Effects of Zearalenone on Ovarian Index and Progesterone Receptor Distribution and Expression in Weaned Gilts (Postprint)

Authors: Yang Lijie, Huang Libo, Li Simeng, Liu Faxiao, Jiang Shuzhen, Yang Zaibin

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Abstract

This study aimed to investigate the effects of diets contaminated with different levels of zearalenone (ZEA) on ovarian index, the distribution of progesterone receptor immunopositive signals, and mRNA expression level in weaned gilts. Forty healthy 25–28-day-old three-way crossbred (Duroc × Landrace × Large White) weaned gilts with an average body weight of [(14.01$±0.86)kg]wererandomlydividedinto4groupsandhousedindividually.Thecontrolgroupwasfedabasaldie mg/kg, respectively. The acclimation period was 10 d, and the experimental period was 35 d. The results showed that with increasing dietary ZEA level, the ovarian index of weaned gilts exhibited a linear increase ($P<0.01$); the ovarian index in the 1.0 mg/kg ZEA group was significantly higher than that in the 1.5 mg/kg ZEA group ($P<0.05$), and the ovarian index in the 1.5 mg/kg ZEA group was significantly higher than that in the 0.5 mg/kg ZEA group and the control group ($P<0.05$). With increasing dietary ZEA level, the integrated optical density (IOD) of ovarian progesterone receptors and relative mRNA expression level both exhibited a linear increase ($P<0.01$); the progesterone receptor IOD and relative mRNA expression level in the 1.5 mg/kg ZEA group were significantly higher than those in the 1.0 mg/kg ZEA group ($P<0.05$), and those in the 1.0 mg/kg ZEA group were significantly higher than those in the 0.5 mg/kg ZEA group and the control group ($P<0.05$). Immunohistochemical results showed that progesterone receptor immunopositive substances in the ovaries of weaned gilts were mainly distributed in the oocytes and granulosa cells of primordial and growing follicles, theca cells, and vascular wall cells. With increasing dietary ZEA level and degree of follicular atresia, the immunopositive reaction was significantly enhanced, but the distribution pattern of progesterone receptor immunopositive substances in the ovary did not change significantly with ZEA level. These results indicate that ZEA (1.0–1.5 mg/kg) can affect the health of the reproductive system in weaned

gilts by regulating high-level expression of progesterone receptors in the ovary, causing the level of progesterone receptors to exceed the threshold for positive regulation of follicular growth, thereby promoting follicular atresia, reducing ovarian index, and altering ovarian development.

Full Text

Effects of Zearalenone on Ovary Index, Distribution and Expression of Progesterone Receptors in Ovaries of Weaned Gilts

YANG Lijie, HUANG Libo, LI Simeng, LIU Faxiao, JIANG Shuzhen, *YANG Zaibin* (College of Animal Science and Technology, Shandong Agricultural University, Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, Tai' an 271018, China)

Abstract

This study investigated the effects of dietary zearalenone (ZEA) at different contamination levels on ovary index and the positive distribution and mRNA expression of progesterone receptors in weaned gilts. Forty healthy crossbred (Duroc × Landrace × Large White) weaned gilts aged 25-28 days were randomly allocated into four groups based on average body weight [(14.01 ± 0.86) kg] and housed individually. The control group received a basal diet, while experimental groups received basal diets supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA. The analyzed ZEA levels in the four groups were 0, (0.52 ± 0.07), (1.04 ± 0.03), and (1.51 ± 0.13) mg/kg, respectively. After a 10-day adaptation period, the formal experimental period lasted 35 days. The results showed that ovary index increased linearly ($P < 0.01$) with increasing dietary ZEA levels. The ovary index in the 1.0 mg/kg ZEA group was significantly higher than that in the 1.5 mg/kg ZEA group ($P < 0.05$), while the index in the 1.5 mg/kg ZEA group was significantly higher than those in both the 0.5 mg/kg ZEA group and the control group ($P < 0.05$). Both the integrated optical density (IOD) and mRNA relative expression of progesterone receptors in ovaries also increased linearly ($P < 0.01$) with rising dietary ZEA levels. The IOD and mRNA expression in the 1.5 mg/kg ZEA group were significantly higher than those in the 1.0 mg/kg ZEA group ($P < 0.05$), which in turn were significantly higher than those in the 0.5 mg/kg ZEA group and control group ($P < 0.05$). Immunohistochemical analysis revealed that progesterone receptor immunopositive substances were primarily distributed in oocytes and granulosa cells of primordial and growing follicles, as well as in theca cells and vascular wall cells. Immunopositive reactions intensified markedly with increasing dietary ZEA levels and progressive follicular atresia, though the distribution pattern of progesterone receptor immunopositive substances in the ovary did not change significantly due to ZEA exposure. These findings indicate that ZEA at 1.0-1.5 mg/kg can promote follicular atresia, reduce ovary index, and alter ovarian development by upregulating progesterone

