CHAPTER IV

DISCUSSION

Prenatal diagnosis is a powerful technique for detection of chromosomal abnormalities in high risk pregnancies especially Down syndrome (trisomy 21). However, standard karyotyping must be performed on metaphase cells, and therefore it takes for culture time several days. Waiting for chromosome analysis can place significant emotional stress on the patient and physician (Lim *et al*., 2002). Apart from being time consuming, traditional cytogenetic analysis is also technically demanding, labor intensive, relatively expensive, and requires highly trained analysts (Feldman *et al*., 2000). Since its introduction in 1992 (Klinger *et al*., 1992) fluorescence *in situ* hybridization (FISH) has become an essential method for the rapid prenatal detection of specific numerical chromosome anomalies on uncultured amniotic fluid samples (Witter *et al*., 2002). The potential application of FISH technology for rapid prenatal diagnosis of the most common aneuploidies has also been established. Early attempts at aneuploidy detection in uncultured amniocytes suffered from significant limitations caused by probe design, sample preparation, and assay conditions (Feldman *et al*., 2000). However, when compared with traditional cytogenetics following amniocentesis, advantages afforded by FISH include rapid results that are generated within 2 days, compared with 7-14 days for traditional cytogenetics. The observation that FISH is less laboring intensive than cytogenetics, and the applicability of FISH to uncultured specimens or to specimens with a low mitotic index. In addition, the results of the FISH studies have also assisted the clinical cytogenetic laboratory in the assignment of priority status for analysis of high-risk specimens. The potential advantages of rapid aneuploidy detection for the health-care provider and patient are as follows. FISH may be the optimal strategy for rapid confirmation of potential numerical chromosome aneuploidies when untrasound reveals fetal abnormalities. FISH coupled with traditional cytogenetics may, in

certain-circumstances, aid genetic counseling; and early receipt of normal results has a positive effect on maternal/fetal attachment and decreases anxiety levels (Ward *et al*., 1993). Furthermore, the quality and characteristics of the probes are the key factors for successful FISH analysis (Feldman *et al*., 2000). Some authors used probes constructed by their own laboratories (Bryndorf *et al*., 1997; Klinger *et al*., 1992; Ward *et al*., 1993). Most cytogenetic laboratories are not qualified to synthesize DNA probes and to perform the necessary quality-control studies. Since 1993, the position of the American College of Medical Genetics (ACMG) has been that prenatal interphase fluorescence *in situ* hybridization (FISH) is investigational. In 1997, the Food and Drug Administration (FDA) cleared the AneuVysion assay (Vysis, Inc.) to enumerate chromosomes 13, 18, 21, X, and Y for prenatal diagnosis (Tepperberg *et al*., 2001). These FISH probes were commercial product and applied according to the manufacturer's instructions. These probes comprised two sets, one set containing alpha satellite probes for the X, Y and 18 chromosomes and the second set containing probes spanning the retinoblastoma gene (RBI) at 13q14 and the region 21q22.13-q22.2 (Pergament *et al*., 2000a). However, many genetic laboratories cannot use commercial DNA specific probe because of expensive cost for direct FISH. Thus, our laboratory is qualified and equipped to produce DNA probes by using the micro-FISH technique.

Several studies reported a strategy for rapid construction of microdissected chromosomal probes. Most of them are the unidentifiable marker chromosomes of unidentifiable unbalanced translocations that frequently prevented complete karyotypic analysis. Meltzer *et al*., 1992 identified the marker chromosome that is derived from the terminal long arm of chromosome 21q21-qter. The regions 21q22-qter was dissected in the studies of Yokoyama and Sakuragawa, 1995. Guan *et al*., 1994 produced WCPs for 15 normal human chromosomes. The cross-hybridization of the acrocentric chromosome (13-15, 21, and 22) was determined. Avoiding dissection of the regions containing highly repetitive sequences can solve this problem. Using this approach, no apparent cross-hybridization was observed between their acrocentric WCPs. There were no reports describing the production of chromosome 21 probes by micro-FISH to apply to uncultured amniocytes for prenatal diagnosis. So, these probes were constructed. At first, the t(21;21) chromosomes

