ACTIVITY OF SOY PHYTOESTROGENS AND ZEARALENONE ON MAMMALIAN REPRODUCTION: A REVIEW*

L. Křivohlávková, K. Hošková, A. Krejcárková, R. Rajmon

Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resourses, Prague, Czech Republic

The present review summarizes the findings about the mechanism and the effects of soy phytoestrogens and zearalenone acting. These substances commonly occur in plant feeds for animals. Owing to their estrogenic effects, they can disturb physiological functions in organism, mainly in the area of reproduction. Consequently, the negative impact of these substances is reflected in the overall economy of the livestock breeding.

endocrine disruptors; estrogenic effects; reproduction; gametes

INTRODUCTION

Endocrine disruptors are substances occurring in the environment which can interfere with hormonal regulation in the organism. These are chemical compounds of either natural or synthetic origin which can variously affect the organism and cause serious health problems. By origin they can be divided into two main groups – synthetically produced substances, which are released into the environment (phenols, pesticides, plastics, etc.), and natural substances. The most important natural endocrine disruptors are natural xenoestrogens (environmental estrogens), structurally similar to molecules of estrogen, which get into the organism through food, and especially phytoestrogens, substances naturally occurring in plants, and zearalenone with its derivates, a product of *Fusarium* mould (R o s s e l l i et al., 2000; B u r t o n , W e l l s, 2002).

The analogous mechanisms of action of xenoestrogens and endogenous estrogens on the human and animal organism suggest that these disruptors affect mainly the area of reproduction (G a l l o et al., 1998; R o s s e l l i et al., 2000). The activity of these substances, especially phenolic compounds, influences the development and function of the genital tract not only in many wild animals, but also in humans (G a l l o et al., 1998; H o y e r, 2001; B u r t o n , W e l l s, 2002).

Phytoestrogens

In their chemical structure and function, phytoestrogens are similar to the endogenous steroidal hormone 17β-estradiol (K n i g h t , E d e n, 1995; T h a m et al., 1998; S t e e n s m a , 2006), which determines their similar effects on the organism. Phytoestrogens

have been identified in more than 300 plant species (K n i g h t , E d e n , 1995; V r z á ň o v á , H e r e s o v á, 2003), where their presence and amount serve the plant as a defence mechanism. In the case of stress conditions the concentration increases (Barrett, 1996) up to 15-fold (K a l a č , M í k a , 1997).

Based on their characteristics and structure, phytoestrogens can be divided into several groups, with a very important subgroup being isoflavones, which include soy phytoestrogens (C o r n w e l l et al., 2004). The estrogenic activity of isoflavones is relatively low; however, after structural changes in the digestive tract it increases significantly (K a l a č , M í k a , 1997). The greatest amount of isoflavones is found in legumes, clovers, beans, and other plants rich in fibre, and their major producers are soybeans (*Glycine max*) and red clover *(Trifolium pratense)* (M o r a v c o v á , K l e i n o v á , 2002; B u r t o n , W e l l s, 2002).

The non-conjugated soy isoflavones – genistein and its structurally similar daidzein, also known as a glycosides genistin and daidzin, belong among the most studied and the most frequent phytoestrogens (K a l a č , M í k a , 1997; K u r z e r , X u , 1997). Other important isoflavones are the metabolic precursors of both the above-mentioned substances – formononetin and biochanin A, respectively.

Genistein belongs to the most effective and the most widely occurring isoflavone. It is unique in its ability to inhibit the group of enzymes of tyrosin protein kinasis and MAPK, which control the cell cycle and are important for meiotic maturation of oocytes, fertilization, and early stages of embryo development (K i n s l e y , 1997; F r i t s c h e , S t e i n h a r t , 1999; R o s s e l l i et al., 2000; M c G i n n i s et al., 2011). It was recorded that genistein inhibits meiotic matura-

tion of murine (M a k a r e v i c h et al., 1997), bovine (B o r z y m et al., 2008), and porcine (Vo d k o v á et al., 2008) oocytes.

Daidzein (4´,7-dihydroxyisoflavon), structurally very similar to genistein, expresses itself in some immune reactions more markedly than genistein, as was confirmed by both *in vivo* and *in vitro* studies. Daidzein increased expression of antioxidative enzymes such as catalase, copper zinc superoxid dismutase (R ö h r d a n z et al., 2002) or CYP1A1 (S h e r t z e r et al., 1999). Furthermore, it affects the metabolism of steroids influencing reproduction (e.g., daidzein inhibits 17β-HSD, 3β-HSD, and to a lesser extent aromatase) (L e B a i l l et al., 2000). Contrary to genistein, daidzein does not affect cell enzymes such as tyrosin kinases or DNA topoisomerases (C o r n w e l l et al., 2004).

Zearalenone

Zearalenone (ZEA) is a mycotoxin which due to its mass occurrence, estrogenic effects, and toxicity for humans and livestock ranks among the strongest. Mycotoxins are toxic non-protein substances with estrogenic effects, originating as products of pathogenic moulds (K a l a č, M í k a, 1997; S t o p p e r et al., 2005), of which nearly four hundred species have so far been identified (W e i d e n b ö r n e r et al., 2000).

