
بسم الله الرحمن الرحيم

Effects of Nutrition and Toxins on Germ Cells; Sperm, Oocyte & Preimplantation Embryo

Dr Mansoureh Movahedin, Ph.D.

Professor of Anatomical Sciences Dept.,

Tarbiat Modares University,

Tehran, Iran

Environmental Toxins

Aflatoxins are of great concern to public health because they accumulate in the body and can be found in edible tissues, such as liver and muscles, as well as in animal food products such as milk, rice, wheat and eggs.

Moreover, aflatoxins has been found in human maternal breast milk, and maternal and cord blood, and can apparently enter the developing fetus in humans and animals.

These unavoidable food contaminants are highly stable chemicals and are usually found as a mixture of aflatoxin B1 (AFB1), AFB2, AFG1 and AFG2.

Environmental Toxins

Aflatoxins were found in **40% of semen samples** collected from ***infertile men***, relative to only **8%** of those collected from ***fertile men.***

The former were associated with abnormal sperm count and morphology, as well as reduced motility (Ibeh et al., 1994; Nduka et al., 2001).

AF1 & Spermatogenesis

Agnes and Akbarsha, 2003

Oral administration of AFB1 (50 µg/kg BW per day) for 35 days resulted in spermatotoxic effects on mouse epididymal sperm, expressed as reduced sperm concentration and motility and increased sperm abnormalities.

Faisal et al., 2008

Intramuscular injection of AFB1 (0.2–0.25 ml/day for 55 days) into male rats resulted in extrusion of the outer dense fibers and axonemal microtubule doublets of the cauda epididymal sperm flagellum, suggesting negative effects on spermatozoa throughout the early stages of spermatogenesis.

Aflatoxin B1 impairs sperm quality and fertilization competence.

A. Komsky-Elbaz, et al., Toxicology, 2018

Spermatozoa were obtained from bull ejaculate and epididymis and capacitated in vitro for 4 h with 0, 0.1, 1, 10 and 100 μM AFB1. Following capacitation, acrosome reaction (AR) was induced by Ca2+ ionophore.

The ***integrity and functionality of sperm*** were examined simultaneously by florescent staining.

A Halosperm DNA fragmentation kit was used to evaluate ***DNA integrity.***

An in-vitro culture system was used to evaluate ***fertilization competence and blastocyst formation rate***, using bovine oocytes.