receptor expression to levels exceeding the threshold for positive regulation of follicular growth, thereby affecting the reproductive health of weaned gilts.

Keywords: zearalenone; weaned gilts; ovary; progesterone receptor

Introduction

Zearalenone (ZEA), also known as F-2 toxin, is a non-steroidal mycoestrogen primarily produced by *Fusarium graminearum* [1]. Survey data indicate that ZEA is one of the most frequently detected mycotoxins in feed ingredients and complete diets in China [2]. The presence of ZEA in feed poses a serious threat to animal health, with pigs being the most sensitive species [1]. Previous studies have demonstrated that ZEA can cause infertility in sows by inducing ovarian dysfunction [3-4], while its metabolites α-zearalenol and β-zearalenol stimulate progesterone synthesis [5]. Dietary ZEA at certain levels can lead to reproductive disorders in sows, including pseudopstrus, reduced litter size, and abortion [6]. Most research on ZEA has been conducted *in vitro*, and no studies have reported the effects of different ZEA levels (0.5–1.5 mg/kg) on ovarian organ index and the distribution and expression of progesterone receptors in weaned gilts. This experiment was designed to investigate the effects of dietary ZEA at varying levels on ovarian organ index and the distribution and mRNA relative expression of progesterone receptors in weaned gilts, aiming to elucidate the molecular mechanisms through which ZEA alters ovarian development by regulating progesterone levels and to provide theoretical basis and insights for understanding ZEA reproductive toxicity and reproductive failure in swine production.

Materials and Methods

The animal feeding trial was conducted at the Animal Science and Technology Park of Shandong Agricultural University from April 25 to June 8, 2016.

1.1 Experimental Material ZEA was purchased from Fermentek (Israel) with chromatographic purity guaranteed at 98%.

1.2.1 Experimental Design and Management Forty healthy crossbred (Duroc × Landrace × Large White) weaned female piglets aged 25–28 days were selected. After 10 days of housing in farrowing crates, piglets were transferred to experimental cages (0.48 m^2). Based on age (35–38 days) and average body weight [(14.01 ± 0.86) kg], they were randomly divided into four groups (n = 10 per group) with no significant difference in initial body weight among groups ($P > 0.05$). The basal diet for weaned gilts was formulated according to NRC (2012) nutrient requirements, with composition and nutrient levels shown in

Table 1 . The control group received the basal diet, while experimental groups received basal diets supplemented with 0.5, 1.0, and 1.5 mg/kg ZEA.

Cages were equipped with plastic slatted floors, nipple drinkers, and feed troughs, allowing ad libitum access to feed and water. The pig house was thoroughly cleaned and disinfected before the trial, with weekly disinfection during the experiment. Infrared heat lamps maintained cage temperature at approximately 30°C during the first week, after which ambient temperature was kept at 26–28°C. Relative humidity was maintained at 65%. The adaptation period lasted 10 days, followed by a 35-day formal experimental period. Routine management and vaccination procedures were followed. All piglets were slaughtered at the end of the experiment.

