Porcine oocytes are most vulnerable to the mycotoxin deoxynivalenol during formation of the meiotic spindle

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Abstract

Deoxynivalenol (DON, vomitoxin) is a secondary metabolite and mycotoxin produced by Fusarium species that occurs with a high prevalence in cereals and grains intended for human and animal consumption. Pigs are considered to be the most sensitive animal species and exposure to DON results in reduced feed intake, reduced performance and cause alterations in the expression of markers of inflammation and cell cycle regulation. The objective of this study was to determine how DON possibly affects the oocyte developmental potential in vitro at concentrations which correspond to those observed in practice. To evaluate DON toxicity during specific stages of oocyte meiosis, cumulus-oocyte complexes were exposed to 0.02, 0.2, or 2 μM DON. Exposure to the highest DON concentration inhibited cumulus expansion and induced cumulus cell death. After exposure for 42 h, DON at all concentrations reduced Metaphase II formation and led to malformations of the meiotic spindle. Despite spindle malformations, exposure to different concentrations of DON did not lead to increased percentages of blastomeres with abnormal ploidy in embryos. Spindle malformation occurred by DON exposure during formation of meiotic spindles at Metaphase I and II, but embryo development was also reduced when oocytes were exposed to DON during Prophase I. Together, these results indicate that exposure to DON via contaminated food or feed can affect oocyte developmental competence by interfering directly with microtubule dynamics during meiosis, and by disturbing oocyte cytoplasmic maturation through other as yet undetermined mechanisms.

Keywords: Deoxynivalenol; Oocyte maturation; Meiotic spindle

1. Introduction

Deoxynivalenol (DON, vomitoxin) belongs to the trichothecene group of mycotoxins produced by different species of the genus Fusarium, particularly F. graminearum. DON contamination occurs worldwide in cereal crops at concentrations ranging from a few microgram to more than 30 mg/kg [1]. Since DON is chemically very stable it is not inactivated during food and feed processing. Pigs are considered to be the most sensitive animal species and ingestion of DON contaminated feed materials results in reduced feed intake and weight gain together with altered immuno-competence and fertility [2–4].

Trichothecene toxicity at the cellular level is characterized by inhibited protein synthesis, impairment of
membrane functions, altered intercellular communication and deregulation of calcium homeostasis [2]. The molecular target of DON in proliferating eukaryotic cells is the 60S ribosomal subunit and binding of DON to ribosomal RNA induces a rapid activation of MAPKs followed by apoptotic cell death in a process that has been termed ‘ribotoxic stress response’ or by enhanced secretion of proinflammatory cytokines, causing injury in the surrounding tissue [5]. Subsequently DON has an anti-proliferative effect on bone marrow cells, the gastrointestinal epithelium, immune cells [6,7] and endometrial cells [8,9]. Originally, Morrissey and Vesonder had already in 1985 suggested on the basis of rodent studies that DON interferes in female fertility as observed by reduced pregnancy [10]. More recent in vitro experiments also suggest that DON may affect porcine fertility by inhibiting oocyte maturation [11,12].

The mechanism of oocyte maturation encompasses interactions between the oocyte and its surrounding cumulus cells, which synchronizes meiosis with structural and molecular changes in the ooplasm enabling the oocyte to support proper fertilization and subsequent embryo development.

When maturation starts the oocyte resumes meiosis from the diplotene stage of Prophase and porcine oocytes enter Metaphase I (M I) 20–22 h after resumption of meiosis, where after it takes the oocyte another 20–22 h to progress towards the Metaphase II (M II) stage [13]. Spindle formation is the result of a dynamic assembly of microtubules and abnormalities in microtubules are associated with abnormal chromosome segregation after fertilization and as a consequence developmental arrest and mosaic aneuploidy in embryos has been reported [14–16]. Reduced embryo development may also be the result of improper fertilization, where multiple spermatozoa penetrate the oocyte and a non-diploid lethal embryo will be formed.

