

CHAPTER I

INTRODUCTION

1.1. Background

The Fragile X Mental-Retardation1 (*FMRI*) gene involves in three disorders, Fragile X syndrome (FXS), premature ovarian failure (FXPOF) and the Fragile X-associated tremor/ataxia syndrome (FXTAS). A full mutation in the *FMRI* gene consists of over 200 CGG repeats in the 5' UTR and as a consequence the gene is hypermethylated. Thus, no mRNA is produced, and the lack of the gene product. The absence of Fragile X Mental Retardation Protein (FMRP), gene product of *FMRI* cause FXS. FXPOF and FXTAS are associated with the premutation (55-200 CGG repeats) in the *FMRI* gene, premutation alleles may become unstable when maternally transmitted to their child (1-4). The risk of expansion into a full mutation depends on the size of the premutation of the mother (5). Tassone et al (2000) found there is a reduction of FMRP levels and elevation of *FMRI* mRNA levels at least fivefold in lymphocytes of premutation male (6). Experiments using premutation mice also display elevated *Fmr1* mRNA levels and decreased *Fmrp* levels (7), this result support a mechanism of involvement in premutation carriers, in which reduced translational efficiency is at least partially compensated through increased transcriptional activity.

Premature ovarian failure (POF) is characterized by early depletion of ovarian follicles before the age of 40 year. It is a heterogeneous disorder affecting

1% of women in the normal population (8-10). It has been proposed that primary ovarian insufficiency (POI) is a more accurate term for the disorder, to describe a long and variable clinical course associated with what used to be classified as POF (11). Therefore the term POI will be used from now in this thesis.

Most cases of POI are idiopathic, thus the underlying mechanisms are largely unknown. Observation of familial cases with POI indicates the role of genetic aberrations in its pathogenesis (12).

Previous studies have shown that the prevalence of POI in *FMRI* premutation women is between 13-26% (10). The underlying mechanisms of POI in premutation women are not clear. The association between *FMRI* repeat size and POI was identified, surprisingly, this relationship is unlinear. Indeed, the risk appears to increase with increasing premutation repeat size between 59 and 99, thereafter the risk of POI plateaus even decreases for women with repeat size over 100 (13).

There are many hypotheses underlying the mechanism of POI associated with the *FMRI* gene, including the *FMRI* mRNA gain-of-function toxicity in the ovary that causes reduction in ovarian reserve (14, 15). The risk for ovarian dysfunction is not increased among full mutation carriers; thus the molecular mechanism underlying this premutation-associated disorder is unrelated to the total absence of FMRP (16).

The strategy of developing a using mouse model with spontaneous or induced mutation to investigate POI is extremely powerful, and has provide

important insights into the basic mechanisms of POI, and subsequent infertility (17, 18).

In order to study POI related to the fragile X premutation in an animal model, an expanded CGG-repeat knock-in mouse model has been generated in which the CGG(8 repeat) allele in the endogenous murine *Fmr1* gene was replaced, via homologous recombination, with a human NheI-XhoI fragment containing a CGG(98 repeat) allele (further named : exCGG mice) (19-21). This knock in mice has similar condition like in human including increase levels of *Fmr1* mRNA in the brain, reduced Fmrp, and instability transmission to next generation. Therefore the premutation mice with repeat length between 100-199 will be used in this thesis.

Understanding the underlying pattern of POI associated with the *FMRI* premutation using the premutation mice model, is important to improve the clinical management and genetic counseling effort of clinician and influence the concept of researchers who are investigating POI associated with the *FMRI* premutation.

1.2. Research Questions

Is there a difference on primordial follicle number, corpus luteum and *Fmr1* mRNA levels in ovaries between homozygous (have two premutation alleles in both X chromosomes) female premutation mice and wild type (wt) mice?

1.3. Research Purposes

1.3.1. General purposes :

To determine primordial follicle number, corpus luteum and *Fmr1* mRNA levels from ovarium of premutation mice, in order to contribute a better understanding for POI associated with the *FMRI* premutation allele.

1.3.2. Specific purposes :

1. To compare primordial follicle number of ovaries from 6-day-old (P6), 25-day-old (P25), 20-week-old (~20wk), and 40-week-old (~40wk) premutation mice to age-matched wt mice.
2. To compare the proportion of presence of recent corpora lutea of ovaries from ~20wk, and ~40wk premutation mice to age matched wt mice.
3. To describe fold change of *Fmr1* mRNA levels in ovaries from premutation mice and wt mice.

1.4. Research Benefits

The results of this research will provide information to understand the underlying pattern of POI among female premutation carriers, to get a better knowledge on pathogenesis of POI associated with the *FMRI* premutation allele.

1.5. Research Originality

To my knowledge, there is yet no published study concerning primordial follicle, corpus luteum and *Fmr1* mRNA levels from ovarium among human or animal carrying *Fmr1* gene premutation allele.

No	Authors	Tissue or Organ	Results
1	Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ	Leukocyte	<i>FMRI</i> mRNA levels were elevated at least fivefold within this same range (6).
2	Brouwer JR, Mientjes EJ, Bakker CE, Nieuwenhuizen IM, Severijnen LA, Van der Linde HC, et al.	Brain	<i>FMRI</i> mRNA levels were elevated fivefold compared to age matched wild type (7).
3	Malter HE, Iber JC, Willemsen R, De Graaff E, Tarleton JC, Leisti J, et al.	Ovaries from a 16-week-gestation fetus carrying a full mutation allele.	The number of oocyte was not markedly reduced (22).