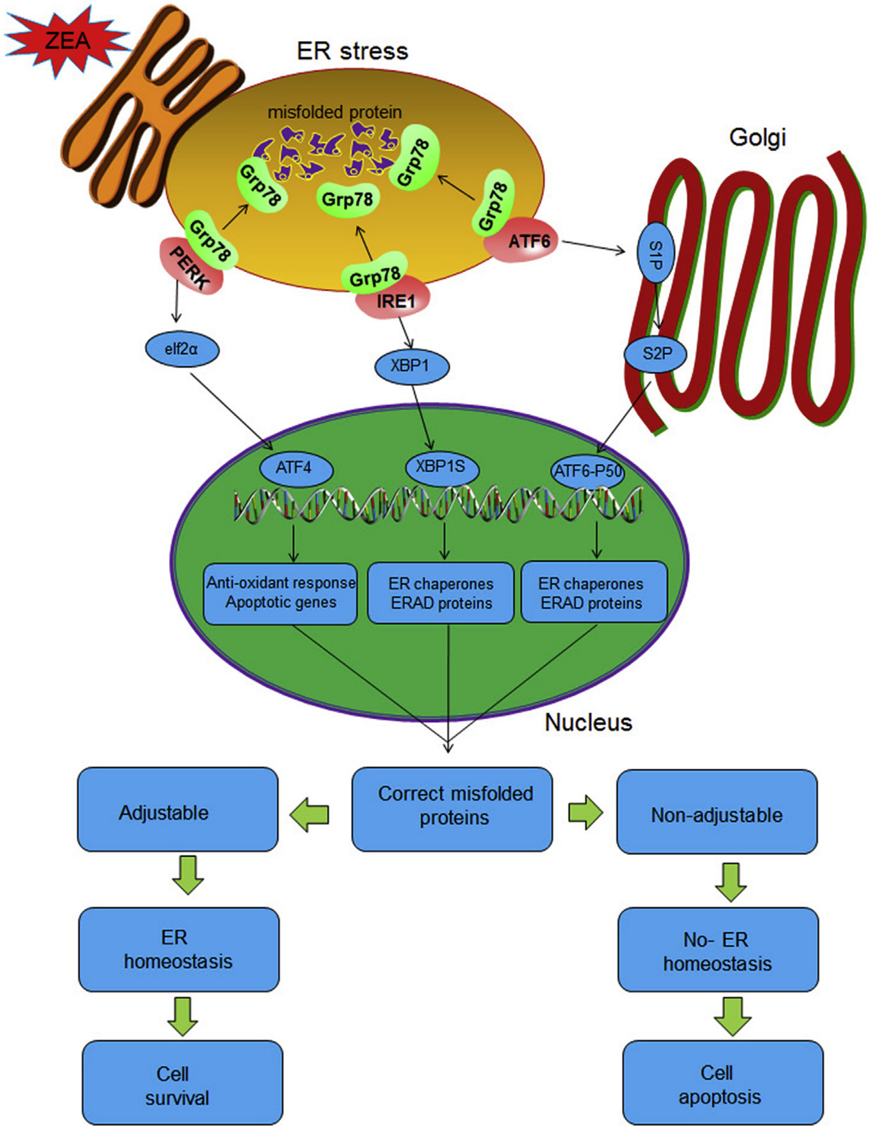


**Fig. 5.** The process of the endoplasmic reticulum (ER) stress or unfolded protein response (UPR). Grp78 dissociates from PERK, IRE1, and ATF6 and is recruited to chaperone the misfolded proteins. Activated PERK could mediate the anti-oxidant repose and apoptotic genes through eIF2α and ATF4. Activated IRE1 could cleave XBP1 into XBP1S. Activated ATF6 translocates to the Golgi and is cleaved to ATF6-p50. Both ATF6-p50 and XBP1s bind ERSE promoters in the nucleus to produce ER chaperones and endoplasmic reticulum-associated degradation (ERAD), which can remove and correct the accumulated misfolded proteins. According to outcome of these of changes, cells can either survive or suffer death through cell apoptosis.

together, ZEA could induce the overproduction of ROS and cause oxidative stress by stimulating leakage electron from the respiratory chain and destroying the antioxidant defense systems in mitochondria.