Surrounding cumulus cells are crucial for the oocyte to acquire developmental competence, since they regulate progression through meiosis and protect the oocyte against oxidative stress by synthesis and transport of glutathione (GSH) to the oocyte. During maturation, cumulus cells expand and undergo cell death and both cumulus cell events are important for acquisition of oocyte developmental competence [17]. Since massive cumulus cell death together with a reduction in oocyte developmental potential was observed after exposure of cumulus-oocyte complexes (COCs) to DON during maturation [12], DON toxicity to the oocyte may be exerted via cumulus cells. On the other hand, oocytes prevent cumulus cell death by secretion of paracrine factors [18].

The objective of this study was to further elucidate the mechanisms involved in effects of DON during oocyte maturation and developmental competence. For these in vitro studies, concentrations were selected that reflect the expected blood levels of DON in pigs following exposure to naturally contaminated feed materials.

2. Material and methods

2.1. Chemicals and culture media

All chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise indicated. DON with a purity of >98% was dissolved in ethanol, diluted 1:10 in PBS to a final concentration of 2 mM, aliquoted and stored at -20 °C until used. Oocyte maturation medium (OMM) was M199 (Gibco BRL, Paisley, United Kingdom) supplemented with 2.2 mg/ml NaHCO3, 0.1% (w/v) polyvinylpiridilone, and 200 μM cysteamine. Recombinant human FSH (Organon, Oss, The Netherlands) was added to a final concentration of 0.05 IU/ml. DON was added to OMM to a final concentration of 0.02, 0.2, and 2.0 μM. OMM without DON (control) or with different DON concentrations all contained 0.01% ethanol (v/v). The IVF medium was modified Tris-buffered medium [19] containing 1 mM caffeine and 0.1% (w/v) BSA (Fraction V, fatty acid free). For washing of presumptive zygotes, NCSU-23 [20] supplemented with 0.4% BSA and 20 mM HEPES was used. Embryos were cultured in NCSU-23 with 0.4% BSA. All media, except media containing HEPES, were equilibrated at 38.5 °C and 5% CO2 for at least 2h before use.

2.2. Collection and culture of cumulus-oocyte complexes

Ovaries were collected from cyclic sows at slaughter and transported to the laboratory in a thermos flask within 2 h. Isolation and selection of COCs was as described previously [13]. Briefly, ovaries were kept at 30 °C in saline and COCs were aspirated from 3 to 6 mm follicles. The follicular contents were allowed to sediment and were washed in Tyrode’s lactate-HEPES medium [21]. Oocytes with compact cumulus cell mass were selected and transferred to a four-well culture dish (Nunc, Roskilde, Denmark) and 35–50 COCs were cultured in 500 μL
OMM with FSH for 21 h, washed in OMM, and further cultured for 21 h in 500 μL OMM without FSH. Culture of COCs was performed in a humidified atmosphere of 5% CO₂ in air at 38.5 °C. DON was present in OMM during the entire culture period (0–42 h), or during the first half (0–21 h) or second half (21–42 h) of the culture period.

2.3. Assessment of cumulus morphology

At 0, 21, and 42 h of culture, COCs were examined for cumulus expansion. The projected surface areas of individual COCs were measured from digitized images by ImageJ v1.30 image analysis software (NIH, Bethesda, MD, USA). Per experimental group, 141–179 COCs were examined in four replicate experiments. After 42 h of culture, unstained COCs were examined by light microscopy and subsequently cumulus cell degeneration was evaluated by Confocal Laser Scanning Microscopy (CLSM; Leica TCS MP, Heidelberg Germany) after live stain of COCs with Ethidium Homodimer-1 (Invitrogen- Molecular Probes, Eugene, OR, USA) and Sytox Green (Invitrogen-Molecular Probes) after fixation. Cumulus cells were considered as degenerated when nuclei were double stained, condensed or fragmented [17].

