Emerging mycotoxins and reproductive effects in animals: A short review

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Minireview

Emerging mycotoxins and reproductive effects in animals: A short review

Running head/Short title: A review of reproductive effects of emerging mycotoxins

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Abstract

Emerging *Fusarium* mycotoxins beauvericin (BEA), enniatins (ENNs) and moniliformin (MON) are gaining increasing interest due to their wide presence especially in cereals and grain-based products. *In vitro* and *in vivo* studies indicate that *Fusarium* mycotoxins can be implicated in reproductive disorders in animals. Of these mycotoxins BEA may affect reproductive functions, impairing the development of oocytes in pigs and sheep. Studies show dramatic inhibitory effects of BEA and ENNA on bovine granulosa cell steroidogenesis. ENNs also inhibit boar sperm motility and cause detrimental effects on embryos in mice and pigs. Although little data are reported on reproductive effects of MON, *in vitro* studies show inhibitory effects of MON on Chinese hamster ovary cells. The present review aims to summarize the reproductive toxicological effects of emerging *Fusarium* mycotoxins BEA, ENNs and MON on embryo development, ovarian function, and testicular function of animals. *In vitro* and *in vivo* toxicological data are reported although additional studies are needed for proper risk assessment.

Short Abstract

Emerging *Fusarium* mycotoxins beauvericin (BEA), enniatins (ENNs) and moniliformin (MON) are gaining increasing interest due to their wide presence especially in cereals and grain-based products. *In vitro* and *in vivo* studies indicate that *Fusarium* mycotoxins can be implicated in reproductive disorders in animals. The present review summarizes the reproductive toxicological effects of emerging *Fusarium* mycotoxins BEA, ENNs and MON on embryo development, ovarian function, and testicular function of animals, but additional studies are needed for proper risk assessment.

Key words: Fusarium mycotoxins, Beauvericin, Enniatin, Moniliformin, Reproduction

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1. Introduction

Mycotoxins are secondary metabolites produced by several species of molds (Behm et al., 2012; Marin et al., 2013; Alshannaq and Yu, 2017) that commonly contaminate different foodstuffs and cereals worldwide especially wheat, barley, corn, and rice (Jestoi, 2008; Medvedova et al., 2011; Jajić et al., 2019). These compounds are endowed with both acute and chronic toxicity and have shown to cause carcinogenic and mutagenic effects as well as reproductive, developmental, and neurological toxicity (Van Egmond et al., 2007; Jestoi, 2008; Cortinovis et al., 2013; Khoury et al., 2019).

Of great concern are the so-called emerging mycotoxins, defined as "mycotoxins, which are neither routinely determined, nor legislatively regulated" (Vaclavikova et al., 2013). Among them, beauvericin (BEA) (Figure 1), enniatins (ENNs) (Figure 2) and moniliformin (MON) (Figure 3), are frequently isolated in food and feed products and pose a serious risk on human and animal health (Jimenez-Garcia et al., 2018; Caloni et al., 2020; Fakhri et al., 2021). A recent study revealed that ENNB and BEA were carried over into eggs at 0.1% and 0.44%, respectively, after 2-3 days of feeding chickens contaminated diets (Emmanuel et al., 2020). Studies in mice show that these mycotoxins accumulate in liver and fat (Rodríguez-Carrasco et al., 2016). Although data are limited, exposure to these mycotoxins has been linked to reproductive disorders (Kalayou et al., 2015; Schoevers et al., 2016; Albonico et al., 2017). The goal of the current review is to summarize *in vitro* and *in vivo* effects of BEA, ENNs and MON on reproductive function in animals.

2. Effects of emerging mycotoxins on ovarian function and embryo development

2.1 Beauvericin

Even if there is currently no sufficient toxico-epidemiological data that confirm effects of BEA on farm animal reproduction, some recent studies established that BEA may affect ovarian function in sows (Santos et al., 2015; Mallebrera et al., 2018) and cattle (Albonico et al., 2017; Perego et al., 2020). Schoevers et al. (2016) reported an impaired development of *in vitro* cultured porcine oocytes and embryos after exposure to BEA. Specifically, Schoevers and cow-workers (2016) reported that concentrations of > 1 μM BEA significantly affected the development of embryos at the 2-4 cell stage (day 2 of embryo culture) (Figure 4) and blastocyst stage (day 6 of embryo culture), and that oocytes exposed to BEA at concentrations exceeding 2.5 μM caused

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reduced embryo developmental capacity. Although BEA affected the rate of developing embryos, no change in their quality (size and apoptotic cell fraction) was detected. Schoevers et al., (2016) also found that BEA at 10 µM reduced cumulus cell (CC) expansion as well as CC viability that decreased to 57% and 37% after 44 h exposure to 5 and 10 µM BEA, respectively. Also, 10 µM BEA applied for 44 h significantly decreased progesterone secretion while increased CYP11A1 expression in CCs (Schoevers et al. 2016).

