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A Study of the of Role Platelet and Fibrinolytic Activity in Pathophysiological Changes in Pre-Eclampsia

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Abstract : *Eight severe pre-eclampsia, 21 mild pre-eclampsia and 12 normal pregnancies were selected from the antenatal care unit of Siriraj Hospital to study their platelet aggregation and fibrinolytic activity both during pregnancy (>20 weeks gestation) and after delivery. It was found that platelets of normal pregnant subjects and both groups of pre-eclamptic patients were hypersensitive to collagen, adrenaline and ADP. After delivery, only normal pregnancy revealed singificant decrease in percent maximal aggregation ($p<0.05$) while pre-eclampsia did not show any significant difference. This may be due to the enhancement of gestational effect on platelet activity which returned to normal after delivery. However, hyperactivity of pre-eclamptic patients may not only be due to the gestational effect alone, but also to the original hyperactive activity of platelet. before pregnancy. Fibrinolytic activity was also found to be decreased significantly in pre-eclampsia because euglobulin lysis time was prolonged over the normal ranges but after delivery it returned to normal. (Thai J Obstet Gynaecol 1993;5: 57-62.)*

Key words : platelet, fibrirolytic activity, pathological changes, pre-eclampsia

In general, pre-eclampsia (PE) is progressively developed to eclampsia which is a major cause of foetal and maternal norbidity and mortality and is often associated with foetal growth retardation. The aetiologies of this disease are still not completely understood, more than one mechanism probably contributes to it. Platelets have been implicated in one of the complex aetiologies of this condition[1] because there is evidence from a number of studies which indicates that anti-platelet therapy given early in high risk pregnancy may protect against PE[2,3]. The alteration in platelet function has been recognized as the important feature of this pathology[4-6] because they may be involved in the formation of microthrombi in the utero-placental circulation which possibly initiates the complication during pregnancy.

Fibrinolytic activity is another

factor which may be involved as aetiopathogenesis of PE. It has been established that fibrinolysis is diminished throughout pregnancy[7,8]. Hypofibrinolysis is also found in PE[9]. This study is, then, designed to determine the degree of platelet aggregation and fibrinolytic activity in the same pre-eclamptic patients compared with the same normal pregnant women, both during pregnancy and after delivery. It is believed that knowledge gained from this study will eventually be of benefit for establishing an approach for early detection and early management of PE.

Materials and Methods

Subjects

Pre-eclamptic patients and normal pregnant subjects were screened by the obstetrician from admitted patients and from the antenatal care unit of Siriraj Hospital.

Pre-eclamptic patients were pregnant subjects who developed hypertension after 20 weeks of gestation, on at least 2 separate occasions, with edema and/or proteinuria. Mild PE is defined as mild hypertension (diastolic blood pressure between 90-100 mmHg) with edema and/or proteinuria (1^+-2^+). Severe PE is defined as severe hypertension (diastolic blood pressure>100 mmHg) with edema and/or proteinuria ($>2^+$). Normal pregnant subjects were normotensive pregnant subjects whose age and gestational age were compatible with the selected mild and severe pre-eclamptic subjects.

Exclusion criteria were patients/subjects who had taken NSAIDs or any drugs known to effect either platelets or prostaglandin synthesis, during 2 weeks before the study or having a history of hypertension before their pregnancy or having suspected renal disease or having diabetes mellitus.

Specimen collection

Venous blood of each subject was collected twice, during pregnancy and after delivery (48 hours). Platelet aggregation was measured by the optical technique[10]. The concentrations of platelets in platelet rich plasma were in the range of 3.00 - 4.00 X 10^8/ml. Fibrinolytic activity was determined as euglobulin lysis times (ELT).

Statistical analysis

Kruskal-Wallis test or Wilcoxon Matched-Pair Signed Rank test was employed to test the differences of variables among/or within subject groups.

Results

The clinical characteristics of the studied subjects are shown in Table 1. During pregnancy, subjects possessing hyper-aggregation platelets were frequently found in all 3 groups

Table 1 Characteristics of studied groups

Characteristics	NP	Mild PE	Severe PE
No. of subjects	12	21	8
Age (yrs.)	22 ± 4	24 ± 6	25 ± 9
Gestational age (wks.)	28 ± 5	36 ± 3	38 ± 3
Blood pressure :			
Systolic (mmHg)	112 ± 10	137 ± 16	156 ± 11
Diastolic (mmHg)	72 ± 8	92 ± 12	108 ± 7
Proteinuria			
(No.of positive subjects)	-	4	8
Edema			
(No.of positive subjects)	-	21	8
Parity :			
Nulliparous	11	14	5
Multiparous	1	7	3
Birth weight (kg)	3208 ± 333	2604 ± 687	1980 ± 730

Table 2 Platelet aggregation patterns in NP and PE

Pattern of platelet aggregation	NP		Mild PE		Severe PE	
	ante-partum	post-partum	ante-partum	post-partum	ante-partum	post-partum
Hyper-	9	8	17	16	6	6
Normal-	2	4	4	4	2	1
Dis-	1	0	0	1	0	1
Total	12		21		8	

Table 3 Percent maximal aggregation of platelets in NP and PE

Maximal aggregation induced by	NP		Mild PE		Severe PE	
	ante-partum	post-partum	ante-partum	post-partum	ante-partum	post-partum
Collagen	62.3 ± 21.4	54.5 ± 8.7	57.2 ± 14.6	51.2 ± 16.1	58.0 ± 11.5	43.3 ± 25.0
Adrenaline	46.7 ± 26.6	18.5 * ± 16.6	43.8 ± 22.3	33.1 ± 21.5	34.0 ± 29.1	38.5 ± 26.2
ADP	61.8 ± 16.3	35.6 * ± 20.6	54.9 ± 17.9	53.5 ± 15.2	43.3 ± 23.4	47.2 ± 24.9
Platelet ($x10^8$/ml)	3.59 ± 0.54	3.66 ± 0.69	3.00 ± 0.68	3.03 ± 1.03	3.16 ± 1.13	3.61 ± 1.23

* $p<0.05$

Table 4 Fibrinolytic activity (euglobulin lysis times) in NP and PE

	NP	Mile PE	Severe PE
Antepartum	267 ± 73	304 ± 66	308 ± 59
Postpartum	227 ± 89	244 ± 75*	254 ± 89

* $p<0.05$ (Normal range<300 minutes)

while subjects possessing dis- or normal-aggregation platelets were less common. In qualitative measurement it was found that after delivery, the number of subjects possessing hyper-aggregation platelets in NP and in mild PE were slightly decreased while there was no change in severe PE (Table 2).

In quantitative measurement, the percent maximal aggregation of platelets after being induced by collagen, adrenaline or ADP in each subject group were averaged and tabulated in Table 3. During pregnancy, there was no significant difference in the percent maximal aggregation between these 3 groups. After delivery, the percent maximal aggregation was decreased significantly in normal group ($p<0.05$) while they did not significantly change in the pre-eclamptic groups ($p<0.05$).

Fibrinolytic activity was expressed as ELT. During pregnancy ELT of NP, mild and severe PE, were not significantly different. After delivery ELT of mild PE was significantly decreased (Table 4).

Discussion

It was found that most of the subjects had hyperactive platelets. In our data collected, hyperactive platelets were found in only 30% of non-pregnant women. It is possible that platelets may be enhanced by gestational effect to be hyperactive during pregnancy then usually return to normal after delivery[11]. However, in this study only maximal aggregation of NP was significantly reduced after delivery while PE was not changed. It might be questioned whether their platelet aggregation showed hyperactivity before pregnancy then initiated PE or PE developed first then hyperactivity followed. The hyperactive platelets might be an important risk factor for the pathogenesis of PE through their involvement in the formation of microthrombi in the utero-placental circulation because it has been reported that thrombi were found in the small blood vessels of patients dying after eclampsia[12]. The concept that subjects with hyperaggregation platelets have a higher risk for developing PE is confirmed by the review of Halushka et al[13] who concluded that diabetic pregnancy had an increased incidence of pregnancy-induced hypertension. Moreover, it has been long known that platelets

of diabetic patients are hyperactive[14].

The study on fibrinolysis of NP and PE revealed that there was some impairment in fibrinolytic activi ty of these patients during pregnancy because their ELT was found not only last longer than ELT after delivery but also prolonged over the normal range. Impairment in fibrinolysis may be associated with fibrin deposition in the vessels of pre-eclamptic patients. By using immunofluorescent method, Vassalli et al[15] and Hustin et al[16] demonstrated that there was indeed some fibrin in the subendothelium of the renal glomeruli and in the decidual segment of spiral arteries of pre-eclamptic patients. Hypofibrinolysis during pregnancy may be due to the effects of placenta-mediated inhibitors on fibrinolytic activity[17]. The evidence supporting this explanation is the increase in fibrinolysis after delivery.

Whether platelets of pre-eclamptic subjects were induced hyperacively by their over synthesis of TXA_2 further investigation is required. The finding should develop more effective management or prevention of this pathology. That is, if the result of the investigation show that more TXA_2 are synthesized in pre-eclamptic pregnancy, easy and safe management could be applied by administration of ASA or any cyclo-oxygenase inhibitors. If TXA_2 synthesis is not significantly changed other types of anti-platelet drugs should be applied to overcome hyperaggregated platelets.

Conclusion

Platelets of both NP, mild and severe PE were hypersensitive to collagen, adrenaline and ADP. After delivery only NP revealed significant decrease of their maximal aggregation. Fibrinolytic activity of PE, was under the normal range, after delivery it returned to normal.

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A Study of the Role of Vaso-Active Prostaglandins (PGI_2 and TXA_2)in Pathophysiological Changes in Pre-Eclampsia

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Abstract : *The plasma levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 were assessed by RIA in 7 severe and 19 mild pre-eclampsia (PE). These levels were compared with the levels in 9 normal pregnancies (NP) , both antepartum and postpartum. It was found that the levels of 6-keto-$PGF_{1\alpha}$, TXB_2 in mild and severe pre-eclampsia were 578.9 ± 26.9, 43.3 ± 16.5 and 517.6 ± 76.6, 26.8 ± 5.0 pg/ml while in normal pregnancy were only 458.4 ± 36.8, 18.6 ± 2.2 pg/ml respectively. However, they did not show any significant difference. (Thai J Obstet Gynaecol 1993;5: 63-66.)*

Key words : prostaglandins, pathophysiological changes, pre-eclampsia

Pre-eclampsia (PE) is an obstetric complication of unclarified aetiopathogenesis. It could be considered as a disorder of platelet and vessel wall interaction during pregnancy. Platelet activation and the imbalance of prostacyclin (PGI_2) and thromboxane (TXA_2) in maternal circulation have been suggested as the important roles in this pathology[1,2]. There is evidence not only for deficient PGI_2 production but also exaggerated TXA_2 synthesis in PE[3,4]. Since PGI_2 promotes vasodilatation and opposes platelet aggregation and adhesion while TXA_2 induces vasoconstriction and enhances platelet aggregation and adhesion to the vascular wall, the combined actions of TXA_2, if unopposed, will lead to maternal hypertension, increased platelet aggregation and decreased utero-placental blood flow which are commonly found in pre-eclamptic patients. Moreover, There is evidence indicating that anti-platelet therapy may protect against PE[5-7]. The roles of platelets and these 2 prostaglandins are, therefore, more reliable. It is worthwhile to study the activity of platelets as well as the plasma levels of PGI_2 and TXA_2 in parallel. However, both PGI_2 and TXA_2 are labile substances. Their stable metabolites, i.e. 6-keto-$PGF_{1\alpha}$ and TXB_2 were studied instead. Platelet aggregation

and plasma levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 in pre-eclamptic patients were then studied and compared with normal pregnant subjects. The results of the platelet study are presented in a separate paper[8], but are also discussed in this paper.

Materials and Methods

Pre-eclamptic patients and normal pregnant subjects were screened by an obstetrician from admitted patients and the antenatal care unit (ANC) of Siriraj Hospital. Two kinds of PE, mild and severe PE, were defined by the development of diastolic blood pressure between 90-100 mmHg with edema and/or proteinuria 1^+ -2^+ and more than 100 mmHg with edema and proteinuria 2^+ or more, respectively during the third trimester of pregnancy. Normal pregnancy was normotensive pregnant subjects with the same compatible age and gestational age as the pre-eclamptic groups. None of the subjects had NSAIDs or were taking any drugs known to effect prostaglandins synthesis for at least 2 weeks before the study. They also should not have any history of hypertension, any suspected renal disease or diabetes mellitus.

