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Gene expression and apoptosis in bovine preimplantation embryo exposed to carbon nanotubes

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Abstract –This study aimed to evaluate the effect of MWNT on expression of transcripts involved in cellular stress and the control of apoptosis, and on programmed cell death in mammal embryo. Blastocysts from control group had higher (P<0.05) total cell number and lower (P<0.05) apoptotic cell index than those exposed to 0.2 µg/mL MWNT for 72h. The number apoptotic cell was similar (P>0.05) between blastocysts exposed and non-exposed, as well as the expression of genes analyzed. In conclusion, MWNT in culture medium may not interfere on expression of the genes evaluated, but induces apoptosis in bovine blastocysts 0.2 µg/ml MWNT after 72h of *in vitro* culture.

Mutiwalled carbon nanotubes (MWNT) can be used in several biological and medical fields, but toxic effects on mammalian's cells and tissues is yet to be fully established. This study aimed to evaluate the effect of MWNT on gene expression involved in cellular stress (Hsp70.1 and PRDX1) and control of apoptosis (Bax), and on cell number and apoptosis in mammal embryo, using *in vitro* produced bovine embryos as a model. Non-functional and non-purified MWNT (size: 40 to 100 µm, diameter: 20 nm) produced by catalytic vapour deposition (ferrocene as catalyst) were used. The embryos were produced after *in vitro* maturation and fertilization of oocytes obtained of ovaries collected from slaughtered cows. At day seven post-fertilization embryos at blastocyst stage were randomly distributed into two culture groups: control group (without MWNT; n=68) and treated group (with 0.2µg/ml MWNT; n=71). Embryos in both groups were cultured in CR2aa medium, supplemented with 10% of fetal calf serum and granulosa cell monolayer, for 72h in microdrops covered by mineral oil and under 5% CO_2 at 38.5° in air. Analysis of the target transcripts were performed using 60 blastocysts (control group =30 and treated group =30) divided into three *pools* for RNA extraction. cDNA was obtained after reverse transcription and was subjected to Real-Time PCR (RT-PCR) using the gene GAPDH as endogenous controls for the subsequent analysis by *REST*® software. The relative amounts of Bax (1.16 ± 0.37), Hsp70.1 (0.75 ± 0.12) and PRDX1 (0.97 ± 0.21) transcripts in embryos cultured with MWNT were not different from control group (P> 0.05). The embryos at eighth day post-fertilization were fixed and permeabilized for TUNEL assay (Promega). Total cell number, apoptotic cell number and apoptotic cell index were analyzed by analysis of variance and means compared by Student Newman Keuls. Blastocysts exposed to MWNT had lower (P<0.05) total cell number and higher (P<0.05) apoptotic cell index than blastocysts from control group (Table 1). The number apoptotic cell was similar (P>0.05) between groups (Table 1). The data showed that viability of bovine embryos at blastocysts stage can be affected by 0.2 µg/ml MWNT after 72h of *in vitro* culture. It was reported recently that MWNT did not induce apoptosis in mouse primary cortical neurons and human neuroblastoma cells after 48 and 72h incubation, respectively [1, 2]. Taking together, those data suggest that embryos are more sensitive to MWNT exposure than other mammalian cells. However, different MWNT concentrations and period of exposure need to be evaluated by further studies. In conclusion, MWNT in culture medium does not interfere on gene expression of Hsp70.1, PRDX1 and Bax, but induces apoptosis in bovine blastocysts treated with 0.2 µg/ml MWNT after 72h of *in vitro* culture.

Table 1. Total cell number and apoptotic cell index (mean±SE) in bovine blastocysts cultured without MWNT and with MWNT.

Group	No.	Total cell no.	Apoptotic cell no.	Apoptotic cell index
Control (without MWNT)	21	425.85±45.69 [a]	93.53±19.84 [a]	19.74±2.83 [a]
MWNT	30	288.08±36.27 [b]	111.64±22.17 [a]	33.01±3.72 [b]

Means with different superscripts within a column differ at P<0.05.