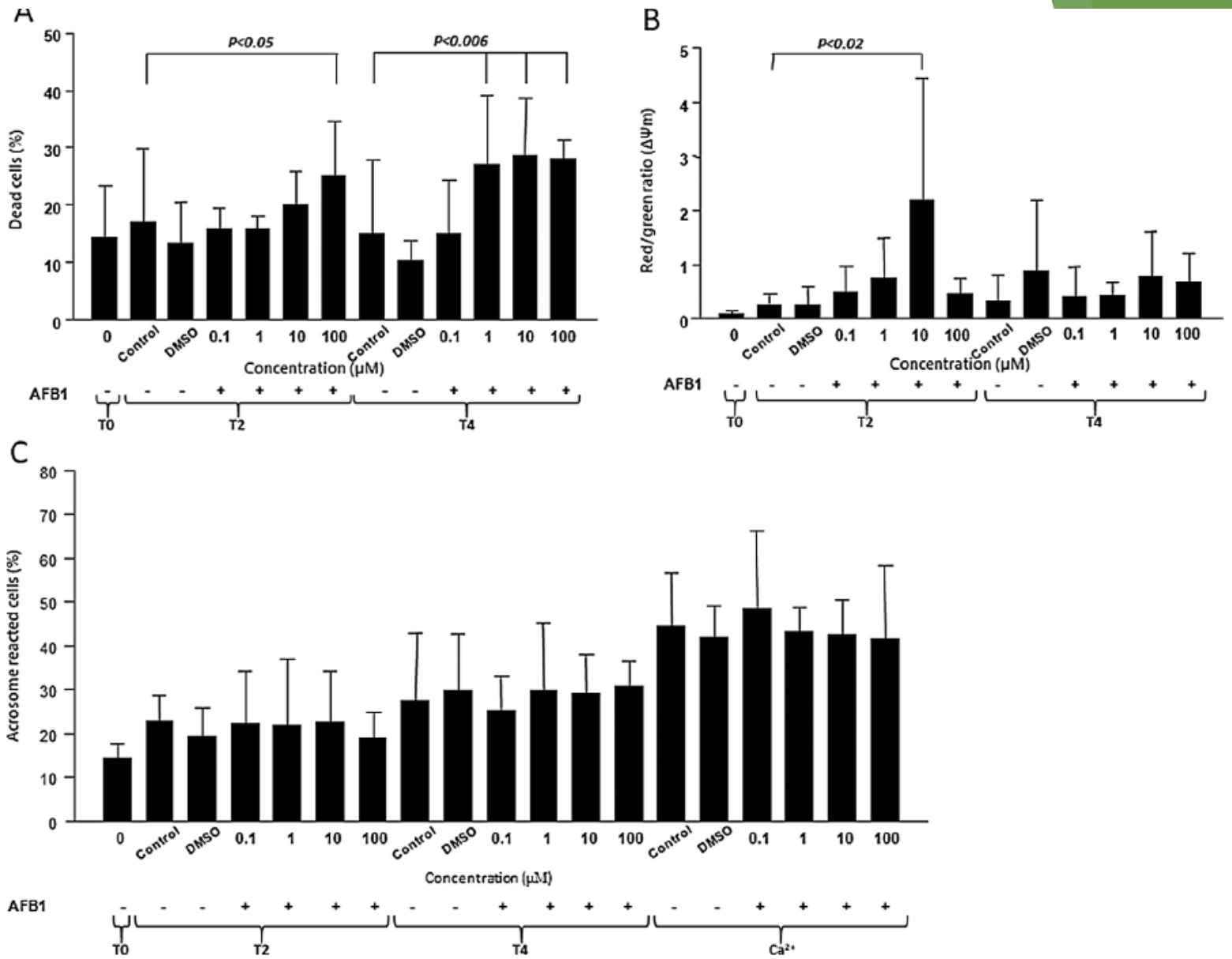


Fig. 2. Effect of AFB1 on ejaculate sperm. Spermatozoa were isolated from fresh ejaculate and incubated for 4 h with 0.1, 1, 10 or 100 µM AFB1 dissolved in DMSO. (A) Sperm viability was determined with PI fluorescent probe. (B) Mitochondrial membrane potential (ΔΨm) was determined with JC-1 fluorescent probe and presented as the mean proportion of red-stained (high potential) to green-stained (low potential) sperm. (C) Acrosome reaction was determined with FITC–PSA fluorescent probe. Data are presented as mean proportion ± SD, calculated for 3 replicates. At least 200 sperm were analyzed per group.