2.4. Assessment of nuclear maturation and microtubule organization

After culture, cumulus cells were removed from oocytes by repeated pipetting. Denuded oocytes were permeabilized for 1 h at 30 °C in a microtubule stabilizing solution, fixed with 4% formaldehyde, stained for microtubules with a monoclonal anti-α-tubulin antibody (DAKO, Glostrup, Denmark) and stained for chromatin with Sytox Green as described previously [17]. Oocytes were classified as follows: Metaphase I (M I) characterized by clumped or strongly condensed chromatin forming a metaphase plate together with spindle shaped structure of MTs; Anaphase-I (A I) stage showing two distinct sets of condensed chromatin inside the spindle; Telophase-I (T I) showing two distinct clumps of chromatin present at the poles of the spindle; Metaphase II (M II) showing condensed chromatin in a metaphase plate with microtubule spindle formation together with a separate polar body. Chromatin-microtubule complexes were considered aberrant when dispersed or condensed chromatin together with no clear microtubule spindle formation was observed. In five replicate experiments 1501 oocytes were examined.

2.5. Assessment of oocyte glutathione concentration

At 0 and 42 h of culture, cumulus cells were removed from oocytes by repeated pipetting. Denuded oocytes were stored at -20 °C and oocyte GSH content was determined as previously described [17]. Briefly, oocytes were lysed in deionized water and GSH was determined by 5,5'-dithio-bis(2-nitrobenzoic acid)-GSH reductase recycling micro-GSH assay. In four replicate experiments 477 oocytes were examined.

2.6. In vitro fertilization

In vitro fertilization of oocytes matured in presence of DON was performed as described previously [13]. Briefly, fresh extended semen from two randomly selected boars was equally pooled, washed and spermatozoa concentration determined. In the absence of DON, groups of 35–50 denuded oocytes were co-incubated with spermatozoa at a ratio of 1000 spermatozoa per oocyte for 24 h. Twenty-four hours after the onset of fertilization, adherent sperm cells were removed from the oocytes by repeated pipetting. Thereafter, oocytes were fixed with 4% formaldehyde in PBS, washed with PBS, stained for 5 min with 0.1 μg/mL 4,6-diamino-2-phenyl-indole (DAPI; Invitrogen-Molecular Probes), and mounted on slides. The nuclear state of the stained oocytes was assessed by fluorescence microscopy. Oocytes with two pronuclei without additional sperm heads or with one pronucleus together with one spermhead, or cleaved embryos with two to four normal blastomeres were considered as monospermic. Oocytes with 3 or more pronuclei or additional sperm heads were considered as being polyspermic. In five replicate experiments 637 oocytes were examined.

2.7. Embryo development

Embryo culture was performed as described previously [13]. Briefly, oocytes matured in presence of DON were fertilized and 24 h after the onset of fertilization presumptive embryos were subsequently transferred to NCSU-23 with 0.4% BSA (wt/vol) and cultured for 5 days in absence of DON. Two and 6 days after the onset of fertilization, development of the embryos was evaluated. To assess a possible dose-dependent effect of DON on embryo development, 1350 oocytes were examined in five replicate experiments. To appraise the sensitivity of oocytes to DON during prophase or formation of meiotic spindles at M I and M II, in four replicates, 829 oocytes and 1614 oocytes were examined after being exposed to 0.2 and 2.0 μM DON, respectively.
2.8. Assessment of aneuploidy in embryos

Embryos at the blastocyst stage were prepared as described for bovine embryos [22]. Fluorescence in situ hybridization (FISH) and cytogenetic analysis were performed as described previously [12]. Clones 192B9, 375B12 (both digoxigenin labeled) and 498D8 (biotin labeled) from a porcine BAC library [23] were used as probes to hybridize with the centromeric regions of chromosomes 7 and 14 and specific hybridization sites were visualized using FITC-labeled avidin and Cy3-labeled mouse-anti-DIG antibodies. Nuclear DNA was counterstained with 20 ng DAPI in 1 mL antifade solution (Vectashield; Vectorlab, Burlingame, CA, USA).

