



# Aflatoxin B1 influences expression of TRPM8 in SKOV, Ovarian Epithelial Cancer cells



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## Introduction

- Aflatoxin B<sub>1</sub> (AFB1) effects fertility in many species by decreasing numbers of follicles in the female (Hasanzadeh et al., 2013) and sperm in the male (Supriya et al., 2016).
- AFB1 decreases steroidogenesis by competitively binding to the steroidogenic acute regulatory (StAR) protein (Supriya et al., 2016).
- A reduction in circulatory testosterone may impact transient receptor potential melastatin 8 (TRPM8) expression.
- TRPM8 acts as an ionotropic testosterone receptor and may play a role in testosterone-induced behaviors including sexual drive, aggressiveness, and fear conditioning (Asuthkar, Demirkhanyan et al., 2015; Asuthkar, Elustondo et al., 2015).

## Objective & Hypothesis

**Objective:** Determine if AFB1 exposure influences TRPM8 expression in gonadal cells.

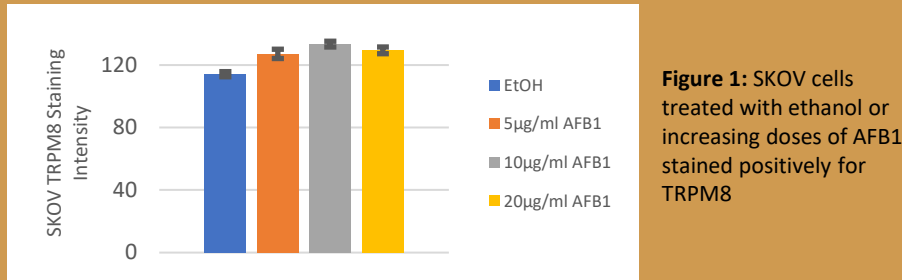
**Hypothesis:** AFB1 exposure may influence expression of TRPM8 channels in gonadal cells due to AFB1's effect on steroidogenesis and TRPM8's role as a testosterone receptor.

## Literature Cited

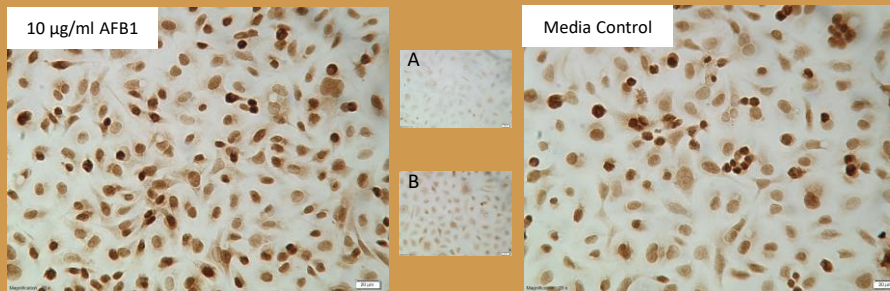
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## Results

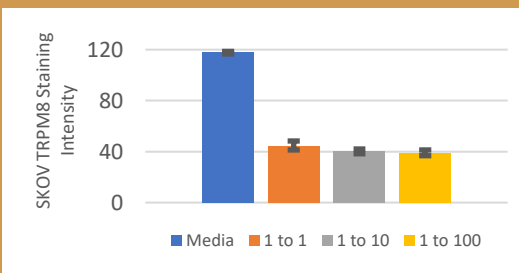
- Relative TRPM8 mRNA did not differ with AFB1 treatments ( $P = 0.2$ ).
- AFB1 treatment resulted in increased TRPM8 staining intensity ( $P < 0.001$ ) compared to ethanol treated cells



**Figure 1:** SKOV cells treated with ethanol or increasing doses of AFB1 stained positively for TRPM8



**Figure 2:** A) Negative control of SKOV staining B) 1:10 rabbit TRPM8 polyclonal antibody:TRPM8 peptide staining. Positive staining appears brown.



**Figure 3:** SKOV cells treated with 1:1, 1:10, and 1:100 molar ratios of rabbit TRPM8 polyclonal antibody:TRPM8 peptide.

- TRPM8 peptide sufficiently blocked the TRPM8 antibody, significantly reducing positive staining ( $P < 0.001$ ).

## Materials & Methods

- Human epithelial ovarian cells, SKOV, ▪ seeded at  $2 \times 10^5$  cells/ml into chamber slides and ▪ treated in triplicate with media containing ethanol, 5 µg/ml AFB1, 10 µg/ml AFB1, or 20 µg/ml AFB1 for 24 hours
- Immunocytochemistry performed using: ▪ Rabbit TRPM8 polyclonal antibody (1:200; Lifespan Biosciences, Inc.; Seattle, WA), ▪ Anti-rabbit HRP conjugated secondary antibody (1:10,000; Jackson Labs; Bar Harbor, ME), ▪ DAB substrate (Vector Laboratories, Burlingame, CA)
- Images captured with Cell Sense software at 200x magnification
- Mean gray scale intensity analyzed using ImageJ software
- SKOV cell lysate was used for mRNA isolation and Real-Time semi-quantitative RT-PCR (SYBR Green procedures of BIORAD)
- Data analyzed using numerical regression (Minitab 18)

## Conclusions

- Blocking the TRPM8 antibody with TRPM8 peptide was successful indicating the TRPM8 antibody used is specific to the TRPM8 channel.
- Treatment of human ovarian epithelial cells, SKOV, with increasing doses of AFB1 resulted in increased TRPM8 expression in immunocytochemistry but did not alter relative mRNA levels.
- Inconsistency between mRNA and protein expression may be due to the timing of mRNA synthesis and translation of the protein.
- Based on these results, expression of TRPM8 in SKOV cells increased in response to AFB1. Although it is not certain if this effect is restricted to these transformed cells, the possibility remains that changes in TRPM8 expression may contribute to the negative reproductive consequences of AFB1 exposure in humans and livestock species.