Comparison of Chromosome Analysis Using Cell Culture by Coverslip Technique with Flask Technique

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Objective: *To determine accuracy rate of chromosome study from amniotic cellculture by coverslip technique compared with flask technique and to compared timing of amniotic cell culture, amount of amniotic cell culture media and cost of amniotic cell culture.*
Study design: *Cross sectional study.*
Setting: *Department of Obstetrics and Gynecology, Phramongkutklao Hospital.*
Subjects: *70 pregnant women who underwent amniocentesis at Phramongkutklao Hospital during November 1, 2007 to February 29, 2008.*
Intervention: *Amniotic cell culture by flask technique and coverslip technique.*
Main outcome measures: *Accuracy of amniotic cell culture for chromosome study by coverslip technique compared with flask technique.*
Results: *Totally 70 pregnant women who underwent to amniocentesis and dividedamniotic fluid to cell culture by flask technique and coverslip technique. 69 samples had similar result from both techniques. The only one sample had cell culture failure inboth methods due to blood contamination. Accuracy in coverslip technique was 100% compared with flask technique. In timing of amniotic cell culture, amount of amniotic cell culture media and cost of amniotic cell culture between 2 methods that coverslip technique was lesser than flask technique.*
Conclusion: *There is statistically significant of accuracy in chromosome result between coverslip technique and flask technique. Coverslip technique was lesser than flask technique in timing, amniotic cell culture media and costs of amniotic cell culture.*

Keywords: *Amniocentesis, Amniotic cell culture, Coverslip technique, Flask technique*

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The prenatal diagnosis is very important for detect fetal abnormalities and also need the cooperation from obstetrician, genetic medicine and technician[1]. Prenatal diagnosis hasmany methods such as 1) chorionic villi sampling 2) amniocentesis 3) cordocentesis 4) preimplantation genetic diagnosis 5) fetal cell in DNA within maternal circulation 6) ultrasound screening[2].

Prenatal diagnosis by amniocentesis has accuracy for detect Down syndrome about 99% and low risk for abortion (0.5%)[3]. Phramongkutklao hospital provide amniocentesis for prenatal diagnosis by using flask method which will give the result within 2-3 weeks. While waiting for result, will make anxious to parents. As we known, amniotic cell culture technique has 2 methods: flask technique and coverslip technique. Coverslip technique can be exclude pseudomosaicism in case of suspected ofmosaicism[4-6]. In Thailand amniotic cell culture by coverslip technique stillhas a few research.

Therefore, the objectives of this research are to determine accuracy rate of chromosome study from amniotic cell culture for karyotypingcompared between 2 methods for the average time of amniotic cell culture, amount of amniotic cell culture media, and cost of amniotic cell culture.

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Material and Method

Because of a few study in comparative between 2 methods, thus in the present study was conducedin pregnant women with indication for amniocentesis who underwent for genetic counseling

and decided to get amniocentesis. From November 1, 2007 to February 29, 2008, 70 women were enrolled in the present study, after getting the study information and informed consent was done. Then pregnant women were appointed to amniocentesis at gestational age 16-20 weeks. After centrifuge the tube at 800-1,000 RPM for 10 minute amnioticyte was devided into 2 parts for 2 methods, flask technique and coverslip technique. Check amniocytes if appropriate cell analysis was done. Chromosome study result, timing of each cell culture, amount of cell media, cost of each cell culture was recorded and statistical analysis was performed (Fig. 1).

Statistics analysis

Use program STATTA/SP version 12.

