**Title:** **Methotrexate Induces a DNA Damage Response in Cultured Primary Sertoli cells *in vitro***

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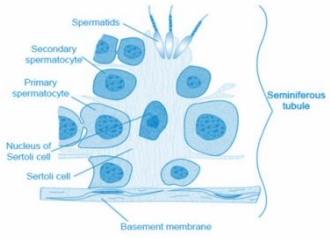
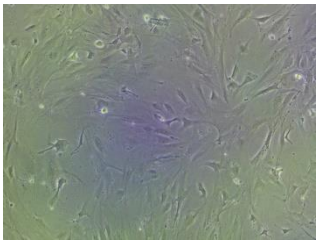
**Abstract (300 word limit)**

Sertoli cells (SCs) are highly differentiated epithelial cells which play an essential role in the functional development of the testis and hence in the expression of the male phenotype. Sertoli cells form the blood-testis barrier making an extraordinary microenvironment where male germ cells develop and are under strict hormonal control. Sertoli cells express the cytokine glial cell line-derived neurotrophic factor (GDNF) under control of the follicle-stimulating hormone. GDNF is an essential regulator of SCs self-renewal and survival in vitro and is required for maintenance of the undifferentiated spermatogonial stem cells population *in vivo*. Various drugs, particularly alkylating agents, have been shown to be gonadotoxic. Methotrexate (MTX) is an anti-metabolite widely used in the treatment of neoplastic disorders, rheumatoid arthritis and psoriasis. The present study explored the mechanism of cytotoxic and genotoxic effects of MTX on a primary culture of Sertoli cells *in vitro*. DNA damage was evaluated using the Comet assay and the cell death was identified as apoptosis using terminal deoxynucleotidyl transferase- (TdT-) mediated dUTP nick end labeling (TUNEL) assay. mRNA expression and proteins levels of GDNF, P53 and ataxia telangiectasia mutated (ATM) were also investigated using quantitative polymerase chain reaction (qPCR) and Western blot methods. Results of the present study clearly showed that MTX -induced DNA damage as evident from the different Comet assay parameters. The GDNF disruption seen in our *in vivo* experiments correlates with the sudden increase of activation of p53 and ATM in Sertoli cells. These results imply that MTX affects the Sertoli cells, inducing GDNF proteins. This disruption signifies the loss of some support mechanism for spermatogenic cells and could be the cause of the increased apoptotic cells.

**Image (if any)**




**Structure of Sertoli Cells**



**Primary Isolated Sertoli Cells**

**Recent Publications (minimum 5) (if any)**

1. Habas, K., Diana Anderson, and Martin Brinkworth (2016) Detection of phase specificity of in vivo germ cell mutagens in an in vitro germ cell system. Toxicology 353: 1-10.
2. Habas, K., Brinkworth, M.H. and Anderson, D (2017). Diethylstilbestrol induces oxidative DNA damage, resulting in apoptosis of spermatogonial stem cells in vitro. Toxicology, 382:117-121.
3. Habas K, Anderson D, Brinkworth MH (2017). Germ cell responses to doxorubicin exposure in vitro. Toxicology letters. 4;265:70-6.
4. Habas, K., Brinkworth, M.H. and Anderson, D (2017). In vitro responses to known in vivo genotoxic agents in mouse germ cells. Environmental and molecular mutagenesis. 58(2), pp.99-107.
5. Habas, K., Brinkworth, M.H. and Anderson, D (2018). Silver nanoparticle-mediated cellular responses in isolated primary Sertoli cells in vitro. Food and Chemical Toxicology. 116, pp.182-188.

## Biography

Diana Anderson (H index 64) holds the Established Chair in Biomedical Sciences at the University of Bradford. She obtained her first degree in the University of Wales and second degrees in the Faculty of Medicine, University of Manchester. She has 500+ peer-reviewed papers, 10 books, has successfully supervised 34 PhDs, is an Editorial Board Member of 10 international journals. She is Editor-in-Chief of a book series on Toxicology for the Royal Society of Chemistry. She gives plenary and key note addresses at various international meetings. She is a consultant for many international organizations, including WHO, EU, NATO, TWAS, UNIDO, OECD.