1.2.2 Preparation of ZEA-Contaminated Diets Chromatographically pure (98%) crystalline ZEA powder was dissolved in ethyl acetate to prepare a stock solution. This solution was sprayed onto a talc carrier, which was left overnight to evaporate the ethyl acetate, yielding a 1,000 mg/kg ZEA premix. The premix was further diluted with ZEA-free cornmeal to produce a 10 mg/kg ZEA premix. Experimental diets were then formulated by substituting corn and carrier in the basal formula with the appropriate amount of ZEA premix to achieve the target ZEA concentrations. All experimental diets were prepared in one batch one week before the trial began and stored in sealed bags in a dry, ventilated area. Diet samples were collected before and after the experiment for nutrient and toxin analysis using the sampling method specified in GB/T 14699.1-1993.

1.2.3 Analysis of Dietary Nutrients and Toxins Dietary nutrients were analyzed according to AOAC (2012) methods. Crude protein was determined by the Kjeldahl method; energy by HR-15 bomb calorimetry; calcium by potassium permanganate titration; phosphorus by molybdenum yellow colorimetry; salt by ammonium thiocyanate back-titration; and amino acids by Hitachi 835-50 automatic amino acid analyzer.

Mycotoxin analysis was performed by Qingdao Entry-Exit Inspection and Quarantine Bureau. ZEA and aflatoxin were quantified by liquid chromatography with fluorescence detection after immunoaffinity column cleanup using external standard calibration. Fumonisin and deoxynivalenol were determined by high-performance liquid chromatography-tandem mass spectrometry with UV detection after immunoaffinity cleanup. The detection limits were 1.0 g/kg for aflatoxin, 0.1 mg/kg for ZEA, 0.1 mg/kg for deoxynivalenol, and 0.25 mg/kg for fumonisin. No other toxins were detected or levels were below detection limits.

1.3.1 Determination of Ovary Organ Index At the end of the experiment, all 10 piglets per group were slaughtered. Ovarian development was observed and recorded, and ovaries were weighed to calculate the ovary organ index. Two ovarian samples were collected from each pig: one fixed in Bouin' s solution for immunohistochemical analysis and one snap-frozen in liquid nitrogen and stored at -80°C for progesterone receptor mRNA expression analysis.

Ovary organ index (mg/kg) = ovary weight (mg) / live body weight (kg)

1.3.2 Immunohistochemistry (Ultra-Sensitive Two-Step Method) Tissue blocks fixed in Bouin' s solution were washed until the original tissue color was restored, then dehydrated through graded ethanol series and cleared with xylene before embedding using a BMJ-23 embedding machine. (1) Sections were cut with a microtome (LEICA RM2135, Germany) and routinely deparaffinized and rehydrated. (2) Antigen retrieval was performed with citrate buffer (0.01 mol/L, pH 6.0), followed by three washes with phosphate-buffered saline (PBS) (0.01 mol/L, pH 7.2) for 5 minutes each. (3) Sections were incubated with 10% H_2O_2 at 37°C for 1 hour in the dark, washed three times with PBS, then blocked with 10% goat serum at 37°C for 1 hour. Rabbit anti-progesterone receptor polyclonal antibody (Santa Cruz: sc-539, Lot: K0714) was applied at 1:50 dilution and incubated overnight at 4°C. After rewarming at 37°C and three PBS washes, sections were incubated with a two-step ultra-sensitive immunohistochemical detection kit for rabbit antibodies (PV-9001, Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.). Diaminobenzidine (TIANGEN: PA110, Lot: P4819) was used for color development, with microscopic monitoring to control reaction time. Sections were counterstained with hematoxylin, differentiated, dehydrated, cleared, and mounted with neutral resin for bright-field observation of immunopositive cell distribution.

1.3.3 Measurement of Integrated Optical Density (IOD) of Ovarian Progesterone Receptors Ovarian tissue sections were observed using an OLYMPUS BX41 (DP25) binocular microscope. For each pig, one section was taken every 10 sections, totaling 5 sections per ovary. Image Pro-Plus 6.0 software was used to analyze the IOD across the cross-sectional area of ovarian slices.