ZEA is produced by *Fusarium* moulds (Z i n e d i n e et al., 2007), and it commonly occurs not only in mild-temperate belt conditions (B e n n e t, K l i c h, 2003). ZEA-producing moulds most often contamine corn, to a lesser degree also other grains (barley, rye, oats), sorghum, and soy (Z i n e d i n e et al., 2007). The toxin production is influenced by the harvest time, storage, kind of pre-crop, and geographical location (G r o m a d z k a et al., 2008; M i n e r v i n i et al., 2011). Global warming caused the *F. graminearum* mould spreading from southern Europe also to the Czech Republic. This mould species is the largest producer of ZEA and represents a major risk of toxicosis (K r m e n č í k, K y s i l k a, 2012).

The danger of mycotoxins follows, a.o., from their high resistance to both physical (high temperatures) and chemical (low pH) influences and from their difficult degradation with common production procedures used in food processing. Use of appropriate agricultural technologies, selection of resistant crop varieties and application of other preventive measures can reduce the negative impacts of ZEA and other mycotoxins on organisms. But the basic presumption is to prevent feeding mouldy foodstuffs (K a l a č, M í k a, 1997).

Mechanism of action of phytoestrogens and zearalenone

Phytoestrogens and zearalenone can have both estrogenic and antiestrogenic effects, due to interaction with transport proteins, membrane and estrogen receptors or with other mechanisms of action.

Biological functions at the cellular and molecular level – proliferation, differentiation and synthesis of proteins in the various target tissues are influenced in this way (B e n a s s a y a g et al., 2002; M o r a v c o v á, K l e i n o v á, 2002; M a l e k i n e j a d et al., 2005).

The estrogenic effectiveness of phytoestrogens and ZEA is significantly lower ($10^{-2}–10^{-3}$) in comparison with that of estrogens. However, they can reach very high concentrations in the organism (100–1000 times higher than the concentrations of endoestrogens) and evoke a response as strong as endogenous estrogens at their physiological level in the organism (B e n a s s a y a g et al., 2002). At a concentration of 1 μM, the estrogenic strength of genistein is actually higher than in 17β-estradiol. At the same time, it is important to take into consideration the influence of the concentration of both phytoestrogens and endogenous estrogens, receptor position and the kind of target organ or cell (T a p i e r o et al., 2002). Considering the key role of estradiol in regulation of the reproductive function, mainly female genital organs are affected at the level of ovarian follicles. It was recorded that genistein and daidzein inhibit viability of follicular cells under *in vitro* conditions (T i e m a n n et al., 2007; G a l l e a t i et al., 2010).

Among the most frequent and the most studied mechanisms of action of phytoestrogens and zearalenone belongs their ability to bind on α- and β-estrogen receptors (ER) (W h i t t e n, P a t i s a u l, 2001; T a p i e r o et al., 2002; D u s z a, C i e r e s z k o, 2006). When binding to estrogen receptors α- and β-, estrogenic compounds exert two basic mechanisms of action – genomic and non-genomic (A n d e r s o n et al., 1999). Natural endogenous estrogens act most often genomically through the estrogen receptors localized in the nucleus, after ligand binding (A n d e r s o n et al., 1999; S i n g l e t o n, S o h a i b, 2003; L i et al., 2004), resulting in the origin of homo- or heterodimer, which interacts with specific palindrome sequences of DNA (estrogen response elements – ERE) and creates a complex ligand – ERE, which is translocated to the nucleus. Therefore, the ER function as an active transcription factor and upregulates or downregulates gene expression of the target genes (K u i p e r et al., 1998; A n d e r s o n, 1999; B e n a s s a y a g et al., 2002; S i n g l e t o n, S o h a i b, 2003). Non-genomical action proceeds more quickly and consists in the ligand binding on an appropriate membrane receptor, by means of which a specific cell response is achieved – activation of transduction signal pathways and production of secondary messengers (e.g. cAMP) (B e n a s s a y a g et al., 2002).

The binding ability of phytoestrogens to ER is closely associated with differences in the affinity of specific estrogen substances to the individual ER. Generally, phytoestrogens have, in comparison with 17β-estradiol, lower binding affinity to both types of ER (K u r z e r, X u, 1997; B e n a s s a y a g et al., 2002), and significantly higher affinity to ERβ than to ERα

(Albertazzi, Purdie, 2002; Matthai et al., 2003; Dusza, Ciereszko, 2006). In general, it can be said that the affinity to ER decreases in the following sequence: estradiol > zearalenone > genistein > daidzein > biochanin A > formononetin (Benassayag et al., 2002; Gruber et al., 2002; Moravcová, Kleinová, 2002). Whereas phytoestrogens bind preferentially to ERβ, ZEA and its metabolites bind to ERα and ERβ with approximately the same affinity. In comparison with estrogens, the effects of ZEA during binding to ERα are fully agonistic; during binding to ERβ its effects are agonistic-antagonistic. With regard to the different distribution of α and β ER subtypes in individual tissues, preference for ERβ plays a key role in the action of phytoestrogens in specific tissues (Cassidy, Faughnan, 2000; Albertazzi, Purdie, 2002).

By their activity natural endogenous estrogens intervene in several enzymatic events through interaction with hydroxysteroid dehydrogenases, enzymes participating in the transduction pathways or the cell cycle (Kurzer, Xu, 1997; Albertazzi, Purdie, 2002; Benassayag et al., 2002; Malekinejad et al., 2005; Dusza, Ciereszko, 2006).