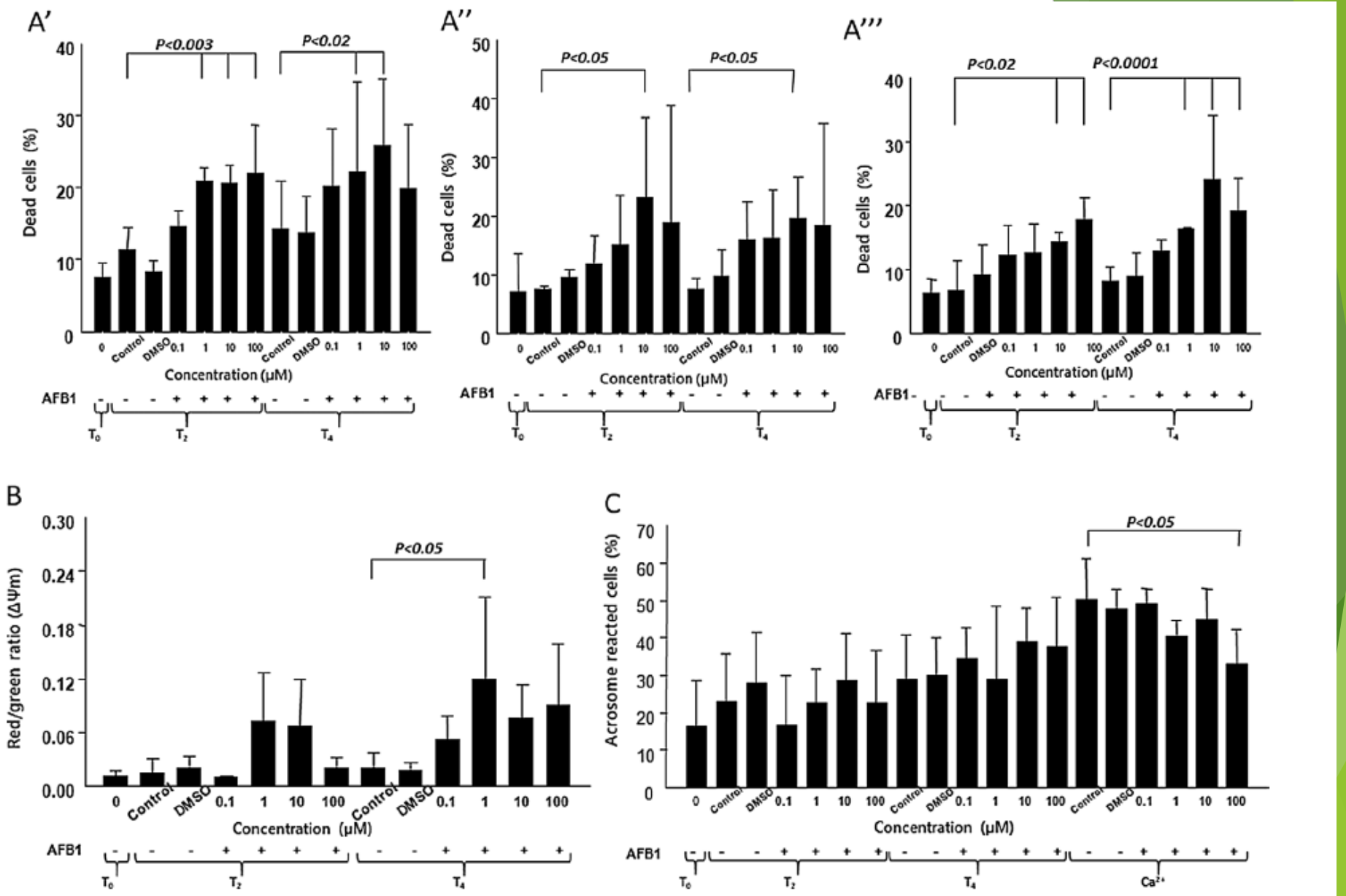


Fig. 3. Effect of AFB1 on sperm isolated from the epididymis. Spermatozoa were isolated from epididymis compartments: head [A'], body [A"] and tail [A"', B, C] and incubated for 4 h with 0.1, 1, 10 or 100 μM AFB1 dissolved in DMSO. (A', A", A"') Sperm viability was determined with PI fluorescent probe. (B) Mitochondrial membrane potential (ΔΨm) was determined with JC-1 fluorescent probe and presented as the mean proportion of red-stained (high potential) to green-stained (low potential) sperm. (C) Acrosome reaction was determined with FITC–PSA fluorescent probe. Data are presented as mean proportion ± SD, calculated for 3 replicates. At least 200 sperm were analyzed per group.

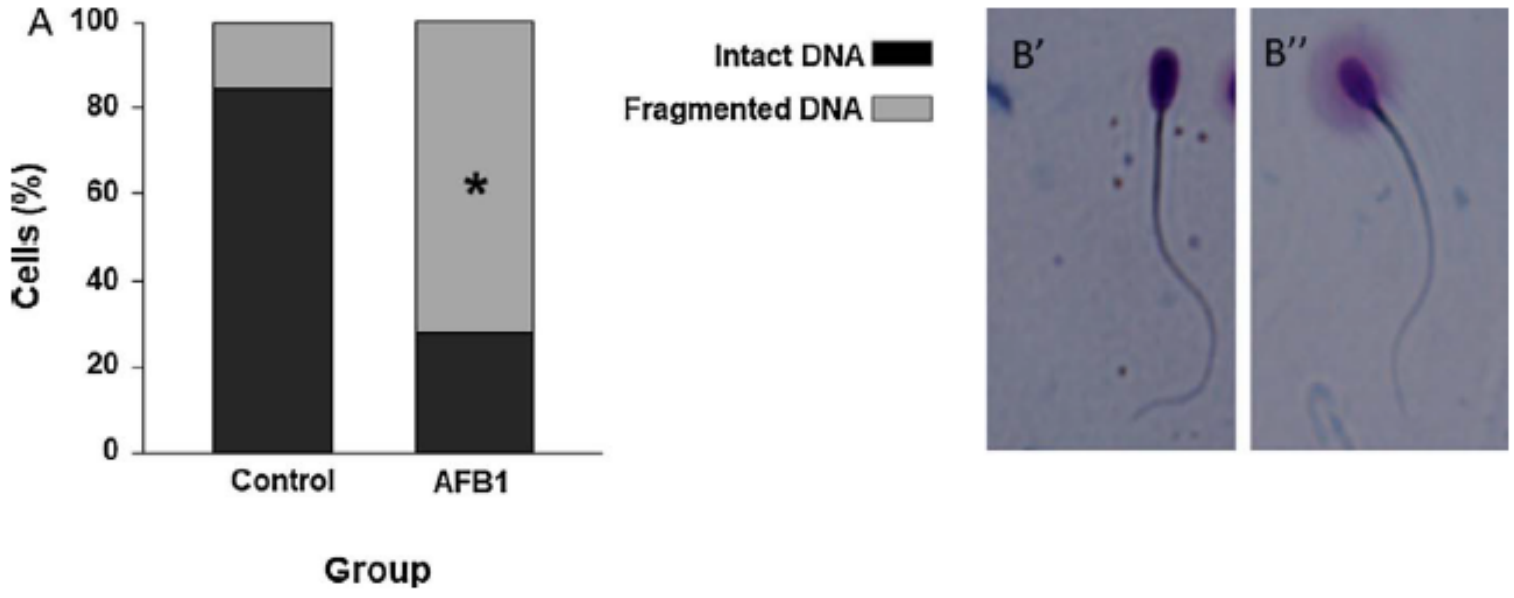


Fig. 4. Effect of AFB1 on sperm DNA fragmentation. (A) Spermatozoa were incubated for 4 h with 10 μM AFB1 dissolved in DMSO. (B) Sperm DNA fragmentation was determined with the Halosperm kit. (B′) Degenerating spermatozoa with small or no halo were considered to have fragmented DNA, while (B″) spermatozoa with large or medium-sized haloes were considered normal, with no fragmented DNA. Data are presented as means ± SD, calculated for 3 replicates, with 200 sperm per group. $^{*}P < 0.0001$.

