STUDY OF PRIMORDIAL FOLLICLE, CORPORA LUTEA AND *Fmr1* mRNA LEVELS FROM OVARIUM OF *Fmr1* GENE PREMUTATION MICE

THESIS

A thesis submitted for the degree of Master of Biomedical Science majoring on Genetic Counseling

by
SANTOSO
G4A007053

POST GRADUATE PROGRAM
DIPONEGORO UNIVERSITY
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On July 6th, 2010
and has been approved by

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text.

Semarang, July 2010

Santoso

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Santoso

ABBREVIATIONS

α	: Alfa
AIRE	: Autoimmune Regulator Polyglandular Failure gene
AMH	: Anti Mullerian Hormone
APECED	: Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy
ATM	: Ataxia Telangiectasia Mutated gene
AT2	: Angiotensin II Subtype 2 gene
β	: Beta
BLM	: BecQ Protein like 3-DNA Helicase gene
Bl/6	: Black 6
BMP15	: Bone Morphogenetic Protein 15 gene
BMP4	: Bone Morphogenetic Protein 4 gene
Cbl	: Cas-Br-M (murine) ecotropic retroviral transforming sequence gene
CGG	: Cytosine-Guanin-Guanin
Ct	: Cycle time
CYP17A1	: 17-α Hydroxylase/17,20-lyase enzyme
Δ	: Delta
dFMR1	: Drosophila FMR1
DIAPH2	: Diaphanous 2 gene
DMSO	: Dimethyl Sulphoxide

DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
Dpc	: Days post coitum
EDTA	: Ethylenediaminetetraacetic Acid
EIFB2B-2	: Eukaryotic Translation Initiation Factor 2B, Subunit 2 gene
EIFB2B-4	: Eukaryotic Translation Initiation Factor 2B, Subunit 4 gene
EIFB2B-5	: Eukaryotic Translation Initiation Factor 2B, Subunit 5 gene
exCGG	: Expanded Cytosine Guanin Guanin
FAD	: Fragile X Associated Disorders
FMRP	: Fragile X Mental Retardation Protein
FMR1	: human Fragile X Mental Retardation 1 gene
Fmr1	: mice Fragile X Mental Retardation 1 gene
FOXL2	: Forxhead Transcription Factor gene
FSH	: Follicle Stimulating Hormone
FSHRH1	: FSH Receptor gene
FXS	: Fragile X Syndrome
FXPOF	: Fragile X Associated Premature Ovarian Failure
FXPOI	: Fragile X Associated Primary Ovarian Insufficiency
FXTAS	: Fragile X Associated Tremor Ataxia Syndrome
γ	: Gamma

GALT	: Galactose 1-Phosphate Uridyl Transferase gene
HCl	: Hydrogen Chloride
HF	: High Fidelity
Homrep	: Homozygous Repeat
HSP	: Stress Response Protein
INHA	: Inhibin α- subunit gene
ko	: Knock Out
LHCGR	: Luteinizing Hormone/Choriogonadotrophine Receptor
Lhx1	: Lim Homeobox 1 gene
LIF	: Leukemia Inhibitory Factor gene
mRNA	: Messanger Ribonucleic acid
NaCl	: Natrium Chloride
PA	: Polyacrilamide
PCR	: Polymerase Chain Reaction
PGCs	: Primordial Germ Cells
POF	: Premature Ovarian Failure
POI	: Primary Ovarian Insufficiency
POLG	: Polymerase γ
P5	: Postnatal 5 (5-day-old)
P25	: Postnatal 25 (25-day-old)
Q and RT PCR	: Quantitative and Reverse Transcriptase PCR
SCF	: Stem Cell Factor gene
SDS	: Sodium Dodecyl Sulfate

Sf1	: Steroidogenic factor 1 gene
SOX3	: SRY (Sex Determining Region Y) Box 3 gene
STAR	: Steroidogenic Acute Regulatory Protein gene
TBE	: Tris-Borate-EDTA
T1A	: Antibody against human FMRP
UV	: Ultraviolet
wt	: Wild type
Wt1	: Wilm's Tumour 1 gene
XIST	: X (Inactive) Specific Transcript gene
2F5	: Antibody against human FMRP
20wks	: 20 week old
40wks	: 40 week old

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF APPENDICES

Abstract

Background : The *FMR1* gene involves in fragile X-associated disorders (FAD), including the fragile X mental retardation syndrome, primary ovarian insufficiency (POI) and Fragile X associated tremor/ataxia syndrome. Primary ovarian insufficiency (POI) affect on 13-26% female premutation carrier, characterized by early depletion of ovarian follicles before the age of 40 years. The underlying mechanisms of POI are still unclear. This study focuses on number of primordial follicles, ovulation and ovarian *Fmr1* mRNA levels in *Fmr1* gene premutation mice as animal models.