1.3.4 Determination of Progesterone Receptor mRNA Relative Expression Specific primers for porcine progesterone receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were designed using Primer 6.0 based on published sequences in GenBank and synthesized by BGI. Primer sequences are shown in Table 2 .

Total RNA was extracted from 50–70 mg of frozen ovarian tissue using the Trizol method. Concentration and purity were assessed using a UV spectrophotometer (Eppendorf, RS323C, Germany), with samples having OD values between 1.8 and 2.0 considered acceptable. Reverse transcription was performed using the PrimeScript™ RT Master Mix kit (RR036A, Takara, Dalian) in a 20 L reaction volume.

Real-time quantitative PCR (qRT-PCR) was conducted in a total volume of 20 L using SYBR Premix Ex Taq (Tli RNaseH Plus) (Takara: RR420A, Lot: AK7502) according to the manufacturer' s instructions, with three replicates per sample. Amplification was performed on an ABI 7500 Real-Time PCR system under the following conditions: initial denaturation at 95°C for 30 s, followed by 43 cycles of 95°C for 5 s and 60°C for 34 s, with a final dissociation stage of 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s.

1.4 Statistical Analysis The 2^(-ΔΔCt) method was used to analyze qRT-PCR data and determine relative progesterone receptor mRNA expression in ovaries. Data were analyzed using one-way ANOVA in SAS 9.2 software. Orthogonal polynomial contrast was applied for linear regression analysis across ZEA gradient treatments, and Duncan' s multiple range test was used for post-hoc comparisons. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$. IOD values for ovarian progesterone receptors were analyzed using IPP 6.0 image analysis software.

Results

2.1 Effects of ZEA on Ovary Index in Weaned Gilts The effects of dietary ZEA at different levels on ovary index are presented in Figure 1 [Figure 1: see original paper]. Ovary index increased linearly ($P < 0.01$) with increasing dietary ZEA levels. Compared with the control group, ovary index was significantly higher in the 1.0 and 1.5 mg/kg ZEA groups ($P < 0.05$). Among the three ZEA-treated groups, the 1.0 mg/kg ZEA group showed a significantly higher ovary index than the 1.5 mg/kg ZEA group ($P < 0.05$), while the 1.5 mg/kg ZEA group had a significantly higher index than the 0.5 mg/kg ZEA group ($P < 0.05$).

2.2 Effects of ZEA on Progesterone Receptor Distribution in Ovaries of Weaned Gilts Immunohistochemical analysis revealed that progesterone receptor immunopositive substances in weaned gilt ovaries appeared brownish-yellow or light yellow. The immunopositive material was primarily distributed in oocytes and granulosa cells of primordial and growing follicles, as well as in theca cells and vascular wall cells, displaying varying shades of brown (Figure 2 [Figure 2: see original paper]). In the control group, healthy primordial follicle oocytes occasionally showed progesterone receptor immunopositive distribution (Figure 2–A1, asterisks), while atretic primordial follicle oocytes exhibited strong positive immunoreactivity in dark brown (Figure 2–A1, arrows). Growing follicle oocytes, granulosa cells, theca cells, and vascular wall cells all displayed progesterone receptor immunopositive material (Figure 2–A2, A3, A4). As dietary ZEA levels increased, immunopositive reactions in oocytes and granulosa cells of primordial and growing follicles gradually intensified (Figure 2–A1, B1, C1, D1). Moreover, with progressive follicular atresia, immunopositive reactions in granulosa cells became significantly stronger (Figure 2–A3, B3, C3, D3), and immunopositive reactions in ovarian vascular wall cells also increased (Figure 2–A4, B4, C4, D4). However, the distribution pattern of progesterone receptor immunopositive substances in the ovary did not change markedly due to ZEA exposure.