Plasma levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 of these subjects were assessed by [^{125}I] 6-keto-$PGF_{1\alpha}$ and [^{125}I] TXB_2 assay system (RIA) after these plasma was extracted by Sep-pak C_{18} cartridge[9]. Extracted plasma could be kept at -20°C until used if RIA process had not yet been performed.

Kruskal-Wallis or Wilcoxon Matched-Pair Signed Rank test were employed to test the difference of variable among/or within subject groups.

Results

Thirty-five subjects were recruited in this study. They were 9 NP, 19 mild and 7 severe PE. Their clinical characteristics are shown in Table 1.

Plasma levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 of NP, mild and severe PE, are shown in Table 2. These levels of each metabolite were not significantly different among the 3 groups of subjects and also were not significantly changed after delivery although the levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 were found slightly higher in mild and severe PE than in NP.

Discussion

The balance between PGI_2/TXA_2 is believed to be important in the control of the hemodynamic changes of pregnancy[1,10]. The imbalance of this ratio in favour of TXA_2 is also thought to be the cause of vasoconstriction of small arteries, activation of platelets and uteroplacental insufficieney in PE as well.[4,11] However, there is still controversy on this point. Our study is also in disagreement with this because the result of this study showed no significant difference in the levels of 6-keto-

Table 1 Characteristics of studied groups

Characteristics	NP	Mild PE	Severe PE
No. of subjects	9	19	7
Age (yrs)	20 ± 3	24 ± 7	26 ± 6
Gestational age (wks)	29 ± 3	36 ± 2	38 ± 3
Blood pressure:			
Systolic (mmHg)	112 ± 10	136 ± 17	156 ± 11
Diastolic (mmHg)	72 ± 8	91 ± 12	107 ± 8
Proteinuria (No.of positive subjects)	-	4	7
Edema (No.of positive subjects)	-	19	7
Parity:			
Nulliparous	8	13	4
Multiparous	1	6	3
Birth weight (kgs)	3281 ± 323	2601 ± 772	1977 ± 789

Table 2 Plasma levels of TXB_2 and 6-keto-$PGE_{1\alpha}$ in NP, mild and severe PE

	TXB_2 ($\bar{x}$ ± SE pg/ml)	6-keto-$PGF_{1\alpha}$ ($\bar{x}$ ± SE pg/ml)
Normal pregnancy	n = 9	n = 9
Antepartum	18.6 ± 2.2	458.4 ± 36.8
Postpartum	18.9 ± 2.4	474.6 ± 62.8
Mild PE	n = 16	n = 19
Antepartum	43.3 ± 16.5	578.9 ± 26.9
Postpartum	43.7 ± 9.8	560.6 ± 31.5
Severe PE	n = 6	n = 7
Antepartum	26.8 ± 5.0	517.6 ± 76.6
Postpartum	25.6 ± 3.9	537.8 ± 48.6

$PGF_{1\alpha}$ and TXB_2 between NP and the 2 groups of PE. Moreover, the plasma levels of these 2 metabolites in all 3 groups were not significantly changed after delivery even though it was found in the previous study on platelet that maximum aggregation of platelets in NP was significantly decreased after delivery[8,12]. Therefore, it seemed that TXA_2 which was proposed to be the important factor involving platelet activation during pregnancy may not be considered. However, in this study PGI_2/TXA_2 was still regarded as being an important factor in PE because their plasma levels of TXB_2 were noticeably higher than plasma levels of TXB_2 in NP but it did not show statistically significant difference which may be due to the

small number of subjects.

Based on our result, the use of anti-platelet durgs which have a primary mode of action such as a cyclo-oxygenase inhibitor i.e. acetylsalicylic acid, in preventing PE seemed not to be very impressive. Further studies on pathogenesis of this disorder as well as other anti-platelet durgs which have other mechanisms of action are still very much needed to establish the prevention and management of PE.

Conclusion

During pregnancy, plasma levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 were higher in pre-eclamptic groups than in the normal group but not significantly different. After delivery, plasma levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 were also insignificantly changed.

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Clinical Evaluation of Prostaglandin E_2 Gel for Preinduction Cervical Ripening in Term Pregnancy with a Low Bishop Score

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Abstract :

Objective : *A prospective clinical trial study investigating the therapeutic effect of prostaglandin E_2 gel in priming the cervix of patients with a low Bishop score.*

Study Design : *Pregnant women with low Bishop scores necessitating labour induction were enrolled in this study. Following the administration of intracervical prostaglandin E_2 gel Bishop scores were assessed at 6,12 and 24 hours thereafter. Repeated dose was given if no satisfactory change of Bishop score was achieved 24 hours later. Oxytocin was given if the Bishop score was > 4 and labour was not established or in some instances to augment inadequate uterine contraction. Mode of delivery, time interval to delivery, delivery outcome and complication were recorded.*

Results : *Of the 28 patients studied, the overall success rate of cervical ripening was 89.28%. Seven patients (25%) had spontaneous deliveries without oxytocin administration. Five patients (17.8%) necessitated repeated dose of prostaglangin E_2 gel and 3 patients (10.72%) failed to achieve satisfactory Bishop scores after second application. Caesarean section rate was 20%. Uterine hyperstimulation, diarrhea, transient fetal tachycardia and bradycardia were the adverse effects encountered.*

Couclusion : *Intracervical prostaglandin E_2 gel can be used successfully for cervical ripening in term and postterm pregnancies with low Bishop scores.*
(Thai J Obstet Gynaecol 1993; 5: 67-71.)

Key words : prostaglandin E_2 gel, cervical ripening

During recent years, there has been much interest in cervical ripening properties of prostaglandin E_2 in patients with unfavourable cervix. Ripeness of the cervix influences the success of the induction[1]. A patient with unfavourable cervix is of great challange when induction is necessary. If the cervical status is not good enough, prolonged induction may result with an increase in both fetal and maternal morbidities.

The present study was carried out to evaluate the efficacy of pros-

taglandin E_2 gel applied intracervically in term pregnancies or beyond. Prostaglandin E_2 gel given by this route yielded satisfactory results for this purpose in many recent reports[2-5]. There is a difference of opinion as to whether prostaglandin E_2 in vivo ripens the cervix by initiating biochemical and structural changes in its connective tissue or by inducing uterine contractions that shorten, soften and dilate the cervix. This dosage of prostaglandin may trigger endogenous prostaglandin synthesis and many patients entered active labour after a latency period of several hours[2,5-8].

Patients and Methods

The study was conducted at Siriraj Hospital, during the year 1992. The characteristics of patients recruited for study included those of primigravidae, singleton pregnancy with the Bishop score of 4 or less. The fetus must be in cephalic presentation and in good condition as evidenced by reactive nonstress test. The uterine contraction must not be in a regular manner. The patients with obvious cephalo-pelvic disproportion or who had previous uterine scar were excluded likewise those with ruptured membranes, antepartum hemorrhage, active asthma, medical complications including a history of hypersensitivity to prostaglandins. Informed consent was obtained from each patient prior to entry into this study.

A sterile speculum was inserted during which cervix and vagina were gently sponged free of mucous and discharge. Prostaglandin E_2 gel (0.5 mg of prostaglandin E_2 with 2.5 ml of gel) stored at 4°C warmed to skin temperature was then installed into the cervical canal under direct visualization. Bishop scores were determined again by the same physician at 6,12,24 hours after application. Oxytocin infusion was given for induction of labour at any time if satisfactory Bishop score (>4) was achieved and no labour was established or given to augment inadequate labour. Artificial ruptrue of membranes was performed when cervix dilated 3 cm and effacement 100%. Repeated dose of prostaglandin E_2 gel was again given if no satisfactory Bishop score was achieved after 24 hours. Surgical difficulty, technical failure and adverse effects were recorded. Continuous uterine activity and fetal heart rate monitoring with electrotocodynamometer were recorded at least for the first two hours after application. Vital signs and venous access were obtained. Failure of treatment was certified if no satisfactory progress of Bishop score was achieved 24 hours after second application.

Mode of delivery, time interval from application to delivery and fetal outcome were recorded, fetal outcome was assessed by Apgar's score. Baseline laboratory studies for complete blood count and differential, renal and liver function tests, urinalysis were obtained and then repeated 24 hours after gel application.

Results

There were 28 patients enrolled in the study. All of the patients had at least 38 complete weeks of pregnancy. Indications for labour induction are shown in Table 1.The average maternal age was 28.25 ± 4.48 yr (17-33 yr) , the average height was 154.86 ± 5.46 cm (145-167 cm) and the average body weight at the time of delivery was 59.72 ± 8.13 kg (50.2-78.3 kg). All patients were primiparous except 2 patients with 1 and 2 previous early spontaneous complete abortions. Bishop scores before treatment are shown in Table 2. Surgical difficulties on application of the gel were found in 2 cases due to posterior-pointed cervix but no technical failure was found.

Table 3 shows the accumulated total number of cases who had Bishop scores of more than 4 after 6,12,24 hours and after second dose application. The overall success rate of cervical ripening was 89.28%. Seven patients (25%) established spontaneous regular contractions and delivered without oxytocin being given. One of these 7 patients had uterine hyperstimulation but with acceptable fetal heart rate pattern during the course of labour. Uterine hyperstimulation also noticed in another patient which lasted for only 1 hour after initial application of gel and eventually returned to normal regular contraction pattern and this patient necessitated oxytocin augmentation 12 hours later. In both cases, fetal outcomes were good without signs of fetal distress.

Three patients (10.71%) showed unsatisfactory changes of cervix (Bishop score ≤ 4) and were judged as failures. Two of the 3 patients had surgical difficulties at the time of both first and second gel applications.

Adverse effects were encountered in 7 patients, 2 uterine hyperstimulation, 1 diarrhea, 2 transient bradycardia and 1 transient tachycar-

Table 1 Indications for induction

Indications	No.(%)
Elective and/or postterm	24 (85.71)
Diabetes mellitus	2 (7.14)
Hydrocephalus	1 (3.57)
Pre-eclampsia	1 (3.57)
Total	28 (100)

Table 2 Bishop scores before treatment

Bishop score	No.(%)
1	9 (32.14)
2	8 (28.57)
3	6 (21.42)
4	5 (17.85)
Total	28 (100)

Table 3 Cumulative successful cases

Cumulative cases with Bishop score>4	No.(%)
After 6 hours	13 (46.43)
After 12 hours	21 (75.00)
After 24 hours	23 (82.14)
After application of 2nd dose	25 (89.14)

Table 4 Outcomes of induction

Mode of delivery	**No (%)**
Spontaneous	19 (76)
Vacuum extraction	1 (4)
Caesarean secton	5 (20)
Average birth weight (g)	3037.60±421.76
Mean Apgar'r scores :	
At 1 min.	8.60±1.91
At 2 min.	9.28±1.10
At 5 min.	9.80±0.50
Time interval from treatment to delivery (hr)	
One dose (n = 23)	26.75 ± 17.88 (6.83-70.66)
Two doses (n = 25)	30.49 ± 21.43 (6.83-74.06)

dia. None of the cases had severe complications during or after deliveries and no postpartum hemorrhage or infection was observed in this study.

Table 4 shows mode of delivery, fetal outcome and time interval from treatment to delivery. Caesarean section was performed in 5 instances. The indications for caesarean section were cephalo-pelvic disproportion in 2 cases, 2 fetal distress and 1 diabetic case with suspicion of chorioamnionitis 24 hours after the first dose. No obvious fetal distress at 5 minutes after birth was observed in all cases.

There were no differences in pre- and post-treatment laboratory tests for complete blood count, urinalysis, renal and liver function tests. These tests were performed before the start of treatment and repeated at 24 hours after the first and second doses of gel application.