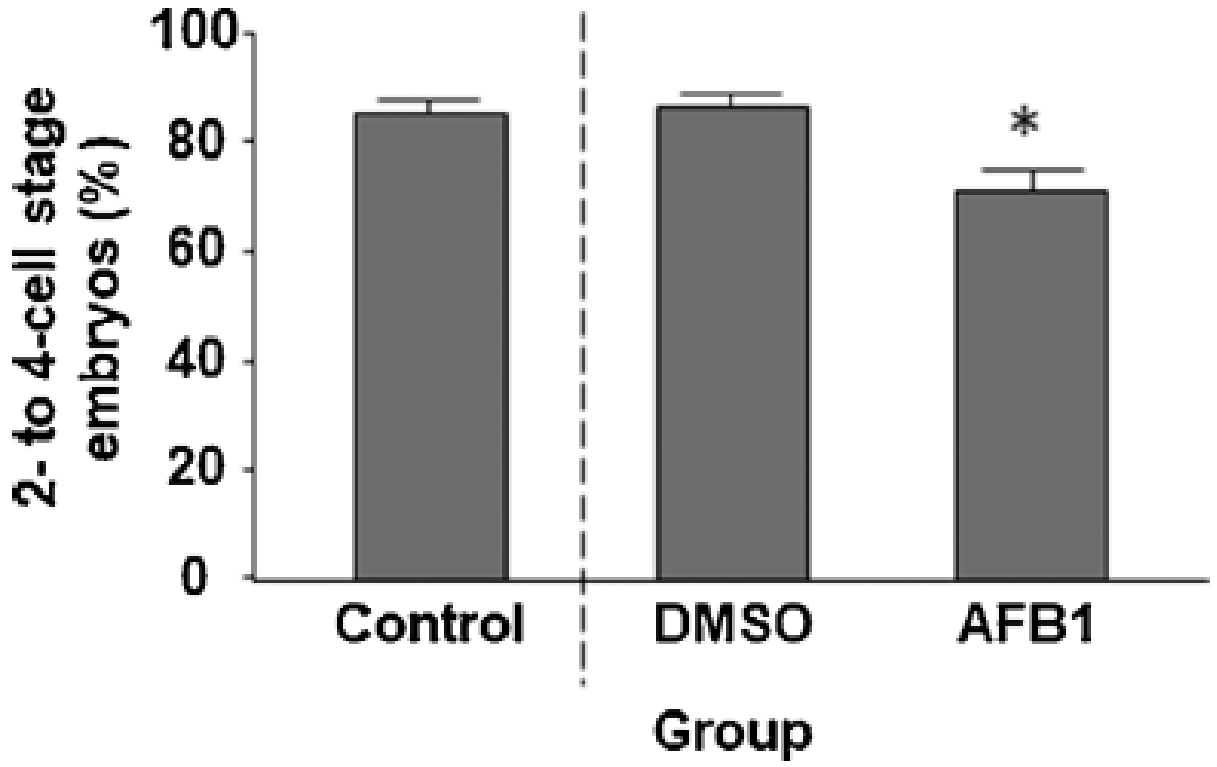


Fig. 5. Effect of sperm exposure to AFB1 on fertilization. Sperm isolated from fresh semen was exposed to 100 μM AFB1 for 4 h. Bovine oocytes were matured for 22 h and fertilized for 18 h with AFB1-treated or control sperm ($\sim 1 \times 10^6$). Presented is the percentage of oocytes that cleaved into 2- to 4-cell stage embryos, 42–44 h postfertilization. The experiment included 6 replicates with 30–60 oocytes per replicate per experimental group. Data are presented as means ± SEM. *$P < 0.005$.

