CHAPTER IV

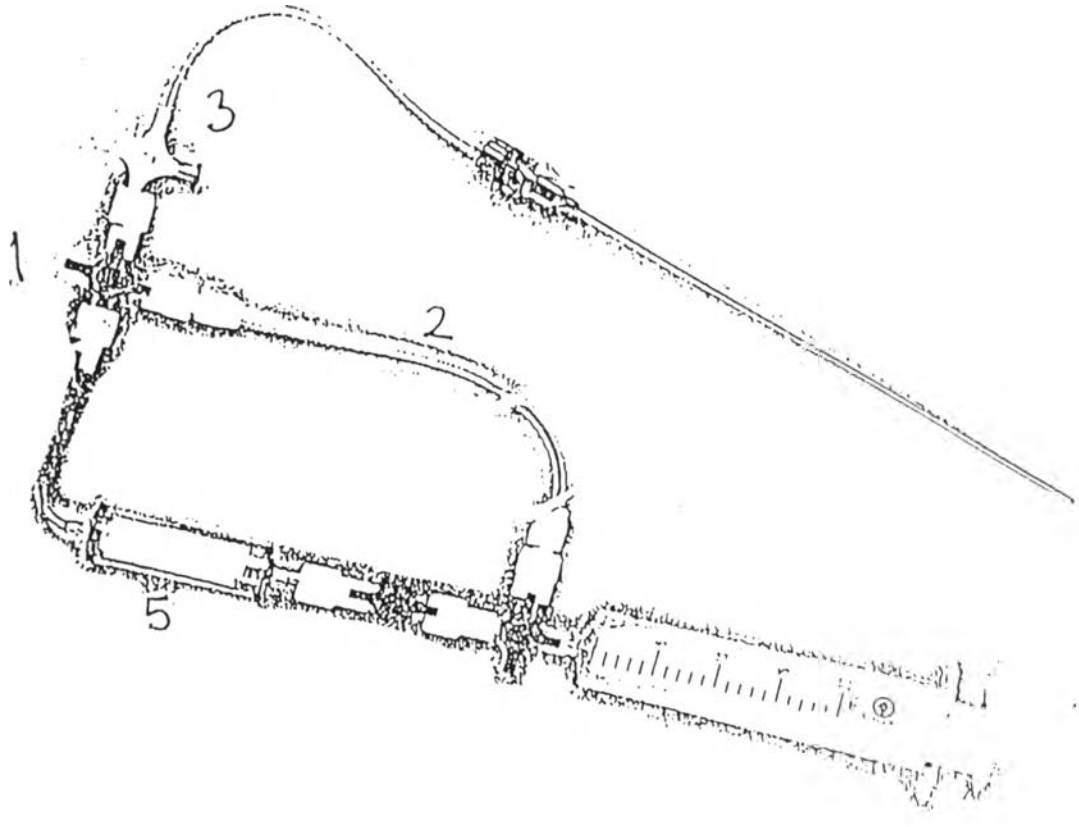
INTERVENTION

Amnifiltration in early amniocentesis is performed by using a filtration device.A circulation system was constructed of commercially available sterile disposable utensils as shown and described in figure 1.The sterivex filter from Millipore with a 0.45 microns pored size filter membrane made of polyvinylidine difluoride was used,because of the nontoxic characteristics of this material.The system was handled by means of three way valves directing the stream of the amniotic fluid.

Sampling procedure: Transabdominal amniocentesis was carried out under ultrasonic guidance using a 20 gauge disposable sterile spinal needle.One cc. of amniotic fluid was initially drawn for Alpha-Fetoprotein study.The filter system was mounted on the needle as in Figure 1. Then 8-10 cc were aspirated through by-pass connecting tube and reinjected through the filter two or three times until

Figure 1

THE FILTER SAMPLING SYSTEM.



1) Three way valves.

2) connecting tube.

5) The sterivex filter from Millipore.

feeling the resistance. The total volume of circulated fluid is 20-40 cc but the total amount of fluid removed came to 8cc.In the control group the amniocentesis were performed in the same fashion but without the filter systems, and the fluid were removed 1 cc per gestational week.

The culture method: The amniotic fluid specimens were sent to cytogenetic laboratory. The specimen from the filter group was flushed with 15 cc.culture medium,CHANG MEDIAM with L- glutamine (2mmol/ml.)penicillin (100 IU/ml.)and Streptomycin (100ug/ml.),and transfered directly into the two sterile slide flaskets. The amniotic fluid specimen from the control group was transfered into the two sterile tubes, and was centrifuged at 1100 RPM for 5 minutes,then the cell pellet was cultureed in two slide flaskets.The cultures were incubated at 37 degree centigrade in the CO2 incubator(5% CO2)for 7 days,the cell growth was then evaluated under an inverted microscope and the medium were changed every two days until harvesting.

The Harvesting: The culture flasks were evaluated under the inverted microscope,after culture medium was changed.When at least 10 medium size coloniies containing mitotic cells by experienced technician were observed,the harvesting was

performed.The mitotic cells were arrested at the metaphase stage in the flasket using colchicine. The cells were treated with hypotonic solution, fixed,spreaded and stained by Trypsin and Giemsa banding technique.

Chromosome analysis: 10-20 metaphases were analysed through the light microscope by counting the chromosome in each metaphase and two metaphases were karyotyped.