Effects of Ochratoxin A on developmental potential of lamb oocytes

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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, grape [1]. OTA plays 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, 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 10 µM OTA (concentration reported as effective in the mouse [4] during in vitro maturation (IVM) for 24 h at 38.5◦C under 5% CO2 [5]). Vehicle controls (IVM medium with 1% methanol) and standard controls (IVM medium without vehicle) were used. After IVM, cumulus cells were removed and oocytes were analyzed for nuclear chromatin. Only those oocyte found in Metaphase II were analyzed by laser scanning confocal microscopy for assessing their cytoplasmic maturation indicated by mt distribution pattern [5]. Data were analyzed by Chi-square test (statistical significance at $P<0.05$). **Experiment 2** Oocytes underwent IVM, in vitro fertilization (IVF) and in vitro embryo culture (IVEC) up to day 7. 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 system. A final concentration of 1×10^6 motile sperm cells/ml was added. Oocytes were partially denuded before incubation with sperm suspension 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]. Cleavage and blastocyst formation rates were recorded (Chi-square test with statistical significance at $P<0.05$).

Results

Table 1: Effects of OTA on oocyte nuclear maturation

Conditions	N° of evaluated oocytes	N° (%) of oocyte showing			
		GV	MI to TI	MII+PB	Abnormal
CTRL	96	13 (14)	10 (10)	62 (65)	5 (5)
1% MeOH	37	7 (19)	5 (14)	23 (62)	2 (5)
10uM OTA	85	22 (26)	8 (9)	39 (46)	11 (13)

Legend: GV= Germinal Vesicle; M= Metaphase; T= Telophase
Chi-square test: Not significant

Commodities affected by OTA and OTA structural formula

Experiment 1: A total of 218 oocytes were analyzed. Lack of vehicle-related effects was noticed (23/37, 62% vs 62/96, 65%, for oocytes cultured with or without vehicle; NS). OTA tended to reduce the maturation rate (39/85, 46% vs 23/37, 62%, for exposed and controls, respectively) even without statistical significance (Table 1). Figure 1 shows nuclear chromatin configuration of oocytes representative of CTRL and OTA-exposed oocytes. OTA reduced the rate of oocytes with healthy perinuclear/pericortical (P/P) mt pattern (4/39, 10% vs 9/23, 39%, $p<0.05$; Figures 2, 3) and increased the rate of oocytes with homogeneous mt distribution in small aggregates (SA).

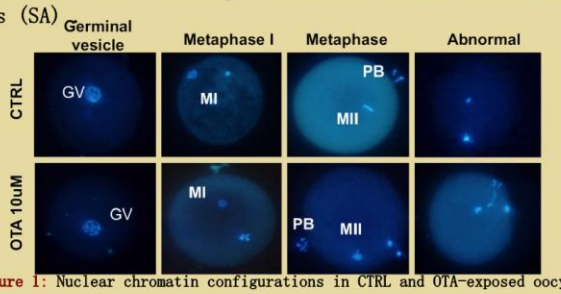

Figure 1: Nuclear chromatin configurations in CTRL and OTA-exposed oocytes

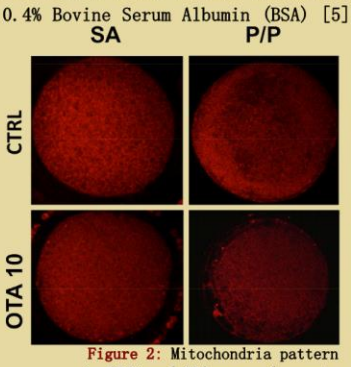

Figure 2: Mitochondria pattern in CTRL and OTA-exposed oocytes

Figure 3: Rates of CTRL and OTA-exposed oocytes with perinuclear/pericortical mitochondria pattern ($P<0.05$)

Experiment 2: A total of 275 oocytes were analyzed. No significant differences were found between controls with and without the vehicle also during IVF/IVEC experiments ($p>0.05$). No effects were noticed on cleavage and blastocyst rates after 10 µM of OTA exposure (Table 2). Figure 4 shows the morphologies of embryos derived from OTA-exposed and control oocytes.

Table 2: Effects of OTA added during IVM development

Conditions	No of evaluated oocytes	No. (%) of cleaved embryos	No. (%) of blastocysts
CTRL	93	31 (33)	2 (2)
1% MeOH	92	24 (26)	1 (1)
10uM OTA	90	22 (24)	4 (4)

Chi-square test: Not significant

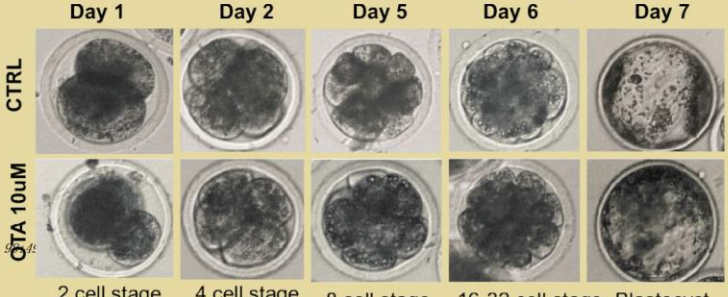

Figure 4: Morphologies of embryos obtained from CTRL and OTA-exposed oocytes

Conclusions

- ✓ OTA hindered oocyte nuclear and cytoplasmic maturation with no apparent effects on embryo morphology.
- ✓ Further studies are in due course to evaluate other embryo quality parameters and the effects of other OTA concentrations.

References

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