Theriogenology 102 (2017) 59–66

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of zearalenone on circulating testosterone concentration, testicular and epididymal morphology and epididymal sperm characteristics in wild boars

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ARTICLE INFO

Article history:
Received 7 August 2016
Received in revised form
13 July 2017
Accepted 16 July 2017
Available online 18 July 2017

Keywords:
Wild boars
Sperm characteristics
Zearalenone
Testis
Epididymis
Biometry
Histology
Testosterone

ABSTRACT

This study investigated the effect of exposure to zearalenone (ZEN) and its metabolites on the characteristics of epididymal spermatozoa, testicular and epididymal biometry and histology, and the concentration of testosterone in blood plasma in male wild boars. The study was performed during more than one year on 18 clinically healthy male wild boars with initial and final body weight, of 39 ± 4 kg and 59 ± 3 kg, respectively. The animals were divided into two experimental groups (group I and group II) and one control group (group C) comprising 6 boars per group. Group I animals were administered *per os* pure zearalenone (ZEN) at 150 μg/kg BW for 7 consecutive days every two months, while group II animals received a dose of 50 μg/kg BW/day via feed that was naturally contaminated with ZEN. These male wild boars were exposed to ZEN over a period of 1 year. Control animals were fed a placebo. Testicles with epididymides of the boars were collected on the last day of the experiment within 3 min after slaughter. Blood samples were collected from each of the male wild boars. Testes and epididymides were measured and sampled for histological examination. Epididymides were dissected and epididymal spermatozoa were harvested. The spermatozoa were diluted with swine-specific BTS extender and stored at 17°C for 144 h. Sperm motility was analyzed with CASA, and other parameters including viability, acrosome integrity, DNA fragmentation index, lipid peroxidation and apoptosis were assessed with flow cytometry. In these wild boars, *per os* exposure to natural sources of ZEN or a combination of ZEN and its metabolites changed the testicular interstitium and led to modification of some epididymal sperm parameters. The interstitial glands in testes of experimental group I were markedly reduced and hyperemic with evident blood stasis in small capillaries. Also in group I were single degenerating seminiferous tubules. In both groups I and II, immediately after dilution of spermatozoa with BTS remarkable decreases in motility rate as well as in progressive motility and the subpopulation of cells with rapid movement were noted compared with the control group ($P < 0.05$). But unexpectedly, after 24 h incubation of boar semen in the BTS diluent, these sperm properties improved and were not significantly different from the control group. Thus, exposure to ZEN has no lasting but only a temporary, reversible effect on wild boar sperm motility. There was no influence of exposure to ZEN and its metabolites on the integrity of membranes, intensity of lipid peroxidation and apoptosis or on sperm chromatin structure. This study is the first using these direct measures of sperm motility and integrity to show a redundant adverse effect of ZEN exposure on wild boar sperm characteristics. There were no

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http://dx.doi.org/10.1016/j.theriogenology.2017.07.015
0093-691X/

effects of exposure to ZEN and its metabolites on body weight, testicular and epididymal biometry, gonadosomatic index and the concentration of testosterone in blood plasma in the male wild boars.

1. Introduction

Wild boars are bred for various purposes (such as meat production, public presentation, hunting and/or dog training) and this species has a wide geographic range [1,2]. The populations and habitats of wild boars are on the rise around the world mainly due to global climate change. In the natural environment, wild boars graze on plants contaminated with fungi that produce undesirable substances, including mycotoxins with estrogenic properties [3]. One of these mycotoxins is zearalenone (ZEN), which is produced by several species of Fusarium and contaminates cereal grains such as maize, wheat, oats and soybeans [4,5]. Zearalenone and its metabolites, α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), are estrogen-like substances, but unlike steroids, they do not have the base structure of sterane [6]. These toxins are almost entirely absorbed following oral administration, and when ingested over prolonged periods, even in NOAEL (no observable adverse effect level), can produce subclinical disease states [6]. ZEN and its metabolites bind competitively to estrogen receptors (ERα and ERβ) because they have sufficient structural similarity to endogenous estrogen, 17b-estradiol [7]. Thus, ZEN acts as an agonist and partial antagonist to estradiol and *via* estrogen gene activation it induces estrogenic effects and reproductive disorders, often reported as hyperestrogenism, in all species tested, particularly in pigs [8]. ZEN and its metabolites can act as potential endocrine disruptors (EDs) at the level of nuclear receptor signaling by altering production of hormones, including testosterone [9]. The toxic effects of ZEN and its metabolites on female reproduction have been well documented. The domestic pig *(Sus scrofa domestica)* is a livestock species that is most sensitive to the presence of ZEN, its metabolites and deoxynivalenol (DON) in feed [10]. Ruhr et al. [11] reported that ZEN, at levels normally found in contaminated feeds, is capable of causing severe reproductive problems in female swine (vulval edema and reddening, increased uterine mass, prolapse of the anus and vagina, pseudopregnancy, early abortion, and smaller and weaker litters). Multiparous female wild boars exposed to feed contaminated with ZEN at the beginning of winter demonstrated pathological changes in the ovaries that prolonged anestrus [3]. In contrast, *per os* administration of ZEN and its metabolites to female wild boars over a period of one year resulted in their accumulation in the animals' tissues, but concentrations were below the typical norms, and the mycotoxins did not to produce clinical symptoms of disease [6].

