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Deoxynivalenol induces toxic effects in the ovaries of pigs: An *ex vivo* approach

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ABSTRACT

Deoxynivalenol (DON) is a frequently found trichothecene mycotoxin that elicits toxic effects on humans and animals. In pigs, DON induces changes in digestive and immune systems. Effects on the reproductive system are scarce and mainly based in *in vitro* models. The aim of this study was to evaluate, using an *ex vivo* model, the effects of DON on the morphology of ovaries of pigs in all stages of follicular development. Six 5-month-old pigs were used for sampling the explants. Thirty-six explants were incubated for 48 hours in culture medium (n = 18) or medium containing 10 μM of DON (n = 18). After the incubation period, the explants were submitted to histologic and immunohistochemical (proliferating cell nuclear antigen [PCNA] expression) analysis. Histologic changes were scored, and a lesional score was established. Oocytes and follicular cells immunostained for PCNA were counted. Explants exposed to DON showed a significant increase in the lesional score ($P = 0.0004$) compared to control explants. The main histologic changes were degeneration of oocytes and granulosa cells, interstitial edema and pyknotic cells. DON induced a reduction in the number of normal follicles in all stages of follicular development: primordial ($P = 0.005$), primary ($P = 0.04$), and growing follicles ($P = 0.04$) compared to control group. Deoxynivalenol also induced a significant increase ($P \leq 0.05$) in the number of pyknotic oocytes in all stages of follicular development; however, no significant change in PCNA expression in oocytes or follicular cells was observed. These results indicated that DON induces toxic effects on the ovaries, affecting follicular development and interfering with reproductive parameters on pigs. Also, the present data indicate that ovarian explants are an adequate model for assessing reproductive toxicity.

1. Introduction

Mycotoxins are secondary metabolites of fungi that may contaminate animal and human feeds. Syndromes caused by mycotoxins range from acute mortality to slow growth and reduced reproductive efficiency [1].

Deoxynivalenol (DON), a mycotoxin of the trichothecene group, is one of the most frequent contaminants of food and feed. In a worldwide survey, DON was found in 55% (17,316 samples) of the cereal samples analyzed with a mean concentration of 967 μg kg^{-1} (250–50,228 μg kg^{-1}) [2]. The global occurrence of mycotoxins is considered an important risk factor for both human and animal health [1]. All animal species evaluated to date are susceptible to the deleterious effects of DON in the following order of susceptibility swine > mice > rats > poultry ≈ ruminants [3]. Differences in metabolism, absorption, distribution,

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and elimination of DON among animal species have been suggested to account for this differential sensitivity [4].

Animals exposed acutely to high doses of DON showed diarrhea, vomiting, leukocytosis, and hemorrhage, whereas chronic exposure induced anorexia, reduced weight gain and nutritional efficiency, and changes in the immune, neuroendocrine, and reproductive systems [5–7]. At the molecular level, DON inhibits protein synthesis by binding to the 28S ribosomal RNA peptidyl transferase site, inducing the phosphorylation of mitogen-activated protein kinases (MAPKs) and promoting apoptosis [8–10]. Deoxynivalenol also induces changes in cytokine gene expression [11] and on steroids hormones production [12].

The potential of trichothecenes to act as endocrine disruptors has been subject of increasing interest [13]. In a previous study, rats exposed to DON showed a reduction in female fertility characterized by reduced pregnancy [14]. In pigs, in *in vitro* models, DON affected the oocyte maturation [15–18] and embryo development [17]. Moreover, DON induced dose-dependent effects on granulosa cell proliferation, steroidogenesis, and steroidogenic gene expression [19,20]. Specifically, DON (0.034 μM and 0.34 μM) induced an increase in the number of granulosa cells, whereas 3.4 μM of DON drastically reduced the cells number [19]. On the other hand, porcine granulosa cells showed an increased expression of cell proliferation markers (cyclin B1 and PCNA) and progesterone production when exposed to 3.4 μM of DON [20].