Discussion

The state of cervix assessed by Bishop score is obviously related to success of a labour induction as Bishop reported in 1964[1]. Prostaglandin E_2 has attracted great attention in terms of preinduction cervical softening. There have been lots of investigation for this purpose. Various routes of administration of prostaglandin E_2 have been investigated for this clinical application and to avoid unwanted adverse effects of larger doses of systemically administered prostaglandin E_2 required to produce cervical ripening. Data obtained from this study showed the effectiveness of prostaglandin E_2 gel intracervical administration for cervical ripening.

In this study only 1 dose of gel was required and all achieved spontaneous vaginal delivery without oxytocin augmentation. Failure of treatment was partly from surgical difficulty at the time of gel application due to cervical position. Multiple gel applications in a situation in which a satisfactory result was not achieved with a single dose were accepted in recent studies[2,3]. A report of a double-blinded study confirmed that prostaglandin E_2, not the gel vehicle, induces cervical changes and labour [4].

Caesarean section rate in this study was not higher than other recent reports[5,6]. Two cases of fetal distress were unrelated to hyperstimulation, both occurred in postmaturity with marked decrease amniotic fluid volume observed at the time of artificial rupture of membranes.

Despite uterine hyperstimula-

tion encountered in 2 patients in this study progress of labour through spontaneous deliveries was achieved with good fetal outcome. Although many previous studies reported no hyperstimulation with the intracervical route,[2,5-7] some had very low or about the same percentages of uterine hyperstimulation as this study[8,9]. Nevertheless, rupture of uterus associated with the use of intracervical prostaglandin gel for induction of labour was recently reported[10].

There is always the possibility that furthur investigations may lead to a better cervical softening agent. The abundance of worldwide information now available makes prostaglandin E_2 as being clinically acceptable in terms of safety and efficacy. Prostaglandin E_2 therapy has low maternal side effects, low percentages of failure, does not increase instrumental deliveries or caesarean section and gives favourable fetal outcome. From the present experience, it is recommended to give a repeated dose after the first 24 hours evaluation of cervical condition. This will give more chance for the cervix to achieve better Bishop scores. Prostaglandin E_2 gel should be used cautiously under the control of electronic fetal monitoring.

Acknowledgement

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Transferrin Concentrations in Amniotic Fluid and Blood During Gestation and Early Puerperium

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Abstract : *The aims of the present investigation were to determine transferrin concentrations in amniotic fluid and in womens' cubital vein blood during gestation as well as in the early postpartal period and to establish referent curves of this very important nonspecific immunologic protective factor. In the period of 3 years serial amniotic fluid specimens were obtained by amniocentesis under ultrasound control with a free-hand technique in aseptic conditions from 91 patients (239 samples) in varying gestational ages. At the same time specimens of cubital vein blood were taken. Transferrin concentrations were determined by method of immunonephelometry using original Boehring kits. All amniotic fluid samples were cultured for aerobic and anaerobic bacteria within 30 minutes after amniocentesis. Our results indicate that transferrin concentrations in amniotic fluid increased gradually with advancing gestational age from 49.90 mg/l in the 13th week of gestation and reached maximum levels of 260.30 mg/l in the 39th gestational week. In the last week transferrin levels significantly decreased to the 95.05 mg/l at term. Peripheral blood transferrin levels increased from 2.58 g/l in the 13th week of gestation and reached maximal values of 4.67 g/l in the 38th week of pregnancy. In the early puerperal period transferrin concentrations were found to increase, with a peak of 5.80 g/l on the second postpartal day. After that levels decreased. (Thai J Obstet Gynaecol 1993;5: 73-79.)*

Key words : transferrin, amniotic fluid, blood, serial amniocentesis

Recent review has given comprehensive accounts on the functional and other properties of transferrins. Three proteins typify the transferrin family; serum transferrin (the iron transport protein), lactoferrin (found in milk and other secretions, as well as leukocytes), and ovotransferrin or conalbumin (from egg white)[1].

Bacterial colonization of the amniotic fluid has been reported in patients with intact membranes during pregnancy, but microbial invasion of the amniotic cavity during labour, prior

to and especially after rupture of membranes was found to occur more frequently[2]. Microbial invasion of the amniotic cavity may lead to maternal infection and fetal/neonatal sepsis. Antimicrobial components in amniotic fluid becomes the last resort to prevent these complications. A lot of reports have been published regarding the antibacterial activity of amniotic fluid. A number of compounds such as lysozyme, transferrin, peroxidase, 7s immunoglobulin, spermine, betalysin, beta 1a/beta 1c globulin and zinc have been found in amniotic fluid and are assumed to exhibit bacterial growth-inhibitory activities[3]. However, how these compounds inhibit the bacterial growth in amniotic fluid is not clearly understood, because investigators have shown contradictory evidence with respect to growth of certain bacterial species, especially in amniotic fluid. The reasons for these differences need to be clarified. An electronic literature search (Medline) has not retrieved any reference about transferrin blood and intraamniotic reference curves (up to December 1992).

Materials and Methods

Serial amniotic fluid samples were obtained under ultrasound control by a free-hand technique in aseptic conditions from patients treated in the Department of Obstetrics and Gynaecology, University Clinical Center in Belgrade, during a period of 3 years, in varying gestational ages. In 91 women, 239 amniocenteses were performed for different purposes. Indications for serial amniocenteses were: Rh-alloimmunisation, diabetes mellitus, polyhydramnios, pregnancy induced hypertension, genetic and amniocentesis at term. Amniotic fluid specimens contaminated with blood, those from patients with ruptured membranes or who were receiving antibiotics were discarded. The primary condition for inclusion in this study was the sterile first amniotic fluid specimen. Each sample was centrifuged at 10° C for 10 minutes at 1000 X g to remove particulate materials and was frozen at -70° C until studied. Transferrin concentrations were determined by method of immunonephelometry using original Boehring kits. All amniotic fluid samples were cultured for aerobic and anaerobic bacteria immediately after amniocentesis. Microorganisms were identified with standard methods. During the amniocentesis and also in the first 5 postpartal days womens' cubital vein blood specimens (3 ml) were taken for analysis. Peripheral cubital vein blood samples of 30 healthy nongravid women were obtained and served as controls. Obtained data were tested by one way variance analysis.

Results

Transferrin intraamniotic concentrations increased gradually with advancing gestational age from 49.90 mg/l in 13th week of gestation and reached maximum levels of 260.30

mg/l in the 39th gestational week (Fig. 1 and Table 1). In the last week of pregnancy, transferrin concentrations significantly decreased.

Transferrin blood concentrations were low in the 13th week of gestation (2.58 g/l) and increased according to the pregnancy progress, with some variations. Maximal concentrations were in the 38th week

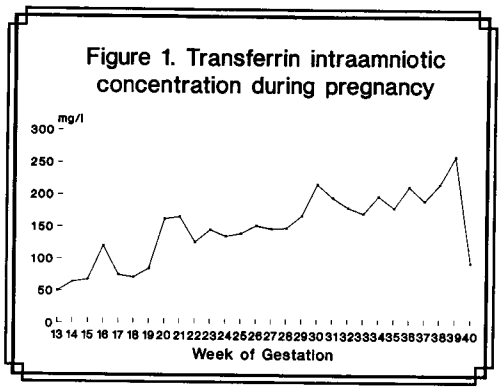

Figure 1. Transferrin intraamniotic concentration during pregnancy

Table 1 Transferrin concentrations in amniotic fluid during pregnancy (mg/l)

Weeks of gestation	$\bar{x}$	SD	Min	Max
13	49.90	-	49.9	49.9
14	63.96	17.91	33.8	82.0
15	68.21	0.22	61.0	75.0
16	120.12	63.23	36.1	267.0
17	75.08	7.97	52.0	80.0
18	77.36	21.59	42.5	125.0
19	85.78	43.16	40.1	156.0
20	162.50	34.64	138.0	187.0
21	166.30	-	166.3	166.3
22	126.50	61.56	75.0	223.0
23	146.02	42.11	62.1	185.1
24	136.00	-	136.0	136.0
25	140.21	32.11	102.1	163.5
26	152.34	28.25	132.2	179.5
27	147.52	28.41	126.1	166.5
28	149.00	-	149.0	149.0
29	168.80	-	168.0	168.0
30	217.00	3.28	214.0	220.0
31	196.52	20.22	172.1	225.4
32	181.00	-	181.0	181.0
33	172.00	-	172.0	172.0
34	199.00	44.91	158.0	240.0
35	180.50	12.59	169.0	192.0
36	213.84	39.20	116.0	259.0
37	191.76	59.58	55.0	290.0
38	217.46	40.28	137.0	272.0
39	260.30	46.59	184.0	308.0
40	95.05	11.30	89.4	112.0

(4.67 g/l). In the puerperium transferrin levels increased and reached a peak on the 2nd postpartal day (5.80 g/l). After that, concentrations decreased and on the 5th day were found to be 3.91 g/1 (Fig. 2 and Table 2). One way variance analysis confirmed statistically significant differences between transferrin concentrations after the 32nd week of gestation

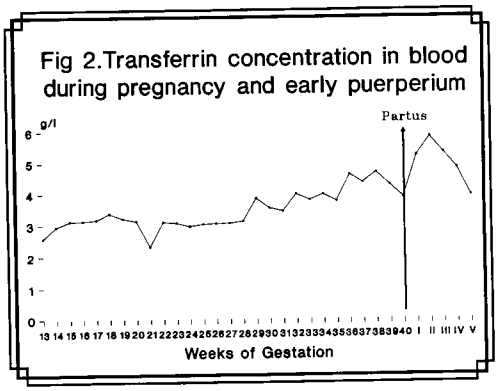

Table 2 Transferrin concentration in blood during pregnancy and early gestation (g/l)

Gestational weeks	x	SD	Min	Max
13	2.58	-	2.58	2.58
14	2.94	0.08	2.9	3.1
15	3.11	0.22	3.0	3.3
16	3.12	0.52	2.2	4.5
17	3.16	0.29	2.8	3.7
18	3.37	0.71	2.5	5.1
19	3.21	0.65	2.2	3.9
20	3.12	0.17	3.0	3.3
21	2.31	-	2.31	2.31
22	3.09	0.41	2.7	3.8
23	3.06	0.24	3.0	3.3
24	2.95	-	2.95	2.95
25	3.02	0.22	2.9	3.3
26	3.04	0.21	3.0	3.2
27	3.05	0.17	3.0	3.3
28	3.11	-	3.11	3.11
29	3.84	-	3.84	3.84
30	3.54	0.36	3.2	3.9
31	3.42	0.42	3.1	4.1
32	3.97	-	3.97	3.97
33	3.78	-	3.78	3.78
34	3.96	0.01	3.8	4.1
35	3.74	0.35	3.4	4.1
36	4.06	0.56	3.9	5.4
37	4.34	0.64	3.5	5.5
38	4.67	0.58	3.6	5.5
39	4.25	0.61	3.4	4.9
40	3.86	0.11	3.7	3.9
I	5.22	0.45	5.1	5.3
II	5.80	0.48	5.6	6.2
III	5.30	0.62	5.2	6.2
IV	4.82	0.51	4.6	5.3
V	3.91	0.31	3.7	4.1

compared to the levels before($p<0.05$). Maximal transferrin blood levels on the 2nd postpartal day confirmed the significance of this nonspecific immunologic defense factor during the period when the antimicrobial protection is the most necessary. In healthy nongravid persons transferrin levels in blood were 2.92 ± 0.57 g/l.

Bacterial growth studies showed proliferation of microorganisms in only 1 amniotic fluid specimen (0.42%), while other samples were sterile. In this patient after the 2nd, repeated genetic amniocentesis in the 18th week of gestation Staphylococcus epidermidis was isolated. Clinical signs of intraamniotic infection were present. She was treated according to the antibiogram and delivered at term without fetal and/or maternal complications. Intraamniotic transferrin concentrations in the 16th and 18th week were 36.1 mg/l and 42.5 mg/l, and at term 55.0 mg/l, while in blood levels were 3.28 g/l, 3.35 g/l in the same gestational ages respectively. This patient had intraamniotic transferrin level at the end of pregnancy significantly lower compared to the other patients without microbiological substrate in amniotic fluid. Also, blood transferrin levels in this patient were significantly lower than in the others ($p<0.05$).

