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REGULAR ARTICLE

RELEASE OF PROGESTERONE AND TESTOSTERONE BY OVARIAN GRANULOSA CELLS AFTER ADDITION OF T-2 TOXIN AND ITS COMBINATION WITH GROWTH FACTOR IGF-I

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ABSTRACT

The aim of the present study was to examine the effect of T-2 toxin and combination of this toxin with growth factor IGF-I on secretion of ovarian hormones progesterone P_4 and testosterone by ovarian granulosa cells (GCs) of gilts. Ovarian granulosa cells were incubated withouth (control) or with treatments at various doses for 48h: T-2 toxin (10, 100 and 1000 ng.ml^{-1}) / T-2 toxin (10,100 and 1000 ng.ml^{-1}) plus IGF-I (100 ng.ml^{-1}). Progesterone and testosterone were determined by RIA. Progesterone release by GCs was significantly (P<0.05) inhibited after addition of T-2 toxin at all doses 10, 100, 1000 ng.ml^{-1}. Release of testosterone was inhibited after addition of T-2 toxin at 10 and 100 ng.ml^{-1}. On the other hand significant (P<0.05) stimulation of testosterone release at the highest dose 1000 ng.ml^{-1} was observed. T-2 toxin in combination with growth factor IGF-I inhibited significantly (P<0.05) progesterone release by GCs at all used doses 10, 100, 1000 ng.ml^{-1}of T-2 toxin with 100 ng.ml^{-1} of IGF-I. Testosterone release was significantly (P<0.05) inhibited after addition of doses 100, 1000 ng.ml^{-1} of T-2 toxin with 100 ng.ml^{-1}of IGF-I. Our *in vitro* results examined

the dose-dependent effect of T-2 toxin and its combination with growth factor IGF-I on release of progesterone and testosterone by ovarian granulosa cells.

Keywords: T-2 toxin, IGF-I, testosterone, progesterone, granulosa cells

INTRODUCTION

T-2 toxin (T-2) which belongs to a trichothecene mycotoxin produced principally by *Fusarium* species, has been detected in a great number of field crops such as maize, wheat and oats **(WHO, 1990)**. T-2 toxin is rapidly absorbed after ingestion in most animal species and it is distributed in the organism with little or no accumulation in any specific organ or tissue **(WHO, 1990; SCF, 2001). Madhyastha *et al.* (1994)** demonstrated that among the 16 trichothecenes studied, T-2 showed the highest relative toxicity. *In vitro* human experiments have shown that T-2 toxin is rapidly metabolized to HT-2 toxin and, consequently, the toxicity of T-2 toxin *in vivo* might partly be attributed to HT-2 toxin **(Konigs *et al.*, 2009).** The chronic toxicity of trichothecenes is characterized by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes, and immunological effects **(Larsen *et al.*, 2004)**. T-2 toxin belongs to mycotoxins which are highly cytotoxic to actively dividing cells in lymphoid and hematopoietic tissues, intestinal crypt epithelium, testis, and ovary **(Saito *et al.*, 1969, Ueno, 1984, Ueno *et al.*, 1973).** In addition, T-2 toxin inhibits DNA, RNA, and protein synthesis in eukaryotic cells, affects the cell cycle, and induces apoptosis *in vivo* and *in vitro* **(Kiessling, 1986). Wu *et al.* (2010)** indicate that moderate to high levels of T-2 toxin cause a variety of toxic effects including immunosuppression, feed refusal, vomiting, weight loss, reduced growth and skin lesions. Mycotoxins as contaminants of animal feed can impair growth and/or reproductive efficiency. This is especially prominent in prepubertal gilts **(Danicke, 2002)**. **D´Mello *et al.* (1999),** demonstrated that trichothecene mycotoxins have been implicated in livestock reproductive disorders such as abortions and ovarian malfunctions. **Caloni *et al.* (2009)** observed in their study that T-2 has potent direct dose-dependent effects on granulosa cell proliferation and steroidogenesis. **Kovács *et al.* (2011)** examined in their study with rabbits that T-2 toxin decreased the basic testosterone level by 45% compared to control ($P < 0.01$) and resulted in lower ($P < 0.05$) GnRH-induced testosterone concentration.

The aim of this study was to examine the dose-dependent changes in the secretion of progesterone (P_4) and testosterone by ovarian granulosa cells after addition of T-2 toxin and T-2 toxin with insuline-like growth factor IGF-I.