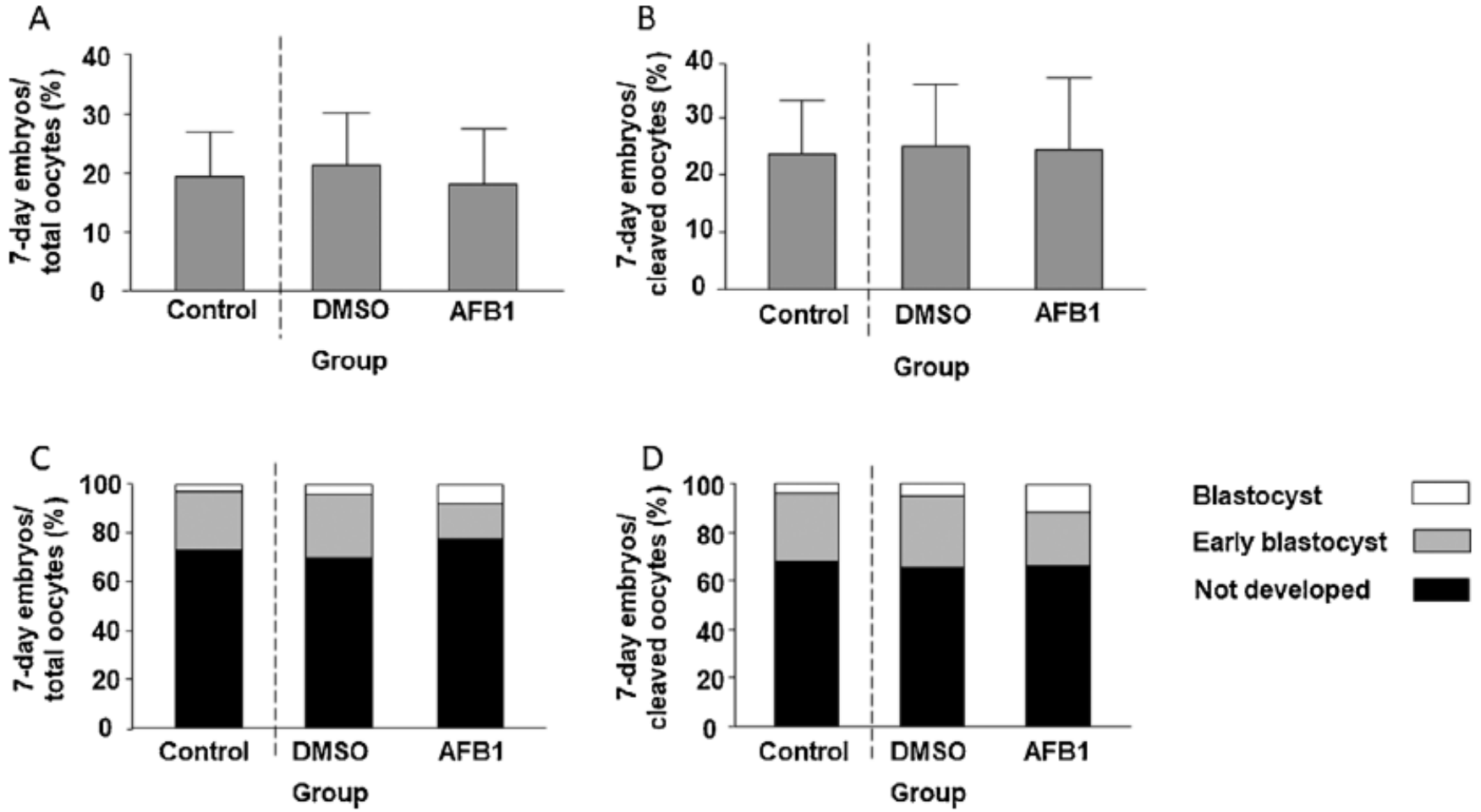


Fig. 6. Effect of sperm exposure to AFB1 on early embryonic development. Bovine oocytes were fertilized for 18 h with AFB1-treated or control sperm and incubated for 7 days. Presented is the percentage of embryos that developed to the blastocyst stage on day 7 postfertilization and the distribution to various embryonic stages, out of total (A, C) or cleaved (B, D) oocytes. The experiment included 6 replicates with 30–60 oocytes per replicate per experimental group. Data are presented as means ± SEM.

The current study provides evidence that exposure to low AFB1 concentrations for a few hours results in **decreased sperm viability**, **hyperpolarization of the mitochondrial membrane** and increased DNA fragmentation.

In light of sperm's sensitivity to AFB1, good-quality semen may be damaged by AFB1 through passage in female reproductive tract. Given the importance of sperm DNA integrity to the developing embryo, the quality of these embryos remains an open question. Further evaluation, at the RNA and protein levels, should be performed to evaluate a possible carryover effect to the blastocyst stage.

Toxicity of beauvericin on porcine oocyte maturation and preimplantation embryo development

Eric J. Schoevers , Reproductive Toxicology , 2016

Mycotoxins are secondary metabolites produced worldwide by fungi under certain environmental conditions. These toxins are common contaminants in raw food materials, have diverse chemical properties and toxicity, and may present a risk for animal and human health.

Beauvericin (BEA) has been increasingly detected as contaminant in cereal products in the past years. The prevalence of feed samples contaminated with beauvericine varies considerably and in a recent survey of multi-mycotoxin analysis the contamination rate reached 98% with maximum concentration amounting to 2326 g/kg.

In consideration of these findings, BEA is classified as an emerging mycotoxin.

Toxicity of beauvericin on porcine oocyte maturation and preimplantation embryo development