Discussion

Transferrins (transferrin, lactoferrin and ovotransferrin) have been found to exert a bacteriostatic effects on a number of bacteria, and these effects could be eliminated by saturating both transferrin and iron-binding sites with iron[4,5]. The presence of lactoferrin in the specific granules of neutrophils suggest an extracellular site of action for lactoferrin[2]. Polymorphonuclear leukocytes exposed to highly purified human lactoferrin exhibit an increased random motility and are primed to produce more superoxide. This action seemed to be specific, because it could be abolished by simultaneous addition of antilactoferrin antibodies. So, polymorphonuclears became more effective after exposure to lactoferrin by having a greater motility and producing superoxide at a faster rate. The bacteriostatic effect of lactoferrin appears related to its ability to deprive bacteria of iron required for growth. Evidence has also been presented suggesting that lactoferrin participates in the alteration of the physicochemical properties of the neutrophil membrane during degranulation, the generation of granulopoiesis, and the modulation of complement function[6]. Besides antimicrobial properties exhibition, transferrins are involved in other processes during pregnancy. It has been confirmed that the liver and the yolk sac stimulate kidney differentiation by producing the soluble factor-transferrin[7]. Tubulogenesis in vitro is influenced and regulated by transferrin[8]. Transferrins specifically stimulate dermatan- and chondroitin-sulphate proteoglycan accumulation around lung cells, and in the extracellular

matrix of lung tissue in vitro[9]. Transferrin mRNA levels increased in liver throughout gestation with maximum expression at term[10].

Results obtained in the current study indicate that transferrin concentrations in the blood of pregnant women are modesty elevated during pregnancy. These findings are in agreement with the results obtained in other investigations[2,3,10].

It is well known that intraamniotic infection plays a role in increased fetal and/or neonatal morbidity and mortality, as well as maternal morbidity. Terzic´ [11] stressed on the fact that antibacterial activity of amniotic fluid may protect patients from chorioamnionitis and resultant preterm delivery. Transferrin is known to be a very important bacterial growth inhibitor[5,12]. A number of authors have previously described transferrin concentrations in amniotic fluid[2-4]. But until now there is no appropriate study that systematically presents transferrin concentrations in amniotic fluid and blood of pregnant women. In our prospective investigation we demonstrated that amniotic fluid transferrin exhibits a pattern of activity related to the gestational age, it increases according to the progression of gestation reaching maximum near term. Our results indicate a strong relationship between high transferrin level and sterile amniotic microenvironment. Up to now, only Nazir et al[13] have published results of antibacterial activity in amniotic fluid specimens obtained by serial amniocentesis. In the cited study in only 4 patients amniotic fluid was taken more than once, and therefore no conclusions could be drawn. The present study showed that incidence of intraamniotic infection after repeated amniocentesis was 0.42%. In the patient with positive microbial finding transferrin blood and intraamniotic levels were lower than in the all other investigated patients.

In conclusion,we can say that transferrin intraamniotic levels increased according to the progression of gestation, reaching maximal values at the 39th week of gestation. Transferrin blood concentrations were found to be increasing in a similar pattern, but it is important to point out that in the puerperium, when the antimicrobial protection is the most important, transferrin had maximal blood levels on the 2nd postpartal day. In addition, we confirmed that this nonspecific factor plays an important role in the antimicrobial activity of amniotic fluid.

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Lysozyme in Amniotic Fluid-Referent Curve

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Abstract : *Lysozyme is known to be the most important bacterial growth inhibitor in amniotic fluid. Although a great number of studies have previously described lysozyme concentrations in amniotic fluid, unfortunately until now there are no investigations that systematically study its intraamniotic concentrations. The aim of the present investigation was to determine lysozyme concentrations in amniotic fluid during gestation and to establish a referent curve of this very important hydrolytic enzyme. Serial amniotic fluid specimens were obtained under ultrasound control by a free-hand technique in aseptic conditions from 91 patients (239 samples) in various gestational ages, from the 13th week of pregnancy until term. Lysozyme activity determination was based on the use of turbidimetry at 546 nm, calculated by a standard curve (phosphate buffer 67 mmol/l, pH 6.3, Micrococcus lysodeicticus 0.2 g/l, sodium azide 8 mmol/l), according to the method of Prockop and Davidson (Lysozyme-Testomer-Boehringwerke). All amniotic fluid samples were cultured for aerobic and anaerobic bacteria within 30 minutes after amniocentesis. Our results indicate that lysozyme concentration increased gradually with advancing gestational age from 2.50 mg/l in 13 weeks of gestation and reached maximum levels of 23.60 mg/l at term. Lysozyme levels at term were almost 10 times higher than at the end of the first trimester. In conclusion we can say that lysozyme plays an important role in the antibacterial activity of amniotic fluid. (Thai J Obstet Gynaecol 1993; 5: 81-86.)*

Key words : lysozyme, amniotic fluid, serial amniocentesis

Bacterial colonization of the amniotic fluid has been reported in patients with intact membranes and during pregnancy, but microbial invasion of the amniotic cavity during labour, prior to and especially after rupture of membranes was found to occur more frequently[1]. Bacterial invasion

of the amniotic cavity may lead to maternal infection and fetal/neonatal sepsis. Antimicrobial components in amniotic fluid becomes the last resort to prevent these complications.

Numerous reports have been published regarding the antibacterial activity of amniotic fluid. A number of compounds such as lysozyme, transferrin, peroxidase, 7s immunoglobulin, spermine, beta-lysin, beta 1a/beta 1c globulin and zinc have been found in amniotic fluid and are assumed to exhibit bacterial growth-inhibitory activities[2]. However, how these compounds inhibit the bacterial growth in amniotic fluid is not clearly understood, because investigators have shown contradictory evidence with respect to growth of certain bacterial species in amniotic fluid. The reasons for these differences need to be clarified[3].

Lysozyme is known to be the most important bacterial growth inhibitor. A number of authors have previously described lysozyme concentrations in amniotic fluid[4,5]. But until now, there are no investigations that systematically present its concentrations in amniotic fluid. So, the purpose of the present study was to determine lysozyme concentrations in amniotic fluid during gestation and to establish referent curve of this very important hydrolytic enzyme.

Materials and Methods

Serial amniotic fluid samples were obtained under ultrasound control by a free-hand technique in aseptic conditions from patients treated in the Clinic of Gynaecology and Obstetrics, University Clinical Center in Belgrade, in various gestational ages. In 91 women, 239 amniocenteses were performed for different purposes. The fluids contaminated with blood, those from patients who were receiving antibiotics or complicated by ruptured membranes were discarded. Each sample was centrifuged at 10° C for 10 minutes at 1000 X g to remove particulate materials and was frozen at -70° C until studied. Lysozyme activity determination was based on the use of turbidimetry at 546 nm, calculated by a standard curve (phosphate buffer 67 mmol/l, pH 6.3, Micrococcus lysodeicticus 0.2 g/l, sodium azide 8 mmol/l), according to the method of Prockop and Davidson (Lysozyme-Testomer-Boehringwerke)[6]. All amniotic fluid samples were cultured for aerobic and anaerobic bacteria within 30 minutes after amniocentesis.

Results

In order to establish a referent curve for lysozyme in amniotic fluid, during the period of three years (1990-1992) in 91 patients 239 amniocenteses were performed, from the 13th week of gestation until term. Indications for serial amniocenteses were: Rh-alloimmunization, diabetes mellitus, polyhydramnios, pregnancy-induced hypertension, genetic and amniocentesis at term. Lysozyme concentration increased gradually with

advancing gestational age from 2.50 mg/l in 13 weeks of gestation and reached maximum levels of 23.60 mg/l at term (Table 1 and Fig. 1). So, lysozyme levels at term were almost 10 times higher than at the end of the first trimester.

Bacterial growth studies showed proliferation of microorganisms in only one amniotic fluid specimen

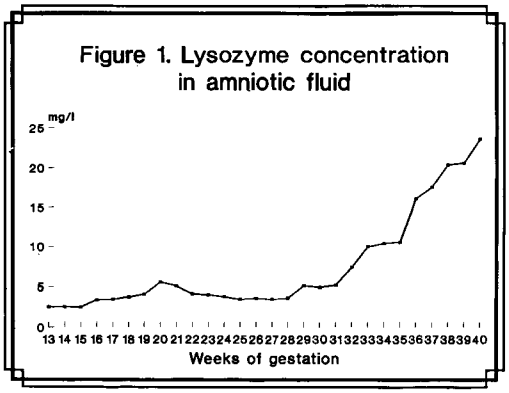

Table 1 Lysozyme concentrations in amniotic fluid (mg/l)

Weeks of gestation	$\bar{x}$	SD	Min.	Max.
13	2.50	-	2.5	2.5
14	2.50	-	2.5	2.5
15	2.50	-	2.5	2.5
16	3.37	0.90	2.5	5.4
17	3.47	0.81	2.5	5.4
18	3.76	0.95	2.5	6.0
19	4.10	2.97	2.5	10.8
20	5.58	2.54	2.5	10.0
21	5.10	2.20	4.0	8.4
22	4.17	0.98	3.0	6.0
23	4.01	0.62	3.1	4.2
24	3.80	-	3.8	3.8
25	3.50	0.21	3.2	3.8
26	3.63	0.25	3.3	3.9
27	3.55	0.21	3.2	3.8
28	3.58	0.22	3.4	3.6
29	5.10	0.21	5.0	5.4
30	4.90	0.98	4.0	5.8
31	5.22	0.42	4.8	5.9
32	7.41	0.22	6.8	8.1
33	10.00	-	10.0	10.0
34	10.40	6.13	4.8	16.0
35	10.50	3.83	7.0	14.0
36	16.18	7.83	4.8	26.0
37	17.59	5.37	2.5	27.5
38	20.41	5.25	12.0	33.0
39	20.63	6.51	12.0	29.0
40	23.60	6.38	14.0	28.0

(0.48%). Other samples were sterile. In this patient after the second, repeated genetic amniocentesis in 18 weeks of gestation Staphylococcus epidermidis was isolated. Clinical signs of intraamniotic infection were present. She was treated according to the antibiogram and delivered at term without fetal and/or maternal complications. Concentrations of lysozyme in the 16th and18th week were 2.5 mg/l, and at term 4.5 mg/l. This patient had a lysozyme level at the end of pregnancy five times lower than in other patients without microbiological substrate in amniotic fluid. Obtained data confirm protective antimicrobial effect of lysozyme in amniotic fluid.

Discussion

Sixty years ago Fleming and Allison[7] confirmed the occurrence in the tissues and secretions of man and animals, and in some vegetable tissues, a bacteriolytic substance to which the name of "lysozyme" has been applied. Lytic action of this substance was especially manifested on certain non-pathogenic bacteria, although it could also be observed with bacteria which were pathogenic to some of the lower animals and to man. When lysozyme-containing material, such as tears, is brought into contact with a susceptible microbe (M.lysodeicticus) a powerful inhibitory, bactericidal and bacteriostatic effects are exerted. Today, we know that lysozyme, one of the best-characterized hydrolytic enzymes, is a major constituent of macrophages, monocytes and polymorphonuclear leukocytes. It hydrolyzes beta-1,4 linkages between N-acetylmuramic acid and 2-acetyl-amino-2-deoxy-D-glucose residues in mucopolysaccharide or mucopeptide components of the cell wall of bacteria. As a result, N-acetyl muramic acid is cleaved from N-acetyl glucosamine. Consequently, the mucopeptide wall is destroyed and the organisms are lysed. The capacity to degrade this commonly occurring component of microbial cells is an important factor in the intracellular and extracellular dissolution of bacteria. Although not identical, lysozymes produced by different species are similar in structure, molecular weight and biologic activity.

The amniotic cavity can be colonized by pathogenic microorganisms by way of maternal circulation before and during labour with intact membranes[1]. In such a case, the fetus depends on maternal host defenses for protection against infectious agents. Bacterial colonization of the amniotic cavity is known to occur following rupture of amniotic membranes by direct ascent of lower genital tract organisms[8]. The fetal membranes serve as a barrier preventing the ascension of lower genital tract infectious agents. It has been shown that invasion of the amniotic cavity is possible even without rupture of membranes[2,9]. In such a case, the amniotic fluid becomes the last resort to prevent fetal sepsis. Intraamniotic infection plays a role in increased fetal and/or neonatal mor-

bidity and mortality, as well as maternal morbidity. Minkoff[10] and Terzic[11] stressed on the fact that antibacterial activity of amniotic fluid may protect patients from chorioamnionitis and resultant preterm delivery.