2.9. Statistical analysis

Statistical analysis was conducted with SPSS software (SPSS Inc., Chicago, IL, USA). The data from nuclear development, fertilization, embryo development followed a binomial distribution and were analyzed by means of logistic regression [24] with the model: \( \ln \left( \frac{p}{1-p} \right) = \alpha + \text{treatment} \), where \( p \) = frequency of positive outcome and \( \alpha \) = the intercept. Diploidy of nuclei in separate blastocysts also followed a binomial distribution and were analyzed by means of a Generalized Linear Model with the model: \( \log \left( \frac{p}{1-p} \right) = \alpha + \text{treatment} + \text{number of cells} \), where \( p \) = frequency of positive outcome and \( \alpha \) = the intercept. In both models, treatment was applied as an independent categorical variable and number of cells as a covariate. Data from COC surface areas were analyzed by a paired t-test, whereas data of glutathione concentration in oocytes and cell number in blastocysts were analyzed by a two-sample t-test with Bonferoni correction.

3. Results

3.1. DON inhibits cumulus expansion at high concentrations

To determine the dose-response effect of DON exposure to cumulus cells, COCs were cultured for 42 h in presence of 0.02, 0.2, or 2 \( \mu \)M DON. At the start of culture, the mean ± SD of the projected surface areas of individual COCs was 0.043 ± 0.013 mm\(^2\) (Fig. 1A). After 21 h of culture, cumulus cells had significantly (\( P < 0.05 \)) expanded their area to 0.083 ± 0.043, 0.078 ± 0.037 and 0.087 ± 0.042 mm\(^2\) when cultured in absence or presence of 0.02 or 0.2 \( \mu \)M DON, respectively (Fig. 1B). However, after exposure to 2 \( \mu \)M DON for 21 h no measurable
expansion could be noted as the surface area of COCs remained unchanged at 0.045 ± 0.018 mm² (Fig. 1C). After 42 h of culture, cumulus cells from the outer layers in the COCs expanded further and attached to the surface of the culture dish in control COCs (Fig. 1D) and those exposed to 0.02 or 0.2 μM DON, whereas cumulus cells cultured in presence 2 μM DON became black and shrunken and detached from the oocyte (Fig. 1E). Further analysis of COCs by ethidium homodimer and sytox green staining followed by CLSM revealed that cumulus cells exposed to 2 μM DON showed more marked signs of apoptosis (Fig. 1FG) than those from control COCs or those exposed to 0.02 and 0.2 μM DON.

3.2. DON reduces progression through meiosis

To evaluate the dose-effect relation between DON exposure and progression through meiosis of oocytes, COCs were continuously exposed to 0.02, 0.2 or 2 μM DON during in vitro maturation and after 42 h culture oocytes were examined for specific stages of meiosis. DON exposure to oocytes during maturation resulted in decreased progression through meiosis: with increasing concentrations of DON significant less oocytes reached the M II stage and more oocytes with chromatin and microtubule aberrations were observed (Fig. 2A). Moreover, the proportions of oocytes at the T I stage were 3 to 4% after exposure to 0, 0.02, or 0.2 μM DON, whereas cumulus cells cultured in presence 2 μM DON became black and shrunken and detached from the oocyte (Fig. 1E). Further analysis of COCs by ethidium homodimer and sytox green staining followed by CLSM revealed that cumulus cells exposed to 2 μM DON showed more marked signs of apoptosis (Fig. 1FG) than those from control COCs or those exposed to 0.02 and 0.2 μM DON.

3.3. DON does not affect glutathione levels in oocytes during maturation

To investigate whether the reduced progression through meiosis in oocytes during exposure to DON is attributable to a reduced production of anti-oxidants by granulosa cells during maturation, oocytes were examined for glutathione content at 0 and 42 h exposure to 0.02, 0.2, or 2 μM DON. In control oocytes, oocyte glutathione content varied from 2.5 ± 0.6 pmol/oocyte at 0 h and significantly increased to 7.3 ± 1.8 pmol/oocyte after 42 h culture. No significant differences in oocyte glutathione content were found between control and DON treated oocytes (7.4 ± 1.4, 5.9 ± 1.9, and 6.4 ± 3.1 pmol/oocyte exposed to 0.02, 0.2, or 2 μM DON) after 42 h in culture.

