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Effect of low-dose zearalenone exposure on reproductive capacity of male mice

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ABSTRACT

Zearalenone (ZEA), a kind of nonsteroidal mycotoxin with estrogenic effects, can influence animal reproductive capacity through interfering with estrogen signaling pathway. Previous studies have shown exposure to ZEA at high doses (higher than No-Observed Effect Level, NOEL) had a significant impact on mouse sperm quality and pregnant rate, but little is known about the effect of exposure to ZEA at low doses (lower than NOEL) on mouse reproductive capacity. This study evaluated the effects of exposure to low-dose ZEA on mouse spermatogenesis and semen quality. Male mice (CD-1) of 21 days were exposed to ZEA at 20, or 40 μg/kg body weight for 14, 28 or 42 days. After exposure to ZEA for 14 days, the spermatogenic cells in seminiferous tubules were declined dose-independently; however in groups treated by ZEA for 28 days, the spermatogenic cells were declined dose-dependently. Moreover, after treatment for 28 days or 42 days, the DNA double stand break (DSB) in spermatogenic cells were increased in a dose-dependent manner in treated groups. Compared with the control group, the sperm concentration, viability, motility, and hyperactive rate in treated groups were decreased dose-dependently and time-dependently. Meanwhile, deformity and mortality rate of sperm in treated groups were increased remarkably dose-dependently too. In conclusion, low dose ZEA impaired male reproductive capacity especially in spermatogenesis and semen quality of mouse.

1. Introduction

Zearalenone (ZEA) is a kind of nonsteroidal mycotoxin having estrogenic effect. It is mainly produced by Fusarium including Fusarium graminearum, Fusarium culmorum, Fusarium equiseti, *etc.* It is widely distributed in maize, wheat, rice, cereal, flour, malt, beer and others (Aiko and Mehta, 2015; Behre et al., 2001; Wei et al., 2010; Zinedine et al., 2007). Recent studies indicated that ZEA could interfere with estrogen signaling pathway through two mechanisms: one is that ZEA is structurally similar to 17β-estradiol (E2) which leads to competitively binding to estrogen receptor (ER) to interfere with estrogen effects (Efsa, 2004; Zwierzchowski et al., 2005); the other is that the structure resemblance with steroids enables ZEA to bind competitively to 3α and 3β-hydroxycorticosteroid dehydrogenase (HSD) which disturbs the sex hormone synthesis, leading to deregulation of the estrogen pathways (Lai et al., 2015). In addition, ZEA induces apoptosis dose-dependently *via* p53 pathway and endoplasmic reticulum stress (Ayed-Boussema et al., 2008; Ben et al., 2015). ZEA can also lead to apoptosis of rat male germ cells through up-regulation of *Fas* and *Fas* ligand, resulting in testis atrophy (Jee et al., 2010; Kim et al., 2003) and apoptosis in testis Leydig cells (Wang et al., 2014).

Spermatogenesis is a critical hormone dependent process influencing male reproductive capacity. The hormones, including follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estrogen and others, regulate spermatogenesis through mediating the interactions between spermatogenic cells and sertoli cells (Chimento et al., 2014, O'Donnell, 2001, Rey, 2003, Weinbauer and Nieschlag, 1990). Previous studies have shown that exposure to ZEA can damage male productive capacity especially in the spermatogenesis and semen quality. Exposure to ZEA led to the decrease in testosterone, testis weight loss, spermatogenesis obstruction, sexual desire inhibition and occurrence of feminization in male pigs (Efsa, 2004). Tsakmakidis et al. found that exposure to 150–250 μM ZEA declined sperm viability, survivability, acrosomal reaction time-dependently and dose-dependently (Tsakmakidis et al., 2006). It was further discovered that the capability of wild pig spermatozoa binding to pellucid zone was

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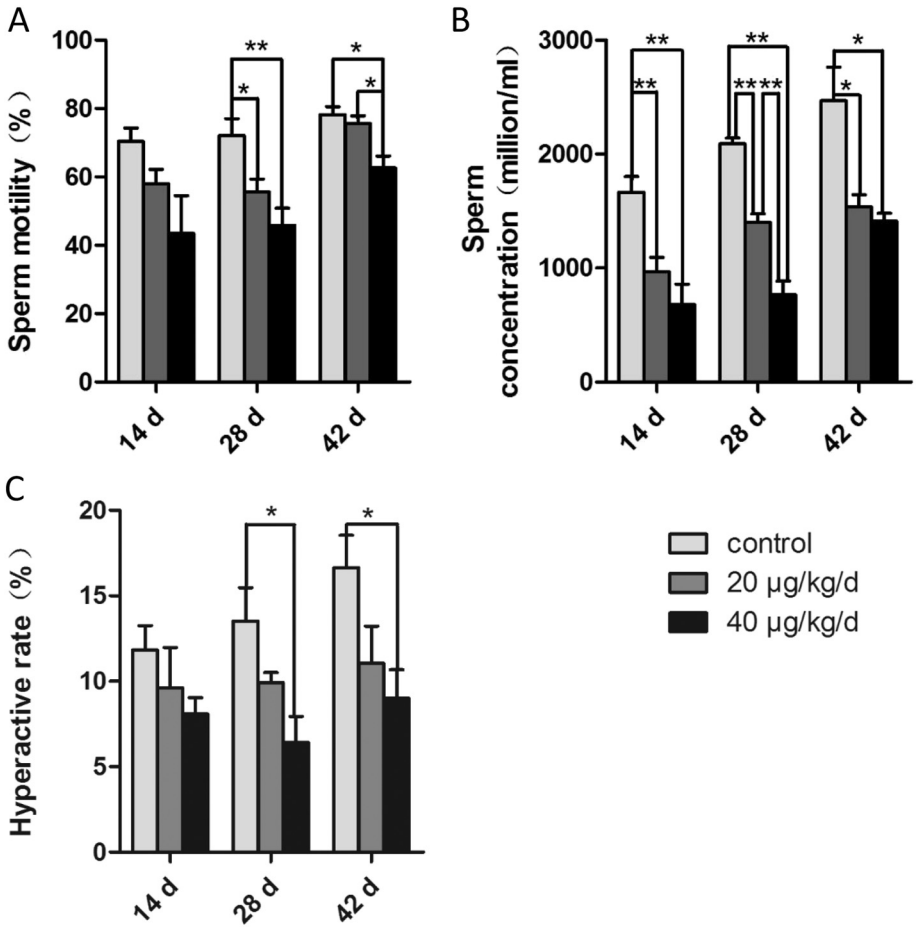