### *4.4.2. ZEA and its derivatives disturb the cell functions and reduced cell numbers by causing endoplasmic reticulum stress*

Recently, increasing numbers of studies have revealed that endoplasmic reticulum stress plays a critical role in the process of ZEA-induced reproductive cytotoxicity (Fig. 5). ZEA induced cell death through endoplasmic reticulum stress in goat and mouse Leydig cells (Lin et al., 2015; Yang et al., 2017a). It was reported that endoplasmic reticulum stress is involved in ZEA-induced apoptosis and cell cycle arrest (Chen et al., 2016). Endoplasmic reticulum stress and caspase-dependent pathways are also involved in ZEA-induced cell death and apoptosis in Leydig cells (Lin et al., 2015). ROS regulates the process of ZEA-induced cell cycle arrest and cell apoptosis via endoplasmic reticulum stress and the ATP/AMPK signaling pathways in mouse Sertoli cells (Zheng et al., 2018c). Endoplasmic reticulum stress mediates a broad range of physiological events including proliferation, apoptosis, and the production and secretion of hormones (Takahashi et al., 2017a). Endoplasmic reticulum stress in the follicles of obese women contributes to progesterone deficiency by inhibiting hCG-induced progesterone production in granulosa cells (Takahashi et al., 2017b).

Several studies have suggested that ZEA induces endoplasmic reticulum stress via ROS (Fig. 4), and after co-treatment with the antioxidant NAC to attenuate ROS, the level of endoplasmic reticulum stress was decreased (Zheng et al., 2018c). The application of two other common antioxidant food components, Quercetin and Crocin, also could protect cells from ZEA by inhibiting endoplasmic reticulum stress (Ben Salem et al., 2015). However, a study has also shown that endoplasmic reticulum stress could promote the level of ROS induced by ZEA. After pre-treatment with the 4-phenylbutyrate (4-PBA) to down regulate the endoplasmic reticulum stress signals, the intracellular ROS content decreased compared with that in cells treated with ZEA alone (Zheng et al., 2018b). It was reported that ROS and endoplasmic reticulum stress could interfere with each other. ROS has a profound effect on the unfolded protein response and endoplasmic reticulum stress. The accumulating misfolded proteins can increase the level of ROS. Disrupting the cellular redox balance could cause the production of misfolded proteins (Chong et al., 2017). ROS can disturb the balance of reduction-oxidation, and recent studies have also reported that ROS increases endoplasmic reticulum stress by increasing the production of unfolded proteins and reducing the efficiency of protein folding pathways (Plaisance et al., 2016). By contrast, during endoplasmic reticulum stress, with the accumulation of misfolded proteins, the level of ROS could increase (Zeeshan et al., 2016). In addition accumulation of misfolded proteins decreases the amount of GSH in the cells, which can stimulate the production of ROS. GSH can reduce the quantities of misfolded proteins by converting non-native disulfide bonds into native disulfide bonds. This event decreases the amount of GSH in the cells. By contrast, as misfolded proteins accumulate, the amount of hydrogen peroxide could increase in cells. During the oxidative protein folding process, PDI receives two electrons from the polypeptide chain substrate via cysteine residues, and is therefore able to oxidize the polypeptide chain substrate and reduce the PDI protein active sites (Chong et al., 2017). However, it has been proposed that other endoplasmic reticulum resident enzymes also assist PDI in the oxidative folding process. Flavin adenine dinucleotide (FAD), which is recruited by endoplasmic reticulum oxidoreductin 1 (ERO1), could conduct a redox reaction on PDI to produce hydrogen peroxide (Ferrari and Soling, 1999).