Eric J. Schoevers , Reproductive Toxicology , 2016

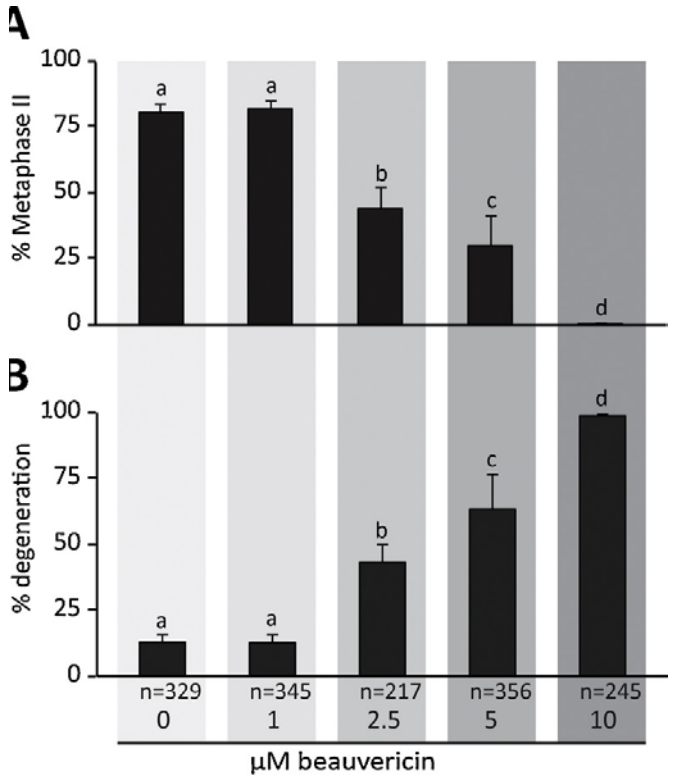
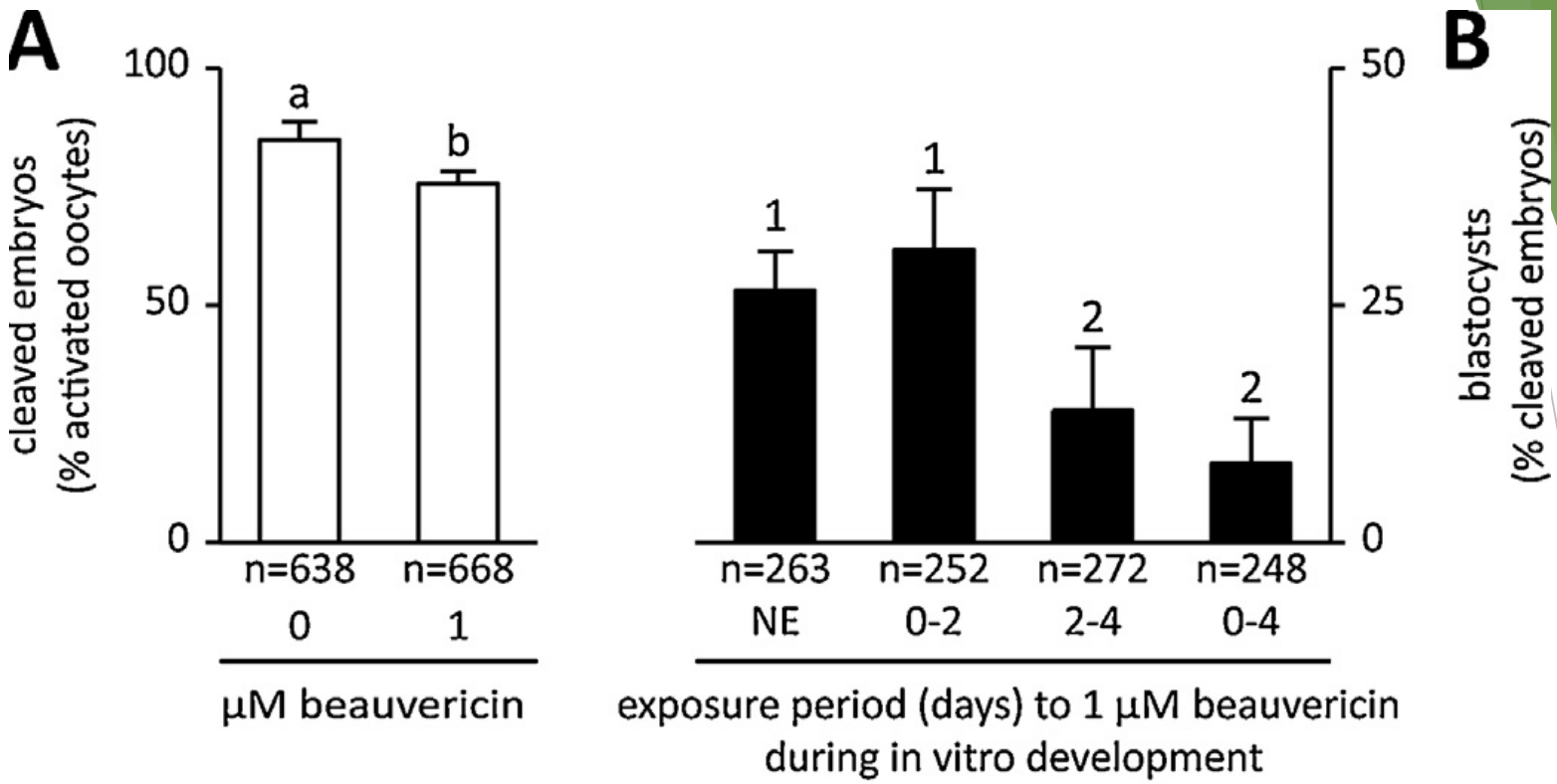


Fig. 2. Progression through meiosis (A), degeneration (B), chromatin structure (C)and mitochondrial activity (D) in porcine oocytes after exposure to beauvericinfor 44 h. A and B, numbers under bars represent the total number of oocytes fromeight replicates. C, chromatin structures in control oocytes (C1-C3) represent Ger-minal Vesicle (C1), Metaphase II (C2) and degeneration by clumped chromatin (C3),whereas in BEA exposed oocytes (C4-6) nuclear degeneration is visible. D, reductionof resazurin by beauvericin exposed oocytes as percentage of that of control oocytes.Identical bars with different letters (abcd) are significantly different ($P < 0.05$).