were dissected with the expectation of no appearance of cross-hybridization. In addition, the two copies of chromosome 21 on the t(21;21) could be usefulness for decreasing the numbers of microdissected chromosome 21 fragments and also improving the quality of DNA amplifications. However, these were unavoidable problem because the repetitive DNA sequences at the centromere remained in the t (21;21). Although historically all such rearrangements were collectively called homologous Robertsonian translocations, the molecular studies have shown that approximately 90% of the chromosomes within this category may actually be isochromosomes. An isochromosome consists of two copies of the same chromosome arm joined in such a way that the arms form mirror images of another. Isochromosome may have a single centromere or may have two centromeres. In which case they are called isodicentric chromosomes (Kaiser-Roger and Rao, 1999). However, these t(21;21) probes indicated the absence of signals along chromosome 14, 15, and 22. Therefore, the second hypothesis was that the cross-hybridization problem could be solved if dissection of the short arm and centromere was not done. This was almost successful because the hybridization signals that at the centromere of chromosome 13 were not so bright. We supposed this resulted from the amplification of contamination from some fragments of short arm and centromere of chromosome 21 in the microdissection step. To collect a chromosome band or region such as the small chromosome 21 certain precautions have to be made. This step was not easy for an inexperienced person but experience and carefulness could achieve an improvement. In addition, the homologous repetitive sequences may be located at the long arm as same as the centromere of the chromosome 21 that further in this study. For the amplification step, we succeed in yielding PCR products from only 8 dissected chromosomal fragments. Furthermore, extraneous contamination of primary DOP-PCR was eliminated problem. The quality of the DOP-PCR product also depends on the preparation of microdissected metaphase chromosomes. DNA damage due to methanol/acetic acid treatment during fixation should be reduced (Engelen *et al*., 1998a). In this study, the cover glass slides holding the metaphase should be washed immediately with distilled water to remove remaining acetic acid and stored at 37°C only 1-2 days until used. When used for microdissection, a slide should not be used for more than one hour.

The reamplification was successful in this study; in spite of the fact that there was endogenous contamination of the previous negative reamplification. However, the experiment was continued and the hybridization signals presented on the target chromosomes that confirmed the efficiency of the reamplification of the DNA of these reamplification only 1 μl of primary DOP-PCR product resulted in 5 hybridization reactions that each containing 20 μl labeled digoxigenin-11-dUTP or biotin-16-dUTP. Therefore, using this scheme the origin of primary DOP-PCR products in the amount 45 μl (after gel electrophoresis 5 μl) could be reamplified about 45 times until its depurination of the chromosomal DNA. Whereas, we could reamplified only 5-10 times from the original product because there were some failure hybridization. However, this method could help us to produce the chromosome 21q derived-probe sufficiency throughout the experiment. Liehr *et al*., 2002 took only 0.5 μl from the original PCR reaction (50 μl) for re-amplified 1 and repeated (re-ampl. 2), followed by a labeling DOP-PCR reaction using 1.5 μl of template and dUTP-hapten instead of dTTP. Using their scheme the original probe can be re-amplified x100x100x66 times. Therefore, we could decrease the amount of template in reamplification and increase the efficiency of the probe, ie, reducing the negative control from the previous reaction by trying decrease number of the cycle parameters to enrich the optimal secondary product. Following this scheme, we may success to produce the probes kit with low cost and commercially available in the future.

Table 8 Summary of greatest number of amniocentesis indications from defferent laboratories.

	References				
Indications	Eiben *et al*., 1999	Chaabouni *et al*., 2001	Luquet *et al*., 2002	Lim *et al*., 2002	Jobanputra *et al*., 2002
Advance maternal age	46.00%	65.05%	14.00%	7.60%	25.00%
Abnormal ultrasound finding	14.00%	8.23%	69.00%	37.90%	29.55
Maternal serum screening	40.00%	1.95%	17.00%	50.00%	3.41%
Previous Down baby	-	11.76%	-	3.00%	35.23%
Congenital anomaly	-	-	-	-	-
Chromosomal disease in close family	-	6.85%	-	-	-
Parental chromosomal abnormality	-	-	-	-	4.55%
Parental balance karyotype	-	0.96%	-	-	-
Rapid sexing (X-linked recessive disease)	-	-	-	1.5%	-
Others	-	5.21%	-	-	2.27%
Total	100%	100%	100%	100%	100%

Most of the indication for prenatal diagnosis in this study was only for advance maternal age. From table 8, we can see that not only advance maternal age but also abnormal ultrasound finding and maternal serum screening. Particularly, the abnormal fetal ultrasound finding (69%) was the greatest indicator of the Luquet *et al*., 2002 study. These can be used for a clinical decision when an aberrant FISH results correspond to an abnormal ultrasound scan. Whenever a bad prognosis FISH result was detected without evident ultrasound changes, they accelerated the management of amniotic fluid culture for the karyotype to confirm the diagnosis.

