Effects of oocyte exposure to Ochratoxin-A on oocyte maturation and embryo development in pre-pubertal lambs

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Introduction

Ochratoxin A (OTA) is a major mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi and has been reported as an ubiquitous natural contaminant found in feed and food products of plants origin, for example, cereals, coffee, cocoa, nuts, peanuts [1]. OTA induces reprotoxic, embryotoxic and teratogenic as well as nephrotoxic, neurotoxic, immunotoxic and carcinogenic activity as reported in either laboratory or farm animals [2]. Major mechanisms of action include inhibition of protein synthesis, toxic effect on mitochondrial (mt) function and calcium homeostasis with consequent oxidative stress, apoptosis induction and DNA adduct formation. Although ruminants are capable of degrading OTA into its matabolite Otα, both OTA and Otα were found in blood samples [3]. Toxic effects of OTA on oocyte maturation have been reported in mice [4]. However, no studies have been reported to date in large animal models, closer to human reproductive physiology than the murine model. The aim of this study was to evaluate the effects of OTA on developmental potential of lamb oocytes.

Methods

Experiment 1 Abattoir-derived lamb ovaries were used. Cumulus-oocyte complexes (COCs) were selected and exposed to 1µM, 5µM and 10µM OTA during in vitro maturation (IVM) for 24 h at 38.5°C under 5% CO2 [5]. In Experiment 1, 60-120 COCs/condition were analyzed (4 to 8 runs/condition); after IVM, cumulus cells were removed, and oocytes were analyzed for meiotic stage. IVM medium with vehicle (1% methanol) was used as control and only those oocytes found in Metaphase II were analyzed by laser scanning confocal microscopy for assessing their cytoplasmic maturation indicated by mitochondria (mt) distribution pattern [5]. Data were analyzed by Chi-square test (statistical significance at P<0.05).

Experiment 2 In Experiment 2, 45-180 COCs/condition were analyzed in 3 to 8 runs/condition; after IVM, oocytes underwent in vitro fertilization and embryo culture up to day 7. Embryo development was monitored by phase contrast microscopy followed by nuclear chromatin evaluation under epifluorescence microscopy . A final concentration of 1×10^6 motile sperm cells/ml was added. Oocytes were partially denuded before incubation with sperm suspension. IVF was performed in Synthetic Oviductal Fluid Medium (SOFM) with sodium bicarbonate for 24h. Ram frozen spermatozoa were thawed and analyzed for concentration and motility by CASA which occurred for 24 hours at 38.5°C under 5% CO2. Presumptive zygotes were cultured for 7 days in SOFM with essential and nonessential amino acids at oviductal concentration and 0.4% Bovine Serum Albumin (BSA) [5]. At the end of culture, cleavage and blastocyst formation rates were recorded after fixing and Hoechst staining (Chi-square test with statistical significance at P<0.05).

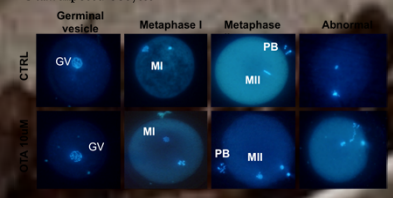
Results

Table 1: Effects of OTA on oocyte nuclear maturation

OTA concentration (µM)	N° of runs	N° of cultured oocytes	N° of evaluated oocytes	Nuclear Chromatin configurations N (%)			
				GV	MI to TI	MII	Abnormal
0 (Ctrl)	8	135	114	19 (17)	10 (9)	73 (64)	14 (12)
0 (1%MeOH)	5	94	81	17 (21) a	9 (11)	51 (63) a	10 (12) a
1	4	80	60	26 (43) b	6 (10)	10 (17) c	18 (30) b
5	4	80	69	28 (40) b	10 (14)	16 (23) c	15 (22)
10	8	142	120	40 (33)	11 (9)	42 (35) c	27 (23)

Legend: GV= Germinal Vesicle; M= Metaphase; T= Telophase Chi-square test: a,b P<0.0001