As shown in Table 3 , the IOD of ovarian progesterone receptors increased linearly ($P < 0.01$) with rising dietary ZEA levels. Compared with the control group, IOD values were significantly higher in the 1.0 and 1.5 mg/kg ZEA groups ($P < 0.05$). Among ZEA-treated groups, the 1.5 mg/kg ZEA group showed significantly higher IOD than the 1.0 mg/kg ZEA group ($P < 0.05$), which in turn was significantly higher than the 0.5 mg/kg ZEA group ($P < 0.05$).

2.3 Effects of ZEA on Progesterone Receptor mRNA Relative Expression in Ovaries of Weaned Gilts The relative mRNA expression of ovarian progesterone receptors increased linearly ($P < 0.01$) with increasing dietary ZEA levels (Figure 3 [Figure 3: see original paper]). Compared with the control group, expression levels were significantly higher in the 1.0 and 1.5 mg/kg ZEA groups ($P < 0.05$). Among the ZEA-treated groups, the 1.5 mg/kg ZEA group showed significantly higher expression than the 1.0 mg/kg ZEA group ($P < 0.05$), which in turn was significantly higher than the 0.5 mg/kg ZEA group ($P < 0.05$).

Discussion

Recent studies on ZEA in animals have primarily used naturally contaminated diets with known ZEA concentrations [7-10]. To avoid confounding effects from other toxins, this study utilized high-purity ZEA to explore the molecular mechanisms of lower ZEA doses (0.5–1.5 mg/kg) on weaned gilt ovaries, building upon our previous findings [7,9-11].

3.1 Effects of ZEA on Ovary Index in Weaned Gilts Reports on ZEA effects on ovarian development in weaned gilts have been inconsistent. One study indicated that 100 mg/kg dietary ZEA promoted maturation of primary and secondary follicles [3], while another suggested that certain ZEA doses (unspecified) inhibited follicle-stimulating hormone secretion and release, stimulated progesterone synthesis, and thereby suppressed pre-ovulatory follicular maturation and affected ovarian development [4]. High ZEA levels can induce ovarian follicular atresia, inhibit granulosa cell proliferation, cause mitochondrial membrane potential loss and elevated reactive oxygen species levels, with apoptosis and necrosis of granulosa cells showing dose-dependent relationships with ZEA concentration [12]. Our results showed that dietary ZEA at 0–1.0 mg/kg caused a linear increase in ovary index; however, at 1.5 mg/kg, ovary index decreased significantly compared with the 1.0 mg/kg ZEA group. This may be because higher ZEA levels promoted excessive progesterone expression in ovaries, exceeding the threshold for positive regulation of follicular growth and thereby inducing follicular atresia and reducing ovary index. These findings suggest that 1.0 mg/kg dietary ZEA may represent a critical dose for promoting ovarian development.

3.2 Effects of ZEA on Distribution and Expression of Progesterone Receptors in Ovaries of Weaned Gilts Successful progesterone expression in animals depends entirely on its corresponding receptor proteins [13-15]. This study assessed ZEA effects on ovarian progesterone expression by measuring progesterone receptor protein content. Recent research indicates that ovarian progesterone levels influence follicle size and oocyte maturity, with follicular diameter positively correlating with ovarian progesterone levels [16]. Additionally, ZEA-receptor complexes may act as estrogen promoters by binding to estrogen-sensitive elements in the nucleus and promoting high-level expression of related genes [17-20]. Progesterone in animals has both synergistic and antagonistic effects with estrogen, with both hormones showing parallel curves during pregnancy [21-22]. Previous progesterone research has focused primarily on factors affecting expression intensity [23-26], with relatively few reports on distribution patterns under different conditions [27]. Our results demonstrate that progesterone receptor immunopositive substances were mainly distributed in oocytes and granulosa cells of healthy primordial and growing follicles, as well as in oocytes of atretic primordial follicles and vascular smooth muscle cells. As dietary ZEA levels increased, immunopositive substances became markedly more abundant, with both IOD values and mRNA relative expression of progesterone receptors in weaned gilt ovaries increasing linearly. However, the distribution pattern of progesterone receptor immunopositive substances did not change significantly with ZEA level. This suggests that 0.5–1.5 mg/kg ZEA can enhance immunopositive reactions and mRNA expression of progesterone receptors in weaned gilt ovaries without affecting their distribution pattern. Although ovary index in the 1.5 mg/kg ZEA group was significantly lower than in the 1.0 mg/kg ZEA group, immunopositive reactions and mRNA expression of progesterone receptors were significantly higher in the 1.5 mg/kg ZEA group, implying that as ZEA levels increase, ovarian progesterone exceeds the threshold for positive follicular growth regulation, leading to follicular atresia and declining ovary index.

