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THE PROTECTIVE ROLE OF TOCOTRIENOL ON CORTICOSTERONE-INDUCED OXIDATIVE STRESS DURING PRE-IMPLANTATION EMBRYONIC DEVELOPMENT IN MICE

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ABSTRACT

Excessive amount of glucocorticoid [cortisol in human or corticosterone (CORT) in rodent] induces oxidative stress (OS) in the cell leading to DNA damage and it has been proven by previous studies. Conversely, it is well documented that tocotrienol (TCT), a potent antioxidant was able to protect cells by neutralizing excessive reactive oxygen species (ROS). Hence, this study was designed to determine the effect of TCT supplementation on the quality and development of embryos and DNA damage level in embryos of CORT-treated mice. Female mice were given TCT orally at three different doses i.e. 30, 60, and 90 mg kg^{-1} BW, concurrent with 10 mg kg^{-1} BW of CORT intraperitoneally (ip) for 14 days. Mice were superovulated and paired individually overnight with stud male mice. After 48 hours post-coitum, female mice were euthanized to collect 2-cell stage of embryos. The morphological observation and *in vitro* development of embryos were accessed and monitored under an inverted microscope and the percentage of DNA damage was analysed via Comet assay. It was found that oral supplementation of 90 mg kg^{-1} BW of TCT in CORT-treated mice were able to normalize the number of fragmented embryos and improve the number of embryos that reach the blastocyst stage. No DNA damage was noted in all CORT-treated groups supplemented with TCT. Supplementation of TCT also suppresses the level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and restored catalase (CAT) activity toward control. The findings of this study indicate that TCT supplementation in CORT-treated mice was able to reverse the effect of CORT-induced fragmentation and oxidative DNA damage in embryos. Thus, the molecular mechanisms by which TCT suppresses oxidative stress and promotes the quality of embryo need to be investigated in detail in future studies.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND AND PROBLEM STATEMENT

Every single organism will encounter stress in its daily routine. Psychological and physical are the factors that can cause stress in human beings. Most of the time it is well managed and dealt with. However, at certain periods, an individual or organism are unable to control the stress response and it may lead to excessive oxidative stress condition (Tsigos & Chrousos, 2002). Previous studies have shown the correlation between the pregnancy failures, abortion and preterm delivery outcomes with the negative life events, where, the outcomes of those studies indicate that the biological responses involve in a psychological stress is known to suppress the reproductive system (Minas, Jeschke, Kalantaridou, et al. 2007; Macklon, Geraedts, & Fauser, 2002).

Stress and anxiety during gestation period were also contributed to the reproductive failure (Symonds, Stephenson, Gardner, & Budge, 2007) via anovulation, implantation failure and dysregulaticataon of placentation (Nakamura, Sheps, & Arck, 2008). For instance, a previous study has documented that 54% of pregnant woman who had miscarried are more likely to have experienced at least one stressful episode in the preceding three months compared to the control group, especially women that face a most stressful period in the fortnight immediately preceding miscarriage (Papagallo, Szabo, & Esposito, 1999). In another study, it has also been revealed that spontaneous abortions cases were strongly associated with psychological stress (Bashour & Abdul Salam, 2001).

The hypothalamus-pituitary-adrenal (HPA) axis together with the sympatho - adrenomedullary system was activated and glucocorticoid hormones, i.e. cortisol and corticosterone (CORT) as well as catecholamines were secreted during stress (Joëls, Karst, Krugers, & Lucassen, 2007). It has been reported that, when the level of CORT is high, it can induce oxidative stress (OS) (Zafir & Banu, 2009). Studies conducted by Lindsay and Nieman (2005) and de Herder and Lamberts (2001), showed that high levels of glucocorticoid suppress the activities of female reproductive system. This is due to high circulation of glucocorticoid that lead to inhibition of luteinizing hormone (LH), ovarian oestrogen and progesterone secretion