In conclusion, while there is currently no epidemiological evidence that BEA exerts direct toxic effects in farm animal sand/or impairs animal productivity and reproduction, the biological potency and the specific effects described in this study on early embryo development clearly indicate that this frequently occurring food and feed contaminant deserves further attention.

-Solanine impairs oocyte maturation and quality by inducing autophagy and apoptosis and changing histone modifications in a pig model

Tao Lin et al., Reproductive Toxicology, 2018

In this study, pig model was used to investigate the effects of -solanine (***a natural toxin found mainly in potato sprouts***) on oocyte maturation, quality and subsequent embryonic development.

Results:

a. -solanine (10 μM) disturbed meiotic resumption
b. increased abnormal spindle formation
c. altered the cortical granule (CG) distribution compared with the untreated group.
d. -solanine triggered autophagy and apoptosis by increasing the expressions of autophagy-related genes (LC3, ATG7, andLAMP2) and apoptotic related genes (BAX and CASP3).
e. -solanine significantly reduced the cleavage and blastocyst formation rates, decreased the total and inner cell mass cells numbers, and increased apoptosis in these porcine embryos.

Evaluating the effects of Escanbil (Calligonum) extract on the expression level of Catsper gene variants and sperm motility in aging male mice

M. Askari Jahromi, et al., Iran J Reprod Med, 2014

The *Escanbil (Calligonum)* Commosum is a plant which has the important antioxidants like Catechin, Epicatechi, Quercetin, kamefrol and Genistin. Catechin is a type of natural phenols and antioxidant which is a plant secondary metabolite. It is often considered to belong to the family of flavonoids.

Table II. Evaluation of different doses of *Escanbil (Calligonum)* extracts on sperm parameters in aging male mice

Groups \ Sperm parameters	Morphology (±SD)	Viability (±SD)	Total motility (±SD)	Count (SD± x×10^6)
Control	57.02 ± 1.16	58.68 ± 0.79	60.72 ± 1.41	4.11 ± 0.27
Sham	58.63 ± 0.92	58.63 ± 0.79	58.7 ± 2.22	4.3 ± 0.21
10 mg/kg	65.46 ±0.41[a]	58.16 ± 0.51	55.7 ± 1.02[abd]	3.7 ± 0.78
30 mg/kg	68.32 ± 0.5[a]	69.38 ± 1.19 [ab]	69.78 ± 0.67 [abc]	4.7 ± 0.37 [ce]
50 mg/kg	65.70 ± 0.84[a]	57.90 ± 1.30	65.96 ± 0.7 [ab]	3.72 ± 0.75

a :Statistical significance with control group (p<0.05)
b :Statistical significance with sham group (p<0.05)
c :Statistical significance with 10 mg/kg group (p<0.05)
d :Statistical significance with 30 mg/kg group (p<0.05)
e :Statistical significance with 50 mg/kg group (p<0.05)

able III. The effect of optimum dose (30mg/kg) of *Escanbil (Calligonum)* extracts on sperm parameters of young and aging male ice

Groups \ Sperm parameters	Morphology (±SD)	Viability (±SD)	Total motility (±SD)	Count (SD± x×10^6)
Aging control	57.02 ± 1.16	58.68 ± 0.79	60.72 ± 1.41	4.11 ± 0.27
Young control	68.54 ± 3.46 [ab]	72.58 ± 1.66 [ab]	74.3± 2.3[ab]	4.6 ± 0. 21 [ab]
Aging sham	58.63± 0.92	58.63 ± 0.79	58.7 ±2.22	4.3± 0.21
Young Sham	67.50 ± 0.17[ab]	71.46 ± 2.97[ab]	72.08 ± 3[ab]	4.7 ± 0.07 [ab]
Aging experiment	69.41 ±3.05 [ab]	68.34 ± 3.50[ab]	67.74 ± 2.14 [ab]	4.5 ± 0.56
Young experiment	76.94 ± 2.03 [cde]	58.82 ± 3.69[cde]	73.30±1.02 [e]	4.90± 0.07

:Statistical significance with aging control group (p<0.05)
b :Statistical significance with aging sham group (p <0.05)
:Statistical significance with young control group (p<0.05)
d :Statistical significance with young sham group (p<0.05)
:Statistical significance with aging experiment group (p<0.05)

Up-regulation of CatSper genes family by selenium

S. Mohammadi, et al., **Reproductive Biology and Endocrinology, 2009**

Data revealed that there was a significant up-regulation of CatSper genes in the experimental groups compared to the control ones.