Effects of phytoestrogens and zearalenone

Phytoestrogens and ZEA can cause various developmental and morphological abnormalities in the female genital tract and changes in the estrous cycle and in sexual behaviour (Chang et al., 1979; D'Mello et al., 1997; Cassidy, Faughnan, 2000; Gromadzka et al., 2008).

Whereas ZEA affects the organism only negatively, phytoestrogens can play also a positive role (Opletal, Šimerda, 2009). Especially in human medicine their positive effects e.g. on bone metabolism and the prevence of occurrence of osteoporosis (Kurzer, Xu, 1997; Matthai et al., 2003; Cornwell et al., 2004) as well as their anticancerogenic effects (Knight, Eden, 1995; Moravcová, Kleinová, 2002; Duncan et al., 2003; Cornwell et al., 2004) are emphasized, and they are widely used as an alternative treatment for suppression of menopause symptoms (Kurzer, Xu, 1997; Romero et al., 2008). Unlike in the human organism, the effects of phytoestrogens in animals are more commonly considered as negative, significantly interfering mainly with reproduction (Adams, 1995b). Phytoestrogens can cause male and female infertility. Of all livestock animals, pigs are the most sensitive to the effects of these substances (Fink-Gremmels, Malekinejad, 2007).

Effects on the female reproductive cycle and juveniles

Phytoestrogens in feed lead to changes in the estrous cycle and in female sexual behaviour and decrease the chance of becoming pregnant (Adams, 1995b;

Opletal, Šimerda, 2006). In general, they have hyperestrogenic effects on the organism (Gajecka et al., 2011), leading to e.g. swelling and reddening of the vulva (Young et al., 1986; Obremski et al., 2003), and to the interference with the level of endogenous estrogens (Adams, 1995a; Opletal, Šimerda, 2006). Markers of estrogenization were also observed in the uterus and vagina (Chang et al., 1979; Whitten et al., 1995; Adams, 1996; Gallo et al., 1998). In ruminants irregular intervals between estrous, occurrence of false estrous, nymphomania, anestrous and decreased number of ovulations were observed (Adams, 1995b; Opletal, Šimerda, 2006). The length of the estrous cycle in rats and mice was prolonged depending on the dosage of phytoestrogens given (Gallo et al., 1998).

Phytoestrogens and ZEA also affect the organism of young animals, both in the prenatal and postnatal period. Transplacentary transmission of phytoestrogens makes it possible to influence the metabolism of estrogens in the uterus (Cassidy, Faughnan, 2000), and in this way they can significantly interfere with the development of estrogen-sensitive tissues and therefore the sexual differentiation of foetuses (Adams, 1996) and neurohumoral direction of ovulation (Barret, 1996; Kurzer, Xu, 1997; Benassayag et al., 2002). Transplacentary transmission of ZEA can also negatively affect embryos, which are especially sensitive 7–10 days after fertilization. Prenatal exposure to a mixture of isoflavones with a predominant share of daidzein and daidzin caused premature puberty in female rats and irregularities in the estrous cycle (e.g. prolongation) in adults (Romero et al., 2008). The toxicity of phytoestrogens and ZEA can be demonstrated by embryonic or later on foetal mortality or possibly by the birth of less viable or dead piglets (Nelson et al., 1994; Romero et al., 2008). Phytoestrogens and ZEA can postnatally affect the progeny via breast milk. The manifestations of toxicosis are similar to those in adult animals – edema and reddening of the vulva and nipples, enlargement of the mammary gland in immature females and males, hypertrophy of the vulva and uterus, possible prolapse of vagina or rectum (Biehl et al., 1993; Adams, 1996; Gallo et al., 1998; Burton, Wells, 2002). ZEA in young gilts negatively affects sexual behaviour, because the reflex of immobility during ovulation is not evoked (Kummer et al., 2001). In prepubertal gilts similar symptoms to those in the cycle of sows occur, but often at lower concentrations of ZEA. In gilts ZEA causes prolongation of the estrous cycle and persistence of *corpus luteum* (Edwards et al., 1987).

Effect on the ovary

These toxins can also cause pathological changes in the ovaries (Opletal, Šimerda, 2006; Schoevers et al., 2012). In cattle feeding red

clover, which contains phytoestrogens, the development of ovarian cysts was observed (A d a m s , 1995b; O p l e t a l , Š i m e r d a , 2006). The syndrome of polycystic ovaries is associated with anovulation, increased secretion of androgens and luteinizing hormone insulin. Abnormal metabolism of IGF was also observed (K n i g h t , E d e n , 1995). Phytoestrogens can cause the development of an enormous number of small and medium-sized follicles, and follicle atresia (A d a m s , 1995a; B u r t o n , W e l l s , 2002), in addition, accompanied by hypertrophy of granulose cells in mice (M a k a r e v i c h et al., 1997). In mice ovaries a lower number of Graafian follicles and the absence of *corpus luteum* were observed. In sheep grazing on clover with isoflavone content, small numbers of follicles mostly without antra and subsequent early atresia were developed (B u r t o n , W e l l s , 2002).