In this investigation we demonstrated that amniotic fluid lysozyme exhibit a pattern of activity related to the gestational age, it increased according to the progression of gestation reaching maximum at term. Our results indicate a strong relationship between high lysozyme level and sterile amniotic microenvironment. Results are also in agreement with previous studies that have shown higher lysozyme concentrations in patients with advanced gestation[5,11-13]. But until now, only Nazir et al[8] have published results of antibacterial activity in amniotic fluid specimens obtained by serial amniocenteses. In the cited study, 4 patients had amniotic fluid taken more than once. Given that amniotic fluid is actively exchanged and that a patient's clinical status may change, cited authors have a standpoint that it seems reasonable to expect that fluid drawn on separate days from the same patient may differ in antimicrobial concentrations. Our study confirms their opinion. Nazir et al[8] also emphasized that serial amniotic fluid samples were obtained in 4 patients only, and therefore no conclusions can be drawn. The present study showed that the incidence of intraamniotic infection after repeated amniocenteses is 0.418%.

In conclusion, we can say that lysozyme levels increased according to the progression of gestation and that this enzyme plays an important role in the antibacterial activity of amniotic fluid.

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First Newborn's Aspirate Microbial Analysis and Lysozyme Activity Determination

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Abstract: *The aim of the present study was to investigate the presence of microorganisms in pregnant women's cervicovaginal regions just before delivery and in the first aspirate of the upper respiratory tract and the oral cavity in the newborns immediately after delivery. Microbial analyses of smears taken two hours postpartally from the throat, oral cavity, stomach and ear of neonates showed totally altered findings. Estimating lysozyme activity in the first newborn's aspirate, by the use of turbidimetry, according to the method of Prockop and Davidson (Lysozyme-Testomer-Boehringwerke), we found it's very high value. Our study indicates that there is a strict relationship between microbiological substratum and lysozyme activity in the first newborn's aspirate. (Thai J Obstet Gynaecol 1993; 5: 87-90.)*

Key words : lysozyme activity, first newborn's aspirate, microbial finding

The removal of the content from the oral cavity and the upper respiratory tract (amniotic fluid, blood, mucus) immediately after the birth is called the primary (first) aspiration. In the first aspirate we may expect microorganisms that are found in the cervicovaginal region[1]. Bacterial colonization of the amniotic cavity has been reported in patients with intact membranes during pregnancy, but microbial invasion of the amniotic cavity during labour, prior and especially after the rupture of membranes was found to occur more frequently. In cases of premature membranes rupture, the ascending infection may lead to maternal infection and fetal/neonatal sepsis[2]. But, one must have in mind that lysozyme, transferrin, peroxidase, beta-lysin, beta 1a/beta 1c globulin, IgA, and zinc-protein complex in neonatal oral and rhinal cavity, throat, pharynx and respiratory tract, are assumed to exibit bacterial growth-inhibitory activities[3,4].

The aim of this study was to investigate the microbiologic and nonspecific immunologic aspect of the first aspirate in newborns immediately

after delivery.

Materials and Methods

The prospective study was carried out during a period of 3 years. Samples of the first aspirate of 107 newborn infants immediately after delivery as well as cervicovaginal region smears taken before the expulsion were analyzed in the Clinic of Gynaecology and Obstetrics, University Clinical Center in Belgrade. Smear samples were taken from throat, pharynx, stomach, skin, umbilicus and ear of the newborns 2 hours after the birth at the Department of Neonatology. Lysozyme activity from the first aspirate content and umbilical cord of the neonates was determined in the Laboratory for immunochemistry.

Each sample was centrifuged at 10° C for 10 minutes at 1000 X g to remove particulate materials and was frozen at -70° C until lysozyme concentration testing. Determination of lysozyme level was based on the use of turbidimetry at 546 nm, calculated by a standard curve (phosphate buffer 67 mmol/l, pH 6.3, Micrococcus lysodeicticus 0.2 g/l, sodium azide 8 mmol/l), according to the method of Prockop and Davidson (Lysozyme-Testomer-Boehringwerke)[5].

All samples were cultured in aerobic and anaerobic conditions immediately after they were obtained.

Oral cavity and pharynx smears as well as peripheral blood samples of 30 healthy nongravid adults served as controls for the lysozyme concentrations estimation.

Obtained data were tested by *Student t test* and one way variance analysis.

Results

The results of our investigation showed microbial presence in the throat aspirates of the newborn infants with predominant of E.coli (24.30%) followed by S. albus (14.95%), Enterococcus (7.48%), Diphtheroids (5.61%), etc.. The incidence of haemolytic streptococcus of "B" group colonization was 2.80%, S. pyogenes 1.87%, Klebsiella-Enterobacter 0.93% and Candida 1.87% in the examined samples. Comparing the results of the microbial analyses of the cervicovaginal smears with the samples of the first newborn's aspirate, we observed the decreased incidence of E.coli (19.63%), but elevated percentage of Enterococcus (27.10%), Candida (6.54%) and Streptococcus beta-haemolytic "B" group (7.48%) in the delivery paths (Table 1).

Microbiological examination of the smears taken 2 hours postpartally from the newborns at the Department of Neonatology showed completely different results: the incidence of E.coli was markedly decreased, that is in general, the presence of microbes was significantly lower in all the examined localizations. Furthermore, 60-120 minutes after the birth sterile media were 4 times more frequent than colonised (Table 2).

Table 1 Comparative analyses of the bacterial flora in the first aspirate and the vaginal smear

Bacterial flora	First aspirate		Vaginal smear	
	No.	%	No.	%
E. coli	26	24.30	21	19.63
Enterococcus	8	7.48	29	27.10
Staph. pyogenes	2	1.87	2	1.87
Strept. haemol. (B)	3	2.80	8	7.48
Strept. haemol.	2	1.87	1	0.93
Staph. albus	16	14.95	57	53.27
Diphtheroides	6	5.61	29	27.10
Klebsiella-Enterobacter	1	0.93	1	0.93
Pseudo. aeruginosa	1	0.93	0	0
Proteus mirabilis	0	0	4	3.74
Acinetobacter	2	1.87	0	0
Candida	2	1.87	7	6.54

Table 2 Bacterial flora present in the smear samples of the newborn infants (Percentage and numeric expression in the examined samples)

Bacterial flora	Throat		Stomach aspirate		Skin		Ear		Umbilicus		Anus	
	No	%	No	%	No	%	No	%	No	%	No	%
E.coli	0	0	2	1.87	0	0	0	0	2	1.87	2	1.87
Strept. haemol. (B)	2	1.87	0	0	2	1.87	1	.93	2	1.87	2	1.87
Enterococcus	1	.93	0	0	0	0	0	0	1	.93		1.93
Klebs.-Enterobact.	0	0	0	0	0	0	0	0	0	0	4	3.74
Staph. pyogenes	7	6.54	1	.93	5	4.67	1	.93	5	4.67	1	.93
Staph. albus	0	0	0	0	6	5.61	1	.93	2	1.87	2	1.87
Acinetobacter	1	.93	0	0	0	0	0	0	0	0	0	0
Diphtheroides	1	.93	0	0	0	0	0	0	0	0	0	0.
Pseudo. aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	0	0
Strepto. haemol.	0	0	0	0	0	0	0	0	0	0	0	0
Candida	0	0	0	0	0	0	0	0	0	0	0	0

Smear samples of the newborns at the Department of Neonatology
(taken 60-120 minutes after birth)

Number of colonised media n= 22 (20.56%)
Number of sterile media n= 85 (79.44%)

Lysozyme activity determination in the first newborn's aspirate was statistically higher than in control group of adult persons ($p<0.01$). Although lysozyme levels were higher in umbilical cord samples than in peripheral blood specimens of healthy nongravid persons, the difference was not found to be statistically significant (Fig.1).

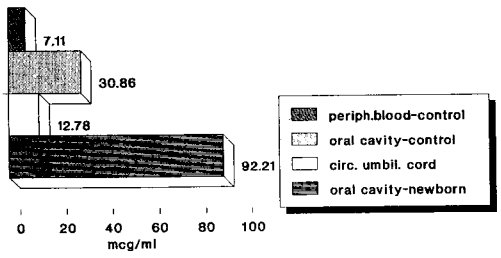

Fig. 1 Lysozyme activity in newborns and adult healthy nongravid persons.

Discussion

The incidence of microbial presence in the first aspirate of the newborn infants is related to the microflora of the maternal cervicovaginal region. However, vaginal flora has variations in different parts of the world. In our study, E.coli was found in 19.63% of cases in the vaginal microflora, and beta-haemolytic streptococcus in 7.48% of cases which is in accordance with the results of other authors[6,7]. The fact is that in the course of delivery a colonization occurs, but a small number of newborns is affected due to the presence of the expressed antimicrobial defence in which beside other factors, lysozyme with its bacteriolytic activity on the peptidoglycans of the microbial walls, has an important role. The analysis of lysozyme values indicated that high levels of this antibacterial enzyme in the investigated substratum can present an important component of the spectrum of nonspecific defence system of the newborn infants, which would probably be confirmed in further clinical and laboratory investigations.

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Comparison of Long and Short GnRH-a/hMG Ovarian Stimulation Protocols in Assisted Reproduction

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Abstract :

Objective: *To evaluate the outcome of long and short GnRH-a/hMG ovarian stimulation protocols in assisted reproduction.*

Design: *A retrospective review.*

Setting: *The study was conducted in a University Hospital in Northern Thailand.*

Patients: *A group of 141 consecutive patients who underwent 186 cycles of ovulation induction for assisted conception at the Infertility Clinic, Maharaj Nakorn Chiang Mai University Hospital, from January 1990 to August 1992.*

Main outcome measures : *Cycle cancellation rates, amount of hMG used, duration of treatment, estradiol levels on the day of hCG injection, number of oocytes retrieved, fertilization rates, preclinical and clinical pregnancy rates, abortion rates and incidence of ovarian hyperstimulation syndrome (OHSS).*

Results: *The long protocol required significantly more hMG, longer duration of treatment, had a higher incidence of OHSS, higher fertilization and abortion rates. No statistically significant differences in cycle cancellation rates, numbers of oocytes retrieved and pregnancy rates were found.*

Conclusion: *Short protocol may be more preferable than long protocol in terms of patients' expense, convenience and side-effects. (Thai J Obstet Gynaecol 1993; 5: 91-98.)*

Key words : assisted reproduction, long and short ovarian stimulation protocols

Natural cycle in vitro fertilization (IVF) has now largely been abandoned in favour of ovarian stimulation cycles for assisted conception because the chance of pregnancy is principally determined by the number of oocytes or embryos replaced[1-3]. In stimulated cycles premature luteinizing hormone (LH) surge, with its deleterious effects on follicular, luteal endocrinology or on uterine environment, accounts for a 10-30% cancel-

lation in IVF cycles[4-6]. To overcome this problem gonadotropin releasing hormone agonist (GnRH-a) has been introduced as the adjuvant controlling ovarian stimulation to reduce the endogenous secretion of LH[6,7]. The use of such agonists has been shown to significantly reduce cancellation rate and to efficiently increase the number of oocytes and embryos when compared with cycles stimulated with clomiphene citrate-human menopausal gonadotropins (CC-hMG) or hMG alone[7-9]. Different protocols for timing GnRH-a have been described[3, 6-10]. In practical terms, these can be reduced to two principle categories: a long (suppression) or a short (flare up) protocols. There is still some controversy regarding which protocol is better than the other in terms of oocyte recovery rates, number and quality of embryos available for transfer, and pregnancy rates.

In the present study, we compared the long and short GnRH-a/hMG ovarian stimulation protocols for assisted reproduction in our infertile patients.

Materials and Methods

The study included 141 consecutive patients who underwent 186 cycles of ovulation induction for assisted conception at the Infertility Clinic, Maharaj Nakorn Chiang Mai University Hospital from January 1990 to August 1992. All patients received complete basic infertility investigations and had failed conventional therapy for their infertility problems before they were considered for assisted reproduction. The details of our program have been described previously[11-13].