### *4.4.3. ZEA and its derivatives reduce cell numbers by causing cell cycle arrest and cell regeneration delay and cell apoptosis*

ZEA could influence the distribution of the cell cycle in Leydig cells and granulosa cells. After treatment with 10 and 20 μg/L of ZEA for 72 h, the percentages of cell distributed in the G1/G0 phase and S phase had changed in Leydig cells (Zheng et al., 2017). Exposure to ZEA and α-ZOL caused a simultaneous increase in the sub-G0 and S + G2/M peaks in cultured granulosa cells from equine ovaries (Minervini et al., 2006). What's more, it was reported that ZEA reduced cell numbers by delaying the regeneration of Leydig cells (Zhou et al., 2018). Study has revealed that the differentiation of progenitor Leydig cells into immature Leydig cells was delayed by ZEA (Zhou et al., 2018). Additionally, several studies have suggested that ZEA and its derivatives reduce cell numbers by inducing cell apoptosis. After exposure to ZEA

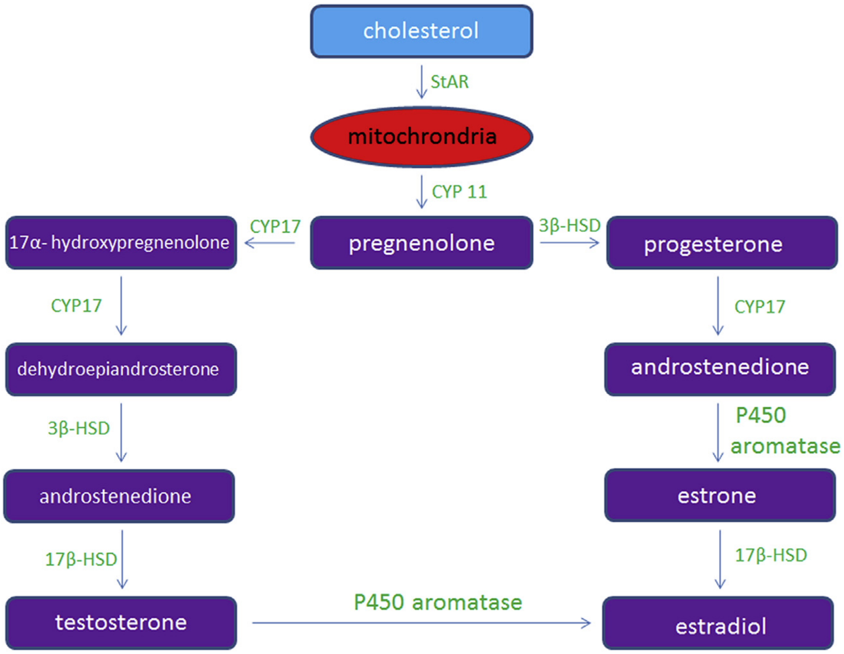


**Fig. 6.** The biosynthetic pathways of sex steroid hormones. The initial step of steroidogenesis is transferring cholesterol into the mitochondria and through the actions of StAR, CYP11, CYP17, 3β-HSD, 17β-HSD and P450 aromatase, the cholesterol is converted to progesterone, testosterone and estradiol.

(30 and 50 μM) for 12 h, early apoptotic cells were detected; while after exposure to ZEA for 24 h, late apoptotic cells were mainly observed *in vitro* (Chen et al., 2015a).

Cells monitor their cell cycle status via cell cycle checkpoints. Cyclins and cyclin-dependent kinases regulate cell progression through the stages of the cell cycle (G1, S, G2, M). Failure of the first two checkpoints (G1 and G2/M) mostly occurs upon DNA damage. DNA damage can arrest cells in G2 before progressing to mitosis, and in G2, the cells can undergo DNA repair mechanisms or apoptosis, similarly to that observed during G1 (Linke et al., 1996). ZEA, as a DNA damaging agent, can cause DNA-DSBs and influenced genome stability in porcine granulosa cells, which further led to the reduction of granulosa cell proliferation and cell cycle arrest in the S and G2/M phases (Liu et al., 2018). Additionally, it was reported that ROS generated in mitochondria could travel into the nucleus and damage the genomic DNA, including base oxidation and SSBs in large DNA molecules (Lee et al., 2016; Scott et al., 2014). Another study showed that ZEA induced cell cycle arrest in the G2/M phase through ROS, and co-treatment with NAC to alleviate ROS reversed the accumulation of cells in the G2/M phase (Zheng et al., 2018c). Thus, oxidative stress may explain why ZEA causes DNA damage and induces cell cycle arrest in different cell types.