In the present application of 12 amniotic fluid samples, there were the alternative FISH results because of the criteria for diagnosis. The major problems found among most studies were the results of unsatisfactory criteria in interpretation of results. The different cut-off points for the proportion of cells with identical pattern of signals needed for diagnosis by FISH were between 50-70% in different studies (Feldman *et al*., 2000; Lim *et al*., 2002). According to the manufacturer's instructions for the interpretation of the FISH analysis, a clinical specimen is considered aneuploid when >60% of the nuclei are aneuploid. This cut-off point was used by most of the authors, and specimens with 10-60% aneuploid nuclei were uninformative cases. The examples of criteria for interpreting FISH results are illustrated in table 9.

Table 9 The criteria for interpreting FISH results and the number of scored nuclei by using commercial specific probe.

References	No. of scored nuclei	Interpretation	
		disomy/normal	trisomy/abnormal
Ward *et al*., 1993	≥ 50	≥ 80%	≥ 70%
Eiben *et al*., 1999	30	> 90%	> 60%
Feldman *et al*., 2000	≥ 50	≥ 85%	≥ 85%
Weremowicz *et al*., 2001	30	≥ 60%/70%	≥ 60%/70%
Jobanputra *et al*., 2002	≥ 50	≥ 80%	≥ 70%
Lim *et al*., 2002	100	≥ 80%	≥ 70%
Luquet *et al*., 2002	50	≥ 90%	-

Note: No cut-off point was fixed for the aneuploides in the Luquet *et al*., 2002 study.

We observed that minimums of 50 nuclei were scored but this cut-off level could be decreased to 30 nuclei. The informative results were defined as normal or trisomy which had ≥80% or ≥70% respectively. However, the cut-off point could have been optimized in their study. For example, Weremowicz *et al*., 2001 increased this cut-off to 70% after the false-negative cases of trisomy 21 and used this point to report a result for the remaining samples. Feldman *et al*., 2000 observed that in the vast majority of hybridization more than 95% of the cells showed an accurate number of signals. These modifications were the basis for the zero uninformative FISH results. Moreover, the relatively small total number of cells needed for diagnosis practically 100, gave a much better option to be more selective in cell quality and were a major factor in the efficiency and accuracy of the test. Jobanputra *et al*., 2002 suggested that more strict reporting criteria could be adopted in the future. The cut-off point could be optimized to as high as 90% for both the disomic as well as trisomic prenatal samples. However, these high cut-off points were optimized for the very reliable commercial specific probes. The microdissected probe that we constructed could not used to interpret results. Thus, we reduced this cut-off to diagnose from only the maximum percentage of the signals. Klinger *et al*., 1992 used the data of statistical analysis to support the assignment of cut-off value such that

samples in which <23% three-signal nuclei designated normal and ≥42% three-signal nuclei designated trisomic. The hybridization patterns that generate 23% - 42% three-signal nuclei are held to be indeterminate. By these criteria, there was no overlap in the confidence intervals predictive of normal or abnormal status at the 99% level. Ward *et al*., 1993 believed that more conservative criteria were appropriate for the initial clinical application of FISH for aneuploidy detection. In our study, an average of 55.14% two-signal nuclei displayed on normal samples and an average of 46.33% three-signal nuclei on trisomy 21.

The important limitation of diagnostic reliability of FISH results was the signal analysis. The FISH probes that we constructed were derived from 21q region whereas the commercial specific probes are derived from 21q22.13-q22.2. This locus specific probe produces smaller hybridization signals and more specific than the 21q-derived probe which we had constructed. Enumeration of FISH spots by human observers reveals difficulties related to objective and correct spot discrimination which require experienced observers (Truong *et al*., 2003). We observed 0, 1, 2, 3, 4, 5, and ambiguous signals in this study. The ambiguous signals were excluded for scoring to decrease the uninformative results that occurred in Horpauphan, 2003 who hybridized microdissected WCPs 22 derived-probe on interphase nuclei and revealed the largest number of unclear or ambiguous signals in almost all their experiment. Interphase FISH probe signal size discrepancy needs to be interpreted with caution, especially if using probes from regions of the genome containing repetitive DNA (Graf *et al*., 2002). There are different types of fluorescent dot counting in interphase nuclei; correct dots, split dots, overlapping dots, missed dots, false dots, out of focus and debris (Netten *et al*., 1997). These may lead to error in the results. A split dot is counted as two dots instead of one dot. Misinterpretation of split dots leads to a lower percentage of disomic cells in a normal specimen. In addition, the other source of false-positive signals comes from the presence of minor binding sites and from split dots (Philip *et al*., 1994). The signal splitting occurred more frequently when alpha-satellite DNA probes were used, but was rarely seen in locus-specific probe detection. Signal splitting can be explained by chromatin extension occurring in the centromeric location and forming a chromatin fiber which links two condensed domains of chromatin. This fiber usually shows very weak signals and it fades faster than a

