CHAPTER I
INTRODUCTION

Infertility: the state of the problem

Infertility is defined as an inability to conceive a child after 12 months of unprotected intercourse. About 10-15% of couples is infertile and seeks medical advice in order to obtain proper diagnosis and treatment. Infertility is a global health issue that affects millions of people worldwide. In fact, no society can escape infertility; some portion of every human population is affected by the inability to conceive during their reproductive lives. Extrapolating this figure to the global population, this means that 50–80 million people may be experiencing infertility at any given time[(1)]. The lack of pregnancy and the resulting childlessness are often highly stigmatizing, leading to profound social suffering for infertile couples and infertile women in particular. Infertility is listed as one of the main reasons for divorce in Xinjiang, China. A large proportion of these couples express psychological problems, due to fertility treatment or to strained relationships with their in-laws[(2)]. Male infertility contributes to at least half of all cases worldwide and is often the most difficult form of infertility to treat[(3)]. Intracytoplasmic sperm injection (ICSI) is the only solution for most cases of male infertility. As there is only one Y chromosome, it may be predicted that large deletions (loss of genetic material) from the Y chromosome will be transmitted to male offspring. The availability of ICSI for the treatment for male infertility has inevitably increased the risk of transmitting male infertility from father to son [(4)]. However, this is likely to be rare in the normal population because, without ICSI treatment, men with very low sperm counts are less likely to father children.

Male infertility

There are several causes of male infertility such as: varicocele, spermatic duct obstruction, endocrine disorders, gonadotoxins, anti-sperm antibodies, infection and ejaculatory dysfunction. Infertilities of undetermined cause are described as idiopathic infertilities and idiopathic male infertility accounts for over 40% of all male infertility cases[(5-8)].Male infertility has been classified on the basis of semen analysis[(9)].

Idiopathic male infertility is characterized by severe defects in sperm production, which are classified as:

1) Azoospermia (absence of sperm)

2) Oligospermia (severe reduction in sperm count)

3) Oligo-astheno-teratozoospermia, OAT (low sperm count, decreased motility and highly abnormal morphology)[10-12].

Genetic defects and spermatogenic failure

It is believed that genetic defects such as chromosome abnormalities and Y chromosome microdeletions are implicated in the pathogenesis of spermatogenic failure. In 1976, the azoospermia factor (AZF) on the long arm of the Y chromosome (Yq) was first reported by Tiepolo and Zuffardi[13]. The AZF was defined by using cytogenetic findings in six azoospermic men and in later studies by molecular techniques. These reports show that microdeletions in AZF are a common cause of male infertility[14]. It has been postulated that Yq11 deletions encompassing three non-overlapping regions (AZFa, AZFb, and AZFc) could be responsible for disruption of spermatogenesis[15]. Recently, a fourth AZF region (AZFd) located between AZFb and AZFc has been proposed[16] but a candidate gene in this region has not yet been identified.

Several cytogenetic investigations have shown that the frequency of chromosomal abnormalities is higher in infertile men[17]. It has been estimated that the overall incidence of chromosome abnormalities in infertile males ranges between 2% and 8% (average 5%) [18, 19]. Klinefelter's syndrome (47,XXY) is the most common chromosome abnormality associated with male infertility and azoospermia[20].

Hormone deficiency and sperm production

Male infertility is rarely caused by a hormone deficiency. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are produced in the pituitary gland and their functions are to stimulate the testis to produce normal amounts of testosterone and sperm. Anything that lowers these hormone levels, for example a pituitary tumor, can result in low or no sperm production and low blood testosterone levels[21-23].

Genetic screening and human assisted reproduction

The ICSI (intracytoplasmic sperm injection) technique is rapidly becoming an accepted procedure in human assisted reproductions, in cases of male infertility. This technique has revolutionized the treatment of males with spermatogenic defects, allowing men who previously would have been unable to have children to achieve biological paternity [(24)]. Although microdeletion of the Y chromosome is not thought to be associated with other health problems, sons of individuals with Y chromosome microdeletion will inherit the mutation and may consequently be infertile themselves [(4)]. Since the development of assisted reproduction technology (ART) chromosomal and genetic causes of infertility have become very important. Genetic screening should be offered to men who have azoospermia or severe oligozoospermia prior to performing ICSI with their sperm. Y chromosome microdeletion screening is important not only to define the etiology of spermatogenic failure but also because it provides precious information for the more appropriate clinical management of both infertile males and their future male offspring.

Several research groups have developed screening methodologies to scan for Y chromosome microdeletion, the majority of methods are based on multiplex PCR and their main focus is to detect a deletion in one of the associated AZF regions [(25)]. A commercial multiplex PCR kit has also been developed (Promega, Madison, USA). There are a few reports which use other protocols, such as Primed *in situ* labeling (PRINS)[(26)], DNA chip technology[(27)] or real-time PCR[(28)]. These protocols are interesting, but they are expensive, complex and require some level of expertise.

Previously, most studies have used sequence-tagged site [(29)] primers to identify chromosome microdeletion using multiplex PCR, and most of the STS primers used thus far amplify anonymous sequences not associated with a specific gene [(10, 30)]. A microdeletion of an STS could, therefore, represent a clinically irrelevant polymorphism rather than the cause of spermatogenic failure [(12, 31-34)]. However, to date, most STS-based studies have incorporated one or two gene-specific primers (*DAZ* and/or *RBM1*) with other STSs associated with anonymous markers. Because the studies used anonymous STSs, individual gene-deletion status was unknown and genotype-phenotype correlation was, therefore, not possible. Thus, the best PCR

markers are derived from intragenic DNA sequences in which an internal deletion or mutation has been shown to cause infertility [35].

In this study, 11 gene-specific markers were used to detect Y chromosome microdeletions in Thai males with spermatogenic failure. The prevalence of the Y chromosome microdeletions in Thai infertile males, which has never been reported, was extensively investigated using an in-house multiplex PCR technique. The method developed for this study is sensitive, specific, inexpensive and can be performed as a routine molecular laboratory assay for analysis of Y chromosome microdeletions. During development, the following parameters were optimized: primer design specific for each designated gene, grouping of suitable primer pairs and multiplex PCR conditions specific for each group of primers. Normal fertile male genomic DNA was used as a 'no deletion' control and an internal control primer was added to each multiplex PCR set.

The established screening protocol, based on multiplex PCR for detection of Y chromosome microdeletions, was used to determine the prevalence of microdeletion in Thai males with azoospermia or oligospermia. The patient's medical history: physical examination details, hormone profiles and karyotype were analyzed together with results from Y chromosome microdeletion analysis. The possible association between these observed conditions and the patient's reduced fertility status were investigated. Furthermore, the information collected, such as the prevalence of Y chromosome microdeletion, abnormal karyotype and abnormal hormone level in Thai infertile males, will provide the background knowledge for further studies.

The objectives of the study

1. To develop an efficient and inexpensive multiplex PCR screening technique for the detection of Y chromosome microdeletions within three regions of the Y chromosome (AZFa, AZFb and AZFc) in Thai males with azoospermia or oligospermia.

2. To investigate the prevalence of Y chromosome microdeletion in Thai infertile males with azoospermia/oligospermia.

3. To determine the frequency of chromosomal abnormalities in Thai infertile males with azoospermia/oligospermia.

4. To evaluate the abnormalities of hormonal profiles level in Thai infertile males with azoospermia/oligospermia.