Another *in vitro* study (Schovers et al., 2021) was carried out to assess the susceptibility of maturating oocytes collected from gilts and sows to different concentrations of BEA and deoxynivalenol (DON), as well as the antioxidant levels in the oocytes' environment. It was observed that oocytes from gilts were more negatively affected by the toxic effects of BEA and DON in comparison to oocytes from sows. Indeed, the nuclear maturation rate was impaired when oocytes from gilts were *in vitro* cultured with 0.5 µM BEA, and this effect was even more evident in the presence of 2.5 or 5.0 µM BEA (Shoevers et al., 2021). Whereas BEA did not influence the maturation rate of oocytes from sows at the assessed concentrations in comparison to controls. The degeneration rate was significantly higher when gilt oocytes were exposed to BEA concentrations, while BEA did not significantly affect the degeneration of oocytes from sows (Shoevers et al., 2021). BEA negatively affected both maturation and degeneration rate in a dose-dependent manner. Moreover, BEA decreased cell viability via the promotion of oxidative stress, and they determined a greater negative impact in cells that are unable to properly eliminate metabolic products like reactive oxygen species (ROS) (Schoevers et al., 2021).

Recently, a study by Mastrorocco and co-workers (2019) was carried out to assess the toxicological effects of BEA on oocyte maturation and embryo development in juvenile sheep, and reported short-term negative impacts on somatic and germinal compartment of the cumulus-oocyte-complexes (COCs) and long-term consequences on developing embryos. When exposed at concentration of 5 µM, BEA significantly decreased oocyte maturation rate (MII stage), compared with controls and when applied at 1 µM, it likewise decreased MII rate (Mastrorocco et al., 2019). Whereas oocytes exposed to 0.5 µM BEA did not show an impairment of maturation rate. Interestingly, a marked cytoplasmic shrinkage in oocytes exposed to 1 µM, 3 µM and 5 µM BEA was observed and when applied at a concentration of 5 µM, BEA caused a significant increment of CCs with multiple nuclear fragments (Mastrorocco et al., 2019). In CCs of matured oocytes, viability was not affected but abnormal mitochondrial distribution patterns as well as gene expression changes after exposure to BEA were observed (Mastrorocco et al., 2019; 2021). In addition, all tested BEA concentrations reduced the mitochondrial membrane potential and ROS levels in MII oocytes during

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in vitro maturation (IVM). Regarding BEA effects on *in vitro* embryo development, 3 μM BEA exposure significantly reduced cleavage rates and increased the number of embryos arrested at 2-3 cell stages in comparison with controls (Mastrorocco et al., 2019). None of the BEA concentrations tested affected blastocyst formation rate, although embryos derived from oocytes treated with the highest concentrations (1 and 3 μM BEA) had not hatched after 8 days. Also, the number of apoptotic nuclei increased significantly after 1 and 3 μM BEA exposure to blastocysts. It was concluded that the presence of BEA in feedstuffs may impact fertility and health of the embryo, therefore food and feed should be carefully monitored (Mastrorocco et al., 2019). Collectively, studies indicate that BEA at doses of ≥ 2.5 μM can significantly impact oocyte and embryo function and development.