normal signal; thus, two signals will be formed from a single centromere (Yan *et al*., 2000). Moreover, during the cell cycle, centromeres have a different appearance in the G_1, S, and G_2 phases. While their morphology in G_0 and G_1 phase is the same, they are decondensed during the S phase and occupy a larger, not clearly defined area. The signals can overlay each other and in part of the cells they cannot be clearly analyzed. During the G_2 phase, when centromeres following replication show double-signals that may not show a difference clear enough from an altered signal number, diagnostic problems may occur (Cannizzaru *et al*., 1997; Raff *et al*., 2001). The others error is overlapping dots that occur due to contact or the overlapping dots are not properly separated; two dots are counted as one dot. A hybridization dot can be missed (missed dots) during segmentation, or is rejected based on it features. Single dots can be separated into two dots or a background signal can be detected as a dot; both errors cause false dots. Autofocusing usually fails because it has focused on debris instead of the nuclei; if a nucleus is out of focus, dots will not be detected. Debris, fluorescent material, or air bubbles cannot always be distinguished from single nuclei based on the measured feature (Netten *et al*., 1997). Split dots and false dots yield a false-positive rate. Overlapping, missed dots and out-of-focus errors give a false-negative rate. Using specimens with less debris and other fluorescent material in the background will reduce the error rates due to fewer false dots, less debris, and fewer focusing errors (Netten *et al*., 1997). These error dots were unavoidable in our experiments, especially the debris that showed on interphase uncultured amniocytes. The morphology of these uncultured amniocyte nuclei is unclear cytoplasm unlike the cultured lymphocytes. This led to the failure of hybridization and uninformative results because of the insufficient nuclei for analysis. The false negative rate (16.66%) FISH result in this study was extremely high. This depends on the number (n) of samples in the study. Luquet *et al*., 2002 summarized the publications using the same kind of probes for rapid FISH prenatal diagnosis. The data indicated that the number of case (n) studies were at least 30 cases (Aviram-Goldring *et al*., 1999) to a maximum of 8,500 cases (Estabrooks *et al*., 1999). The false negative rate of their studies was only 0-5 cases whereas our study presented 1 case out of a total 6 trisomy 21 cases. Moreover, when a false negative or an uninformative case is observed in a female fetus, most of the authors explain the discrepancy as possible maternal

contamination, although it could be a hybridization failure, especially when the aneuploidy involved chromosomes tested with alpha satellite probes (Luquet *et al*., 2002). Therefore, some teams chose to exclude all macroscopically hemorrhagic specimens from their studies. Many studies reported the maternal contamination in their studies; however, this did not affect test interpretation. The maternal contamination may occur either in hemorrhagic specimens or in apparently clear specimens. Eiben *et al*., 1999 reported a false negative case because of maternal cell contamination in clear amniotic fluid. Luquet *et al*., 2002 think that FISH may be performed on hemorrhagic specimens, but its interpretation requires some caution: exclude the lobulated nuclei, increase the number of scored nuclei proportionally to the maternal contamination in male fetuses, and, when FISH is apparently normal XX, wait for the karyotype. Morris *et al*., 1999 tested all heavily and some moderately bloodstained samples with X and Y probes simultaneously, and hybridization with the autosomal probes was performed only if ≥ 80% of the scored nuclei were male. In addition, some hemorrhagic specimens fail to grow in culture, and in these cases, FISH can give invaluable information (Luquet *et al*., 2002).