Data about the toxicity of DON on reproductive system have used mainly cell line models focusing changes on oocytes or granulosa cell maturation [17,21]. In addition to cell line models, ovary explants culture has been used as an *in vitro* system for identifying endocrine-active compounds that alter steroid biosynthesis [22]. The main advantage of the explant model is the possibility to evaluate tissue morphology, maintaining the complex patterns of differentiation seen *in vivo* and bringing different results from that obtained with isolated single cell types. Therefore, the purpose of this study was to determine, through ovarian explant culture, the effects of DON on porcine morphology of ovaries. We also aimed to evaluate the effects on the integrity and proliferation of the ovarian follicles from the earliest (primordial and primary) to most advanced (secondary and tertiary follicles) stages.

2. Materials and methods

2.1. Ovaries samples

Ovaries (n = 6 pairs of ovaries) were collected at a local abattoir from six adult, nonpregnant sows (Landrace × Large White × Duroc), 5-month-old. Immediately postmortem, the ovaries were washed in 70% alcohol followed by two washes with Dulbecco Eagle Modified Medium (DMEM) (GIBCO, NY, USA). The ovaries were transported in DMEM at 20 °C, within 1 hour to the laboratory after slaughter.

2.2. Experimental protocol

In the laboratory, the ovaries were stripped of surrounding fat tissue and ligaments and then cut in half. Subsequently, the ovarian cortex of each ovary was divided into six fragments approximately 3 × 3 × 1 mm. The explants were transferred to six-well culture plates (Easy-Path, São Paulo, Brazil) containing 3 mL of culture medium (3 explants/well). The culture was performed for 48 hours, at 39 °C under a CO_2-controlled atmosphere, and all the media were incubated for 1 hour prior to use. The basic culture medium (control medium) consisted of DMEM supplemented with fetal bovine serum (10%), glutamine (0.4 μL/L), gentamicin (10 μL/mL) and penicillin/streptomycin (10 mL/L). For the experimental condition, the medium was supplemented with 10 μM of DON. The dose used in this experiment is based in previous *in vitro* [23] and *ex vivo* [24] studies that have shown toxic effects with this concentration for pig intestinal cells and tissue. This dose (10 μM) corresponds to an ingestion of 3 mg kg^{-1} of contaminated feed. The aim of this tissue incubation was only to allow the tissue to preserve its normal morphology, temperature, and metabolism [25]; there was no intention to promote cell growth.

2.3. Histological analysis of the ovarian explants

After the incubation period, explants were fixed in 10% buffered formalin solution, dehydrated in increasing alcohol solutions, diaphanized in xylene, and embedded in paraffin. Sections of 5 μm were stained with periodic acid of Schiff and hematoxylin and eosin for histological analysis. The histological changes were evaluated using a tissue score in which the intensity of lesions was considered. The lesional score was calculated by taking into account the extent of each lesion (according to intensity or observed frequency, scored from 0–3). The maximum score (24) indicates the overall lesion of the ovarian tissue. The criteria used to determine the lesional score are shown in Table 1. Therefore, each ovarian explant was analyzed according to eight endpoints (Table 1).

In addition, histological changes were evaluated according to the stages of follicular development. Follicles were classified into three categories as described previously [26]: (1) primordial follicles characterized by an

Table 1
Establishment of the lesional score–endpoints used to assess pigs histologic changes.

Tissue	Type of lesion	Intensity factor[a]	Maximum score
Granulosa cells	Detachment of the granulosa cell layer from the follicular basal membrane	0–3	24
	Disorganization of granulosa cells	0–3	
	Nuclear pyknosis	0–3	
Oocytes	Flattening or deformity of the pellucid zone	0–3	
	Cytoplasmic vacuolation	0–3	
	Nuclear pyknosis	0–3	
Ovarian stroma	Interstitial edema	0–3	
	Nuclear pyknosis	0–3	

[a] The extent of each lesion (intensity or observed frequency) was evaluated and scored as 0 = no lesion, 1 = low extent, 2 = intermediate extent, and 3 = large extent. The organ score was obtained by the sum of each lesion score.