MATERIAL AND METHODS

Ovaries (n=12) from cycling pigs were obtained from healthy Slovakian White gilts without visible reproductive abnormalities. Ovaries were transported to the laboratory in containers at 4ºC and washed in sterile physiological solution. Follicular fluid was aspirated from 3–5 mm follicles. Granulosa cells were isolated by centrifugation for 10 min at 200xg followed by washing in sterile DMEM/F12 1:1 medium (BioWhittaker™, Verviers, Belgium) and resuspended in the same medium supplemented with 10% fetal calf serum (BioWhittaker™, Verviers, Belgium) and 1% antibiotic–antimycotic solution (Sigma, St. Louis, Mo, USA) at a final concentration of 10^6 cells.ml^{-1} (as detected by haemocytometer). Portions of the cell suspension were dispensed to 24-welled culture plates (Nunc™, Roskilde, Denmark, 1 ml/well; for RIA). The well plates were incubated at 37.5ºC and 5% CO_2 in humidified air until a 75% confluent monolayer was formed (5 days), at this point, the medium was renewed and ovarian granulosa cells were incubated with the similar supplements (DMEM/F12 1:1 medium, 10% fetal calf serum, without 1% antibiotic–antimycotic solution) and without (control) or with T-2 toxin (10, 100, 1000 ng.ml^{-1}) (Romer Labs Division Holding GmbH, Tulln, Austria) and T-2 toxin in combination with IGF-I (100 ng.ml^{-1}) (Sigma – Aldrich, USA) for 48h. After 48h of culture the media from wells were removed and the culture media from well plates were aspirated and kept at –20ºC for subsequent assay. Concentrations of progesterone and testosterone were determined in 25-100 µl samples of incubation medium by RIA. The concentrations of progesterone and testosterone were assayed using Radioimmunoassay (RIA) according to the manufacture`s instructions. All RIAs were validated for use in samples of culture medium.

Statistical Analysis

Each experimental group was represented by four culture wells of granulosa cells. Assay of substances in incubation medium were performed in duplicate. Significance of differences between the control and experimental groups were evaluated by t-test using statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as

means ± SEM. Differences were compared for statistical significance at the P - level less than 0.05 (P<0.05).

RESULTS

Release of progesterone after addition of T-2 toxin (10, 100, 1000 ng.ml^{-1}) to ovarian GCs.

P_4 was released by the ovarian granulosa cells in the control group withouth T-2 toxin and in the experimental groups with T-2 toxin addition. Release of P_4 was (P<0.05) significantly inhibited in all experimental groups (10, 100, 1000 ng.ml^{-1}) in comparison with the control group (Fig. 1).

Release of testosterone after addition of T-2 toxin (10, 100, 1000 ng.ml^{-1}) to ovarian GCs.

Testosterone was released by the ovarian granulosa cells in the control group without T-2 toxin and in the experimental groups with T-2 toxin addition. After addition of 1000 ng.ml^{-1}of T-2 toxin we observed significant (P<0.05) stimulation in testosterone release (Fig. 2).

Release of progesterone after addition of T-2 toxin (10, 100, 1000 ng.ml^{-1}) *with growth factor IGF-I (100 ng.ml^{-1}) to ovarian GCs.*

P_4 was released by the ovarian granulosa cells in the control group without T-2 toxin plus IGF-I and in the experimental groups with T-2 toxin plus IGF-I addition. We observed significant (P<0.05) inhibition in progesterone release in all experimental groups after addition of combination of T-2 toxin with IGF-I (Fig. 3).

Release of testosterone after addition of T-2 toxin (10, 100, 1000 ng.ml^{-1}) with growth factor IGF-I (100 ng.ml^{-1}) to ovarian GCs.

Testosterone was released by the ovarian granulosa cells in the control group without T-2 toxin plus IGF-I and in the experimental groups with T-2 toxin plus IGF-I addition. Release of P_4 was significantly (P< 0.05) inhibited by addition of 100 ng.ml^{-1}of T-2 toxin with 100 ng.ml^{-1} of IGF-I and 1000 ng.ml^{-1} of T-2 toxin with 100 ng.ml^{-1} of IGF-I respectively in comparison with the control group (Fig. 4).

The effect of T-2 toxin on release of progesterone by ovarian granulosa cells

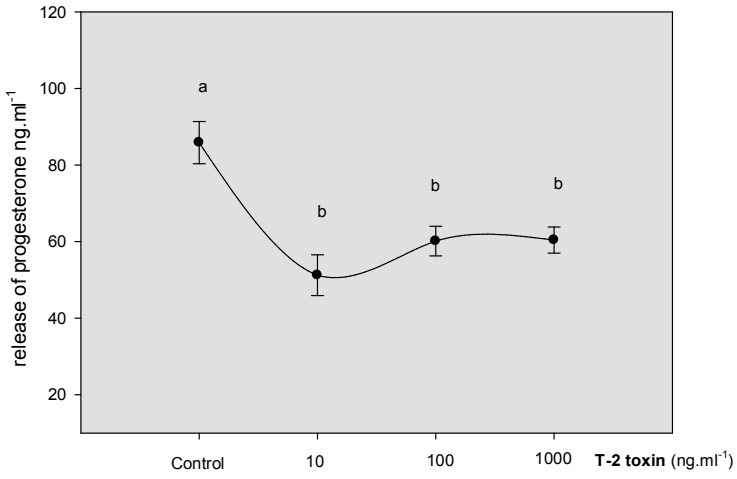

Figure 1 The effect of T-2 toxin on progesterone release by porcine ovarian granulosa cells

Control represents culture media without T-2 toxin addition; the other groups represent culture medium with T-2 toxin (10, 100, 1000 ng.ml^{-1}). Signs *a*,*b* denote values significantly different from control group (P<0.05) evaluated by paired t-test. RIA.