Figure 1: Nuclear chromatin configurations in CTRL and OTA-exposed oocytes



Experiment 1: at any tested concentration, OTA affected oocyte maturation rates (17%, 23% and 35% versus 63%; for 1 µM, 5µM and 10µM OTA respectively vs vehicle control; Table 1, P<0.0001; Figure). In MII-oocytes, 10 µM OTA significantly reduced the rate of oocytes showing healthy perinuclear/subcortical mitochondria distribution pattern (16% vs 50% for 10µM OTA and control respectively; P<0.01) while 1µM and 5µM OTA did not affect this ooplasmic parameter (62% and 25% for 1µM and 5µM OTA, respectively; not significant) (Table 2). OTA reduced the rate of oocytes with healthy homogeneous perinuclear/pericortical (P/P) mt pattern(16% vs 50% for 10µM OTA and control respectively; P<0.01) while 1µM and 5µM OTA did not affect this ooplasmic parameter (62% and 25% for 1µM and 5µM OTA, respectively; not significant; Figure 2) and increased the rate of mt distribution in small aggregates (SA).

Figure 2: Mitochondria pattern in CTRL and OTA-exposed oocytes

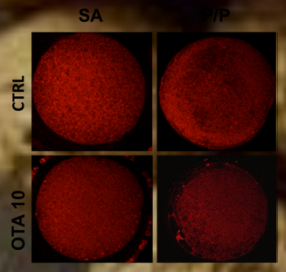


Figure 3: Nuclear chromatin configurations in embryos from CTRL and OTA-exposed oocytes

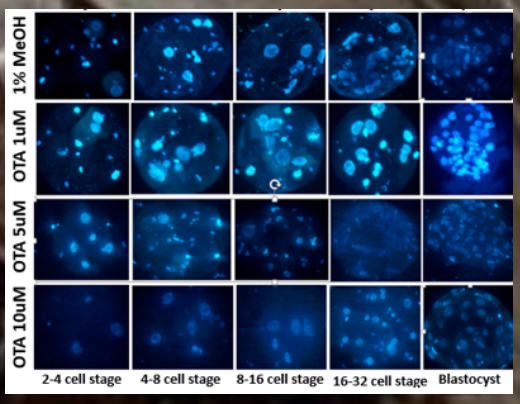


Table 2: Effects of in-vitro exposure to OTA during IVM on bioenergetic/redox status of juvenile sheep oocytes

OTA concentration (µM)	N° of evaluated MII oocytes	Mitochondria distribution pattern N (%)	
		Perinuclear/ subcortical	Small aggregates + abnormal
0 (Ctrl)	73	33 (45)	40 (54)
0 (Ctrl+1%MeOH)	54	26 (48)	28 (52)
1	8	5 (62)	3 (37)
5	12	3 (25)	9 (75)
10	44	7 (16) b	37 (84) b

Table 3: Effects of in vitro exposure to OTA during IVM on embryo developmental competence of juvenile sheep oocytes

OTA concentration (µM)	N° of cultured oocytes	N° of evaluated oocytes	Embryo developmental stages Fluorescence microscopy-based nuclear chromatin evaluation N (%)								Uncleaved oocytes N (%)
			2-4 cell	4-8 cell	8-16 cell	16-32 cell	Morula	Blast.	Deg. Chrom.	Total	
0 (Ctrl)	198	183	43 (23)	17 (9)	6 (3)	0 (0)	0 (0)	5 (3)	12 (6)	83 (45)	100 (55)
(Ctrl +1%MeOH)	154	134	25 (19)	15 (11)	9 (7)	1 (1)	0 (0)	1 (1)	11 (8)a	62 (46)	72 (54)
10	99	89	20 (22)	9 (10)	5 (6)	0 (0)	0 (0)	4 (4)	1 (1)b	39 (44)	50 (56)
5	89	79	15 (19)	13 (16)	4 (5)	1 (1)	0 (0)	1 (1)	4 (5)	39 (49)	40 (51)
1	56	46	13 (28)	3 (6)	2 (4)	1 (2)	0 (0)	2 (4)	3 (6)	24 (52)	22 (48)

Experiment 2: No significant effects were noticed on total cleavage (45 to 52%) and blastocyst formation rate (20 to 24%) at any OTA concentration (Table 3). Figure 3 shows nuclear chromatin configurations of embryos derived from OTA-exposed and non-exposed oocytes.

Conclusions

- ✓ OTA affected oocyte maturation and mitochondria pattern but had no apparent effects of embryo development. Further studies are in progress to evaluate additional embryo quality parameters and effects of oocyte exposure to lower environmental (nanomolar) OTA concentrations.

References

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