3.4. DON impairs oocyte fertilization

To understand whether reduced oocyte developmental potential by DON is caused by improper fertilization, DON exposed oocytes were fertilized and examined for sperm penetration and pronucleus development 24 h after the onset of fertilization. No difference in sperm penetration was observed between control oocytes (67%) or oocytes exposed to 0.02 or 0.2 μM DON (67 and 71%). However, exposure to 2 μM DON significantly (P < 0.001) reduced the percentage of sperm-penetrated oocytes to 43%. Expressed as percentage of sperm-penetrated oocytes, control oocytes and those exposed to 0.02 μM DON exhibited significant (P < 0.05) lower polyspermia rates (47 and 56%, respectively) than oocytes exposed to 0.2 or 2.0 μM DON (73 and 74%), respectively.

3.5. DON reduces embryo development

To evaluate the dose effect of DON-exposure during oocyte maturation on the developmental capacity, COCs were continuously exposed to 0.02, 0.2, or 2 μM DON after which oocytes were fertilized and presumptive embryos cultured in the absence of DON. When maturing oocytes had been exposed to DON, reduced percentages of both four-cell embryos and blastocysts were recorded after fertilization, in a dose-dependant manner (Fig. 3). Since DON induced spindle abnormalities and increased polyspermia in oocytes, the resulting embryos were expected to carry altered ploidy or display more nuclei in one of
Fig. 2. Progression through meiosis of porcine oocytes after culture for 42 h in the absence (control) or presence of 0.02, 0.2, or 2 μM deoxynivalenol (DON) (A) and after exposure to 2 μM DON from 0–21h during Germinal Vesicle breakdown or during 21–42h when the oocyte progressed towards Metaphase II (B). After culture, oocytes were stained for chromatin and microtubules and percentages of oocytes at Metaphase II (black bars), Anaphase I and Telophase I (gray bars) or with chromatin-microtubule aberrations (white bars) were determined. Data are from 5 replicate experiments. Within identical bars, bars with different symbols (abc, *, **, or 1234) are significantly different (P < 0.05). (C) Confocal laser scanning photomicrographs of chromosomes and microtubules in porcine oocytes. Red: microtubules; Green: chromatin; Yellow: overlap. Control oocytes: Metaphase I with chromatin inside the spindle (C1), Anaphase I with chromatin being pulled towards the spindle poles (C2), Telophase I with chromatin located at both ends of the spindle (C3) and Metaphase II with chromatin inside spindle and polar body with remnants of microtubules (C4). Oocytes matured in the presence of 2 μM DON: reduced microtubule formation at Prometaphase I (C5), disorganized chromatin and microtubule structures during Metaphase I (C6), arrested at Telophase I with reduced size of the spindle and with condensed chromatin at the spindle poles (C7) and aberrant Metaphase II plate with condensed chromatin and disorganized microtubules (C8).
its blastomeres, possibly leading to premature loss of blastomeres during early embryo development [12,14]. Therefore, four-cell embryos and blastocysts from control and DON exposed oocytes were examined by FISH for diploidy of chromosomes 7 and 14 and for numbers of nuclei. With four-cell embryos derived from control oocytes, 70% of cells were diploid for chromosomes 7 and 14, whereas 46, 43, and 45% of cells from four-cell embryos from oocytes exposed to 0.02, 0.2, or 2 μM DON respectively were diploid for chromosomes 7 and 14. In blastocysts a significantly lower percentage of diploid blastomeres and a reduced number of nuclei were observed when these embryos were derived from oocytes matured in the presence of 2 μM DON (Fig. 3).