The toxicological effects of BEA, alone and in combination with fumonisin B1 (FB1), were assessed on bovine granulosa cells (Albonico et al., 2017). In this study, cell proliferation, steroid production and gene expression were evaluated. The highest BEA concentration tested (10 μM) significantly decreased granulosa cell numbers by 72% and concentrations of 3 and 6 μM BEA significantly reduced steroid production. Indeed, both progesterone (Figure 5A) and estradiol (Figure 5B) production decreased after 48 h exposure to 3 and 6 μM BEA. Regarding combined effects of FB1 and BEA, a significant reduction of cell proliferation (57%), estradiol (97%) and progesterone (80%) production was observed (Albonico et al., 2017). Concerning gene expression, 30 μM BEA exposure inhibited FSH plus insulin-like growth factor 1 (IGF1)-induced *CYP11A1* and *CYP19A1* mRNA abundance. However, concentrations of BEA at ≤ 1.5 μM had no effect on steroid production by bovine granulosa cells (Albonico et al., 2017). Perego and coworkers (2020) reported that 30 μM BEA completely inhibited the FSH plus IGF1-induced *UHRF1* mRNA expression in bovine granulosa cells. BEA has been detected in feed products in concentrations ranging from few units to hundreds of μg/kg with a prevalence of 62% (Tolosa et al. 2019), suggesting that BEA may represent a risk for animal health affecting reproductive functions.

In a preliminary study (Caloni et al., 2018), *in vitro* effects of BEA and glyphosate in Roundup formulation on bovine ovarian cell proliferation and steroid production were investigated. Granulosa and theca cells were collected and cultured for 48 h using 10% fetal bovine serum-containing medium followed by 48 h of serum-free medium, control solvent, FSH and IGF1. After 48 h treatment with 3 μM BEA, IGF1-induced cell numbers, estradiol production, and progesterone production were inhibited by 50%, 97% and 97% respectively. A similar effect after glyphosate (10 μg/mL) 48 h exposure was observed. These results confirm that BEA may potentially affect reproductive function in cattle (Caloni et al., 2018). Collectively, studies indicate that BEA at doses

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of ≥ 2.5 μM can significantly impact granulosa cell, oocyte and embryo function. However, the mechanism of action of BEA needs further clarification. Using *in vitro* assays, BEA exhibits weak antagonistic effects on the androgen receptor but not the estrogen receptor (Garcia-Herranz et al., 2019). In another study, BEA (1 μM) showed antagonistic activity on progestogen and glucocorticoid receptor transcriptional activity (Fernández-Blanco et al., 2016). Studies using *Xenopus* oocytes (Tang et al., 2005) and guinea-pig smooth muscle cell preparations (Nakajyo et al., 1987) indicate that BEA may also act to alter extracelluar Ca^{2+} influx.

2.2 Enniatins

Concerning enniatins (ENNs), few data on toxicity, concentration levels, occurrence and metabolism are available (Juan et al., 2013; Fakhri et al., 2021; Kˇrížová et al., 2021). However, some studies regarding the toxicokinetic parameters were carried out (Jestoi et al., 2008). In pigs a rapid gastrointestinal absorption was observed after oral administration of enniatin B1 (ENNB1) at a concentration of 0.05 mg /kg body weight (Devreese et al., 2014). Whereas after intravenous administration of ENNB1, a high clearance and moderate distribution were reported in both pigs and chickens (Devreese et al., 2014; Fraeyman et al., 2016; Bertero et al., 2018). According to Rodríguez-Carrasco et al. (2016), no acute toxicological effects during lifespan or pathological changes in mice after intraperitoneal administration of enniatin B (ENNB) were reported. Moreover, ENNB was detected in all tissues especially in the lipophilic ones.

Recently, Wang et al. (2021) reported detrimental effects of ENNB1 on porcine embryos with the developmental competence of early embryos being significantly decreased after 10, 25 and 50 μM ENNB1 exposure, reduced cleavage rate, blastocyst rate and blastocyst cell number compared with the controls. When ENNB1 was applied for 12 h, no significant developmental differences were observed compared with the control group, whereas the percentage of blastocysts formed were significantly higher than that of embryos treated for 24 h (Wang et al., 2021). Indeed, 10 μM ENNB1 applied for 24 h significantly decreased the developmental rate and quality of embryos. Wang et al. (2021) also demonstrated that ENNB1 affected nuclear remodelling progress, induced apoptosis in blastocyst cells, downregulated the expression of the antioxidant genes Sod1 and Gpx4 at the 4-cell and blastocyst stages, significantly disrupted the transcription levels of Dnmt1, Dnmt3a, Tet1 and Tet3, and decreased the expression of Eif1a, Oct4, Nanog and Sox2 demonstrating that ENNB1 alters the expression of genes in early embryos. When melatonin was added to embryos treated with ENNB1, defects induced by ENNB1 were significantly reduced (Wang et al., 2021). Whether ENNA has similar effects on embryo development will require further study.