Under the conditions of this experiment, ZEA significantly increased ovary index, immunopositive reactions, and mRNA relative expression of progesterone receptors in weaned gilts, suggesting that dietary ZEA at 1.0–1.5 mg/kg affects reproductive health in weaned gilts by regulating progesterone expression and altering follicular development in ovaries.

References

[1] Jiang SZ, Yang WR, Yang ZB. Metabolism, toxicity and preventive measures

of zearalenone [J]. *Chinese Journal of Animal Nutrition*, 2011, 23(2): 196–202.

[2] Rong XP, Zhao LH, Ji C, et al. Investigation on zearalenone contamination in feed ingredients and complete feeds in some regions of China [J]. *Chinese Journal of Animal Science*, 2015, 51(22): 20–24.

[3] Huang LN, Peng SQ, Liu N. Research progress on reproductive and developmental toxicity of zearalenone [J]. *Chinese Journal of Endemic Disease Control*, 2014, 29(3): 181–184.

[4] Minervini F, Dell' Aquila ME. Zearalenone and reproductive function in farm animals [J]. *International Journal of Molecular Sciences*, 2008, 9(12): 2570–2584.

[5] Alm H, Greising T, Brüssow KP, et al. The influence of the mycotoxins deoxynivalenol and zearalenol on *in vitro* maturation of pig oocytes and *in vitro* culture of pig zygotes [J]. *Toxicology in Vitro*, 2002, 16(6): 643–648.

[6] Jiang SZ, Yang ZB, Yang WR, et al. Effects of purified zearalenone on growth performance, organ size, serum metabolites, and oxidative stress in postweaning gilts [J]. *Journal of Animal Science*, 2011, 89(10): 3008–3015.

[7] Chen XX, Yang WR, Zhang CY, et al. Effects of Fusarium toxins on growth performance, small intestinal disaccharidase activity and antioxidant capacity of weaned piglets [J]. *Chinese Journal of Animal Nutrition*, 2015, 27(6): 1875–1882.

[8] Döll S, Gericke S, Dänicke S, et al. The efficacy of a modified aluminosilicate as a detoxifying agent in Fusarium toxin contaminated maize containing diets for piglets [J]. *Journal of Animal Physiology and Animal Nutrition*, 2005, 89(9/10): 342–358.

[9] Yang LJ, Niu QS, Zhang CY, et al. Short-term feeding of Fusarium toxin-contaminated diets induces changes in vulva size, serum metabolites, and oxidative stress indices in weaned piglets [J]. *Swine Industry Science*, 2016, 33(3): 85–88.

[10] Niu QS, Yang WR, Huang LB, et al. Effects of Fusarium toxins on vulva size, reproductive organ indices, and distribution and expression of uterine estrogen receptors in weaned gilts [J]. *Chinese Journal of Animal Nutrition*, 2016, 28(5): 1525–1533.

[11] Dai M, Jiang S, Yuan X, et al. Effects of zearalenone-diet on expression of ghrelin and PCNA genes in ovaries of post-weaning piglets [J]. *Animal Reproduction Science*, 2016, 168: 126–133.