In the present study, no evidence of mosaicism obtained by karyotyping. Mosaicism, or the presence of two or more cell lines in culture, is one of the most complex and challenging issues in prenatal diagnosis. There are three levels of mosaicism. Level I is defined as a single-cell abnormality. Level II is defined as a multiple-cell abnormality or a whole-colony abnormality in one culture not seen in any other cell cultures. Level III is true mosaicism that the presence of a second cell line in two or more independent culture (Randolph, 1999). When mosaicism is found in cultured fetal cells there may be problems in interpretation, both as to whether the fetus is truly mosaic and as to the clinical significance of the observation (Thompson *et al*., 1991c). Jobanputra *et al*., 2002 reported that in the samples with karyotypical mosaicism specific for the tested chromosomes, the results obtained by FISH (on uncultured cells) and karyotype did not match. In the case with mos 47,XX,+21 [5]/46,XX[15] karyotype, 25% of the metaphase spreads showed trisomy for chromosome 21 whereas using interphase FISH 68% of the nuclei showed three signals (trisomy) for chromosome 21. Corresponding to Luquet *et al*. (2002) reported that there was not always a direct correlation between the degree of mosaicism

according to FISH on uncultured amniocytes and conventional cytogenetics. Therefore, the diagnostic mosaicism still depends on the karyotypical results.

In this study, the efficiency of detection and hybridization should be improved. There are several factors either probes or target DNA preparations. The probes that we constructed remained the cross-hybridization which led to miss scoring FISH signals. This problem may be solved by increasing the amount of cot-1 human DNA to block the repetitive DNA, whereas the signals on the target DNA should not dim or decrease. The other choice with the complex method will be the dissection the region as same as the commercial probe or at the terminal long arm of chromosome 21 which the dim signals or failure hybridization may occur. For the uncultured amniocytes preparation, particularly the nuclei covered with cytoplasm should be reduced, in which case the hybridization signals appear too weak. These problems occurred from inaccurate fixation. The fixation procedure can cause either the overlapping signals which occur more often when the diameter of the fixed nucleus is small or the split signals which stretched nuclei (Munné *et al*., 1998). There are different methods for preparing uncultured amniocytes that we had tried. Finally, we dicided to use the protocol that adopted from Klinger *et al*., 1992 and Lerner *et al*., 2002a. However, the aim of these target cells was the methods that digested cytoplasm. We may try or adopt the hypotonic or fixation treatment by decrease/increase the time or the concentration of reagents when treating. In addition, the target DNA on the slides should be treated carefully while doing FISH that help them decrease the signal-to-noice-ratio, the high background, the non-specific hybridization, and also improve cytoplasm digestion, so the hybridization efficiency could be afforded.

Even though the results of many studies demonstrate that FISH could provide a rapid and accurate clinical method for prenatal identification of chromosome aneuploidies. This powerful FISH technique should generally be used as an adjunct to the traditional karyotyping and not as its replacement (Feldman *et al*., 2000; Lim *et al*., 2002). Furthermore, the detection of aneuploidies with micro-FISH probe was initiated in this study. The sensitivity, specificity and accuracy must be improved to achieve the reliable results. In the future, the micro-FISH probe for chromosomes 13, 18, X, and Y should be produced to detect the aneuploidies of equal quality the

commercial probe. Most important the cost of micro-FISH probes should be determined prior to apply for prenatal diagnosis.

However, these conventional cytogenetics and either FISH or micro-FISH techniques are invasive. The development of non-invasive techniques which minimize the risk of pregnancy loss would offer an ideal means of prenatal diagnosis. For example of these techniques are the triple test, the detection of fetal cells in maternal circulation, and the preimplantation genetic diagnosis.

Triple Test

The triple test is a maternal serum screening test that looks for three specific substances: AFP, hCG, and Estiol. Alpha-fetoprotein (AFP) is a protein that is normally produced by the fetus. Human chorionic gonadotropin (hCG) is a hormone produced within the placenta. Estriol is an estrogen produced by both the fetus and the placenta. The triple test is performed between 15^{th} and 17^{th} week of pregnancy. Accurate results can still be obtained through 20 weeks gestation, in rare cases it may be performed up to 22 weeks.

The results for AFP, estriol and hCG are usually phrased as high or low values. High levels of AFP may suggest that the developing baby has a neural tube defect. The most common neural tube defects are spina bifida and anencephaly. Low levels of AFP may indicate that the developing baby has Down syndrome. Abnormal levels of hCG and estriol may indicate that the developing baby may have chromosome abnormalities. The combination of a low AFP, low estriol and a high hCH suggests further screening for Down syndrome (American Pregnancy Association, 2005).