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In mouse blastocysts, ENNB1 exerted cytotoxic effects and induced a significant increase of oxidative stress (Huang et al., 2019). Exposure of 5 and 10 μM ENNB1 resulted in a high apoptotic cell content and induced a reduction of inner cell mass (ICM) cells in blastocysts, whereas no significant differences in trophectoderm cell number compared with the untreated group were observed. The rate of morulas that developed into blastocysts after 5 and 10 μM ENNB1 treatment was significantly lower than that of the untreated group, and exposure of blastocysts with 5 and 10 μM ENNB1 reduced the development score (in accordance with the shape of ICM and trophoblast layer) resulting in a lower post-implantation developmental potential (Huang et al., 2019). In addition, detrimental effects on *in vivo* embryonic development after ENNB1 exposure were observed. Intravenous treatment for 4 days of 1, 3, and 5 mg/kg body weight/day ENNB1 caused apoptosis of embryos at blastocyst stage and affected embryonic development from the zygote to blastocyst stage. After 10 μM ENNB1 exposure, degradation of embryos was reported and fetal weight was significantly lower than that of the untreated group at 13 days post-transfer (Huang et al., 2019). Also, intracellular ROS levels after ENNB1 exposure were investigated. Specifically, the ENNB1 treated group showed a significantly higher ROS profile involving caspase-9 and -3 compared with the control group. Taken together, these results suggest that ENNB1 can be considered a risk factor in embryonic development and should be classified as an embryotoxic agent (Huang et al., 2019). Collectively, the studies reviewed indicate that ENNB at doses of ≥ 5 μM can significantly impact embryo development.

Recently, enniatin A (ENNA) at 1 and 3 μM was found to decrease granulosa cell numbers by 30% and 60%, respectively, from large (> 8 mm) follicles, whereas ENNA reduced cell numbers by 10, 90, and 95% when applied at 1, 3 and 5 μM to granulosa cells from small (1-5 mm) follicles, respectively (Chiminelli et al., 2022). After 1 and 2 days of treatment, ENNA at 0.3, 1 and 3 μM significantly inhibited estradiol production by large-follicle granulosa cells by over 80% and progesterone production by over 70% (Chiminelli et al., 2022). In small-follicle granulosa cell cultures, ENNA at 1, 3 and 5 μM significantly inhibited estradiol production by over 99% after 1- and 2-day exposure, and similarly, progesterone production was inhibited by over 90% after exposure to ENNA at 1, 3 and 5 μM (Chiminelli et al. 2022). Large-follicle granulosa cells are more differentiated than small-follicle granulosa cells as measured by estradiol producing ability (Stewart et al., 1996; Spicer and Aad, 2007). The authors concluded that ENNA at doses ≥ 1 μM significantly affects bovine granulosa cell growth and steroidogenesis in a dose-dependent manner suggesting its potential to impair reproductive function in cattle (Chiminelli et al., 2022). Additional research is needed to evaluate and compare ENNA and ENNB effects in other species. Also, the mechanism of

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ENNA or ENNB effects remain to be elucidated, but a recent study using transcriptional activation assays indicates both ENNA and ENNB exhibit antagonism to estrogen and androgen receptors (Park and Lee, 2021).

2.3 Moniliformin

Data on adverse effects of moniliformin (MON) in animals are lacking for the majority of the species. However, reduced weight gain, adverse haematological effects, cardiotoxicity, heart lesions and mortality were identified as the main adverse effects in pigs (EFSA, 2018). In poultry, the heart was described as the primary target organ (EFSA, 2018). Regarding developmental and reproductive toxicity of MON, an *in vivo* study conducted in mink (*Mustela vison*) was performed by Morgan and co-workers (1998). Female minks were exposed to <0.2 (control), 8.1 (low-dose) and 17 (high-dose) ppm MON contained in feed from 2 weeks preceding the breeding season until their offspring were 8 weeks of age. Interestingly, body weight of adult females treated with the low-dose MON significantly increased in comparison with the control group at 3 weeks (1,092 ± 27.0 g vs. 991 ± 28.4 g) and at 6 weeks postpartum (923 ± 25.0 g, vs. 814 ± 29.3 g) (Morgan et al., 1998). Vulvar swelling scores for the females were not significantly different among the groups. Although 17 ppm MON was not lethal to adult female minks, neonatal mortality, and reduced offspring body weights at birth as well as at 3 and 8 weeks of age were observed (Morgan et al., 1998). Whereas no significant body weight differences among the groups at 6 weeks of age were reported. Moreover, offspring mortality after 17 ppm MON administration significantly increased between 6 and 8 weeks and no liver, heart, and lungs lesions or alteration in both control and high-dose treated groups of 8 weeks old were observed. The toxic effects reported in the offspring seemed to be due to MON placental transfer (Morgan et al., 1998). Also, in laying hens fed MON 100 mg/kg for 28 days, egg weights and egg production significantly decreased by 5% and 14%, respectively, however, 50 mg/kg MON had no effect (Kubena et al., 1999).