Long-term exposure to low concentrations of ZEA leads to impairment of proliferative activity of the follicle granulose cells and connective tissues of the ovarian stroma in prepubertal gilts (G a j e c k a et al., 2011). In gilts, ZEA caused follicle atresia and apoptotic changes in granulose cells (O b r e m s k i et al., 2003). In pregnant and lactating sows exposed to the action of ZEA, the number of follicles with normal morphology was decreased. This reduction of reserves of the early stages of follicles can cause the premature depletion of healthy follicles and shorten the reproductive period in sows (S c h o e v e r s et al., 2012). The derivates of ZEA, α- and β-zearalenol, inhibit progesterone synthesis in porcine granulose cells (T i e m a n n et al., 2003).

Effects on oocytes

The effects of phytoestrogens and ZEA are also manifested at the level of oocytes. The degree of impact on the morphology of oocytes, markers of meiotic and developmental competence, varies in specific estrogenic substances depending on the dose, estrogenic effectiveness, animal species or the particular cultivation system. Generally, compared to phytoestrogens, the effects of ZEA are more prominent and marked at lower concentrations (A d a m s , 1995a; O p l e t a l , Š i m e r d a , 2006). Genistein, due to its effects, is a very often monitored phytoestrogen which affects, a.o., meiotic maturation and early embryogenesis in cattle, rabbits, and pigs. In bovine oocytes genistein does not cause blockage of Germinal Vesicle Break-Down (GVBD), but the meiotic maturation is altered and in a statistically significant portion of oocytes, meiotic maturation stops stage or prometaphase I in the late diakinesis (M a k a r e v i c h et al., 1997). In pigs and mice, *in vitro* experiments proved the ability of genistein to block, in a dose-dependent manner, GVBD at meiotic maturation of oocytes (J u n g et al., 1993; V o d k o v á et al., 2008). Contrary to this study, M a k a r e v i c h et al. (1997) recorded that

in low concentrations genistein stimulated nuclear maturation of pig oocytes.

Jung et al. (1993) explain the effects of genistein ability to inhibit tyrosine protein kinases (TPK), whereas some studies suggest that the effects of phytoestrogens are not dependent only on this inhibition. V a n C a u w e n b e r g , A l e x a n d r e (2000) tested the effects of another inhibitor of TPK – lavendustin A – and concluded that resumption of meiosis in mice oocytes was not inhibited. Simultaneously, V o d k o v á et al. (2008) recorded, although at higher concentrations and with substantially lower efficiency, similar inhibitory effects of genistein also in genistin – a phytoestrogen, which does not inhibit the activity of TPK. The results of these experiments suggest that inhibition of meiotic maturation of oocytes is also achieved in other ways than just by inhibition of the activity of TPK. This fact is supported by the results of *in vitro* experiments which describe the ability to inhibit meiotic maturation in pig oocytes at the level of GVBD, also by 17β-estradiol (B i n g et al., 2001; L i et al., 2004).

On the other hand, low doses of another soy phytoestrogen, daidzein, did not affect nuclear maturation, rapidness of fertilization, or even the number of fertilized oocytes like in the subsequent embryonal development in pigs (the part of the embryos at the blastocyst stage or the blastomer/blastocyst ratio). However, decrease of progesterone synthesis by cumulus cells was determined (G a l l e a t i et al., 2010).

The effects of ZEA on pig oocytes cultivated *in vitro* caused a decrease in the number of oocytes capable of undergoing GVBD (S c h o e v e r s et al., 2012). The cultivation of porcine cumulus oocyte complex in the presence of ZEA caused a significant decrease in the number of oocytes which achieved MII stage after 44 h. An increased incidence of nucleus abnormalites in oocytes, which leads to aneuploidy, was also observed (M a l e k i n e j a d et al., 2007). The defects in spindle apparatus and aneuploidies in porcine oocytes are most frequently induced by bivalent separation failure or by premature segregation of sister chromatids, and they have negative effects on the subsequent fertilization and early embryonal development (A l m et al., 2002, 2006; M a l e k i n e j a d et al., 2007; H o r n a k et al., 2011). At the cytoplasmic level ZEA induced vacuolization of ooplasma, which is considered a manifestation of autophagia (M a l e k i n e j a d et al., 2007; S c h o e v e r s et al., 2012). In pig oocytes cultivated in IVM medium supplemented with ZEA (1000 μg/l) the impairment of *zona pellucida* sperm penetration after *in vitro* fertilization was observed (S a m b u u et al., 2011).

Phytoestrogens also inhibit *in vitro* expansion of cumulus cells surrounding the oocyte. T i r o n e et al. (1997) determined that a concentration of 60μM genistein in a cultivation medium completely blocked the FSH and EGF stimulation effect on expansion in

mice oocytes. The same effect in pigs, at a concentration of 148µM, was confirmed by Vodková et al. (2008). The ability to suppress the expansion of cumular cells of porcine oocytes by 17β-estradiol was also demonstrated (L i et al., 2004).

Effect on the male

Although most of the effects described concerned females, the effects of environmental estrogens on males were demonstrated as well. ZEA in prepubertal boars caused a reduction of libido and a decrease of testosterone level in the plasma; however, the development of sexual behaviour was not affected. In young castrates it can result in the hypertrophy of the mammary gland or edema of the prepucium. In boars ZEA can cause reduced mass of the testicles. Long-term exposure to a low concentration of ZEA can cause the reduction of epididymis and seminal vesicles development in young males (B e r g e r et al., 1981; T s a k m a k i d i s et al., 2006; B e n z o n i et al., 2008; M i n e r v i n i , D e l l´ A q u i l a , 2008; K a n o r a , M a e s , 2009; B h a t et al., 2010).