Fig. 1. The effect of ZEA on mice which had been treated with ZEA of different doses for 14, 28, 42 days on sperm motility (A), sperm concentration (B) and hyperactive rate (C). Bars are mean ± SD; asterisk (* or **) indicates significant difference ($P < 0.05$ or $P < 0.01$).

significantly lowered when exposed to 60–80 μg/ml ZEA and zearalenol (ZOL) (Tsakmakidis et al., 2007). Benzoni et al. reported that exposure to ZEA and its derivatives could decrease the sperm viability, survivability, and chromosome stability in pigs (Benzoni et al., 2008). With subcutaneous injection of 0–75 mg/kg ZEA and its derivatives to adult male mice, sperm mortality and malformation rate of mice were increased significantly, while sperm concentration, intact acrosome rate and testosterone level were decreased dose-dependently (Yang et al., 2007b). Exposed to 0–20 μg/ml ZEA to rats for 24 h, the cytoskeleton damage and secretion dysfunction were declined in sertoli cells and consequently reproductive capacity was reduced (Zheng et al., 2016). When sheep pituitary cells were cultured with ZEA *in vitro*, FSH secretion was reduced and LH secretion was elevated (Arispe et al., 2013). When testis Leydig cells were cultured with ZEA *in vitro*, ZEA inhibited testosterone biosynthesis and secretion *via* the crosstalk of ER signaling and orphan nuclear receptor Nur77, as well as down-regulating the transcriptional level of 3β-HSD-1, P450scc and StAR (steroidogenic acute regulatory) (Liu et al., 2014, Savard et al., 2016, Yang et al., 2007a).

Meiosis is an extremely important process in bisexual reproduction for maintaining genetic diversity, of which any abnormity occurrence might lead to aneuploidy formation causing infertility, miscarriage, or birth defect (Hunt and Hassold, 2008). In male germ cells, when proceeding to pachytene stage, un-repaired or accumulated DNA double-strand breaks (DSBs) may cause meiosis disruption which impairs spermatogenesis and leads to male infertility (Longhese et al., 2009). Previous studies indicated that DNA fragmentation rate and micronucleus generation in CaCo2, Vero and DOK cell lines were significantly increased dose-dependently by the exposure to ZEA (Abid-Essefi et al., 2003; Ouanes et al., 2003). Therefore, we set out to testify if ZEA has an impact on DNA-DSBs, which marked by γH_2AX expression (Blanco-Rodriguez, 2009).

Massive investigations have revealed the acute and chronic impacts of high dose ZEA on male reproductive capacity, while the effects of low dose ZEA was unknown. Therefore, the objective of this investigation was to explore the effects of exposure to low dose ZEA on mouse spermatogenesis, semen quality and underlying mechanisms.

2. Materials and methods

2.1. Ethics statement

All procedures applied in this study were reviewed and approved by the Ethics Committee of the Third Military Medical University. All experimental protocols were performed in accordance with the guidelines of the Third Military Medical University. CD1 mice were provided by Vital River Laboratory Animal Technology Co. LTD (Beijing, China) fed at stationary temperature (21–22 °C) and humidity (31%–39%), with 12 h light-dark cycle.

2.2. Animal model establishment and ZEA dosage application

According to literature (Creppy, 2002), ZEA under 40 μg/kg body weight (b.w.) is defined as safe dosage to female reproductive capacity, while no safe dosage is defined for male. In order to testify the effect of low dose ZEA (Sigma-Aldrich, Z2152, USA) on male mouse reproductive capacity, two ZEA dosages of 20 and 40 μg/kg b.w./day (dissolved with 0.1% DMSO) were used in this investigation. The mice in control group were given water containing 0.1% DMSO. Ninety CD1 male mice at 21 day of age were randomly divided into 3 groups (30 mice in each group): control, 20 and 40 μg/kg b.w./day. Solution with different concentration of ZEA was given by daily oral gavage at set