It was reported that ZEA induces cell apoptosis through many pathways, including the intrinsic pathway, extrinsic pathway, and endoplasmic reticulum stress pathway (Xu et al., 2016). ZEA could induce apoptosis and necrosis in rat Sertoli cells via the extrinsic and intrinsic apoptotic pathways (Xu et al., 2016). ZEA can induce apoptosis in RAW 264.7 macrophages through the endoplasmic reticulum stress pathway (Chen et al., 2016). In the intrinsic pathway, it was reported that ZEA could trigger apoptosis through the overproduction of ROS and mitochondrial damage (Gupta et al., 2012; Zhu et al., 2012). It was also suggested that the extrinsic pathway is involved in the process of ZEA-induced apoptosis (Xu et al., 2016). ZEA induces apoptosis in germ cells of male rats and that this toxicity of ZEA is partially mediated through modulation of the Fas cell surface death receptor (FAS) and the FAS ligand (FASL) systems (Jee et al., 2010). The expression of FASL and caspase-8 increased in a dose-dependent manner upon ZEA treatment (Xu et al., 2016). Additionally, several studies have shown that ZEA could induce apoptosis through endoplasmic reticulum stress (Chen et al., 2016). The activation of the endoplasmic reticulum stress pathway plays a key role in ZEA-induced apoptosis in mouse Leydig cells (Lin et al., 2015). Endoplasmic reticulum stress is involved in the cell apoptosis induced by ZEA via activating the ATP/AMPK signaling pathway in Sertoli cells (Zheng et al., 2018c).

### 4.5. ZEA and its derivatives can disturb the functions and structure of mitochondria

Mitochondria play indispensable and highly specialized roles in the production of steroid hormones, which are essential for life-sustaining homeostasis and reproduction in all vertebrates (Fig. 6) (Papadopoulos and Miller, 2012). All steroid hormones, including estrogens, progestins, androgens, glucocorticoids, mineralocorticoids, and neurosteroids are made from cholesterol (Papadopoulos and Miller, 2012). The initial step of steroidogenesis is transferring cholesterol into the mitochondria through the steroidogenic acute regulatory protein (StAR) (Stocco, 2001). Through the action of cytochrome P450 side-chain cleavage enzyme (P450scc), cholesterol is converted to pregnenolone in the inner mitochondrial membrane, which is the rate-limiting step in the synthesis of steroid hormones (Minervini and Dell'Aquila, 2008). Through the actions of cytochrome P450 family 17 (CYP17), 3 beta-hydroxysteroid dehydrogenase (3β-HSD), and 17 beta-hydroxysteroid dehydrogenase (17β-HSD), pregnenolone was also converted to testosterone (Haider, 2004). Pregnenolone is also transferred to the microsomal compartment in which it is converted into progesterone by 3β-HSD (Liu et al., 2014). The androgen could be converted into estrogen by cytochrome p450 aromatase in two different ways, including the transformation of testosterone into estrogen and androstenedione into estrogen (Cherradi et al., 1997; Minervini and Dell'Aquila, 2008).

However, an increasing number of studies have revealed that ZEA could influence mitochondrial functions, which might disturb the synthesis of steroid hormones (Adibnia et al., 2016; Zhang et al., 2018). After treatment with ZEA (1, 2, and 4 mg/kg, b. w) for 28 continuous days, the mitochondrial content was decreased in male rats (Adibnia et al., 2016). Disruption of mitochondria was observed after treatment with ZEA, and the disruption was more severe with increasing doses (Zheng et al., 2016). Rh123 fluorescence of porcine granulosa cells treated with ZEA was statistically decreased in a dose-dependent manner compared with that in the control, implying that ZEA causes loss of the mitochondrial transmembrane potential in porcine granulosa cells (Zhu et al., 2012). ZEA and its metabolites decreased the mitochondrial membrane potential (MMP), and caused cytoplasmic release of cytochrome c in RAW264.7 cells (Lu et al., 2013a). In PC3 cells, ZEA might cause changes in the oxidative activity of mitochondria and induce mitochondrial depolarization (Kowalska et al., 2017).

