CHAPTER III

RESEARCH DESIGN OVERVIEW

HYPOTHESIS:

Increased amount of the living cell through the filter technique will allow an earlier harvesting which resulting in an early result of chromosome analysis.

RESEARCH QUESTION:

Will the filter technique in early amniocentesis improve the culture quality, by 50% reduction in culture time and decrease the failure rate for prenatal diagnosis of chromosome abnormality ?

RESEARCH OBJECTIVE:

1.To improve the culture quality of early amniocentesis by using the filter technique,to reduce the duration of culture time by 50% and also improve the success rate of cell culture to yield the result of chromosome analysis.

2. To minimized the pregnancy and fetal complication by using the technique of recirculated amniotic

fluid passing through the filter system which reduce the removal of amniotic fluid volume.

RESEARCH DESIGN:

Randomized clinical trial with single blinding and Stratified randomization.

RESEARCH METHODOLOGY:

Pcpulation and Sample:

The target population is the patients who are referred for prenatal diagnosis of chromosome abnormality at Pramonkutklao Hospital because of the following indication:

1.Maternal age 35 years old and older.

2.Previous aneuploidy offspring.

3.Known familial chromosome rearrangement.

4.Sex Linked recessive carrier.

5.Abnormal maternal serum Alpha-Fetoprotein screening.

The patients who are at the early stage of gestation e (12-14 complete weeks of gestation estimated from time of last menstrual period and also confirmed by ultrasound measurement). were stratified in three strata according to gestational age: 12,13 and 14 weeks. Blocked randomization was used to allocate patients within each strata in one of

two groups for early amniocentesis with and without the filter technique. The number and size of the block in each strata was calculated on the basis of the incidence of the gestational age at the first antenatal visit of the studied population. The amniotic fluid specimens from both groups was sent to the cytogenetic laboratory for usual culture and analysis.

One technician performed the setting up of the culture from both groups.Each specimen was set up in three slide flasket.Another technician who carried on the study including cell growth observation,harvesting, analysis and karyotyping ,blinded from the filter or nonfilter technique.The first technician will be the person who interpret the result of the cytogenetic study and report to the investigator.

The Inclusion Criteria:

1. Singleton pregnancy without medical nor obstetrical complications.

2. The patient and husband decide to have an early prenatal diagnosis test after genetic counselling.

3. Informed consent for participation in the study was obtained.

The Exclusion Criteria:

1. Multiple pregnancy.

2. The pregnancy with the medical or obstetrical complications.

3. The patient and husband decide against the prenatal diagnosis.

SAMPLE SIZE :

Estimation of possible magnitudes of effects can be obtained from the previous cytogenetic studies of amniotic fluid taken before the 15th week of pregnancy for earlier prenatal diagnosis. A review of 114 consecutive cases by Rubello in 1991 (n = 109), the mean harvest day was 12.2 with the range of 8 - 30 days. From the study of Sundberg et al in 1993 who performed the filter technique at 11-14 weeks of gestation (n = 100) the mean harvest day was 8.0 and the range was 6-13 days.

From these two studies, to obtain power for 90% with the alpha error for 5% using the formula

$$N = \frac{2(Za + Z\beta)^2}{(X1 - X2)^2} o^2$$

N = number per group

X1 = mean of the harvest days in the group with standard early amniocentesis (taken as 12.2 days.)

X2 = mean of the harvest days in the group of early amniocentesis with the filter technique (taken as 8 days.)
0^2 = pooled variance (taken as 7.82.)
The result is the estimated sample size of N = 8 per group.

In order to evaluate the improvement of the culture success rate, the sample size determination should be calculated by the difference of the culture failure between the amnifiltration group and the standard early amniocentesis group.

From the study by Rooney et al[8] in 1989, early amniocentesis in 40 patients yielded the result of chromosome analysis in 32 patients so the failure rate was 20%. The study by Sunberg et al[23] of the amnifiltration in early amniocentesis in 100 patients achieved a successful result in all cases (failure rate was 0%).

Using these results to obtain the power for 80 % with the alpha error for 5% using the formula

$$N = 2(Z\alpha+Z\beta)\ \pi(1-\pi)/(p1-p2)^{-}$$

$$\text{with } \pi = \frac{P1+P2}{2}$$

P1 = Failure rate in standard early amniocentesis(taken as 20%).

P2 = Failure rate in amnifiltration(taken as 0%).

yields N = 28 per group.

In order to answer the research question on the improvement of the culture quality in both reduction of the culture time and decrease in the failure rate, the sample size for this study should be at least 28 patients in each group.