A study by Cetin and Bullerman (2005) conducted on a mammalian cell line reported that MON moderately exerted cytotoxic effects on Chinese hamster ovary cells (CHO-K1). After 48 h exposure of 100 µg/mL (1.02 mM) MON, proliferation of CHO-K1 cells was inhibited by 10% (Figure 6A) and after 72 h exposure MON inhibited CHO-K1 cell proliferation by 30% (Figure 6B) (Cetin and Bullerman., 2005). In another *in vitro* study MON treatment for 24 and 96 h was found to cause 25% death of CHO-K1 cells at concentration of 5 µg/mL (51 µM) (Vesonder et al., 1993). Thus, MON does not appear to be as toxic as BEA, ENNA or ENNB1, but additional studies are needed to confirm this. Indeed, due to the large uncertainties in the risk assessment of humans, farm,

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and companion animals, the Panel on Contaminants in the Food Chain (CONTAM Panel) recommends more studies of the toxicokinetic and adverse effects of MON in different species to set up a comprehensive risk assessment for humans, farm, and domestic animals (EFSA, 2018).

3. Effects of emerging mycotoxins on testicular function

3.1 Beauvericin

Data regarding *in vivo* effects of BEA in males are few, and studies have mainly been conducted on birds using different combinations of mycotoxins (Bertero et al., 2018, Caloni et al., 2020). Two *in vivo* studies were performed on broiler chickens to evaluate BEA toxicity and no influence on growth performances and carcass characteristics were observed after the administration of feed mainly contaminated with MON and BEA (Leitgeb et al., 2003, Zollitsch et al., 2003). Moreover, an integrated *in vivo* approach (EFSA, 2018) was applied in mice to give information of BEA and ENNB oral toxicity. In this study *in vitro* and *in vivo* acute genotoxicity as well as reproductive and developmental toxicity assessments were performed. The reproductive toxicity screening was conducted on 10 male mice/group and 10 female mice/group after 42 days of BEA and ENNB administration. Adult mice were exposed to 0 mg/kg body weight., 0.1 mg/kg body weight, 1 mg/kg body weight and 10 mg/kg body weight BEA dose levels. In the male reproductive system, atrophic tubules with germ-cell- disorganization and tissue alteration in testicles were observed after exposure to 10 mg/kg body weight BEA. However, no change in sperm numbers was reported (EFSA, 2018).

An *in vitro* study performed by Tonshin and co-workers (2010) on *Fusarium* mycotoxins demonstrated that ENNs and BEA induce mitochondrial impairment in rat liver, human neural (Paju), murine insulinoma (Min-6) cells and boar spermatozoa. BEA and mixture of ENNs negatively influenced the basic mitochondrial functions at micromolar and submicromolar concentrations due to their potassium-selective ionophoric properties (Tonshin et al., 2010). Previously, BEA has been reported to be a non-competitive Ca^{2+} entry inhibitor (Nakajyo et al., 1987). Thus, the conducted studies suggest that BEA may impair male reproductive functions, but additional research is needed to ascertain the effects of BEA on testicular steroidogenesis.

3.2 Enniatins

In 2003, Hoornstra and co-workers investigated the effects of different toxins on boar spermatozoa. It was observed that 500 ng/mL (0.73-0.78 μM) ENNA, ENNA1, ENNB and ENNB1 inhibited sperm motility by depolarising the mitochondria and hyperpolarizing the plasma membrane of sperm

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cells (Hoornstra et al., 2003). These results are in agreement with the findings of Tonshin et al., (2010) who reported that ENNB, a mixture of ENNs (3% A, 20% A1, 19% B, 54% B1) and BEA caused mitochondrial function impairment by affecting the mitochondrial transmembrane potential, inhibiting the oxidative phosphorylation, and reducing calcium retention capacity of the mitochondria in boar sperm. After 10–20 min exposure of boar sperm to 0.6 µg/mL (0.94 µM) ENNB in media with 4 mM (physiological) or 1 mM (low) concentration of K+, hyperpolarization of the plasma membrane and depolarization of mitochondrial membrane potential were observed (Tonshin et al., 2010). The exposure of ENNB in a medium with physiological potassium concentration (4 mM) induced an efflux of potassium from the cytoplasm towards the exocellular space and a flux of potassium from the cytoplasm into the mitochondria, depleting the cytoplasm of potassium and destroying the ion homeostasis of cells (Tonshin et al., 2010).