Furthermore, the results of sperm analysis showed that the sperm parameters were improved in the aging as well as young adult male mice following Se treatment.

Se treatment in the aging subjects could up-regulate the expression of CatSper genes, and therefore results in elevation of sperm motility.

Furthermore, Se treatment improved sperm parameters, especially morphology and viability rates.

In vitro development of embryos from experimentally Kerack-addicted Mice

E. Mohammadzadeh, Int J Reprod BioMed, 2017

Mouse embryos from addicted mothers

Table I. Development of mouse morulae from addicted and non-addicted mothers in T6 media following 96-h cultivation

Groups	Control	Vehicle	Exp. I (5 Mg/kg)	Exp. II (35 mg/kg)	Exp. III (70 mg/kg)
No.	88	56	53	51	67
24 hr					
M	3 (3.41)	20 (35.71)	25 (47.17)	7 (13.73)	31 (46.27)
Eb+Lb	67 (76.14)	35 (62.50)	18 (33.96)	37 (72.55)	34 (50.75)
Hgb+Hdb	17 (19.32)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
D	1 (1.13)	1 (1.79)	10 (18.87)	7 13.73)	2 (2.99)
48 hr					
M	0 (0.00)	0 (0.00)	1 (1.89)	0 (0.00)	0 (0.00)
Eb+Lb	28 (31.82)	22 (39.29)	22 (41.51)	30 (58.82)	30 (44.78)
Hgb+Hdb	53 (60.23)	30 (53.57)	19 (35.85)	14 (27.45)	24 (35.82)
D	7 (7.95)	4 (7.14)	11 (20.75)	7 13.73)	13 (19.40)
72 hr					
M	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Eb+Lb	16 (18.18)	7 (12.50)	5 (9.43)	13 (25.49)	8 (11.94)
Hgb+Hdb	58 (65.91)	39 (69.64)	37 (69.81)	28 (54.90)	41 (61.19)
D	14 (15.91)	10 (17.86)	11 (20.75)	10 (19.61)	18 (26.87)
96 hr					
M	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Eb+Lb	12 (13.64)	7 (12.50)	1 (1.89)	3 (5.88)	4 (5.97)
Hgb+Hdb	62 (70.45)	39 (69.64)	38 (71.70)	32 (62.75)	42 (62.69)
D	14 (15.91)	10 (17.86)	14 (26.42)	16 31.37)	21 (31.34)

Data presented as n (%).
Control, embryos from non-addicted mothers in T6 medium
Vehicle, received only normal saline and lemon juice;
Exp. I, experimental group I received Kerack at a dose of 5 mg/kg after addiction to Kerack;
Exp. II, experimental group II received Kerack at a dose of 35 mg/kg after addiction to Kerack and
Exp. III, Experimental group III received Kerack at a dose of 70 mg/kg after addiction to Kerack.
M: morula Eb: early blastocyst Lb: late blastocyst Hgb: hatching blastocyst
Hgd: hatched blastocyst D: degenerated embryo.

Table II. The effect of Kerack on blastomer, ICM and apoptotic cell number in the blastocyst stage

Groups	Total Cell	ICM	Apoptotic cell
Control	65.08±8.42	26.15±5.34	1.46±1.45
Vehicle	56.29±6.16	23.29±3.15	0.29±0.49
Experimental I (5 mg/kg)	46.17±4.15[ab]	18.17±3.07[ab]	2.92±2.71[ab]
Experimental II (35 mg/kg)	46.44±6.46[ab]	18.11±2.32[a]	5.11±4.86[ac]
Experimental III (70 mg/kg)	40.92±7.09[a]	17.17±3.21[ab]	7.17±2.89[abd]

Control, embryos from non-addicted mothers in T6 medium
Vehicle, received only normal saline and lemon juice;
Exp. I, experimental group I received Kerack at a dose of 5 mg/kg after addiction to Kerack;
Exp. II, experimental group II received Kerack at a dose of 35 mg/kg after addiction to Kerack and
Exp. III, Experimental group III received Kerack at a dose of 70 mg/kg after addiction to Kerack.
[a] Significant difference versus control and vehicle groups ($p<0.001$).
[b] Significant difference versus vehicle groups ($p<0.05$).
[c] Significant difference versus vehicle groups ($p<0.01$).
[d] Significant difference versus exp. 1 (5mg/kg) ($p<0.01$).
ICM: Inner Cell Mass

The Kerack addiction during pregnancy retards preimplantation development and induces apoptosis.

Conclusion

- Considering the parameters which can affect on quality and quantity of sperm, oocyte & preimplantation embryos
- ***Epigenetics***
- Environmental Toxins
- Healthy Nutrition (Organic Foods ???)
- Quality of Life
- Physical Activities
- Overcome on Stresses
- Smoking, Alcohol, Drug …..
- Mobile Waves
- Physical Parameters of Embryology Lab like: PH, Temperature, **VOC**, Light, CO2 Incubator, Culture Medium, Period of Culture, Freezing Technique, ……

Thank You for Your Attention
Any Question??