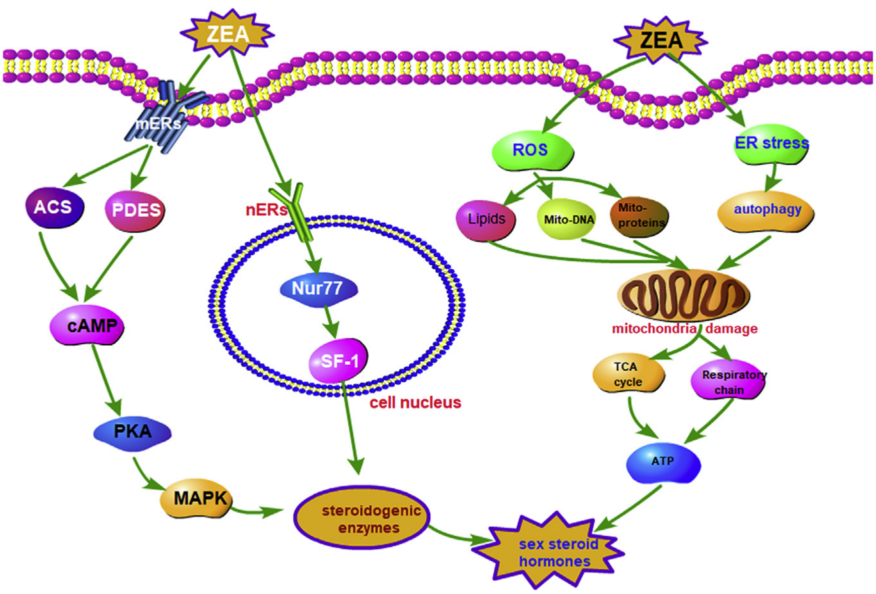


**Fig. 7.** Zearalenone (ZEA) disturbed the synthesis of sex steroid hormones by affecting the expression and activities of related steroidogenic enzymes and causing damage to the mitochondrial structure and functions. ZEA mediated the expression and activities of related steroidogenic enzymes through the ER membrane and nuclear receptors. The disturbance to mitochondrial functions and the damage to the mitochondrial structure caused by ZEA could be attributed to the overproduction ROS and the excessive action of autophagy.

### *4.6. The molecular mechanisms by which ZEA and its derivatives disturb the functions and structure of mitochondria*

The main method by which ZEA disturbs the functions and structure of mitochondria may its induction of ROS overproduction, leading to oxidative stress (Fig. 7). Growing evidence suggests that overwhelming production of ROS could destroy bio-molecules and the structure of organelles (He et al., 2017). Several studies have shown that co-treatment with antioxidants, including NAC, Vitamin E, and Proanthocyanidin, to alleviate the oxidative stress could partially reverse the disruption of the cytoskeletal structure and ultrastructure caused by ZEA (Zhang et al., 2018). ROS cause oxidative damage to mitochondrial lipids, DNA, and proteins, making mitochondria further prone to the production of ROS. ROS impair the ability of mitochondria to synthesize ATP and carry out their wide range of metabolic functions, including fatty acid oxidation, the TCA cycle, the urea cycle, and amino acid metabolism (Zorov et al., 2006). The potential pathological outcomes of ROS production also include the formation of mutations or deletions to the mitochondrial DNA (mtDNA), oxidative damage to the respiratory chain, lipid peroxidation, and overall mitochondrial dysfunction.

