UNIVERSITI TEKNOLOGI MARA

THE QUALITY OF OOCYTES AND EMBRYO IN ASSOCIATION WITH METABOLIC CHANGES FOLLOWING TOCOTRIENOL-RICH FRACTION (TRF) SUPPLEMENTATION IN AGING MICE

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Ovarian aging has been associated with oxidative stress and loss of ovarian function. Tocotrienol has been proven to exert beneficial effects on the female reproductive system. The role of tocotrienol in affecting metabolism in the ovary and subsequently improves the quality of oocytes and embryos in aging mice remains unknown. Therefore, the relationship between metabolic changes in the ovary and the quality of oocytes and embryo in aging mice following tocotrienol-rich fraction (TRF) supplementation was investigated. This study was divided into Study 1; determination of oocyte and embryo quality and Study 2; metabolic changes in ovary and serum. Young female mice at the age of six weeks old (Group A) (n=16) and aged-matched control aging mice of eight months old were used (Group B1) (n=16). Sixty-four mice of six months were divided equally into four groups (n=16); one group was given tocopherol-stripped corn oil as vehicle control (Group B2), three groups were given TRF supplementation orally daily at the dose of 90, 120 and 150 mg/kg BW (Group C1, C2 and C3) respectively for two months. After two months duration, mice were superovulated and euthanised to collect the oocytes (n=8) and embryo (n=8) for quality assessment. Oocytes retrieved were further analysed for the pro-apoptotic factor (Bax) and anti-apoptotic factor (Bcl-2). In Study 2, mice were euthanized to collect the ovary and serum for non-targeted metabolomics analysis using liquid chromatography tandem mass spectrometry of quadrupole time-of-flight (LC-MS Q-TOF). Results showed that aging negatively affected the quality of oocytes and embryos. In aging group (Group B1), the percentage of normal oocytes was significantly lower (78.2%) and the abnormal oocytes were significantly higher (21.8%) ($p<0.001$), the expression of Bax (3.265 ± 0.016) was significantly higher ($p<0.001$), the expression of Bcl-2 protein was significantly lower (0.064 ± 0.001) ($p<0.001$), Bax/Bcl-2 ratio measured (0.064 ± 0.001) was significantly higher ($p<0.001$), the percentage of normal embryos (29.4%) was significantly lower and the percentage of abnormal embryos (70.6%) were significantly higher ($p<0.001$), the percentage of embryos that reached the blastocyst stage in the aging group was significantly lower ($p<0.001$). From metabolomics analysis, pathway analysis revealed significantly altered metabolic pathways for fatty acid and amino acid metabolism in aging that might influence the quality of oocytes. Meanwhile, the TRF supplementation was able to improve the oocytes quality and embryonic development at the dose of 150 mg/kg BW. In the group supplemented with TRF 150 mg/kg BW (Group C3), the percentage of normal oocytes was significantly higher (87%) ($p<0.01$) and the abnormal oocytes were significantly lower (13%) ($p<0.05$), the expression of Bax protein (1.924 ± 0.071) was significantly lower ($p<0.01$), the expression of Bcl-2 protein was higher (0.148 ± 0.019) ($p<0.001$), the Bax/Bcl-2 ratio (0.013 ± 0.001) was significantly lower, the percentage of normal embryos was significantly higher (66.3%) and the abnormal embryos were significantly lower (13%) ($p<0.01$) and the percentage of embryos that reached blastocyst stage was improved and development was higher as compared to vehicle control group ($p< 0.01$).TRF supplementation also causes metabolic changes in the ovary that delay the consequences of aging, thus improving the quality of oocytes in aging mice. Consequently, it is recommended that TRF-supplementation delay the consequences of aging that lead to infertility by protecting the reproductive organs from further deterioration.

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TABLE OF CONTENTS