It has been demonstrated that phytoestrogens and ZEA also affect male gametes – spermatozoa. The action of phytoestrogens during incubation results in a decrease in the induction of acrosomal reaction in sperm and inhibition of the binding of sperm to the *zona pellucida*, whereas in bovine spermatozoa motility, viability and capacitation are not affected (H ä g e l e et al., 1998; H i n s c h et al., 2000; M e n z e l et al., 2007). Studies by M o h a m e d et al. (2011) showed that the effects of phytoestrogen genistein on the capacitation of porcine sperm are comparable to those of endogenous estrogens. On the contrary, in human sperm the phytoestrogen genistein significantly accelerates capacitation and acrosome loss, which can lead to the inability to fertilize the oocyte (F r a s e r et al., 2006; C e d e r r o t h et al., 2010). Genistein, in comparison to 17β-estradiol, has stronger effects on stimulation of acrosomal reaction in pig sperm (M o h a m e d et al., 2011). On the other hand, F u r u y a et al. (1992) and P a r k et al. (2011) mention that the addition of genistein strongly inhibits capacitation in mice sperm.

The above-mentioned authors agree that genistein has no effect on motility; however, K u m i - D i a k a , T o w n s e d (2001) recorded that higher concentrations of genistein result in significant interference with the number of motile spermatozoa, whereas morphology is not affected. P a r k et al. (2011) recorded that genistein (100µM in medium) decreased the reactivity of murine sperm acrosome after short-term exposure (15 min) contrary to prolonged exposure (30 min), which had a stimulative effect. In the case of bovine sperm, after 25 min of genistein exposure (7.4µM in medium), acrosome reaction was inhibited (H i n s c h et al., 2000).

ZEA can negatively affect the stability of chromatin and the motility of boar spermatozoa (B e n z o n i et

al., 2008) as well as damage the plasmatic membrane of sperm, and reduce the percentage of sperm with reactive acrosome (T s a k m a k i d i s et al., 2006).

The effect of ZEA on qualitative parameters for IVF systems was examined by a long-term (one week) incubation of boar spermatozoa with this contaminant, and no significant changes in penetration as a whole, or in monospermy and polyspermy oocyte fertilization, were recorded. In the same way, there were no negative effects on the cleavage and development of blastocystes after *in vitro* fertilization. After three weeks of incubation the motility, viability, and plasma membrane integrity were significantly reduced compared with non-stored sperm (S a m b u u et al., 2013).

CONCLUSION

Phytoestrogens and zearalenone affect the reproduction of animals at the level of changes in the function of the reproductive organs, even at the level of gametes – oocytes and spermatozoa. Spermatozoa can be affected by these estrogen substances not only in male gonads during their own formation and maturation, but also in the female genital tract after ejaculation.

The effects of these substances in the form of impact on reproductive efficiency are especially prominent in pigs, which are often exposed to phytoestrogens and zearalenone in feedstuffs.

REFERENCES

Adams NR (1995a): Organizational and activational effects of phytoestrogens on the reproductive tract of the ewe. Processes of the Society for Experimental Biology and Medicine, 208, 87–91.

Adams NR (1995b): Detection of effects of phytoestrogens on sheep and cattle. Journal Animal Science, 73, 1509–1515.

Adams NR (1996): Phyto-estrogens: lessons from their effects on animals. Proceedings of the Nutrition Society of Australia, 20, 156–162.

Albertazzi P, Purdie D (2002): The nature and utility of the phytoestrogens: a review of the evidence. Maturitas, 42, 173–185.

Alm H, Greising T, Brussow KP, Torner H, Tiemann U (2002): Influence of the mycotoxins deoxynivalenol and zearalenol on *in vitro* maturation of pig oocytes and *in vitro* culture of pig zygotes. Toxicology in Vitro, 16, 643–648.

Alm H, Brussow KP, Torner H, Vanselow J, Tomek W, Danicke S, Tiemann U (2006): Influence of fusarium-toxin

contaminated feed on initial quality and meiotic competence of gilt oocytes. Reproductive Toxicology, 22, 44–50.

Anderson JJB, Anthony M, Messina M, Garner SC (1999): Effects of phyto-oestrogens on tissues. Nutrition Research Reviews, 12, 75–116.

Barrett J (1996): Phytoestrogens. Friends or foes? Environmental Health Perspectives, 104, 478–482.

Benassayag C, Perrot-Applanat M, Ferre F (2002): Phytoestrogens as modulators of steroid action in target cells. Journal of Chromatography B, 777, 233–248.

Bennett JW, Klich M (2003): Mycotoxins. Clinical Microbiology Reviews, 16, 497–516.

Benzoni E, Minervini F, Giannoccoro A, Fornelli F, Vigo D, Visconti A (2008): Influence of *in vitro* exposure to mycotoxin zearalenone and its derivatives on swine sperm quality. Reproductive Toxicology, 25, 461–467.

Berger T, Esbenshade KL, Diekman MA, Hoagland T, Tuite J (1981): Influence of prepubertal consumption of zearalenone on sexual development of boars. Journal Animal Science, 53, 1559–1564.

Bhat R, Ravishankar VR, Karim AA (2010): Mycotoxins in food and feed: present status and future concerns. Comprehensive Reviews in Food Science and Food Safety, 9, 57–81.