The effect of T-2 toxin on release of testosterone by ovarian granulosa cells

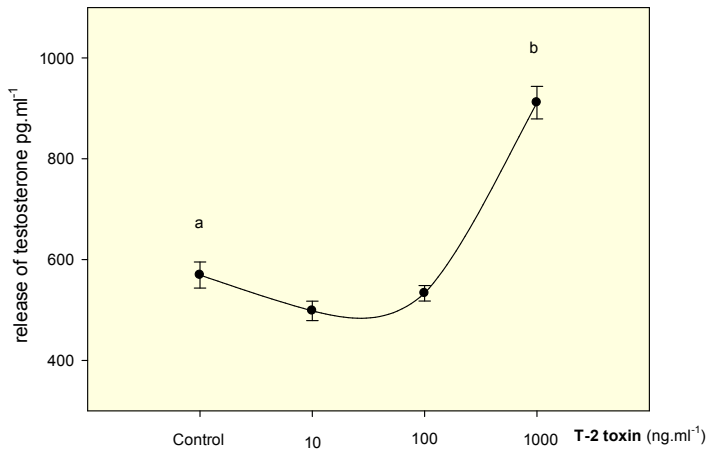

Figure 2 The effect of T-2 toxin on testosterone release by porcine ovarian granulosa cells

Control represents culture media without T-2 toxin addition; the other groups represent culture medium with T-2 toxin (10, 100, 1000 ng.ml^{-1}). Signs *a*,*b* denote values significantly different from control group (P<0.05) evaluated by paired t-test. RIA.

The effect of T-2 toxin with growth factor IGF-I on release of progesterone by ovarian granulosa cells

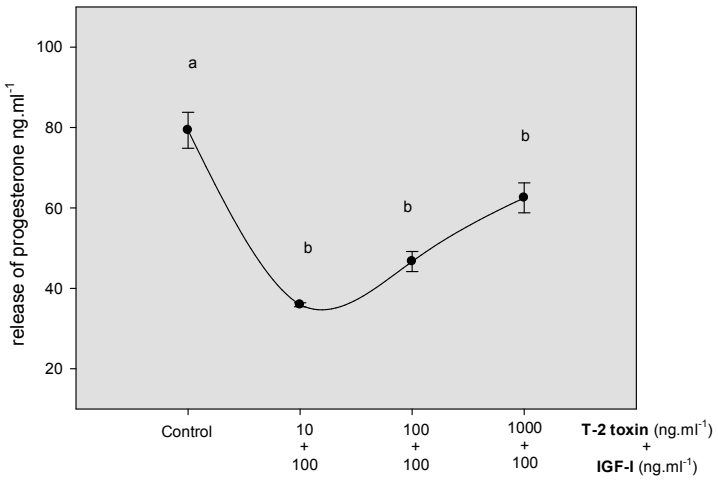

Figure 3 The effect of T-2 toxin in combination with IGF-I on progesterone release by porcine ovarian granulosa cells

Control represents culture media without T-2 toxin plus IGF-I addition; the other groups represent culture medium with T-2 toxin (10, 100, 1000 ng.ml^{-1}) plus IGF-I (100 ng.ml^{-1}). Signs *a b* denote values significantly different from control group (P<0.05) evaluated by paired t-test. RIA.

The effect of T-2 toxin with growth factor IGF-I on release of testosterone by ovarian granulosa cells

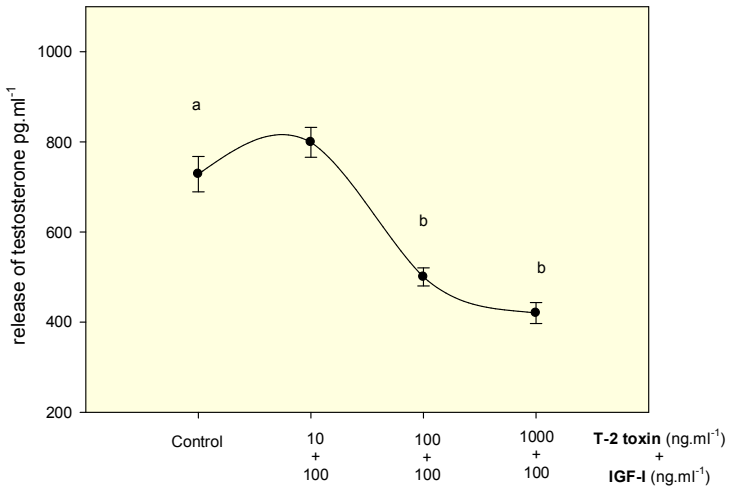

Figure 4 The effect of T-2 toxin in combination with IGF-I on testosterone release by porcine ovarian granulosa cells