To investigate whether DON reduces oocyte developmental competence by formation of aberrant spindles or by cumulus cell degeneration, COCs were exposed to 0.2 or 2 μM DON during Prophase I (0–21 h) or during formation of meiotic spindles at M I and M II (21–42 h). The oocytes were subsequently fertilized in vitro and embryos cultured. Development of four-cell stage embryos was not reduced when oocytes were exposed for 0–21 h or 21–42 h to 0.2 μM DON, but blastocyst development was significantly reduced when exposed to DON during 0–21 h or 21–42 h of maturation (Fig. 4A). Oocytes exposed to 2 μM DON during 0–21 h of maturation revealed significantly reduced embryo development at the four-cell and blastocyst stages and DON reduced embryo development even further when it was present during 21–42 h of culture (Fig. 4B). Following these results it can be assumed that DON not only interferes with meiotic spindle formation but also with processes that precede spindle formation, since exposure to DON during 0–21 h of maturation already reduced the developmental potential of the oocytes without affecting meiotic spindle formation.

4. Discussion

Here we report findings resulting from in vitro exposure of COCs to different concentrations of the mycotoxin deoxynivalenol (DON). For the in vitro experiments three exposure levels were selected, i.e. 0.02, 0.2, and 2 μM. The data show that DON can adversely affect maturation of porcine oocytes. In particular, DON caused abnormalities of the meiotic spindles. However, abnormal spindle formation does not exclusively account for the reduced oocyte developmental competence since exposure to DON during germinal vesicle breakdown resulted in normal progression through meiosis, but reduced embryo development after fertilization.

Cumulus expansion was completely inhibited by DON at 2 μM, but not at lower concentrations,
indicating a threshold level for DON toxicity in cumulus cells. Cumulus expansion results from the deposition of hyaluronic acid by cumulus cells in the extracellular space and is under control of GDF-9, a protein secreted by oocytes [25,26]. DON is known to inhibit protein synthesis in different cell types, but it remains unclear whether absence of cumulus expansion is caused by reduced GDF-9 or hyaluronic acid production. In addition to reduced cumulus expansion, cumulus cells showed an increased apoptotic response as indicated by the morphology of their nuclei when COCs were exposed to 2 μM DON (data not shown). Oocyte-secreted proteins can prevent cumulus cells from undergoing apoptosis [18] and therefore cumulus cell apoptosis may have resulted from reduced secretion of such proteins. The increased number of apoptotic cells following exposure to 2 μM DON was confirmed by the alamar Blue® assay (data not shown). These findings are in line with previous investigations devoted to the toxic mechanism exerted by DON in human lymphocytes [7]. In contrast, cultured porcine endometrial cells have been reported to become necrotic after exposure to 3.76 μM DON [8].

DON interferes with oocyte progression through meiosis by inducing malformation of the meiotic spindles. When oocytes matured in the presence of 2 μM DON, spindle aberrations at the M I stage were observed, and oocytes maturing in the presence of 0.2 or 0.02 μM DON exhibited spindle aberrations after reaching the M II stage. This is in agreement with our previous results [12]. The observations that (a) maturing oocytes were most sensitive for DON between the M I and the M II stages and (b) meiosis was interrupted at earlier stages when exposed to higher DON concentrations imply that DON directly affects microtubule polymerization. DON may also affect the function of kinetochore proteins, which form the spindle checkpoint switch, controlling the dynamics of the meiotic spindle during meiosis. Improper function of kinetochore proteins will deactivate the spindle checkpoint switch leading to meiotic arrest at the M I or T I stages [15].

DON impairs oocyte developmental potential as demonstrated by reduced embryo development at several levels: (a) before meiotic spindle formation, (b) during meiotic spindle formation, and (c) during fertilization. Meiotic spindle formation and embryo development was affected at all concentrations of DON, with a linear dose-response following exposure during the entire incubation period of 42 h.
However, limited exposure of COCs to 0.2 or 2 μM DON during Prophase I allowed normal progression through meiosis but reduced further embryo development. Absence of cumulus cell expansion and induction of cumulus cell death negatively influence oocyte maturation by altering glutathione levels in the oocyte [27,28,17]. Since DON only inhibited cumulus expansion and induced cumulus cell death at 2 μM, but both 0.2 and 2 μM DON reduced embryo development it is suggested that abnormal cumulus cell function cannot be the only cause for reduced embryo development. This is supported by the observation that continuous exposure to different DON concentrations did not influence oocyte glutathione content. During Prophase I, DON may inhibit the synthesis and the secretion of GDF-9 and BMP-15 in the oocyte and it is demonstrated that these factors predispose for proper embryo development [29].