Biehl ML, Prelusky DB, Koritz GD, Hartin KE, Buck WB, Trenholm HL (1993): Biliary-excretion and enterogepatic cycling of zearalenone in immature pigs. Toxicology and Applied Pharmacology, 121, 152–159.

Bing YZ, Nagai T, Rodriguez-Martinez H (2001): Effects of cysteamine, FSH and estradiol-17beta on *in vitro* maturation of porcine oocytes. Theriogenology, 55, 867–876.

Borzym E, Bobowiec R, Kosior-Korzecka U, Martelli F, Burmunczuk A (2008): Disturbances of cow oocyte maturation by phytoestrogens. Medycyna Weterynaryjna, 64, 1107–1111.

Burton JL, Wells M (2002): The effects of phytoestrogens on the female genital tract. Journal of Clinical Pathology, 55, 401–407.

Cassidy A, Faughnan M (2000): Phyto-oestrogens through the life cycle. Proceedings of Nutrition Society, 59, 489–496.

Cederroth ChR, Auger J, Zimmermann C, Eustache F, Nef S (2010): Soy, phyto-oestrogens and male reproductive fiction: a review. International Journal of Andrology, 33, 304–316.

Chang K, Kurtz HJ, Mirocha CJ (1979): Effects of the mycotoxin zearalenone on swine reproduction. American Journal of Veterinary Research, 40, 1260–1267.

Cornwell T, Cohick W, Raskin I (2004): Dietary phytoestrogens and health. Phytochemistry, 65, 995–1016.

D'Mello JPF, Porter JK, MacDonald AMC, Placinta CM (1997): Fusarium mycotoxins. In: D'Mello JPF (ed.): Handbook of plant and fungal toxicants. Xxx Ed. CRC Press. Boca Raton, 287–301.

Duncan AM, Phipps WR, Kurzer MS (2003): Phyto-oestrogens, best practise and research. Clinical Endocrinology and Metabolism, 17, 253–271.

Dusza L, Ciereszko R (2006): Mechanism of phytoestrogen action in reproductive processes of mammals and birds. Reproductive Biology, 6, 151–174.

Edwards S, Cantley TC, Day BN (1987): The effects of zearalenone on reproduction in swine. 2. The effect on puberty attainment and postweaning rebreeding performance. Theriogenology, 28, 51–58.

Fink-Gremmels J, Malekinejad H (2007): Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone. Animal Feed Science and Technology, 137, 326–341.

Fraser LR, Beyet E, Miligan SR, Adeoya-Osiguwa SA (2006): Effect of estrogenic xenobiotics on human and mouse spermatozoa. Human Reproduction, 21, 1184–1193.

Fritsche S, Steinhart H (1999): Occurence of hormonally active compounds in food: a review. European Food Research and Technology, 209, 153–179.

Furuya S, Endo Y, Oba M, Nozawa S, Suzuki S (1992): Effects of modulators of protein-kinases and phosphatases on mouse sperm capacitation. Journal of Assisted Reproduction and Genetics, 9, 391–399.

Gajecka M, Rybarczyk L, Zwierzchowski W, Jakimiuk E, Zielonka L, Obremski K, Gajecki M (2011): The effect of experimental, long-term exposure to low-dose zearalenone mycotoxicosis on the histological condition of ovaries in sexually immature gilts. Theriogenology, 75, 1085–1094.

Galleati G, Vallorani C, Bucci D, Bernardini Ch, Tamanini C, Parmeggiani A, Spinaci M (2010): Daidzein does affect progesterone secretion by pig cumulus cells but it does not impair oocytes IVM. Theriogenology, 74, 451–457.

Gallo D, Cantelmo F, Distefano M, Ferlini C, Zannoni GF, Riva A, Morazzoni P, Bombardelli E, Mancuso S, Scambia G (1998): Reproductive effects of dietary soy in female wistar rats. Food and Chemical Toxicology, 37, 493–502.

Gromadzka K, Waskiewicz A, Chelkowski J, Golinski P (2008): Zearalenone and its metabolites: occurrence, detection, toxicity and guidelines. World Mycotoxin Journal, 1, 209–220.

Gruber CJ, Tschuggel W, Schneeberger C, Huber JC (2002): Production and actions of estrogens. The New England Journal of Medicine, 346, 340–350.

Hägele W, Hinsch E, Muller-Schlosser F, Brummer A, Schill WB, Hinsch KD (1998): The influence of phytoestrogen genistein on sperm function. Tierärtzliche Umschau, 53, 437–441.

Hinsch KD, Aires V, Hägele W, Hinsch E (2000): *In vitro* tests for essential sperm functions using the phyto-oestrogen genistein as a test substance. Andrologia, 32, 225–231.

Hornak M, Jeseta M, Musilova P, Pavlok A, Kubelka M, Motlik J, Rubes J, Anger M (2011): Frequency of aneuploidy related to age in porcine oocytes. PLoS ONE, 6, doi: 10.1371/journal.pone.0018892.

Hoyer PB (2001): Reproductive toxicology: current and future. Biochemical Pharmacology, 62, 1557–1564.

Jung T, Fulka Jr J, Lee C, Moor RM (1993): Effects of the protein phosphorylation inhibitor genistein on maturation of pig oocytes *in vitro*. Journal of Reproduction and Fertility, 98, 529–535.

