THE INFLUENCE OF TOCOTRIENOL ON THE DEVELOPMENT OF PREIMPLANTATION EMBRYOS, IMPLANTATION SITES AND PREGNANY OUTCOMES IN CORTICOSTERONE-TREATED MICE

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2. Letter of Offer (Research Grant)

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Tuan/Puan,

TAJUK PROJEK FRGS : "THE INFLUENCE OF TOCOTRIENOL ON LIPID PEROXIDATION IN THE DEVELOPMENT OF PREIMPLANTATION EMBRYOS IN STRESSED RATS"

Dengan hormatnya perkara di atas adalah dirujuk.

Sukacita dimaklumkan Kementerian Pengajian Tinggi Malaysia telah meluluskan projek seperti tersebut di atas.

'Kelulusan ini juga tertakluk kepada syarat-syarat seperti berikut :

i. Tempoh projek penyelidikan ini ialah 2 tahun, iaitu bermula **15 September 2007.**

ii. Kos yang diluluskan ialah sebanyak **RM 87,620.00** sahaja. Sila kemukakan bajet yang baru mengikut kos yang diluluskan dengan menggunakan borang Bahagian E – Budget/Belanjawan. Perbelanjaan hendaklah mengikut butiran belanjawan yang telah diluluskan.

iii. Semua pembelian bahan/peralatan adalah diminta agar tuan/puan mematuhi prosedur perbendaharaan di mana pembelian melebihi RM 500.00 hendaklah mengemukakan sebutharga.

iv. Tuan/Puan dikehendaki mengemukakan laporan prestasi secara ringkas 2 kali setahun dan juga laporan prestasi projek tahunan secara terperinci dengan menggunakan format yang ditetapkan.

v. Tuan/Puan dikehendaki mengemukakan laporan prestasi kewangan tahunan secara terperinci dengan menggunakan format yang ditetapkan.

5.2 Enhanced Executive Summary

Stress is a common problem encountered by females that could influence fertility. An increased in corticosterone (CORT) levels is an indication of stress in rodents. Corticosterone administration resulted in impaired reproductive performance in human as well as in other species, as a consequence of excessive production of reactive oxygen species. Tocotrienol (TCT), on the other hand has been reported to play a great role as an antioxidant. This study aims to investigate the effects of tocotrienol supplementation on the development of preimplantation embryo, the number of implantation sites and delivery rate in corticosterone-treated mice. Mice were given CORT, TCT or combination treatment of CORT and TCT for seven consecutive days. In Experiment I, the dose of tocotrienol that were administered are 30, 60 and 90 mg/kg BW while in Experiment II, tocotrienol at doses of 60, 90 & 120 mg/kg BW were respectively administered by gavaging to animals. Exogenous corticosterone was administered by intraperitoneal injection (ip) at the dose of 10mg/kg BW. The *in vitro* development of preimplantation embryos were monitored daily. The numbers of embryos implanted in the uterus and the number of pups delivered were counted. Results showed that corticosterone administration significantly reduced the the number of embryos that reached hatched blastocyst stage, implantation sites and delivery rate as compared to control mice. On the other hand, tocotrienol supplementation in CORT-treated mice returned all the above parameters towards normal value. This finding revealed that tocotrienol supplementation at the dose of 60 mg/kg body weight is able to reverse the adverse effect of CORT in all parameters studied. However, further study is still required in order to elucidate the mechanism of action of tocotrienol in reversing the effect of CORT.

5.3 Introduction

Stress is an attribute of modern life style. Stress is a daily problem encountered by men and women where it affects reproductive system and could influence fertility (Senders & Bruce, 1997; Vilar, 1993). Fertility issues on couples have been widely discussed around the world. It has been estimated that in 2002, about 35 to 70 million couples worldwide are infertile and have turned to Assisted Reproductive Technology (ART) to overcome their infertility (Schultz & Williams, 2002). Stress results in enhanced release of glucocorticoid due to activation of sympathoadrenals and hypothalamic-pituitary-adrenal axis. Increased glucocorticoid levels, i.e. cortisol in human and corticosterone (CORT) in rodents are indication of stress (Joels et al., 2007).

Several stages of normal reproductive process could be disturbed by stress (Euker & Riegle, 1973). Adrenocortical activation during the preimplantation phase may inhibit fertility in women (Rowel, 1970). "Stress" was defined as teratogenic and CORT is the most probable adrenal steroid hormone involved in mouse under stress condition (Hackman & Brown, 1972). In human, early pregnancy loss is attributed by maternal stress as indicated by increased cortisol levels (Nepomnaschy *et al*., 2006). Exogenous CORT administration during second term of pregnancy in rodents resulted in an increased frequency of totally resorbed litters, where the effect seen is directly proportional to the dose of CORT administration (Hackman & Brown, 1972). However, the mechanisms that affect the reproductive system in that study remain unclear.

Stress resulted in excess formation of free radicals or reactive oxygen species (ROS) which will eventually to lead to oxidative stress condition. Stressful conditions cause endogenous cellular antioxidant mechanisms to downplay, thus enhancing free radical generation and subsequently develop oxidative stress (Sikka, 2001). Corticosterone as a stress hormone have a causal role in impacting oxidative processes induced during the adaptive response to physical and psychological stress (Zafir & Banu, 2009). Recent study has shown that high blood corticosterone concentrations increase the oxidative stress level (Cote *et al*., 2010). Decline in endogenous antioxidant status and rise in oxidative stress markers by corticosterone parallel to the effects of restraint stress. (Zafir & Banu, 2009). Restraint stress in rats induced both psychological and physical stress resulting in oxidative stress condition (Kashif *et al*., 2003). On the other hand, oxidative stress following immobilization stress in rats lead to oxidative damage in the brain (Liu *et al*., 1996).

Conditions associated with oxidative stress can result in elevated levels of toxic molecules i.e free radicals that can cause cellular malfunction and even cell death. Many health problems including cardiovascular diseases, diabetes mellitus and cancers are reported to be due to increase in free radical activities (Clarkson & Thompson, 2000). The cellular