Fertilization of oocytes matured in the presence of DON resulted in increased percentages of polyspermic zygotes. Various environmental chemicals are known to affect the distribution and exocytosis of cortical granules in oocytes after fertilization [30]. DON did not show a comparable effect (results not shown) hence the mechanisms involved in the formation of polyspermic oocytes remains to be elucidated. Direct impairment of the zona pellucida by residual amounts of DON during the process of fertilization can be excluded because fertilization took place in a DON-free medium and DON is rapidly removed by passive diffusion from all cells [31,32].

Presence of DON during oocyte maturation predisposed for abnormal chromosome numbers in blastomeres during early embryo development, probably as a result of aberrant spindles in matured oocytes and polyspermic penetration during fertilization. Only in blastocysts, derived from oocytes exposed to 2 μM DON, the increase in cells with abnormal chromosome numbers was also accompanied with reduced cell numbers. The relation between blastocyst cell number and diploidy has been described earlier and anomalies in microfilament distribution would impair blastomere cell division [16].

In this study oocytes have been exposed to DON for a maximum period of 42 h. In contrast, in vivo (farm) animals or humans are exposed to DON during a longer period, since DON is ingested via contaminated feed or food. It has been described that in pigs chronic DON exposure does not alter progesterone and oestradiol production by granulosa cells, and does not impair follicular development [33,34]. In our study oocytes were exposed to DON specifically during maturation, but since DON interacts with microtubules, effects of DON can certainly be expected when oocytes and embryos are exposed to DON during fertilization or during early embryonic development. Interestingly, when four-cell embryos that originated from control oocytes (not exposed to DON) were cultured in the presence of DON, development to the blastocyst stage was completely inhibited in the presence of 2 μM DON. Exposure of embryos to lower concentrations of DON (0.02 and 0.2 μM) did not affect blastocyst development (data not shown), indicating that the maturing oocyte is particularly sensitive to DON exposure as oocytes maturing in the presence of 0.2 or 0.02 μM DON exhibited spindle aberrations after reaching the M II stage.

This study provides evidence that different mechanisms of DON toxicity impair oocyte developmental potential. DON is known to inhibit protein synthesis and to promote apoptosis. Moreover, DON has been described to induce the expression of pro-inflammatory cytokines as TNF-α, IL-1β, IL-6 in lymphoid tissues [35], and these pro-inflammatory cytokines can impair fertility [36–38]. Particularly TNF-α has been demonstrated to impair oocyte developmental potential in both pigs and cattle [39,40].

In conclusion, our results indicate that DON can impair oocyte developmental competence by interfering with microtubule dynamics during meiosis, and by disturbing oocyte cytoplasmic maturation. The outcome of the analysis of the specific stages of meiosis in relation with (a) cumulus cell viability, (b) spindle malformation, (c) altered fertilization, and (d) diploidy of embryos indicate that DON affects oocyte developmental at different levels of oocyte maturation. These effects are relevant for the risk assessment of DON, as the in vitro test were conducted with toxin concentrations that are likely to occur under in vivo conditions. Indeed, plasma levels of 0.06–0.09 μM have been determined in pigs fed a DON contaminated diets for a period of 5–8 weeks [41]. In humans, urine samples analysed for DON to quantify exposure levels of normal consumers of grains and cereal products, showed that DON can be detected in more than 90% of urine samples obtained from healthy consumers at concentrations up to 0.125 μM [42]. These findings underline the importance of further research on the effects of DON on human and animal reproduction.
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