The endocrine disrupting activity of ENNB was investigated by Kalayou et al., (2015) using the H295R model, a neonatal porcine Leydig cell model, and reporter gene assays (RGAs), and showed that in H295R cells, only 100 µM ENNB caused a loss in cell viability following 48 h incubation compared to control cells. In Leydig cells, 0.01–10 µM ENNB did not influence cell viability, whereas 100 µM ENNB caused a significant loss of viable cells by 20 and 21% in the unstimulated and LH-stimulated Leydig cells respectively. Moreover, 15.6 µM ENNB was cytotoxic on the RGA cell lines (Kalayou et al., 2015). In unstimulated Leydig cells, ENNB did not affect basal testosterone production. In LH-stimulated cells 0.01 and 10 µM ENNB did not affect testosterone production whereas 100 µM ENNB significantly reduced both estradiol and testosterone production. In addition, gene transcription analyses in H295R cells were performed, and twelve of the sixteen genes were significantly modulated by 10 µM ENNB as compared to the control (Kalayou et al., 2015). Genes downregulated by ENNB included *HMGR, STAR, CYP17A1,* and *CYP11A1*, whereas genes upregulated by ENNB included *CYP19A1*. From these studies it can be concluded that ENNB affects cell viability (i.e., toxic) and modulates hormone production, but only at levels of 10 µM to 100 µM. Moreover, considering that ENNB co-occurs with other mycotoxins in food and feed, further studies using mycotoxin mixtures are needed (Kalayou et al., 2015; Maranghi et al., 2018).

3.3 Moniliformin

Toxicity and toxicokinetic information are limited in experimental and farm animals, however haematotoxicity and cardiotoxicity as well as reduced body weight are the main reported adverse effects caused by MON (Kubena et al., 1999; Morris et al., 1999; Harvey et al., 2001; Harvey et al., 2002., EFSA, 2018). A study (Javed et al., 2005) on FB1, fumonisin B2 and MON in combination

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was performed to assess the pathologic effects in broiler chicks. Organs were collected from chicks that died during the treatment period, survivors and controls were examined and dose–response lesions as ascites, hydropericardium, hepatopathy, nephropathy, cardiomyopathy, pneumonitis, and gizzard ulcerations were observed in all examined groups (Javed et al., 2005). After exposure to 27 and 154 ppm of MON, 70% and 100% of chicks had liver lesions, therefore future studies should evaluate effects of MON on egg production of mature hens. In male chicks, testicles collected from toxin-fed birds appeared small and elongated compared to control groups, but no details of specific toxins were reported (Javed et al., 2005). Therefore, further studies are needed to evaluate possible direct effects of MON on testosterone production in male poultry and other species, as well as to evaluate the effects of MON on ovarian function.

4. Conclusions

The emerging *Fusarium* mycotoxins BEA, ENNs and MON have been demonstrated to cause reproductive effects in all the animal species studied, both in females and males. *In vitro*, BEA and ENNs can alter reproductive function impairing oocyte maturation and embryo development and inhibiting granulosa cell proliferation as well as steroid production and gene expression. *Fusarium* mycotoxins can also impair sperm function and affect testicular hormone synthesis. Moreover, some reproductive effects demonstrated *in vitro* have been confirmed in *in vivo* studies. In females, detrimental effects on embryonic development and increased offspring mortality were observed. Whereas in males, effects on testicles were reported. However, occurrence, toxicity and toxicokinetic data of these emerging mycotoxins are still lacking for the majority of animal species, particularly ruminants. Therefore, further studies with particular regard to mycotoxins in mixture are required to set up a proper risk assessment.

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Figure Legends:

Figure 1. Chemical structure of beauvericin.

Figure 2. Chemical structure of enniatins.

Figure 3. Chemical structure of moniliformin.

