THE PREVENTIVE EFFECT OF TOCOTRIENOL ON DELAYED PREIMPLANTATION EMBRYONIC DEVELOPMENT IN AGING MICE

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ABSTRACT

Oxidative stress induced by the aging process was known to cause impairment in female reproductive and antioxidant defense system. Tocotrienol (TCT), through its antioxidant properties has been suggested to play a role in reducing oxidative stress and promoting fertility. The present study evaluates the protective effect of TCT on the ovarian function which was assessed from the response for ovulation process, the quality and developmental competence of embryos produced, and the activity of catalase (CAT), a component of antioxidant defense system. Twelve weeks old female mice were subjected to daily oral gavages of either corn oil (vehicle) or TCT at different doses of 60, 120 and 240 mg kg^{-1} body weight (BW) for 8 weeks duration. Age control groups consisted of young mice (7 weeks old) and aging mice (20 weeks old). Animals were then superovulated and cohabited with a fertile male at a ratio of 1:1. After 48 hours, animals were euthanized, the fallopian tubes were excised and embryos were flushed out. The embryos recovered were then be counted and graded based on its morphology. Only normal embryos were cultured to observe the developmental competence from 2 cells to expanded blastocyst stage. Counting the total number of embryos recovered quantified the ovarian response for ovulation process whereby grading the embryo morphology determined the embryo quality. We found out that the age related decline of embryo count, quality and *in vitro* development from 2 cells to 4 cells stage was improved with TCT treatment at the dose of 120 and 240 mg kg^{-1} BW. The embryos recovered from 240 mg kg^{-1} BW TCT treatment group also denoted to have a higher progression rate at 4 cells to morula stage when compared with all groups. There is no significant difference in CAT level in plasma and ovarian tissue samples in all TCT treatment groups as compared to aging control. Therefore, TCT is found to have a beneficial effect on the ovulation response, embryonic quality and *in vitro* development from 2 cells to morula stage in aging mice.

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CHAPTER 1: INTRODUCTION

1.1 General Background

It is a well known scenario that female fecundity rate decreases with advancing age. The critical turning point of female fertility is said to be at middle age when the rate of loss of the non-renewable pool of oocyte-containing follicles is persistently decreasing in an accelerated manner with time (Broekmans et al., 2009; Miao et al., 2009; Tarin et al., 2001). Although there were studies showed that female mice have germline stem cell which can replenish the primordial follicle pool during adult life (Niikura et al., 2009; Tilly et al., 2009), these premeiotic germ cells recovered from aged mice were found to be functionally defective causing progressive loss of oocyte input back into the ovarian reserve (Niikura et al., 2009). The negative effect of female aging not only in decreasing the quality and developmental competence of embryos but also raising the probability of obstetric and/or perinatal complications (Broekmans et al., 2009; McGee and Hsueh et al., 2000; Miao et al., 2009; Tarin, 1996; Tarin et al., 2001; Wallace and Kelsey et al., 2010). As the current trend, women tend to postpone their first childbearing due to family financial status and career purposes, the subsequent infertility due to aging process have become a serious matter (Broekmans et al., 2009). In fact, the total fertility rates (TFR) or the estimated average number of children born per family for many Western societies are critically low. The TFR is important to ensure replacement of the population (Broekmans et al., 2009). Thus, any methodological advance that can delay the infertility event due to aging process is in critical demand.

Despite the complex interplay of multiple causal mechanisms, the free radical theory which postulates that gradual oxidative damage induced by the accumulation of reactive oxygen species (ROS) over time found to be a major contributor to the aging process (Broekmans et al., 2009; Miao et al., 2009;