Control represents culture media without T-2 toxin plus IGF-I addition; the other groups represent culture medium with T-2 toxin (10,100,1000 ng.ml^{-1}) plus IGF-I (100 ng.ml^{-1}). Signs *a b* denote values significantly different from control group (P<0.05) evaluated by paired t-test. RIA.

DISCUSSION

Results of our *in vitro* study indicate that the release of steroid hormones progesterone and testosterone were affected by addition of T-2 toxin and T-2 toxin with IGF-I. We observed that 1. the release of P_4 was (P<0.05) significantly inhibited by *Fusarium* toxin at doses 10, 100, 1000 ng.ml^{-1}, 2. the release of testosterone was significantly (P<0.05) stimulated after addition of 1000 ng.ml^{-1} 3. after addition of combination T-2 toxin with IGF-I we analysed significant (P<0.05) inhibition in progesterone release in all experimental groups 4. the release of P_4 was significantly (P<0.05) inhibited by addition of 100 ng.ml^{-1}of T-2 toxin plus 100 ng.ml^{-1} of IGF-I and 1000 ng.ml^{-1}of T-2 toxin plus 100 ng.ml^{-1}of IGF-I respectively.

T-2 toxin is a mycotoxin from A-trichothecenes group which is mainly produced by *Fusarium* species. In many observations authors indicate that *Fusarium* mycotoxins infuence oocyte quality in gilts **(Alm *et al.*, 2006)**, steroidogenesis and proliferation and apoptosis of porcine ovarian GCs **(Medvedova *et al.*, 2011, Kolesarova *et al.*, 2011)**. In another studies author showed that the peroral T-2 intake can significantly retard the folliculus maturation and

ovulation and perhaps the subsequent luteinisation also in ruminants kept on concentrate-rich diet **(Huszenicza *et al*., 2000)**. **Glavits *et al*. (1983)** examined in their study with sows during the last third of gestation an inhibitory effect on the ovaries, with histological degeneration and accompanying atrophy after feeding contaminated feed of 1-2 ppm. Another authors **Yang *et al*. (2012)** demonstrated in their study with T-2 toxin that this toxin can directly decrease the testosterone biosynthesis in the primary Leydig cells derived from the mouse testis.

Previous mentioned studies indicated that *Fusarium* mycotoxins have a great impact on reproductive performances. In our experiments we observed that T-2 toxin alone as well as in combination with growth factor IGF-I inhibited progesterone secretion by ovarian granulosa cells when compared with control group. Similarly **Caloni *et al*. (2009)** in their results revealed that T-2 toxin had potent inhibitory effects on IGF-I and FSH-induced estradiol and progesterone production. Another authors indicated in their study with B-trichothecene mycotoxin deoxynivalenol (DON) that this mycotoxin had inhibitory effects on IGF-I-induced progesterone production **(Ranzenigo *et al*., 2008).**

T-2 toxin added to ovarian granulosa cells caused the significant testosterone stimulation after addition of 1000 ng.ml^{-1} but after 10, 100 ng.ml^{-1} the release was slightly below that of the control cells which is in accordance with **Medvedova *et al*. (2011)** who observed in their study that fusarium toxin DON at the dose of 1000 ng.ml^{-1} significantly stimulated ($P< 0.05$) progesterone release by GCs. No significant ($P > 0.05$) differences were found among control and experimental groups with doses of 10 and 100 ng.ml^{-1}of DON on the progesterone release by GCs. After addition of T-2 toxin 10, 100 ng.ml^{-1}with IGF-I 1000 ng.ml^{-1} we observed inhibition in testosterone secretion by ovarian GCs. Another authors in their study determined that testosterone production was dose-dependently inhibited ($P<0.05$) after addition of T-2 toxin to H295R cells **(Ndossi *et al*., 2012).**

Currently, the study of mycotoxins and their impact on animal health is very required issue because of their frequently occurence in various agricultural commodities and feeds. In conclussion, there is little data about the toxic effect of T-2 toxin on secretion activity of ovarian granulosa to produced steroid hormones and therefore our obtained results will be beneficial by examination of toxic aspects of this toxin on cellular level.

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