The other method by which ZEA can damage mitochondria is the induction of aberrant and excessive autophagy. Autophagy is triggered as the degradation and dynamic recycling system, and the materials are delivered to double membrane vacuoles inside lysosomes for degradation. To maintain cellular homeostasis and adjust to changing physiological demands, cells get rid of damaged, dysfunctional, and superfluous cytoplasmic components via autophagy (Wang and Klionsky, 2011). Mitochondrial degradation by autophagy might have an essential role in maintaining mitochondrial functions (Kim et al., 2007). Accumulating evidence suggests that ZEA could trigger the autophagy in different cell types including Leydig cells, RAW264.7 macrophages, cardiomyoblast H9c2 cells, and Sertoli cells (Ben Salem et al., 2017; Chen et al., 2016; Wang et al., 2014b). ZEA destroyed the structure and affected the expression of cytoskeleton associated proteins through the overproduction of autophagy in TM4 cells (Zheng et al., 2018b). The PI3K-Akt-mammalian target or rapamycin (mTOR) signaling pathway is involved in ZEA-induced autophagy. ZEA triggered autophagy in TM4 cells through the ERK signaling pathway to suppress the phosphorylation of mTOR. Endoplasmic reticulum stress stimulates autophagy by inhibiting the ERK signaling pathway to suppress the phosphorylation of mTOR (Zheng et al., 2018b). Thus, the disruption of mitochondrial functions and the damage to mitochondrial structures caused by ZEA might be attributed to the overproduction of ROS and the excessive action of autophagy.

### *4.7. ZEA and its derivatives can affect the expressions and activities of the steroidogenic enzymes*

Several studies have revealed that ZEA affects the expression of steroidogenic enzymes (Fig. 7). After treatment with 1, 5, 10, and 20 μg/mL ZEA for 24 h, the mRNA levels of P450scc, 17β-HSD, and P450c17 were decreased in mouse Leydig cells (Liu et al., 2014). ZEA can interfere with steroid metabolism by altering the enzyme activity of 3β-HSD and StAR, which are key enzymes involved in the biosynthesis of gonadal steroid hormones (Bravin et al., 2009). The use of a ZEA-contaminated (0, 5, 10 and 20 mg/kg) diet during the whole gestation period caused a significant decrease in 3β-HSD and StAR expression at both the mRNA and protein levels in F1weaned and adult testis (Gao et al., 2018). ZEA and α-ZOL could suppress the secretion of HCG-induced testosterone, which correlated with the decrease in 3β-HSD-1, P450scc, and StAR levels in mouse Leydig cells *in vitro* (Yang et al., 2007b). In addition to disturbing the expression of steroidogenic enzymes, many studies have also suggested that ZEA is one of substrates for 3α-HSD and 3β-HSD. Its structural resemblance to steroids means that ZEA has the ability to bind competitively to 3α and 3β- HSD, which disturbs the synthesis of sex hormones (Malekinejad et al., 2006a). *In vitro*, ZEA inhibited androgen production and steroidogenic enzyme activities in immature Leydig cells by downregulating expression levels of cholesterol side cleavage enzyme (Cyp11a1), 3β-hydroxysteroid dehydrogenase 1 (HSD3b1), and steroid 5α-reductase 1 (SRD5a1) at a concentration as low as 50 nM (Zhou et al., 2018).

### *4.8. The molecular mechanisms by which ZEA and its derivatives disturb the expression and activities of steroidogenic enzymes*