The triple test can allow the detection of trisomy 21 pregnancies in women either older or younger 35 years. This test can detect approximately 60% of the pregnancies affected by trisomy 21, with a false-positive rate of about 5%. In women older than 35 years, the triple test fails to detect 10-25% of pregnancies affected by trisomy 21. Guidelines published by the American College of Obstetricians and Gynecologists state that maternal serum screening or triple test may be offered as an option for those women who do not accept the risk of amniocentesis or chorionic villus sampling or who wish to have this additional information prior to making a

decision about having amniocentesis (American College of Obstetricians and Gynecologists, 1996; David and Newberger, 2000).

Detection of fetal cells in the maternal circulation

Isolation of fetal cells from the maternal circulation is a promising method for the prenatal diagnosis of chromosomal and genetic disorders without the need for invasive procedures (Al-Mufti *et al*., 2001). Three major fetal cell types have been sought in maternal blood, including lymphocytes, trophoblasts, and erythroblasts. Recent efforts to isolate fetal cells have focused on fetal erythroblasts because they have a short lifespan, are present in significant numbers in the peripheral blood of early fetuses and have a full complement of nucleogenes, Fetal cells are present in extremely low concentration (1 fetal cell in $1x10^4$ to $1x10^7$ nucleated maternal cells) (Bischoff *et al*., 1998). A combination of physical and immunological methods is used to isolate fetal nucleated cells from maternal blood. Physical methods include density gradient centrifugation and micromanipulation techniques while immunological methods include the use of monoclonal antibodies (Department of Chemical Pathology, The Chinese University of Hong Kong, 1999). The proportion of fetal cells can be enriched to about 1 in 10-100 by techniques such as magnetic cell sorting (MACS) or fluorescence activated cell sorting (FACS) after attachment of magnetically labeled or fluorescent antibodies on to specific fetal cell surface markers. Enrichment procedures have used monoclonal antibodies to the antigens CD71 and glycoprotein A. These antigens were selected because they have been found to be expressed on fetal erythroblasts and CD71 antigen is the one most widely and successfully used.

The resulting sample is unsuitable for traditional cytogenetic analysis because it is still highly contaminated with maternal cells. However, with the use of chromosome-specific DNA probes and fluorescence *in situ* hybridization (FISH), it is possible to suspect fetal trisomy by the presence of three-signal nuclei in some of the cells to the maternal blood enriched for fetal cells.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is a technique used to identify genetic defects in embryos created through in vitro fertilization (IVF) before transferring them into the uterus. Because only unaffected embryos are transferred to the uterus for implantation, PGD provides an alternative to current postconception diagnostic procedures, ie, amniocentesis or chorionic villus sampling, which are frequently followed by pregnancy termination if results are unfavorable. PGD is performed in conjunction with IVF and is offered for both fertile and infertile couples (Marik, 2005). In this procedure, an egg is removed from the female partner and fertilized in vitro using techniques developed for couples undergoing in vitro fertilization for infertility. The fertilized oocyte is cultured in the laboratory up to the eight-cell stage of the early blastocyst. A single cell, which is known as a blastomere is removed and analysed by PCR to see if the zygote is affected by the disorder for which the couple is at risk. In addition, fluorescence *in situ* hybridization (FISH) was preferred because PCR bears the risk of misdiagnosis caused by contamination. Although FISH is widely used for genetic analyses, its reliability depends on the types of probes cells and their fixation (Munné *et al*., 1998).

Termination of Pregnancy

If diagnostic testing reveals fetal trisomy 21, the parents should be provided with current, accurate information about Down syndrome and assistance in deciding on a course of action. Their options include continuing the pregnancy and raising the child, continuing the pregnancy and seeking adoption placement for the child or terminating the pregnancy. Consultation with a genetic counselor, a medical geneticist or a developmental pediatrician can be helpful to address the parents concerns and facilitate their decision-making process (Stein et al., 1997; David and Newberger, 2000).

The presence of a serious abnormality in a fetus in the majority of developed countries is an acceptable legal indication for termination of pregnancy. This does not mean that this is an easy choice for a couple to make. It is essential that all couples undergoing any form of prenatal diagnosis investigation, whether invasive or non-invasive, be provided with information about the practical aspects of termination of

pregnancy before the prenatal diagnosis procedure is carried out. This should include a practical explanation that termination in the first trimester is carried out by surgical means under general anaesthetic, whereas a woman undergoing a mid-trimester termination will have to experience labour and delivery (Muller and Young, 2001b).

In the other hand, the legal for termination of pregnancy in the developing country like Thailand is an unacceptable. Therefore, providing the genetic counseling is an important for management of the pregnancy and delivery with respect to the future pregnancies.