References

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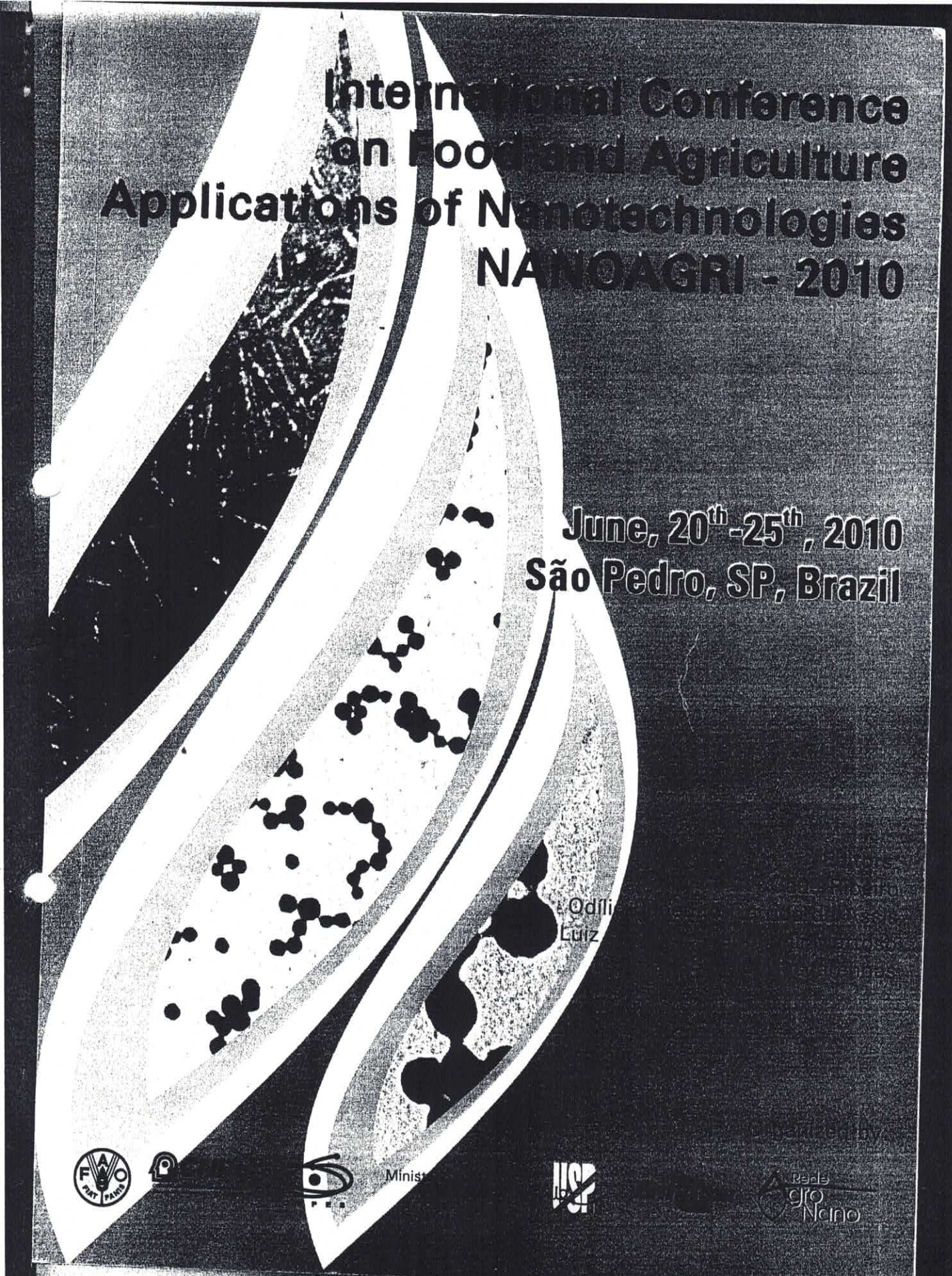

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