Figure 4. Development of embryos after exposure to beauvericin. Reprinted from Reproductive Toxicology, 65, Schoevers E.J., Santos R.R., Fink-Gremmels J., and Roelen B.A.J., Toxicity of beauvericin on porcine oocyte maturation and preimplantation embryo development, pages 159-169, 2016, with permission from Elsevier.

Figure 5. Effect of various doses of BEA progesterone (Figure 5A) and estradiol (Figure 5B) production by bovine granulosa cells. Modified from Toxicon, 128, Albonico M., Schutz F.L., Caloni F., Cortinovis C., and Spicer, L.J., *In vitro* effects of the *Fusarium* mycotoxins fumonisin B1 and beauvericin on bovine granulosa cell proliferation and steroid production, pages 38-45, 2017, with permission from Elsevier. Asterisks indicate mean differs from controls (0 µM BEA) ($P < 0.05$).

Figure 6. The cytotoxic effects MON at concentrations of 0.2–100 µg/ mL (2 µM – 1 mM) on proliferation of CHO-K1 (◆), Caco-2 (■), C5-O (▲), V79 (□), and HepG2 (△) cell lines following 48-h (Panel A) or 72-h (Panel B) exposure as determined by the MTT bioassay. Reprinted from Food and Chemical Toxicology 43, Cetin Y., and Bullerman L.B., Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay, pages 755-764, 2005, with permission from Elsevier.

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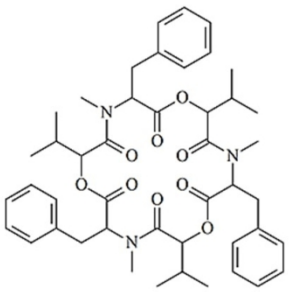

Figure 1

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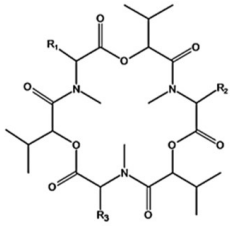

	R1	R2	R3
enniatin A	*sec*-but	*sec*-but	*sec*-but
enniatin A_1	*i*-pr	*sec*-but	*sec*-but
enniatin B	*i*-pr	*i*-pr	*i*-pr
enniatin B_1	*i*-pr	*i*-pr	*sec*-but

Figure 2

374x209mm (96 x 96 DPI)

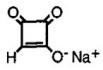

Figure 3

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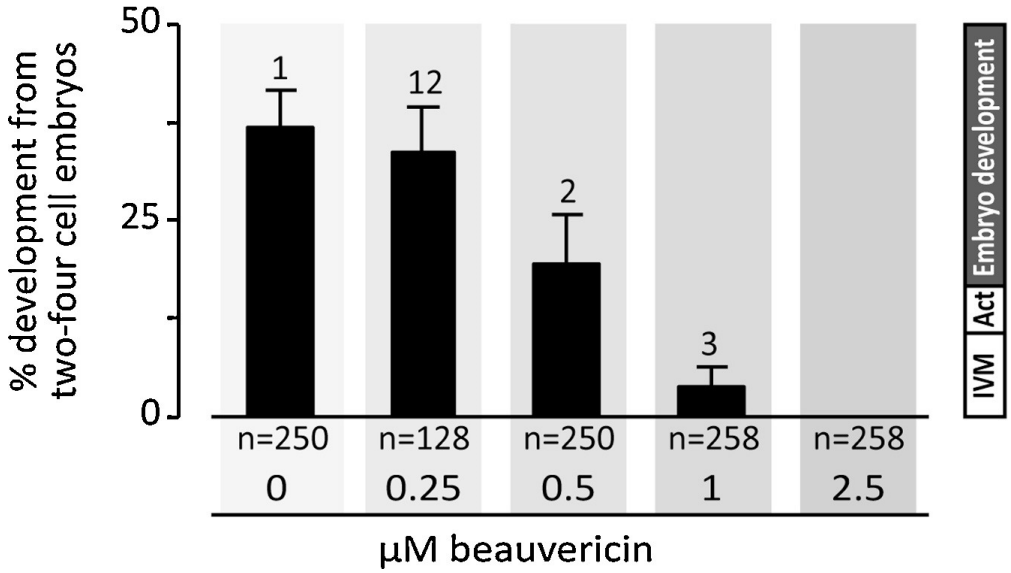