Method : This research was a cross sectional study using a *Fmr1* gene premutation mice model (exCGG mice) and wt mice. The number of primordial follicles were determined at different ages (P6, P25, ~20wk, and ~40wk). The presence of recent corpora lutea (CL) were determined at ~20wk and ~40wk. *Fmr1* mRNA levels in ovaries from P25 premutation mice were determined and compared to wild type (wt) mice.

Results : Approximately a four fold elevated level of *Fmr1* mRNA was found in ovaries of premutation mice. No differences in number of primordial follicles was found in ovaries from P6 and ~20wk premutation mice compared to wt mice ($p=0.052$ and $p=0.515$, respectively). Significant higher number of primordial follicles was found at P25, and ~40wk premutation mice compared to wt mice ($p=0.018$, and 0.006, respectively). Furthermore reduced ovulation, determined by presence of recent corpora lutea, in 20 and 40-week-old premutation mice was found.

Conclusion : The fragile X premutation does not cause a reduction in the initial pool of primordial follicles from birth but might be involved in mechanisms influencing the folliculogenesis results in decreased ovulation..

Keyword : Primary ovarian insufficiency (POI), *FMR1* gene, FMRP, *FMR1* mRNA, primordial follicle, folliculogenesis

Abstrak

Latar Belakang : Gen *FMR1* merupakan penyebab *Fragile X-associated disorders* (FAD), yang meliputi *Fragile X Syndrome* (FXS), *Primary ovarian insufficiency* (POI) dan *tremor/ataxia syndrome*. 13-26% wanita pembawa alel premutasi mengalami POI, yang ditandai dengan berkurangnya jumlah cadangan folikel dalam ovarium sebelum berusia 40 tahun, sementara itu patogenesis dari POI pada wanita premutasi sampai sekarang masih belum diketahui. Penelitian ini bertujuan untuk mengetahui jumlah cadangan folikel primordial, ovulasi dan konsentrasi *Fmr1 mRNA* pada ovarium tikus premutasi gen *Fmr1*.

Metode : Penelitian ini merupakan penelitian *cross sectional* dengan menggunakan tikus premutasi dan *wild type* (wt). Jumlah folikel primordial dihitung dari kelompok tikus pada usia yang berbeda (6 hari, 25 hari, ~20 minggu, dan ~40 minggu). Adanya ovulasi dideteksi dengan adanya *recent corpora lutea* pada kelompok tikus usia ~20 minggu dan ~40 minggu. Konsentrasi *Fmr1 mRNA* diukur dari ovarium kelompok tikus premutasi usia 25 hari yang dibandingkan dengan kelompok wt.

Hasil : Terdapat peningkatan empat kali lipat konsentrasi *Fmr1 mRNA* pada ovarium tikus premutasi dibandingkan dengan kelompok wt. Tidak terdapat perbedaan jumlah folikel primordial pada kelompok tikus premutasi usia 6 hari ($p=0.052$) dan ~20 minggu ($p=0.515$) dan terdapat peningkatan jumlah folikel primordial pada usia 25 hari ($p=0.018$) dan ~40 minggu ($p=0.006$). Selain itu, ditemukan penurunan jumlah ovulasi yang dideteksi dengan adanya *recent corpora lutea*, pada kelompok usia ~20 minggu dan ~40 minggu.

Kesimpulan : Premutasi gen *Fmr1* tidak menyebabkan pengurangan jumlah folikel primordial. Ditemukan gangguan folikulogenesis yang menyebabkan pengurangan jumlah ovulasi pada tikus premutasi.

Kata Kunci : Primary ovarian insufficiency (POI), gen *FMR1*, FMRP, *FMR1* mRNA, folikel primordial, folikulogenesis.