In brief, patients were allocated to either the long or short GnRH-a ovarian stimulation protocols at the discretion of their attending physicians. Buserelin acetate (Suprefact®, Hoechst) was administered intranasally at a dose of 100 ug 6 times per day, starting in the midluteal of the preceding cycle in the long protocol, or on the first day of the current cycle in the short protocol. In both regimens, hMG (Pergonal®, Serono) and/or follicle stimulating hormone (FSH, Metrodin®, Serono) were given intramuscularly according to the patients' age and previous responses, from cycle day 3 onward. Pelvic sonogram was done on the first day of the cycle as baseline, and then on a daily basis from day 8 of the cycle to monitor follicular growth. Daily measurement of serum estradiol by specific radioimmunoassay (Estradiol Maia Kit, Biodata Products) was done from day 6 of the cycle. Human chorionic gonadotropin (hCG, Profasi®, Serono) 5000-10000 IU was given intramuscularly when ultrasound demonstrated 2 or more ovarian follicles exceeding 17 mm in diameter and the estradiol level reached ≥ 300 pg/ml per dominant follicle. Buserelin was stopped on the day of hCG injection and oocyte retrieval was scheduled 34-36 hours later, followed by gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT) or in

vitro fertilization and embryo transter (IVF & ET). Luteal phase was supported with hCG 1500 IU intramuscularly on days 2, 5, 8, and 11 dating from oocyte recovery.

Criteria for cycle cancellation included an insufficient ovarian response (poor estradiol increase), a decrease in estradiol level (falling levels ≥ 20% of previous levels before fulfilment of criteria for hCG administration), and excessive ovarian response.

Statistical analyses were performed using Student's t-test, Fisher exact test and Chi-square test when appropriate. The results were considered significant at value $p < 0.05$.

Results

Fifteen ovarian stimulation cycles out of 186 were cancelled before oocyte retrieval (Table 1), leaving 171 cycles for analysis. Of these, 87 were assigned to short and 84 to long protocols. The mean ages of the patients, duration and aetiologies of infertility, as well as types of assisted reproduction were comparable in the two groups (Table 2).

Table 1 Reasons for cycle cancellation before oocyte retrieval

Causes	Short protocol	Long protocol
Insufficient ovarian response	4	8
Excessive ovarian response	-	1
Falling E2 levels	1	1
Total cancellation*	5/92	10/94

* p = 0.2817, no significant difference by Fisher exact test.

Table 2 Patients profiles

	Short protocol (n=87)	Long protocol (n=84)	p value
Mean ages (years)[a]	35.6 ± 7.9	34.9 ± 3.3	NS[b]
Duration of infertility (years)	7.1 ± 4.2	7.0 ± 3.9	NS
Aetiologies of infertility[c]			
Tubal obstruction[d]	37 (42.5%)	45 (53.6%)	NS
Endometriosis[d]	24 (27.6%)	17 (20.2%)	NS
Male factor[d]	23 (26.4%)	18 (21.4%)	NS
Unexplained[e]	9 (10.3%)	7 (8.3%)	NS
Type of assisted reproduction			
IVF & ET[d]	47 (54.0%)	55 (65.5%)	NS
GIFT[d]	27 (31.0%)	21 (25.0%)	NS
ZIFT[e]	3 (3.4%)	1 (1.2%)	NS
GIFT + IVF & ET[e]	10 (11.5%)	7 (8.3%)	NS

a=Unpaired t-test
b=NS, nonsignificant
c=Some patients had more than one infertility factors
d=Chi-square test
e=Fisher exact test

Table 3 Comparison of short and long GnRH-a superovulation protocols

	Short protocol (n=87)	Long protocol (n=84)	p value
Numbers of hMG ampoules	21.2 ± 9.2	30.1 ± 13.4	0.0030
Days of GnRH-a/hMG treatment	11.3 ± 2.1	19.8 ± 3.6	<0.000001
E2 level on the day of hCG injection (pg/ml)	1791.5 ± 801.4	2007.9 ± 883.9	NS[a]
Numbers of oocytes retrieved	7.1 ± 4.8	8.8 ± 5.2	NS
Fertilization rates (%)	226/447 (50.6%)	301/496 (60.7%)	0.002
Numbers of oocytes/embryos replaced			
GIFT	5.7 ± 2.6	6.5 ± 2.9	NS
IVF & ET	3.9 ± 2.4	4.4 ± 2.6	NS
Numbers of pregnancy per retrieval cycle	19 (21.8%)	14 (16.7%)	NS
Biochemical pregnancy[b]	4	6	
Clinical pregnancy[c]	14	7	
Ectopic pregnancy	1	1	
Numbers of abortion[d]	6 (31.6%)	14 (100%)	0.001
Biochemical pregnancy	4	6	
Clinical pregnancy	1	7	
Ectopic pregnancy	1	1	
Ovarian hyperstimulation syndrome[d]	0	5	0.0269
Mild	0	1	
Severe	0	4	

a=NS, nonsignificant
b=Biochemical pregnancy is diagnosed when the level of B-hCG > 25 mIU/ml, followed by a higher level in a subsequent assay 2 days later.
c=Clinical pregnancy is diagnosed when a gestational sac is visualized under ultrasound.
d=Fisher exact test

Serum estradiol levels on the day of hCG injection, the numbers of oocyte retrieved, numbers of oocytes/ embryos replaced and pregnancy rates were comparable in the two groups. However, there were significant differences in the requirement of hMG, the duration of GnRH-a/hMG treatment, fertilization and abortion rates, and in the incidence of ovarian hyperstimulation syndrome (Table 3).

Discussion

Various approaches for the clinical use of GnRH-a as adjuvant to control ovarian stimulation have been described. The main parameter subjected to variation is the duration of analogue administration before starting the stimulation with hMG[3, 6-10]. In the long protocol, GnRH-a is first administered for at least 10 days to induce a state of temporary hypogonadotropic hypogonadism. This is then followed by hMG administration when full ovarian suppression is achieved. In contrast, the short protocol makes use of the initial stimulatory phase of GnRH-a to augment ovarian response to hMG, which is started 2-3 days after the analogue[3,6,7,10]. This may

explain the findings in our study that a lower amount of hMG is required in the short protocol than in the long one. In this regard, short protocol may be more advantageous in term of expense. Moreover, the short protocol may be better tolerated as it involves a significantly shorter mean duration of treatment (11.3 vs 19.8 days in this study).

As can be seen in Table 1, cycle cancellation rates due to insufficient ovarian response seems to be slightly higher in the long protocol than in the short protocol. However, overall cancellation rates are not statistically different among the two protocols. It is also noticeable that cancellation due to falling E2 levels before hCG administration, which presumably signifies premature luteinization, occured in only 2 out of 186 cycles (1.1%).

Although our data show that fertilization rate is significantly higher in the long protocol than in the short one, the pregnancy rates are comparable. According to Loumaye et al[14], the "flare-up" effect in short protocol exposes the ovaries to a relatively high level of LH during the first half of the follicular phase. In their study, pregnancy rates are similar but oocyte fertilization rate and embryo quality are reduced in the short when compared with the long protocol. They postulated that high LH level in the early follicular phase may have deleterious consequences upon oogenesis. Mettler et al[15] and Dirnfeld et al[16] reported better follicular maturation and a higher pregnancy rate using the long compared with the short protocol. On the contrary, Lippitz et al[17], Zorn et al[18], Frydman et al[19] and Acharya et al[20] cannot demonstrate any advantage of the long over the short protocol regarding folliculogenesis, oocytes recovered and pregnancy rates. Obviously, a consensus has not been reached and further evaluation by means of a large enough randomized trial is needed.

Our overall clinical abortion rate of 8/21 or 38.1% is rather high when compared with 22% for IVF and 19% for GIFT as reported by the United States IVF-ET Registry in 1990[21]. It is noticeable that abortions occur significantly more often in the long than in the short protocol (7 in 7 or 100% versus 1 in 14 or 7.1%). The reason the abortion rate is much higher in the long than of that in the short protocol is not apparent. We postulate that the suppression phenomenon with a decrease in the synthesis and storage of pituitary LH and desensitization of granulosa cells may be more prolonged and more profound in the long protocol. Therefore, the rescue of luteal function by four doses of 1500 IU hCG given every three days may be adequate in the short but not in the long protocols. Further research is needed to either confirm or refute this postulation. Indeed, in a randomized controlled study of luteal support, Yovich et al[22] concluded that combined hCG/progesterone regimen provides a better outcome than hCG or progesterone alone.

It is also possible that endogenous hCG produced by the conceptus may not be sufficient to sustain luteal function and hCG supplementation should be given well beyond the implantation period when long stimulation protocol is used. Although they present no direct evidence to support their recommendation, Brinsden and Asch[23] continued progesterone supplementation until 10 weeks' gestation if their GIFT pregnancies were normal. Our data suggest that such supplementation may be necessary in the long protocol, but its value in the short protocol should be further evaluated.

Ovarian hyperstimulation (OHSS) occurred in 5/171 stimulated cycles, giving an incidence of 2.9%. In this report, all cases of OHSS occurred in the long protocol group. This may be related to the finding that more follicles are recruited as evidenced by a higher level of E2 on the day of hCG injection and the higher mean number of oocytes retrieved. The details of this serious complication in our patients have been described previously[24].

This study has limitations in that it is a retrospective review and patient allocation is not randomized. Moreover, it is possible that significant differences in pregnancy rates among the two GnRH-a protocols exist but we do not have a large enough sample size to show the difference. However, based on our limited data short protocol may be more preferable than long protocol in terms of patients' expense and convenience. A further randomized trial is needed to evaluate the two protocols. Luteal phase support should also be a subject for future evaluation.

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Uterine Hydatid Cyst : An Extremely Rare Localization

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Abstract : *Hydatid cyst is a parasitic disease caused by Echinococcus. Although the first and most important site for this parasite is the liver, it can be seen in pelvic organs as well. However, the primary involvement is very rare. We report a case with a primary involvement of the uterus which was operated on and was proven by microscopic studies. (Thai J Obstet Gynaecol 1993;5: 99-102.)*

Key words : hydatid cyst, uterus, localization

Hydatid cyst (Echinococcosis) is characterized by worldwide distribution and the incidence among human beings is dependent on the incidence in intermediate hosts including sheep, pigs, and cattle. The most common unilocular hydatid cyst is caused by Echinococcus granulosus while the alveolar type is caused by Echinococcus multilocularis[1]. A new species of Echinococcus, Echinococcus vogeli, has been identified in South America, and it may be responsible for most human hydatid cysts in the region[2].

Patients with simple or uncomplicated multivesicular cyst are usually asymptomatic. The cyst increases in size at the rate of about 1 cm/year. It is this gradually enlarging mass that compresses adjacent host structures leading to the signs of echinococcosis in humans. Abdominal pain and tenderness are the most common complaints. Jaundice and ascites are uncommon. If any secondary infection be added, tenderness, hepatomegaly, chills, and spiking temperatures will occur. Urticaria and erythema offer evidence of a generalized anaphylactic reaction. Vomiting with passage of hydatid membranes in the emesis (hydatidemesia) and passage of membranes in the stool (hydatidentria) may also occur. Intrabiliary rupture represents the most common complication

and occurs in 5 to 10% of cases. Suppuration, the second most common complication, is caused by bacteria from the biliary tract. The formation of the purulent material results in the death of the parasite and conversion into a pyogenic abscess[3,4].

Hydatid cyst is suggested by the presence of a symmetrical tumour mass and it is detected by palpation, routine roentgenograms of the abdomen or chest, radioactive scans, or sonography. An enzyme immunoassay, complement fixation, hemagglutination, latex agglutination, and bentonite flocculation tests are available in diagnosis. Diagnostic aspiration of intact cyst should not be performed because of the danger of rupture and spillage of cyst contents[3,4].

Surgical removal of an intact cyst is the preferred form of therapy. If this is impossible, marsupialization and sterilization of the cyst contents with 2% formalin, hypertonic solutions, and 1% iodine may be effective[3]. The use of imidazoles as an adjunct to surgery or in inoperable cases of echinococcosis has been advocated[4].