Figure 4

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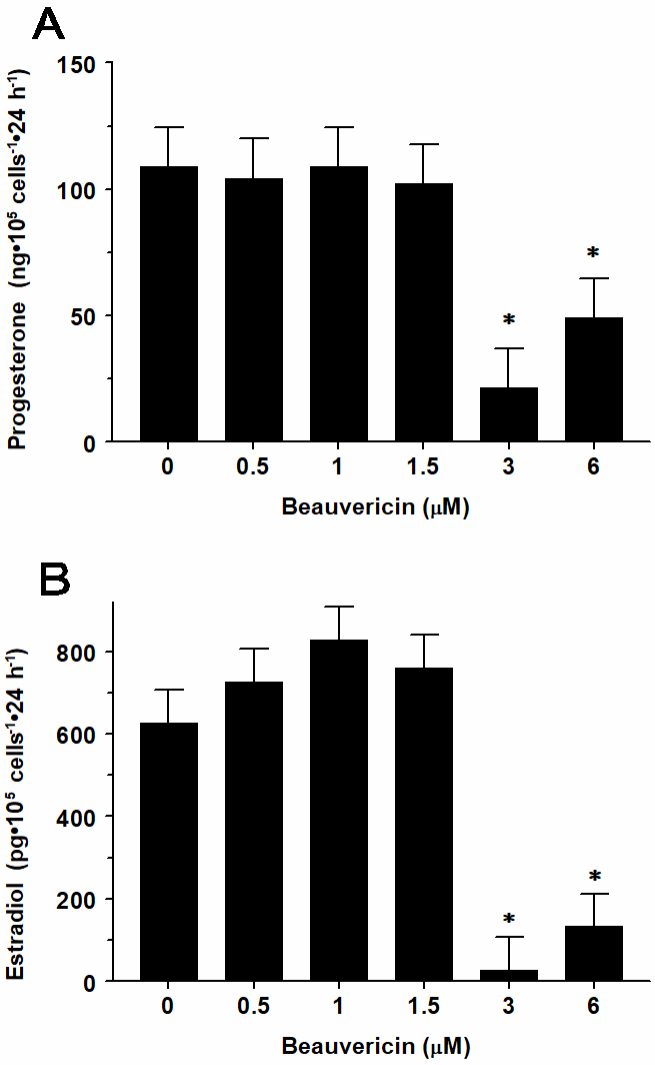

Figure 5

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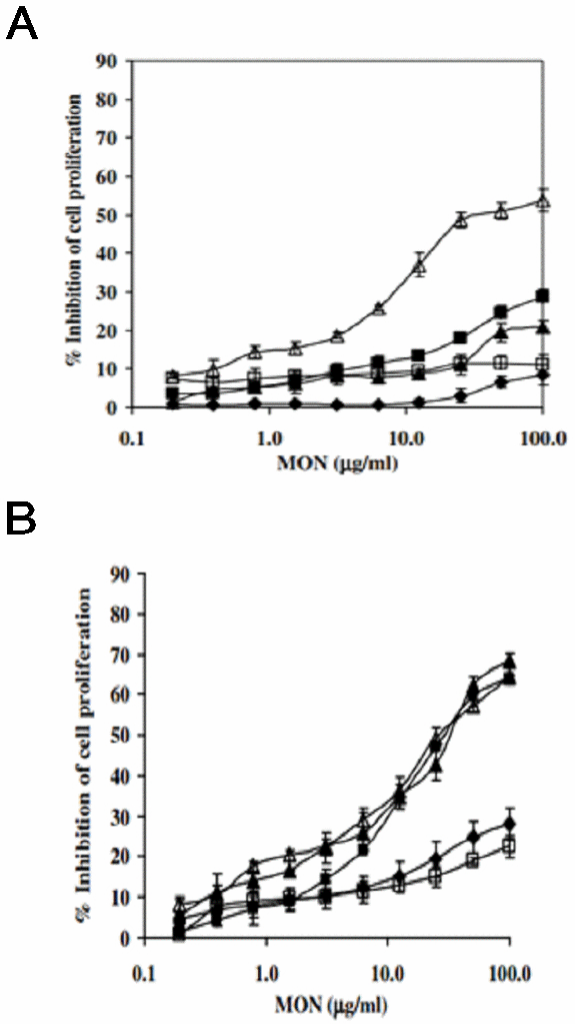

Figure 6

169x254mm (120 x 120 DPI)