Results

All of 70 pregnant women who underwent for amniocentesis and divided amniotic fluid to cell culture by flask technique and coverslip technique. In Table 1 shows that age group that underwent to amniocentesis

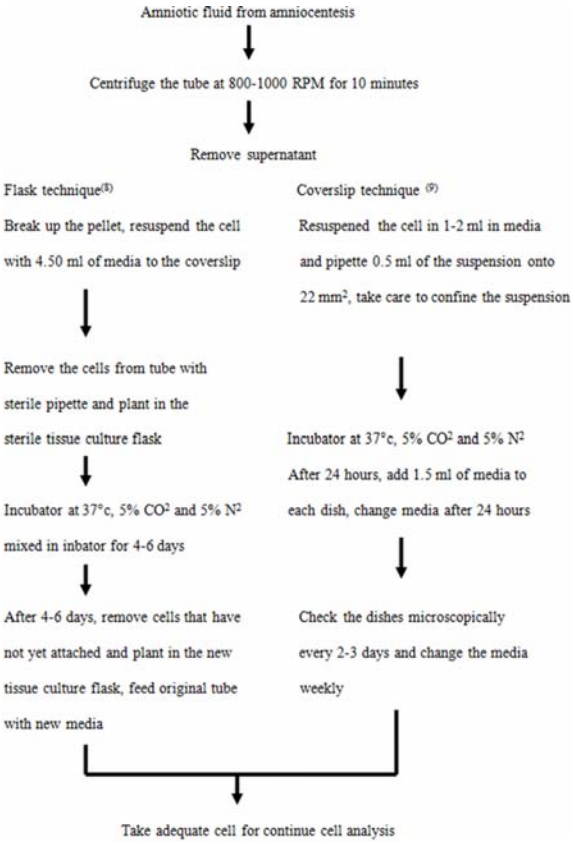

Fig. 1 Cell culture method.

of the present study is 31-45 years. In 2 cases were below 34 years old, one was 30 years old has Down's syndrome in previous child, the other was 33 years old was twin pregnancy. In 1 woman was more than 20 weeks on the date for amniocentesis was 31 weeks gestational age underwent for amniocentesis because of abnormal ultrasound, absent of cerebellavermis and the result of karyotype was 47, xy+18. No complications of amniocentesis process. In 69 amniotic fluid samples was clear or straw color, but 1 sample was blood contamination and cellculture failure in this case. 69 samples had result similarly between 2 methods, it was significant (by kappa: K = 1.000, p<0.001) and use McNemar test Chi-square test for detect the result was not correlate or not that conclusion was result correlation between two method (p = 1.000). As the result normal karyotype was 66 samples and abnormal chromosome was 3 samples. Sensitivity of coverslip is 100%, specificity is 100%, positive predictive value is 100%, negative predictive value is 100% compared with gold standard (flask technique) (Table 2).

In coverslip technique has different process from flask technique in drop of suspension, amount of media in each change. Coverslip drop only on coverslip that 22 mm^2 and flask drop in the bottle. In amount of media of coverslip technique use 1-1.5 ml, flask use 1.5-2 ml for each change. Amount of amniotic cell media per sample in coverslip technique in the present study

Table 1. Demographic data

Data	Numbers
Age	
31-33 years	2
34-36 years	36
37-39 years	22
40-45 years	10
Parity	
No have children	18
Have 1 child	41
Have 2 children or more	11
Gestational age	
16-18 weeks	60
19-20 weeks	8
More than 20 weeks	1
Indication of PND	
Advanced maternal age	69
Previous abnormal chromosome	1
Complications of PND	
Amniotic fluid sample	0
Clear or straw color	69
Blood contamination	1

Table 2. Accuracy of amniotic cell culture

	Flask technique (gold standard)		Total
	Abnormal chromosome	Normal chromosome	
Coverslip technique			
Abnormal chromosome	3	0	3
Normal chromosome	0	66	66
Total	3	66	69

lesser than flask technique (3.5 ml and 6 ml respectively). Cost per person in amniotic cell culture by coverslip technique lesser than flask technique (66.50 baht and 114.00 baht respectively)

The average time of amniotic cell culture between 2 methods were 7.97±0.54 days (6-9 days) in coverslip technique and 9.82±0.64 days (9-11 days) in flask technique. It showed that average time of amniotic cell culture by coverslip technique lesser than flask technique in statistically significant.

Discussion

Amniotic cell culture by coverslip technique is the another method of amniotic cell culture, besides flask technique that exist in now.