[12] Zhu L, Yuan H, Guo CZ, et al. Zearalenone induces apoptosis and necrosis in porcine granulosa cells via a caspase-3- and caspase-9-dependent mitochondrial signaling pathway [J]. *Journal of Cellular Physiology*, 2012, 227(5): 1814–1820.

[13] Li W, Sun LM, Liu YC, et al. Research progress on hormone receptors and ovarian cancer prognosis, pathogenesis and treatment [J]. *Modern Oncology*

Medicine, 2017, 25(4): 657-660.

[14] Shi B, Liu XZ, Xu T, et al. Expression characteristics of a novel membrane progesterone receptor gene (mPRL) during oocyte maturation in half-smooth tongue sole (*Cynoglossus semilaevis*) [J]. *Progress in Fishery Sciences*, 2017, 38(1): 10-17.

[15] Liu C, Feng J. Expression of estrogen and progesterone receptor genes in ovarian cancer tissues [J]. *Chinese Journal of Clinical Obstetrics and Gynecology*, 2006, 7(1): 28-32.

[16] Bi Y, Gao L, Tian L, et al. Relationship between estrogen and progesterone levels in follicular fluid, follicle size, and oocyte maturity [J]. *Progress in Obstetrics and Gynecology*, 2011, 20(12): 948-951.

[17] Zhou HC, Liu XQ, Yang MQ, et al. Effects of zearalenone on porcine ovarian granulosa cells cultured *in vitro* [J]. *Chinese Journal of Veterinary Science*, 2017, 37(2): 297-302.

[18] Gao S, Liang Z, Deng JL, et al. Reproductive toxicity and mechanisms of zearalenone and deoxynivalenol in female animals [J]. *Chinese Journal of Animal Nutrition*, 2016, 28(4): 1042-1049.

[19] Wu YC, Yang WR, Yang ZB, et al. Effects of low-dose zearalenone and adsorbents on growth performance, serum biochemical indices, and antioxidant indices in growing layers [J]. *Chinese Journal of Animal Nutrition*, 2016, 28(4): 1137-1144.

[20] Zhu BL, Zhang L, Yang WL, et al. Effects of zearalenone on blood biochemical indices, liver oxidative damage, and toxin residues in broilers [J]. *Animal Husbandry and Veterinary Medicine*, 2016, 48(6): 10-14.

[21] Durlej M, Tabarowski Z, Slomczynska M. Immunohistochemical study on differential distribution of progesterone receptor A and progesterone receptor B within the porcine ovary [J]. *Animal Reproduction Science*, 2010, 121(1/2): 167-173.

[22] Peralta LE, Olarte MR, Argañaraz M, et al. Progesterone receptors: their localization, binding activity and expression in the pig oviduct during follicular and luteal phases [J]. *Domestic Animal Endocrinology*, 2005, 28(1): 74-84.

[23] Qiao ZH, Li DJ, Pang LE, et al. Effect of elevated serum progesterone duration before hCG injection on IVF-ET outcomes [J]. *International Journal of Reproductive Health/Family Planning*, 2017, 36(1): 9-13.

[24] Hua KQ, Yao LQ, Cao Q, et al. Estrogen and progesterone regulate nm23-H1 protein expression in ovarian epithelial carcinoma cells through activation of phosphorylated signaling pathways [J]. *Chinese Journal of Obstetrics and Gynecology*, 2006, 41(11): 756-761.

[25] Davidson B, Kjæreng ML, Førsund M, et al. Progesterone receptor expression is an independent prognosticator in FIGO stage I uterine leiomyosarcoma

[J]. *American Journal of Clinical Pathology*, 2016, 145(4): 449-458.

[26] Mohammed H, Russell IA, Stark R, et al. Progesterone receptor modulates estrogen receptor-α action in breast cancer [J]. *Nature*, 2015, 523(7560): 313-317.

[27] Tian XY, Yang JH, Li F. Immunohistochemical study on distribution of estrogen and progesterone receptors in malignant ovarian tumors [J]. *Chinese Journal of Practical Medicine*, 2001, 28(3): 40-41.

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