Kalač P, Míka V (1997): Natural harmful substances in vegetable fodder. Institute of Agricultural and Food Information, Prague, 317 p. (in Czech)

Kanora A, Maes D (2009): The role of mycotoxins in pig reproduction: a review. Veterinarni Medicina, 54, 565–576.

Kinsey WH (1997): Tyrosine kinase signaling at fertilization. Biochemical and Biophysical Research Communications, 240, 519–522.

Knight DC, Eden JA (1995): Phytoestrogens – a short review. Maturitas, 22, 175.

Krmenčík P, Kysilka J (2012): Bacterial superantigens . http://www.biotox.cz/toxikon/bakterie/superantigeny.php. Accessed 12 January, 2012 (in Czech)

Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg P, Gustafsson JA (1998): Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology, 139, 4252–4263.

Kumi-Diaka J, Townsed J (2001): Effect of genistein isoflavone (4′,5′,7′-trihydroxyisoflavone) and dexamethasone on functional characteristics of spermatozoa. Journal of Medicinal Food, 4, 39–47.

Kummer V, Faldíková L, Herzig I, Laníková A (2001): Effects of mycotoxins on health and reproduction of animals; diagnostics and prevention of mycotoxicoses. Veterinary Research Institute, Brno. (in Czech)

Kurzer MS, Xu X (1997): Dietary phytoestrogens. Annual Review of Nutrition, 17, 353–381.

Le Baill JCh, Champavier Y, Chuliaa AJ, Habrioux G (2000): Effects of phytoestrogens on aromatase, 3p- and 17p-hydroxysteroid dehydrogenase activities and human breast cancer cells. Life Sciences, 66, 14.

Li Q, Niwa K, Hunter MG (2004): Effets of 17β-estradiol on *in vitro* maturation of pig oocytes in protein-free medium. Journal of Reproduction Development, 50, 305–313.

Makarevich A, Sirotkin A, Taradajnik T, Chrenek P (1997): Effects of genistein and lavendustin on reproductive processes in domestic animals *in vitro*. The Journal of Steroid Biochemistry and Molecular Biology, 63, 329–337.

Malekinejad H, Maas-Bakker RF, Fink-Gremmels J (2005): Bioactivation of zearalenone by porcine hepatic biotransformation. Veterinary Research, 36, 799–810.

Malekinejad H, Schoevers EJ, Daemen I, Zijistra C, Colenbrander B, Fink-Gremmels J, Roelen BAJ (2007): Exposure of oocytes to the fusarium toxins zearalenone and deoxynivalenol causes aneuploidy and abnormal embryo development in pigs. Biology of Reproduction, 77, 840–847.

Matthai C, Rohr UD, Metka M, Huber JC, Imhof M (2003): Estrogen receptor modulation by plant hormones. GYNE, 44., 1–20. (in Czech)

McGinnis LK, Carroll DJ, Kinsey WH (2011): Protein tyrosine kinase signaling during oocyte maturation and fertilization. Molecular Reproduction and Development, 78, 831–845.

Menzel VA, Hinsch E, Hägele W, Hinsch KD (2007): Effect of genistein on acrosome reaction and zona pellucida binding independent of protein tyrosin kinase inhibition in bull. Asian Journal of Andrology, 9, 650–658.

Minervini F, Dell'Aquila ME (2008): Zearalenone and reproductive function in farm animals. International Journal of Molecular Sciences, 9, 2570–2587.

Minervini F, Dell'Aquila ME, Maritato F, Minoia P, Visconti A (2011): Toxic effects of the mycotoxin zearalenone and its derivatives on *in vitro* maturation of bovine oocytes and 17 beta-estradiol levels in mural granulosa cell cultures. Toxicology in Vitro, 15, 489–495.

Mohamed ESA, Park YJ, Song WH, Shin DH, You YA, Ryu BY, Pang MG (2011): Xenoestrogenic compounds promote capacitation and an acrosome reaction in porcine sperm. Theriogenology, 75, 1161–1169.

Moravcová J, Kleinová T (2002): Phytoestrogens in nutrition – do they bring or involve risk? Chemické Listy, 96, 282–286.

Nelson PE, Dignani MC, Anaissie EJ (1994): Taxonomy, biology and clinical aspects of Fusarium species. Clinical Microbiology Reviews, 7, 479–504.

Obremski K, Gajecki M, Zwierzchowski W, Zielonka L, Otrocka-Domagala I, Rotkiewicz T, Mikolajczyk A, Gajecka M, Polak M (2003): Influence of zearalenone on reproductive system cell proliferation in gilts. Polish Journal of Veterinary Sciences, 6, 239–245.

Opletal L, Šimerda B (2006): Toxic agents of natural origin (secondary metabolites of plants and fungi) in raw materials for production of feedstuffs and possibilities of their determination. Institute of Animal Science, Prague-Uhříněves, 1–79.

Opletal L, Šimerda B (2009): Flavanoids in animal nutrition. http://www.vuzv.cz/old/vyziva/studie29.pdf. Accessed 8 March, 2009 (in Czech)

Park YJ, Mohamed ESA, Kwon WS, You YA, Ryu BY (2011): Xenoestrogenic chemicals effectively alter sperm functional behaviour in mice. Reproduction Toxicology, 32, 418–424.

