

**TOCOTRIENOLS MAY REVERSE THE DELETERIOUS EFFECTS OF  
NICOTINE-INDUCED OXIDATIVE STRESS ON EMBRYO  
DEVELOPMENT IN MICE  
(600-RMI/ST/DANA 5/3/DST(100/2010))**

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## 1.0 INTRODUCTION

The adverse effects of smoking on reproduction are well documented, yet the current trend of tobacco smoking among women still remains. Approximately 4% of Malaysian population aged  $\geq 15$  years are female smokers (WHO Policy, Strategy Advisory Committee for Tobacco Free Initiative Estimated, 2000). In general, people smoke cigarettes for the psychoactive effects of nicotine where it is believed that nicotine may enhance the sense of well-being, produce arousal or relaxation, help maintain vigilance, or reduce anxiety (Benowitz, 1988). However, nicotine has shown many potential adverse health consequences in reproductive or perinatal disorders including low birth weight, prematurity and spontaneous abortion (Benowitz, 1988), delay in conception (Zenzes, 1995) and the onset of menopause in female smokers (Midgett and Baron, 1990). Therefore, certain components in cigarette smoke may directly or indirectly interfere with embryo development and viability.

Nicotine is present in cigarettes in amounts varying from 0.8 to 1.8 mg per cigarette depending on the brand and size of cigarette (Benowitz et al., 1983; Rosa et al., 1992). As much as 1.0 mg of nicotine is recorded to be absorbed by smoking a single cigarette (Barbieri et al., 1986). Nicotine is a toxic alkaloid and is quickly absorbed through the respiratory track, mucosa of the mouth and skin (Gandini et al., 1997). It has also been reported that lung appears to serve up as a reservoir for nicotine, which slows its entry into the arterial circulation, implying that even though almost all of the nicotine inhaled in each puff being absorbed in a few seconds, it may require 30–60 seconds or longer for the nicotine to be fascinated (Brewer et al., 2004).

Nicotine also induces oxidative stress (OS), a condition arises when the generation of reactive oxygen species (ROS) and other radical species overrides the scavenging capacity by antioxidants, either due to the excessive production of ROS or an inadequate availability of antioxidants. This is accompanied by an alteration of antioxidant enzymes in various tissues including blood plasma, ovary, liver as well as heart (Sies, 1993). This alteration of antioxidant enzymes is reflected by an increased level of malondialdehyde or MDA, an oxidative stress biomarker (Spiteller, 1993). It has been reported that various tissues of mice exposed to side-stream cigarette smoke show elevated oxidative DNA damage (Howard et al., 1998) with a concurrent increase in lipid peroxidation and

decreased level of antioxidant enzymes (Helen et al., 2000). Furthermore, increased lipid peroxidation in blood of smokers has also been shown to be linked with oxidative damage on DNA with carcinogenesis (Kalra et al., 1991). It seems that people who smoke or exposed to cigarette smoke are also subjected to nicotine-induced oxidative stress (Suleyman et al., 2002).

Under physiological conditions, biomolecules are comprised of stable bonds formed by paired electrons. Weakened and disrupted bonds generate free radicals which are unstable and highly reactive (Attaran et al., 2000; Pierce et al., 2004). These free radicals gain stability by acquiring electrons from nearby nucleic acid, lipids, proteins, and carbohydrates, causing cellular damage and disease (Van Langendonck et al., 2002; Szczepanska et al., 2003). At controlled levels, free radicals exert some physiological effects and mediating processes such as tissue remodelling, hormone signalling, oocyte maturation, folliculogenesis, tubal function, ovarian steroidogenesis and cyclical endometrial changes (Agarwal et al., 2005).

Reactive oxygen species (ROS) are formed endogenously during aerobic metabolism as a result of either metabolic pathways of oocytes and embryos or body's defence mechanisms. Apart from being formed endogenously, ROS also can arise from exogenous sources such as alcohol, tobacco and various environmental pollutants (Agarwal et al., 2008). Some examples of ROS include hydroxyl radicals, superoxide anions, hydrogen peroxide and nitric oxide (Agarwal et al., 2008). Reactive oxygen species are capable of inflicting significant damage to cell structures when they increase to pathological levels.

Antioxidants, on the other hand, act to scavenge ROS and free radicals and repair the ROS-induced damage to cell structures (Agarwal and Allamaneni, 2004). There are two types of antioxidants; enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase, and non-enzymatic antioxidants or natural antioxidants including Vitamin E, Vitamin C, selenium, beta carotene, zinc, taurine, hypotaurine, cycteamine and glutathione.