Recently, it was reported that ZEA mediates the expressions of the steroidogenic enzymes through nerve growth factor IB (NGFIB, also known as NUR77) (Sadovsky and Dorn, 2000). NUR77 is a member of the NUR nuclear receptor family and is one of the primary transcription factors that can regulate the expression of steroidogenic enzyme genes. After exposure to ZEA, the expression of NUR77 was decreased in mouse Leydig cell *in vitro* (Liu et al., 2014). Studies have shown that ER signaling pathways could downregulate NUR77 protein levels by suppressing the transcription of its gene (Lee et al., 2012). ERα and ERβ are expressed in Leydig cells and the ER signaling pathways play a distinct role in mediating the expressions of steroidogenic enzymes. In ERα or ERβ knockout mice, the expression levels of P450scc, 17β-HSD, and StAR were increased (Akingbemi et al., 2003). Thus, ZEA may mediate the expression of steroidogenic enzymes via crosstalk with estrogen receptor signaling and orphan nuclear receptor NUR77. NUR77 binds as a monomer to a regulatory element similar to that recognized by the nuclear receptor steroidogenic factor 1 (SF-1), which is encoded by the *NR5A1* gene. It was suggested that NUR77 could constitute a synthesized transcription factor involved in the LH/cAMP-dependent activation of the *STAR* promoter in steroidogenic cells (Martin et al., 2008). In Leydig cells, the cAMP accumulated by LH signaling stimulates PKA and activates many transcription factors that stimulate the expression and activities of StAR (Manna et al., 2009). The catalytic activity of adenylyl cyclase could increase the production of cAMP. In turn, cAMP is hydrolyzed to 5-adenosine monophosphate via the action of phosphodiesterase. Studies have shown that ZEA can bind membrane ERs and disrupt the balance of adenylyl cyclase and phosphodiesterase, which can affect the balance of cAMP synthesis and degradation (Lee et al., 2012). Thus, ZEA mediates the expressions and activities of the related steroidogenic enzymes through the membrane ERs and nuclear receptors.

## 5. Conclusions and future perspective

There are many factors which can contribute to the widely different susceptibility among various species. Several studies have suggested that the susceptibility depends on the rate of α-ZEL and β-ZEL converted from ZEA in the metabolic process. Both of these reduction metabolites have different estrogenic activities, in which α-ZEL is almost 500 fold stronger in comparison to ZEA, while β-ZEL is 16 times lower than ZEA (Yang et al., 2017b). Thus the animals that can convert ZEA into α-ZEL more than β-ZEL are more sensitive to ZEA such as rats and pigs, while the animals that mainly convert ZEA into β-ZEL are lower sensitive to ZEA such poultry, birds, and sheep. What's more, the rate of conjugation in the metabolic phase II can also contribute to the different susceptibility. These conjugates are hydrolyzed into free forms by intestinal microbes and then reabsorbed by the intestine, resulting in two consecutive peaks in plasma concentration of ZEA after oral administration in swine: a typical enterohepatic circulation. Additionally, besides the different production of derivatives, the development of the reproductive organs is considerably different in different animal species, which may also influence the sensitivity of ZEA. Rat testicles are very different form boar testicles, and the Leydig stem cells and their development into adult Leydig cell populations are also widely different in different specious. However, the molecular mechanisms underlying this association remain unclear and require further investigation.

For a long time, tremendous efforts have been made to investigate the toxicological mechanism of ZEA on the human and animal reproductive system. However, several studies have indicated that besides the reproductive toxicity, ZEA also exhibits immunotoxicity and can modulate most aspects of immune responses and impair lymphoid organs, resulting in thymus atrophy; the mycotoxin can alter thymus and spleen lymphocyte phenotypes and decrease peroxide production by peritoneal macrophages (Hueza et al., 2014). Interesting, it was indicated that estrogens are important modulators of the immune system, and estrogen receptor alpha is expressed in nearly every immune cell and it can be detected in thymocytes, bone marrow non-hematopoietic cells, T cells, B cell precursors, and circulating B cells (Corradetti et al., 2018; Cunningham and Gilkeson, 2011). However, studies on the role of estrogen receptors in the immunotoxicity induced by ZEA are lacking. Thus, further research should be designed to explore the relationship between estrogenic effects and immunotoxicity induced by ZEA. Futhermore, growing evidence indicated that ZEA has the carcinogenic property. ZEA was involved in the increasing the risk of hormone-dependent tumors and might exert a crucial role in the increasing incidence of cancer in different organs (Belli et al., 2010). The oncogenic effects of ZEA are due mainly to ERα-mediated transcriptional activation of genes that promote cell proliferation or alleviate apoptosis. However, the detailed effects of ZEA on oncogenes have not yet been completely explained. It is predicted that in the future, the related research about the effects of ZEA and its derivatives on oncogenes and MicroRNAs will become a trend.

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## Conflicts of interest

The authors declared that they have no conflict of interest.

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