Romero V, Cruz CD, Pereira OCM (2008): Reproductive and toxicological effects of isoflavones on female offspring of rats exposed during pregnancy. Animal Reproduction, 5, 83–89.

Röhrdanz E, Ohler S, Tran-Thi QH, Kahl R (2002): The phytoestrogen daidzein affects the antioxidant enzyme system of rat hepatoma H4IIE cells. The American Society for Nutritional Sciences, 132, 370–375.

Rosselli M, Reinhart K, Imthurn B, Keller PJ, Bubey RK (2000): Cellular and biochemical mechanisms by which environmental oestrogens influence reproductive function. Human Reproduction, 6, 332–350.

Sambuu R, Takagi M, Namula Z, Otoi T, Shiga S, Santos RR, Fink-Gremmels J (2011): Effects of exposure to zearalenone

on porcine oocytes and sperm during maturation and fertilization *in vitro*. Journal of Reproduction and Development, 57, 547–550.

Sambuu R, Takagi M, Namula Z, Nii M, Taniguchi M, Uno S, Kokushi E, Tshering C, Dos Santos RR (2013): Effects of long-term *in vitro* exposure of ejaculated boar sperm to zearalenone and α-zearalenol in sperm liquid storage medium. Animal Science Journal, 84, 28–34.

Schoevers EJ, Santos RR, Colenbrander B, Fink-Gremmels J, Roelen BA (2012): Transgenerational toxicity of zearalenone in pigs. Reproduction Toxicology, 34, 110–119.

Shertzer HG, Puga A, Chang Ch, Smith P, Nebert DW, Setchell KDR, Dalton TP (1999): Inhibition of CYP1A1 enzyme activity in mouse hepatoma cell culture by soybean isoflavones. Chemico-Biological Interactions, 123, 31–49.

Singleton DW, Sohaib AK (2003): Xenoestrogen exposure and mechanisms of endocrine disruption. Frontiers in Bioscience, 8, 110–118.

Steensma A (2006): Bioavailability of genistein and its glycoside genistin. Dissertation, Wageningen University.

Stopper H, Schmitt E, Kobras K (2005): Genotoxicity of phytoestrogens. Fundamental and Molecular Mechanisms of Mutagenesis, 574, 139–155.

Tapiero H, Ba GN, Tew KD (2002): Estrogens and environmental estrogens. Biomedical Pharmacotherapy, 26, 36–44.

Tham DM, Gardner CD, Haskell WL (1998): Clinical review 97 – potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. Journal of Clinical Endocrinology and Metabolism, 83, 2223–2235.

Tiemann U, Tomek W, Schneider F, Vanselow J (2003): Effects of the mycotoxins alpha- and beta-zearalenol on regulation of progesterone synthesis in cultured granulosa cells from porcine ovaries. Reproductive Toxicology, 17, 673–681.

Tiemann U, Schneider F, Vanselow J, Tomek W (2007): *In vitro* exposure of porcine granulosa cells to the phytoestrogens genistein and daidzein: effects on the biosynthesis of reproductive steroid hormones. Reproductive Toxicology, 24, 317–325.

Tirone E, Dálessandris C, Hascall VC, Siracusa G, Salustri A (1997): Hyaluronan synthesis by mouse cumulus cells is regulated by interactions between follicle-stimulating hormone (of epidermal growth factor) and a soluble oocyte factor (or transforming growth factor β1). Journal of Biological Chemistry, 272, 4787–4794.

Tsakmakidis IA, Lymberopoulos AG, Alexopoulos C, Boscos CM, Kynakis SC (2006): *In vitro* effect of zearalenone and α-zearalenol on boar sperm characteristics and acrosome reaction. Reproduction of Domestic Animals, 41, 394–401.

Van Cauwenberg A, Alexandre H (2000): Effect of genistein alone and in combination with okadaic acid on the cell cycle resumption of mouse oocytes. The International Journal of Developmental Biology, 44, 409–420.

Vodková Z, Rajmon R, Petr J, Klabanová P, Jílek F (2008): Effects of genistein and genistin on *in vitro* maturation of pig oocytes. Czech Journal of Animal Science, 53, 1–8.

Vrzáňová M, Heresová J (2003): Fytoestrogens. Interní medicína pro praxi, 9, 448–451. (in Czech)

Weidenbörner MJ, Wieczorek C, Appel S, Kunz B (2000): Whole wheat and white wheat flour – the mycobiota and potential mycotoxins. Food Microbiology, 17, 103–107.

Whitten PL, Patisaul HB (2001): Cross-species and interassay comparisons of phytoestrogen action. Environmental Health Perspectives, 109, 5–20.

Whitten PL, Lewis C, Russell E, Naftolin F (1995): Potential advesrse effects of phytoestrogens. Journal of Nutrition, 125, 771–776.

Young LG, King J (1986): Low concentrations of zearalenone in diets of boars for a prolonged period of time. Journal of Animal Science, 63, 1197–1200.

Zinedine A, Soriano JM, Molto JC, Manes J (2007): Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food and Chemical Toxicology, 45, 1–18.

Corresponding Author:

Ing. Lenka K ř i v o h l á v k o v á , Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Animal Husbandry, Kamýcká 129, 165 21 Prague 6-Suchdol, Czech Republic, tel.: +420 224 382 796, e-mail: krivohlavkova@af.czu.cz