We report here a case of uterine hydatid cyst which is an extremely rare involvement.

Case Report

A 55-year-old woman, gravida 5, para 5, was referred with irregular vaginal bleeding for 2 months. Previous medical history was unremarkable. General examination showed no abnormality. On pelvic examination, the mass (8 cm in diameter) was on the right side of the uterus. It was not possible to differentiate from the right parametrium. The left parametrium was free.

On ultrasonography, a large septated and cystic mass (8x5 cm) was identified on the left side of the uterus (Fig.1). Ultrasonic examination demonstrated no other pathology in the abdomen.

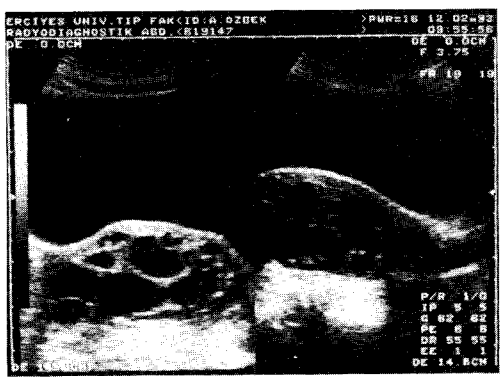

Fig. 1 Ultrasonic examination showing septated cyst.

Histological examination of the specimens from endometrium and cervix did not reveal any pathology. The remainder of the physical, gynaecological, and laboratory analyses, such as blood pressure, pulse, fasting plasma glucose, electrolytes, renal and liver function tests and chest X-ray were normal.

Laparotomy revealed that the right ovary was normal in size and adhered to the subserous mass of the uterus. Abdominal hysterectomy with

bilateral salpingo-oophorectomy was performed.

During macroscopic analysis the uterus weighed 160 g and measured 12x6x4 cm. On the right side of the uterus under the serosa and inside the myometrium a yellow colored irregular cystic cavity sized 6x4x3 cm appeared (Fig. 2). In this cavity there were a lot of chitinous layers of cysts, and the distance had no relation with the endometrial cavity. Macroscopic analyses of cervix, endometrium, fallopian tubes and ovaries revealed no other pathology.

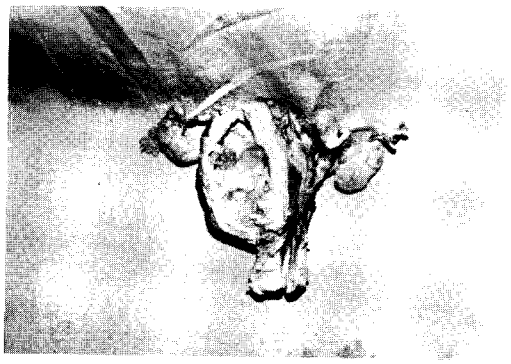

Fig. 2 Uterine specimen showing hydatid cyst.

Histologic examination of the cystic cavity showed a pericyst composed of hyalinized connective tissues. Inside the lumen there were pieces of chitinous layers composed of basophylic laminars and scolices were observed in some sections (Fig.3). Between the myometrial cells mononuclear cells infiltration was seen (Fig.4). Histologic analysis of endometrium and ovaries showed no other pathology.

After histological diagnosis we performed indirect hemagglutination test, and it was positive in 1/256 titration. During the operation the cyst was perforated. Therefore, after defi-

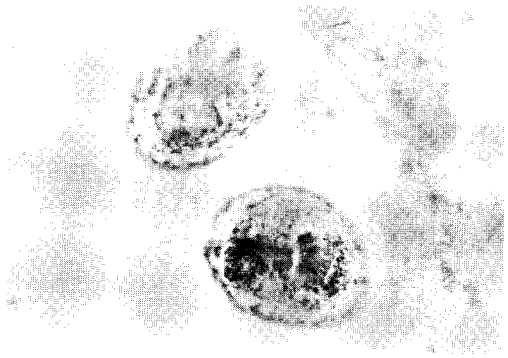

Fig. 3 Basophylic laminars and scolices. HE. X250.

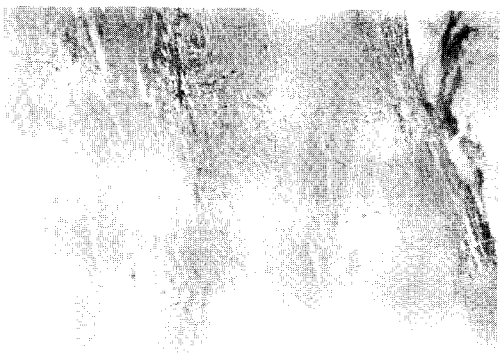

Fig. 4 Mononuclear cell infiltration in the myometrium. HE. X 32.

nite diagnosis postoperatively we started albendazole therapy in doses of 10 mg/kg/day for 8 weeks for prophylactic purpose.

The patient did well postoperatively and was discharged on the 10th postoperative day. The patient was asymptomatic for 3 months postoperatively.

Discussion

Hydatid cyst is a parasitic disease caused by Taenia Echinococcus. Approximatelly 70% of hydatid cysts are located in the liver. The next important site for this parasite is the lung. Splenic, renal, cerebral, ocular and osseous hydatids have been described[1,4]. Patiroglu et al[5] reported hydatid cyst localizations of their 8 years experience. Of 190 hydatid cysts, 35 were located outside the liver and lung. The location of those patients were as follows: 7 omentum, mesentary, and peritonium, 5 brain, 5 muscle, 3 ligamentum latum, 3 spleen, 2 ovary, 2 thyroid, 2 bone, 2 subcutaneous tissue, 1 kidney, 1 the lumen of arteria femoralis and 1 breast.

Secondary involvement of the pelvic organs was seen. However, primary involvement is very rare. Hangval et al[6] reported an ovarian hydatid cyst in 1979.

Primary uterine hydatid cyst is an extremely rare condition. We found only two reports in the literature[7,8]. Diagnosis of the hydatid cyst with frequent localization is easy, but in infrequent localization like ours, the diagnosis is not easy.

We think that gynaecologists should remember hydatid cysts when they find septated cystic mass in the pelvis. Also, we think that if a hydatid cyst is diagnosed as in our case after an operation, one should start albendazole postoperatively.

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Uterus Didelphys with Unilateral Obstructed Hemivagina and Renal Agenesis on the Same Side : A Case Report

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Abstract : *A rare condition, complete or incomplete duplication of the uterus and cervix with unilateral vaginal obstruction, is usually associated with ipsilateral renal agenesis. Clinical presentations are various such as pelvic pain, dysmenorrhea, abnormal vaginal bleeding or vaginal discharge. This patient complained of recurrent foul smelling vaginal discharge for many years, that was initially treated with many antibiotics and failed to recognize the true diagnosis. The menstrual and fertility functions were normal. The case, diagnosed and treated at Chonburi Hospital, is presented for awareness of this syndrome. (Thai J Obstet Gynaecol 1993; 5: 103-106.)*

Key words : uterus didelphys, renal agenesis, vaginal discharge

The specific association of uterus didelphys, obstructed hemivagina and ipsilateral renal agenesis was recognized as early as 1922[1]. In a minority of cases, a communication connecting the right and left sides at the level of the vagina was present[2]. The presenting symptom of this patient was recurrent vaginal discharge which did not respond to medical treatment.

Case Report

Mrs BR, a 25-year-old multiparous Thai woman presented to the Gynaecologic Out-patient Division with the complaint of foul smelling purulent vaginal discharge off and on which did not respond to many courses of various antibiotics for 2-3 years. Other symptoms such as dysmenorrhea, abdominal pain, fever or urinary symptoms were not found in this patient. Her first menstruation began at 15 years old, and the menstrual cycle was normal. She had 2 children delivered by normal vaginal delivery. Her past and family histories were negative.

Systemic physical examinations were normal. Pelvic examination re-

vealed normal external genitalia. During a speculum examination, a slightly protruding left vaginal fornix and foul smelling purulent discharge drainage from a small pinpoint area just anterolateral to the cervix was apparent. The uterus and both adnexae were normal.

Wet smear, Gram-stain revealed numerous white blood cells and mixed organisms, neither Trichomonas vaginalis nor fungus was found. On the endovaginal ultrasonogram, small hypoechoic density cystic space measuring 1.8 x 2.2 cm was found at the left lateral vaginal fornix. The uterus and adnexae were normal. No abnormal pelvic mass could be demonstrated.

Intravenous pyelography revealed an absent left urinary system and compensatory hypertrophic change of right kidney. No vesico-vaginal or uretero-vaginal fistulas could be demonstrated (Fig. 1).

Barium enema was performed revealed normal large bowels and no demonstrable recto-vaginal fistula.

The patient underwent hysterectomy because of persistent markedly foul smelling vaginal discharge and her family was complete. At laparotomy, no left kidney and ureter were present, confirming the findings of previous examinations. There were double uteri, the right side was normal in size but the left one was rather small. The fallopian tubes and ovaries were normal. The small left hemivagina was connected to the left small uterus and filled with purulent discharge. Transabdominal hysterectomy was done with no immediate or late complications.

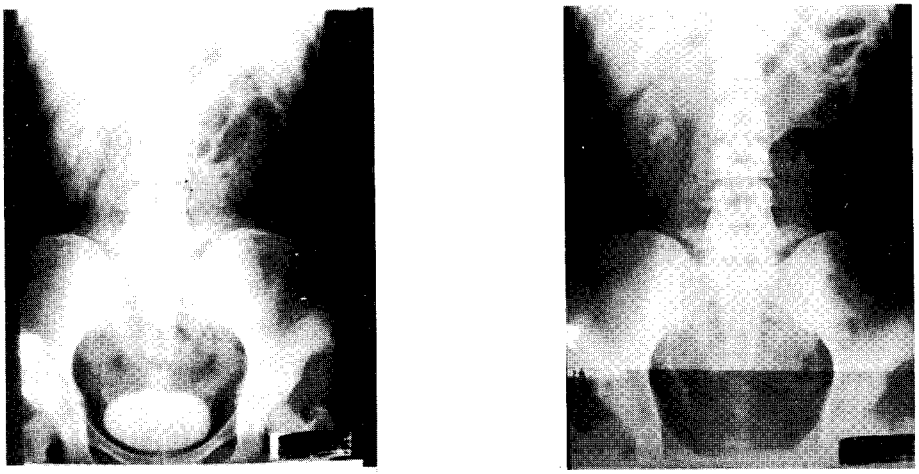

Fig. 1 IVP revealed renal agenesis of left kidney and ureter.

Discussion

At the sixth week of development, both male and female embryos have two pairs of genital ducts[3]. The first pair are mesonephric ducts which run along the lateral side of the mesonephros to the cloaca. The second pair are paramesonephric ducts which run first lateral to the mesonephric duct, but then cross it ventrally to grow caudomedial in direction. In the female embryo, the paramesonephric duct comes to full development and develops into the main genital duct of the female. Its cephalad part develops into the Fallopian tube. Its caudal part fuses with one on the opposite side by the end of the 7th week and finally differentiates into the uterus and upper vagina[3,4]. The growing paramesonephric duct is completely dependent on the mesonephric duct[4]. The ureteric bud is formed by an outgrowth of the mesonephric duct near the opening into the cloaca and penetrates the metanephric blastema which finally develops into metanephros or permanent kidney[3,5]. The failure to form the ureteric bud or make contact of the bud with metanephric blastema may result in agenesis of the kidney on that side[6].

So it is possible that abnormality in the development of the caudal portion of one mesonephric duct may result in failure of ipsilateral kidney development and also involvement of the ipsilateral paramesonephric duct, like in this patient. The incidence of unilateral renal agenesis with paramesonephric duct anomalies was reported as 1/2300 autopsies[7].

The abnormal laterally displaced paramesonephric duct cannot come into contact with the urogenital sinus in the center to form a normal vagina and only a blind sac, i.e. an imperforated or obstructed hemivagina is formed. In this case, we found uterus bicornis bicollis with unilateral hemivagina and the little communication between the two sides of vagina at the level of the vagina. The communication is rather small so it can cause obstruction of the discharge in the blind pouch vagina and become secondarily infected. The communication may be acquired in nature[5].