In the present study, compared with flask technique as gold standard, in 66 pregnant women had normal chromosome and 3 pregnant women had abnormal chromosome in both methods. That was similar result in both methods; it was accuracy in chromosome result by coverslip technique compared with flask technique. Sensitivity, specificity, positive and negative predictive value were 100% together. In the present study will be concluded that coverslip technique canbe used substitute flask technique. In coverslip technique use more experience of technician because it culture only on coverslip which 22 mm^2 in size. Therefore changing media and transfer from incubator use more carefully. There is study about coverslip technique can exclude pseudomosaicsm[5] because of amniotic cell attach to coverslip in clone, but in flask technique amniotic cell floating in amniotic cell media. It might be misdiagnosis to mosaicism therefore it use the other flask bottle for assistant in diagnosis.Although this study is small study (70 samples), but the result is imply to accuracy of the coverslip technique compared with flask technique because sensitivity, specificity, positive predictive value and negative predictive value is 100%.

In the present study, 1 sample that did not have enough amniotic cell for continue chromosome analysis from blood contamination. In this case amniocentesis was done through placenta and had bloody in amniotic fluid, make get the erythrocyte was the large amount, until cause sediment fall of the erythrocyte greatly until had no the space for amniotic cell can grow up. Thus, the limitation of amniotic cell culture in the case of large amount of erythrocyte was similarly by both methods. In case of large amount of erythrocyte in amniotic fluid maybe treated by irrigate surface of culture after amniotic cell attach about 24 hours. If the initial cell pellet contains clots, these clots can be removed and cultured separately on the chance that some viable amniotic cells are trapped in the clot. Supernatant amniotic fluid, after a separate low speed (less than 1,000 rounds per minute) to pellet the red cells frequently remain in suspension and can be cultured, through the yield of primary colonies low[10].

Generally, if there is absolutely no growth in any cultures by 8-10 days, notification of culture failure is given and the cultures are kept a minimum of three weeks before they are discarded[10]. In this case, after 10 days there are not have amniotic cell in both method. Therefore advice patient for repeat amniocentesis or follow-up ultrasound for screening fetal anomalies. Patient decided to repeat amniocentesis and chromosome result was normal female karyotype (46,xx).

About comparing average time of both amniotic cell culture, coverslip technique was less than flask technique.

Conclusion

Amniotic cell culture by coverslip technique has accuracy when compared with flask technique. In comparative in average time of culture, amount of culture media per sample and cost of amniotic cell culture per person that coverslip technique was lesser than flask technique. Therefore coverslip technique is the

another method for amniotic cell culture, which would be substitute flask technique. But coverslip technique use more experience for this test.

Potential conflicts of interest

None.

References

1. Thompson MW, McInnes RR, Willard HF. Prenatal diagnosis. In: Thompson MW, McInnes RR, Willard HF, editors. Thompson & Thompson genetics in medicine. 5th ed. Philadelphia: W.B. Saunders; 1991: 411-24.
2. Jenkons TM, Wapner RJ. Prenatal diagnosis of congenital disorders. In: Creasy RK, Resnik R, editors. Maternal-fetal medicine. 5th ed. Philadelphia: Saunders; 2004: 235-73.
3. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC 3rd, Wenstorm KD. Prenatal diagnosis and fetal therapy. In: Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC 3rd, Wenstorm KD, editors. Williams obstetrics. 22nded. New York: McGraw-Hill; 2005: 314-35.
4. Zhang YJ, Garver KL, Marchese SG, Diggans GR, Muracca-Clemens M, Flecker D. Mosaicism or pseudomosaicism: the problem of hypermodal cells in amniotic fluid cell culture. Prenat Diagn 1984; 4: 99-108.
5. Cheung SW, Spitznagel E, Featherstone T, Crane JP. Exclusion of chromosomal mosaicism in amniotic fluid cultures: efficacy of in situ versus flask techniques. Prenat Diagn 1990; 10: 41-57.
6. Featherstone T, Cheung SW, Spitznagel E, Peakman D. Exclusion of chromosomal mosaicism in amniotic fluid cultures: determination of number of colonies needed for accurate analysis. Prenat Diagn 1994; 14: 1009-17.
7. The College of American Pathologists (CAP). Commission on laboratory accreditation. Cytogenetics checklist. Northfield, IL: CAP; 2007.
8. Leff A, Kaffe S, Hsu YF. Amniotic fluid I: Flask culture, harvesting and staining. In: Barch MJ, editor. The act cytogenetics laboratory manual. 2nd ed. New York: Raven Press; 1991: 166-67.
9. Peakman DC. Amniotic fluid II: In situ culture and harvesting, coverslip processing method. In: Barch MJ, editor. The act cytogenetics laboratory manual. 2nd ed. New York: Raven Press; 1991: 172-3.
10. Priest JH. Prenatal chromosomal diagnosis and cell culture. In: Barch MJ, editor. The act cytogenetics laboratory manual. 2nd ed. New York: Raven Press; 1991: 157-64.