Data concerning effects of ZEN on the reproductive biology of male wild boars are scarce, although they represent an attractive model for studying testis function in male domesticated pigs [12]. Therefore, the results of scientific research and physiological data obtained with wild boars, which were domesticated for several thousands of years and are the ancestors of domestic pigs, can be extrapolated between wild boars and pigs without the risk of major errors [13,14].

Likewise, few studies have reported the influence of ZEN and its metabolites in male boars, possibly due to the lack of adverse effects on the reproductive potential of mature boars *in vivo*, with the exception of a previously reported reduction in plasma testosterone levels [15]. In pre-pubertal boars, ZEN consumption resulted in reduced libido and depressed plasma testosterone and delayed, but did not eliminate, development of sexual behavior [16]. However, numerous studies have revealed that the testes of other species are sensitive to ZEN. Yang et al. [17], showed that ZEN can reduce testosterone secretion by mouse Leydig cells *in vitro* and *in vivo*. ZEN at a concentration of 0.1 nM can also adversely affect sperm parameters in stallions after only 2 h of exposure [18]. However, to the best of our knowledge, there is a lack of available information on ZEN toxicity against the spermatozoa of male wild boars.

Therefore, this study aimed to analyze the effect of prolonged feeding of male wild boars with pure ZEN from natural sources or with feed naturally contaminated with this mycotoxin on the biological characteristics of epididymal spermatozoa. During the study, wild boar semen was diluted and stored in BTS extender for 144 h at 17 °C.

Other objectives of this study included evaluation of the effects of exposure to ZEN and its metabolites on the body weight, testicular and epididymal biometry and histology, gonadosomatic index and concentration of testosterone in blood plasma in male wild boars.

2. Materials and methods

2.1. Ethical principles

All experimental procedures involving animals were carried out in compliance with Polish legal regulations determining the terms and methods for performing experiments on animals (opinion of the local Ethics Committee for Animal Experimentation No. 35/2010 22.02 2010).

2.2. Experimental animals and feed analysis

The experiment was conducted at the Centre for Research into Forests and Game Breeding of the Wrocław University of Environmental and Life Sciences, Poland, on clinically healthy wild boars with an initial body weight of 39 ± 4 kg. The animals were penned in groups with *ad libitum* access to water. The feed administered was tested for the presence of mycotoxins: ZEN, α-ZEL and DON. Mycotoxin levels in the diets were estimated by common separation techniques using immunoaffinity columns (Zerala-Test™ Zearalenone Testing System, G1012,VICAM, Watertown, USA and DON-Test™ DON Testing System, VICAM, Watertown, USA) and high performance liquid chromatography. The following chromatographic conditions were applied: a Poroshell 12 SB-C18 column, 2.7 μm, 3.0 × 100 mm (Agilent P/N 695975-302), temperature −40 °C, phase A (10 mM $AcNH_4/H_20$), phase B (10 mM $AcNH_4$/MeOH + ACN), gradient: 5%B to 100%B in 15 min, flow − 0.8 ml/min, time of analysis − 15 min, sample volume − 1 μl, source: ESI, gas temperature − 300 °C, gas flow − 5 l/min, nebulizer − 45, capillary − 3500 V, Hewlett Packard liquid chromatograph (HPLC), 1050 and 1100, with fluorescent and/or UV detection [19].

In toxicological analyses in the diets Deoxynivalenol (DON) values were found to be below the sensitivity of the method.

2.3. Experimental design

The animals were divided randomly into two experimental