The presenting symptoms depend on the site of communication such as : progressive dysmenorrhea, foul smelling vaginal discharge, abdominal pain and urinary symptoms. In this case there is only the symptom of persistent vaginal discharge. This symptom and sign may suggest an abnormally developed paramesonephric duct with a communication[5]. No dysmenorrhea can be explained by nonfunctioning endometrium of the affected side.

The anomalies that had unilateral hemivagina were more difficult to be diagnosed. This case had a small cystic mass at the vaginal wall requiring differentiation from more common lesions of the lateral vaginal wall, Gartner's duct cyst. Purulent discharge from the small opening at the vagina or fornix require differentiation from recto-vaginal fistula or

other infection with fistula to vagina.

In cases of unilateral hemivagina, the ultimate goal of treatment is adequate excision of vaginal septum to create a common vagina. The septum should be totally removed or made as wide as possible in one procedure. But in cases of superimposed infection, simple excision of the bulging vaginal septum for drainage the pus must be done initially, and should be followed by complete excision of the septum later on. This procedure is important to facilitate the examination and treatment later on because the patient may be pregnant in the defective side of the uterus. If abortion or term pregnancy does occur, it is difficult to do curretage or vaginal delivery through a small opening or an inadequately excised vaginal septum.

In patients with a single kidney, there is a higher risk than normal. During pregnancy there is a higher incidence of urinary tract infection, this will increase the maternal mortality and the fetal wastage. The remaining kidney is nearly always affected with some chronic renal disease[8].

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Special Article

Cryopreservation of Human Embryos

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Principles of Cryopreservation of Cells

Principles of cryopreservation are similar for living cells. The procedure of cryopreservation includes initial exposure to and equilibration with cryoprotectants, cooling to sub-zero temperatures, storage, thawing, and finally, dilution and removal of the cryoprotectants, with return to a physiologic environment that will allow further development[1,2]. Cells must maintain structural integrity throughout the cryopreservation procedure. Major factors known to affect survival of cryopreserved cells include the species, the developmental stage, the cryoprotectants, and the method of cryopreservation[3].

For the slow cooling method, probably the single most important principle of cryopreservation is that it is necessary to reduce damage caused by intracellular ice formation. The most common method is to remove most of the water from cells before they are cooled. If dehydration is inadequate, large, intracellular crystals of ice may form, which damage cells severely[2,3].

The rapid cooling method normally refers to the techniques of vitrification and ultrarapid freezing. The rapid cooling techniques are still being developed and refined, and may ultimately replace the conventional high-cost and time-consuming slow cooling methods.

Methods for Cryopreservation of Human Embryos

Slow Cooling

The original human embryo freezing techniques involved slow cooling in cryoprotectant solutions to low sub-zero temperatures to avoid the formation of intracellular ice[4]. The cryoprotectants used were dimethyl sulphoxide (DMSO) and glycerol, and the methods were based on those used to cryopreserve embryos of laboratory and domestic animals. Pregnancies and births were reported when early cleavage stage embryos (4-cell to 16-cell) were cooled slowly in 1.5 M DMSO to -40°C[5] or to -80° C[6], and when blastocyst stage embryos were frozen in 8% glycerol to-36°C[7]. The cryoprotectant 1,2 propanediol

(PROH) was also reported to be very successful for freezing pronucleate and early cleavage stage embryos (2-cell to 4-cell) when they were slow cooled in 1.5 M PROH and 0.1 M sucrose to -30° C[8]. While it is difficult to determine which method produces the best result, the majority of IVF clinics presently freeze pronucleate and early cleavage stage embryos by slow cooling in PROH or DMSO[9].

The method for cryopreservation of pronucleate and early cleavage stage embryos in PROH involves exposure of the embryos, at room temperature, to medium containing 1.5 M PROH for 10-15 minutes, and then loading the embryos into freezing straws in medium containing 1.5 M PROH and 0.1 M sucrose[10]. The straws are sealed and cooled at -2°C/minute to -7°C. The straws are seeded at -7°C, slow cooled at -0.3° C/minute to -30°C and then cooled rapidly at -50.0°C/minute to -190°C, before plunging into liquid nitrogen. Embryos are thawed rapidly (30.0°C/minute) by removing the straws from liquid nitrogen and keeping them at room temperature for 40 seconds. The straws are then placed in a water bath at 30° C for 1 minute. The cryoprotectants are removed by stepwise exposure for 5 minutes at room temperature to medium containing 0.2 M sucrose and 1 M PROH, 0.2 sucrose and 0.5 M PROH, 0.2 M sucrose, and finally medium supplemented with 20% human serum. Embryos are then cultured in medium supplemented with 15% human serum. Early cleavage stage embryos are cultured for 2-4 hours prior to replacement, and pronucleate stage embryos are cultured overnight and replaced approximately 24 hours post-thaw.

DMSO may be used to freeze preimplantation embryos of all developmental stages[11]. The embryos are then cooled to -7°C at -2°C/minute, seeded and cooled slowly (-0.3°C/minute) to either temperatures around -30 to -40°C[5] before rapid cooling and storage in liquid nitrogen, or temperatures of -60 to -80° C[6] before storage in liquid nitrogen. Embryos slow cooled to higher sub-zero temperatures are thawed rapidly in a warm water bath (30-37°C), and those slow cooled to lower temperatures are thawed slowly from -80°C to around 0°C at 5-15°C/minute. DMSO is then removed by gradual dilution.

Glycerol is used for later stage embryos, preferably after reaching the blastocyst stage. Embryos can be frozen using the method described by Cohen et al[7]. This involves a stepwise exposure, at room temperature, of the blastocysts to gradually increasing concentrations of glycerol. The blastocysts are then loaded into freezing straws in medium containing 8-10% glycerol. The straws are cooled at -1°C/minute to -7°C, seeded and then cooled at a rate of -0.3°C /minute to -36°C, before being plunged directly into liquid nitrogen. The straws containing blastocysts are thawed by removing them from liquid nitrogen and placing them in a water

bath at 30°C for 1 minute. The cryoprotectant is removed by stepwise exposure of the blastocysts to medium containing gradually decreasing concentrations of glycerol.

The results of human embryo cryopreservation by slow freezing techniques, from 25 member institutes of the Society of Assisted Reproductive Technology in USA, showed the mean number of pronuclear oocytes, early cleavage-stage and blastocyst-stage embryos transferred per pregnancy was 11.5, 16.0 and 46.0, respectively (pregnancy rate per transfer : 17.4, 12.5 and 4.3%, respectively)[12]. These results indicate that pronuclear-stage oocytes have a higher survival rate after freezing than cleavage-stage embryos, a conclusion which has general support among IVF embryologists[13,14]. Concerning the cryoprotectants, it is very difficult to conclude that there is any difference in the success rate of cryopreservation by slow cooling in PROH or DMSO. Comparable results (12.3-14.5% and 16-17% pregnancy rate per transfer)[15,16] were obtained with PROH and DMSO for the cryopreservation of early cleavage-stage embryos, while that of pronuclear oocytes, PROH may produce better results[8]. In addition, combination of sucrose to PROH significantly increased survival of embryos after thawing to 61% compared with 46% without sucrose[17].

Vitrification

A relatively recent approach to achieve rapid freezing without the use of freezing machines is called vitrification. Vitrification is defined as the physical process by which a highly concentrated solution of cryoprotectants solidifies during cooling without the formation of ice crystals. The solid retains the normal molecular and ionic distribution of the liquid state and is called a glass and can be considered to be an extremely viscous supercooled liquid[18]. Vitrification has certain advantages over freezing because it avoids the damage caused by intracellular ice formation and the osmotic effects caused by extracellular ice formation[19]. The theory behind vitrification as a method for cryopreservation has been reviewed thoroughly by Fahy et al[20]. Basically, the vitrification solution needs to consist of one or more cryoprotectants in excess of 40% (v/v)[9]. The original vitrification solution which consisted of 20.5% DMSO, 15.5% acetamide, 10% PROH and 6% ethylene glycol allowed a successful cryopreservation of mouse 8-cell embryos[18]. These solutions are toxic to cells at ambient temperature, so embryos are usually placed in the final concentrated solutions at low temperatures (0-4°C). Revision of the initial composition of vitrification solutions, i.e. combinations of glycerol (6.5 M) and polyethylene glycol (6%) or glycerol (25%) and PROH (25%), have reduced their toxicity and have made the method a little easier to use. Very recently, mouse embryos have been successfully vitrified in a solution composed of 40% ethylene

glycol, 30% ficoll and 0.5 M sucrose[21].

Most of the studies have been done in animal embryos, especially in mice, with encouraging results but there has not been any reports to date on the success of human embryo vitrification. The only report of vitrification of human embryos has been by Quinn and Kerin[22]. They vitrified 22 embryos and of 11 embryos warmed,only one survived and was transferred, but no pregnancy occurred.

Ultrarapid Freezing

In contrast to vitrification, this procedure involves crystallization of extracellular and, maybe, intracellular water. The freezing method is simple and quick, involves no expensive freezing machines. A prerequisite for successful ultrarapid freezing is the presence of a permeating cryoprotectant such as DMSO and a nonpermeating compound usually sucrose[23]. A simple ultrarapid freezing technique developed by Trounson et al[24-26] requires a 3 minute equilibration of embryos in high concentrations of DMSO (3.0 to 4.5 M) and sucrose (0.25 M) before plunging into liquid nitrogen. Embryos are thawed rapidly in a warm water bath (37° C) and the continued development of frozen-thawed embryos in vitro and in vivo is not significantly different to non-frozen embryos[27]. The outcome of ultrarapid freezing is mostly influenced by the cryoprotectant(s) and the time and the temperature of exposure. One-cell mouse embryos, for example, seemed to survive and develop well after being frozen-thawed either in 4.5 M DMSO for 3 minutes at 22°C or in 4.5 M PROH for 5 minutes at 4°C[28]. Concerning chromosomal abnormalities in mice associated with ultrarapid freezing using DMSO, Shaw et al[29] found that 4.5 M DMSO was safe and efficient, while lower concentrations had this detrimental effect.

In human embryos, the results of ultrarapid freezing from the Monash IVF programme, using 3.0 M DMSO and 0.25 M sucrose, have been disappointing; no live birth was obtained in spite of high survival and developmental rates after thawing (7-12% pregnancy rates)[9,30]. However, these success rates have been reproducible and reported from other groups[31,32]. Very recently, ultrarapid freezing using 3.5 M DMSO and 0.25 M sucrose has also been reported to produce comparable results to the conventional controlled rate technique (slow cooling in 1.5 M PROH)[33]. Concerning the cell stage of the human embryos being rapidly frozen, Diotallevi et al[34] reported higher survival rates of 2- 4-cell stage than 6-8 cells stage (82.3% vs 41.8%). Interestingly enough, the first live birth, using an ultrarapid two-step embryo freezing method, has just been reported[35]. The protocol utilized a permeation 1st step of low concentration (1.5 M DMSO) cryoprotectant for 5 minutes followed by a dehydration 2nd step of high concentration (3.5 M DMSO) cryoprotectant for 2.5 minutes, and then

plunged directly into liquid nitrogen. Following storage in liquid nitrogen, embryos were thawed rapidly (6 seconds at 37° C) and removed from cryoprotectant in a gradual stepwise fashion at room temperature and allowed to culture for 24 hours prior to transfer.

Conclusion

Cryopreservation has been widely incorporated into clinical IVF and presently based on slow cooling methods using DMSO and PROH. optimum strategy at the present time is to freeze pronuclear oocytes in PROH, as this achieves pregnancy rate of around 20% of patients transferred embryos. Slow cooling of early cleavage stage embryos in DMSO results in pregnancy rates of 10-15% of patients transferred embryos. For the human blastocyst, slow cooling in glycerol and rapid thawing is the only method reported with success. The rates of survival from freezing and thawing blastocysts are not sufficiently high, however, to justify the losses associated with prolonged in vitro incubation.

New methods of rapid freezing have been developed using mouse embryos. These methods include vitrification and ultrarapid freezing. It is too early to assess their value in human IVF but they are replacing slow cooling methods for cryopreserving animal embryos due to their simplicity and good results.

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