ความแม่นยำของผลการตรวจโครโมโซมด้วยการเลี้ยงเซลล์น้ำคร่ำโดยวิธีการเลี้ยงเซลล์น้ำคร่ำโดยวิธี Coverslip technique เปรียบเทียบกับวิธีการเลี้ยงเซลล์น้ำคร่ำโดยวิธี Flask technique

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วัตถุประสงค์: เพื่อศึกษาความแม่นยำ (accuracy) ของการตรวจโครโมโซมจากการเลี้ยงเซลล์โดยวิธี coverslip technique เปรียบเทียบกับวิธีมาตรฐานคือ flask technique ในโรงพยาบาลพระมงกุฎเกล้า

วัสดุและวิธีการ: ภายหลังได้รับการอนุมัติจากคณะอนุกรรมการพิจารณาโครงการวิจัยและความยินยอมจากผู้ป่วย จำนวน 70 ราย ที่นัดมารับการตรวจโครโมโซมโดยการเจาะน้ำคร่ำ แล้วนำน้ำคร่ำที่ได้มาแบ่งเลี้ยงเป็น 2 วิธี คือการเลี้ยงแบบ Flask technique กับ Coverslip technique แล้วนำผลจากการวิเคราะห์โครโมโซมมาเปรียบเทียบกันและจะเปรียบเทียบในด้านปริมาณน้ำยาที่ใช้, เวลาที่ใช้ในการเลี้ยงเซลล์ของแต่ละวิธี ตลอดจนค่าใช้จ่ายที่ใช้ของแต่ละวิธี

ผลการศึกษา: ผู้เข้าร่วมการวิจัยทั้งสิ้น 70 รายที่มาตรวจโครโมโซมด้วยการเจาะน้ำคร่ำและเลี้ยงเซลล์โดยวิธี Coverslip technique และวิธี Flask technique พบว่าน้ำคร่ำ 69 ตัวอย่างมีผลการตรวจโครโมโซมที่สอดคล้องกันแต่อีก 1 ราย ไม่สามารถอ่านผลโครโมโซมได้ เนื่องจากมีเลือดปนอยู่ในน้ำคร่ำมาก ทำให้มีความแม่นยำเท่ากับร้อยละ 100 และการเลี้ยงเซลล์น้ำคร่ำแบบ Coverslip technique ใช้เวลาในการเลี้ยงเซลล์น้อยกว่าการเลี้ยงเซลล์น้ำคร่ำแบบ Flask technique อย่างมีนัยสำคัญคือใช้เวลาเฉลี่ย 8 กับ 10 วัน ตามลำดับ ตลอดจนจำนวนน้ำยาที่ใช้และค่าใช้จ่าย น้อยกว่าการเลี้ยงเซลล์แบบ Flask technique คือ 3.5 กับ 6 มิลลิลิตร ตามลำดับ

สรุป: การเลี้ยงเซลล์แบบ Coverslip technique มีความแม่นยำในการตรวจโครโมโซมเท่ากับการเลี้ยงเซลล์แบบ Flask technique แต่สามารถประหยัดเวลาและค่าใช้จ่ายมากกว่าแบบ Flask technique